NUTRIENT REQUIREMENTS OF DAIRY CATTLE

Eighth Revised Edition

Committee on Nutrient Requirements of Dairy Cattle

Board on Agriculture and Natural Resources

Division on Earth and Life Studies

A Consensus Study Report of

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Preface

The Committee on Nutrient Requirements of Dairy Cattle, in accordance with the Statement of Task (see Appendix A) developed the eighth revised edition of the *Nutrient Requirements of Dairy Cattle* and accompanying software model. Although extensively revised and in many cases expanded, most chapters in the previous edition (NRC, 2001) are included in this version. Although nutrient interactions abound, the committee maintained the approach of separating discussion (chapters) mainly by nutrient (e.g., energy, protein, minerals), but some chapters discuss specific classes of animals (calves or transition cows). New chapters on production systems, feed by-products, additives, toxic agents, and feed analysis were added. Chapters include a review of the literature (mostly on papers published after 2000) with an emphasis on justification of requirements and equations. The software model was extensively revised from the previous edition to include all of the revisions discussed in the text.

Information in some chapters is not directly used in the software, but they are in-depth reviews of topics related to the nutrition and feeding of dairy cattle. Most chapters include equations that were incorporated into the software to estimate nutrient supply, requirements (or responses), and other outputs that may have value to nutritionists and other users. The availability of the needed inputs was paramount when deciding on which equations to include. The inputs required are usually available from on-farm data or from commercial feed testing laboratories. For some outputs, published equations were evaluated and, if appropriate, incorporated directly into the model (e.g., estimated water consumption). When multiple published equations were available, the committee evaluated the inference space of the equations, the availability of the needed inputs, and fit statistics and chose the ones that it thought were best. In some cases, users are allowed to choose specific equations. The committee attempted to describe strengths and weaknesses of various equations. For other outputs, data from mostly published sources were collated, and new equations were derived from the database

(e.g., milk protein yield responses). Last, in situations where very little data were available, equations may represent simple mean responses (e.g., some mineral requirements). Adequate information is in the text so that users can determine how equations were derived.

As with previous editions, changes were not made to requirements (or recommendations) unless new data or a reanalysis of older data indicated changes were necessary. However, most nutrient requirements underwent at least minor revision. The greatest changes occurred with protein. The protein/amino acid supply and requirement system was completely revised compared to the seventh edition, with much greater emphasis on amino acids rather than protein. Dry matter intake equations were developed for all classes of cattle, and in some cases, include feed factors in addition to animal characteristics. The calf requirement system for protein, energy, and minerals underwent extensive revision. To estimate environmental impact, methane production is estimated, as is manure excretion of nitrogen and phosphorus. The feed composition database is completely revised and includes estimates of variation and ranges for many common feeds. Although this edition is a significant and comprehensive update, substantial gaps in knowledge still exist, and these were pointed out in specific chapters. This was done to not only encourage research in those areas but also indicate why requirements or supply functions were not presented for certain nutrients.

The software does not use stochastic processes; however, estimates of variance for equation coefficients and various fit statistics are included. Users can use that information to determine the amount of confidence they assign to specific estimates. A major goal in the development of the feed composition data tables was to generate accurate estimates of variation by rigorously screening data. For many minerals and vitamins, inadequate data were available to derive accurate estimates of variation, and to indicate the level of uncertainty in those situations the term *dequate Intake* was used in place of *requirement*.

The software model is integral to the book. The interface is similar to the 2001 model, but output has been extensively revised and provides more information than previously and in a user-friendly format. As with all software, the output is only as accurate as the inputs, and users are encouraged to use actual data, rather than defaults, whenever possible. This revision and its accompanying software should be of value to teachers and students of dairy cattle nutrition, field nutritionists and veterinarians, nutrition scientists, and ultimately producers and consumers of dairy products.

Richard A. Erdman and William P. Weiss, *Co-Chairs*
Committee on Nutrient Requirements of Dairy Cattle

Acknowledgments

Summary[1]

Since the publication of the first booklet on animal nutrient requirements in 1945 (the dairy section consisted of 21 pages), the National Research Council's series on the *Nutrient Requirements of Dairy Cattle* has become an essential tool for students, teachers, researchers, and the dairy industry. The seventh revised edition was more than 380 pages. The information, equations, and, more recently, the software included in these publications have helped the dairy industry improve the efficiency of nutrient utilization, reduce the environmental impact of milk production, and improve the health of dairy cows. The updates to this publication generally reflect incremental improvements that match the pace at which new data are generated. Occasionally, some larger-scale changes are made when versions are updated, but the data needed to make these larger changes may have required numerous years to accumulate.

The overarching task for the committee (see Appendix A for the complete Statement of Task) was to develop "a comprehensive analysis of recent research on the feeding and nutrition of dairy cattle including . . . amounts of amino acids, lipids, fiber, minerals, vitamin and water needed by . . . dairy cattle." The committee's goal was to develop a report that would improve the accuracy of predicting animal performance from nutrient inputs and to consider variables that affect requirements. Other specific charges to the committee included developing accurate feed composition tables, reviewing the literature on the value of coproducts of biofuel production for dairy cattle, improving methods to estimate energy values of feeds, and reviewing and proposing strategies to minimize nutrient losses in manure and reduce greenhouse gas production. Last, the committee was charged with updating and expanding the previous computer model.

The committee responsible for producing the eighth revised edition of the *Nutrient Requirements of Dairy Cattle* committed substantial time and effort to reviewing scientific literature; collating, screening, and analyzing data; and finally synthesizing this report and accompanying software. The seventh revised edition served as the starting point, but the committee revised all chapters and added some new sections. Per its charge, the committee evaluated all of the equations and updated or completely replaced them when appropriate. This summary is only an outline of the major changes and updates. To appreciate the full scope of the changes, see the specific chapters.

In Chapter 1, for the first time in the Dairy Requirement series, the meaning of requirement as used in this publication is defined. The term *Adequate Intake* (AI) is introduced, which is used when adequate data were not available to define an actual requirement. The importance of variation in feed and diet nutrient composition and in animal responses is discussed.

Chapter 2 discusses feed intake regulation in dairy cattle at the physiological and biochemical level. Dietary and management factors that influence intake are also discussed. This chapter contains revised equations to estimate intake of growing heifers and lactating cows. A change from the previous version is that some equations contain not only animal factors, such as body weight and milk production, but also dietary factors. The software provides intake estimates using animal only or animal plus dietary factors.

Energy supplies and requirements are discussed in Chapter 3. The net energy system was retained, but the method to estimate dietary concentrations of net energy was modified, with the greatest change from the previous edition being the discount factor related to level of feed intake. Digestible energy is calculated on a feed basis, but metabolizable and net energy are only calculated for the complete diet. Energy requirements for maintenance were increased by about 20 percent, and smaller refinements were made to energy requirements for pregnancy, growth, and lactation.

Chapter 4 discusses fat metabolism with an emphasis on quantifying factors that affect digestibility of fatty acids. Digestibility coefficients for feeds and common fat supplements were rederived based on newer data. The chapter also includes

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[1] Minor factual corrections were made subsequent to the prepublication version of this report on pp. 41, 64, 74, 87, 257, and 434. The changes were made to provide additional clarity on equations and assumptions relative to the model used in this report.

a review of recent literature on production and reproductive responses to fat supplementation. Last, dietary factors affecting milk fat concentration and milk fatty acid profile are discussed along with potential human health responses.

Major dietary carbohydrates (predominantly starch, simple sugars, and neutral detergent fiber) are discussed in Chapter 5. Because carbohydrates are the predominant energy source for dairy cattle, factors affecting both ruminal and total tract digestibility are discussed in detail. Recommendations for total dietary neutral detergent fiber (NDF) and forage NDF concentrations have been updated and are based on dietary starch concentration. A new physically adjusted NDF (paNDF) system is introduced to incorporate diet particle size into fiber recommendations.

Chapter 6 discusses protein and amino acid metabolism, responses, and requirements. This chapter arguably represents the greatest changes from the previous version. The equations to generate microbial protein were revised extensively, and essentially all estimates of rumen-degradable and digestible rumen undegradable protein were updated. Similar to the previous edition, protein requirements were factorialized, but in this revision, requirements for essential amino acids are included. Requirements are presented for net essential amino acids and net protein, which can be converted to metabolizable amino acids and protein using variable efficiencies. Rather than a requirement for milk protein synthesis, response functions were developed that estimate protein yields based on intakes of specific amino acids and energy.

Mineral requirements are in Chapter 7. For most minerals, requirements are expressed on an absorbed mineral basis, and both absorption coefficients and requirements were reevaluated for all minerals. For the first time, absorption coefficients for some minerals (magnesium, phosphorus, and copper) are adjusted based on user inputs rather than using ingredient-specific constants. Generally, most mineral requirements were refined rather than undergoing substantial changes. For some trace minerals, AI rather than requirements is used.

Vitamins are discussed in Chapter 8. AIs are presented only for vitamins A, D, and E, but other vitamins are discussed in detail. The AI for vitamin D is greater than the requirement presented in the previous version, and the AI for vitamin A includes an adjustment for milk yield.

Chapter 9 consists of a discussion of factors affecting water intake, quality guidelines for water, and effects of water composition (e.g., salinity, concentrations of other minerals, and nitrate) on water intake and cow production and health. The chapter includes an extensive review of available equations that can be used to predict water intake, and the software now includes estimated water intake as an output.

Calf nutrition and management are discussed in Chapter 10. Calves are generally defined as animals less than 18 percent of mature body weight or about 125 kg for a Holstein calf. Animals larger than that are considered heifers

and are discussed in Chapter 11. The nutrient requirement system for calves was completely overhauled. Equations for requirements for metabolizable protein, metabolizable energy, and minerals were derived from newer data and more accurately predict animal response than those in the previous version. An equation to estimate starter intake was also derived. Maintenance requirements are now adjusted for environmental temperatures outside the thermoneutral zone of the calf. Equations to estimate the metabolizable energy value of feeds when fed to young calves are included.

Chapter 11 is dedicated to a discussion of growing replacement heifers, which are defined as animals with a body weight >18 percent of mature weight (approximately 125 kg) until either 60 or 21 days prepartum depending on user inputs. Structural errors in equations to predict body composition were eliminated, and the overall equations were simplified. A much larger database was available to develop body composition equations than was available to the previous committee. Hence, estimates of changes in body composition (fat and protein) will be more accurate, resulting in more accurate estimates of energy and protein requirements for growth.

Chapter 12 discusses physiological and metabolic changes that occur as cows or heifers transition from the gestating state to the lactating state. Nutrient requirements unique to this class of animals are in this chapter along with equations to estimate intake during the dry and late-gestation period. The equations to estimate intake have been improved compared to the last version and incorporate some dietary factors in the prediction. The chapter includes an up-to-date review of health disorders that occur in the peripartum period and nutritional and management practices that can be used to reduce the prevalence of those disorders. The impact of colostrum synthesis in late gestation on nutrient needs is discussed but not incorporated into the model.

Various dairy production systems are discussed in Chapter 13, including organic milk production, grazing systems, and robotic (or automatic) milking systems. The emphasis in this chapter is on how the various systems may influence nutrient requirement or nutrient supply differently from what is discussed in previous chapters. For example, organically managed dairy cows do not have different requirements than other cows, but because of economics and available ingredients, supply of nutrients may differ. Energy requirements are greater for grazing cows than cows housed in confinement (assuming equal body weight and milk production) because of the energy expended walking and gathering food (pasture). These equations were changed from the previous version because of a larger database, and the equations should be more accurate. Best nutritional management practices for automatic milking systems are discussed, as is the effect of genetically modified feeds on milk production and composition.

The direct environmental impacts of milk production and mitigation strategies are discussed in Chapter 14. Equa-

tions to estimate methane production (also used to estimate metabolizable energy) for both nonlactating and lactating animals are included in the text and the computer model. Equations to estimate nitrogen and phosphorus excretion in manure are included (excretion of many other minerals is estimated based on estimated mass balance). Equations do not include functions related to mitigation methods, but the chapter contains information on current best practices to reduce the environmental impact of dairy farming.

Material in Chapters 15, 16, and 17 is not directly used in the model; however, these chapters are up-to-date reviews on important topics relevant to the nutrition and management of dairy cattle. Production methods, nutrient composition, and animal responses to important by-product feed ingredients are in Chapter 15. Numerous by-products are discussed, but feed ingredients derived from biofuel production are emphasized, including various types of distillers grains. Chapter 16 discusses commonly used feed additives that are allowed to be fed to dairy cattle in the United States (as of 2020). Some additives such as monensin, essential oils, and direct fed microbial can affect digestibility and metabolic efficiency in some situations, but these effects were not included in the model. Users can use the information in that chapter to manually adjust output if desired. Chapter 17 discusses naturally occurring compounds found in some feedstuffs that can have adverse effects on dairy cattle. Examples include alkaloids (e.g., fescue toxicity), mycotoxins, and gossypol. Toxicities derived from consumption of feeds contaminated with certain bacteria such as *Clostridium botulinum* and *Escherichia coli* O157:H7 are also discussed. Effects of anthropogenic agents and excess mineral intake are not included.

Chapter 18 is a new addition. It includes information on assays needed for inputs in the model. The chapter includes appropriate citations with detailed descriptions of recom-mended assays along with potential sources of error. It also includes chemically based assays (e.g., crude protein), mechanical-based assays (e.g., particle size to calculate physically adjusted neutral detergent fiber), and biologically based assays (e.g., in situ protein digestibility kinetics).

The feed composition data in Chapter 19 is completely new and was generated specifically for this publication. Data were provided by four major commercial laboratories (see Acknowledgments) and then underwent a rigorous screening method to identify mislabeled feeds. Many of the mean nutrient concentrations for individual feed ingredients in Chapter 19 will be similar to other feed composition tables reported in previous revisions. However, the standard deviations may differ greatly from other tables. Because of the rigorous screening that was applied to these data, the standard deviations found in this chapter are likely more accurate representations of the true variation in nutrient composition.

Chapter 20 lists all of the equations in the model used to calculate nutrient supplies, requirements, and responses. For some equations, additional text is included describing how the equations were derived. However, for most equations, that information can be found in the specific nutrient chapters. Model evaluation is also discussed in this chapter.

Last, Chapter 21 includes tables of recommended nutrient supplies and dietary concentrations for different classes of dairy cattle. Because numerous factors affect requirements and nutrient supply, the data in those tables represent very specific situations. Nutrient supplies are calculated assuming "typical" diets with model-estimated dry matter intake. Requirements are for very specific animal conditions (e.g., milk yields, body weight, growth rates). The software, with user-specific dietary and animal inputs, will yield more accurate assessments of nutrient supply and requirements than the tables.

1

Defining Requirements

INTRODUCTION

Previous editions of the *Nutrient Requirements of Dairy Cattle* (e.g., NRC, 2001) reported requirements for various nutrients in dairy cattle without specifically defining what the term "nutrient requirement" actually meant. A simple definition of a dietary nutrient requirement is the daily amount of a nutrient necessary to meet a healthy animal's needs for maintenance, activity, growth, reproduction, and lactation without any change in body reserves or status. That definition implies that the requirement for each nutrient is based on physiological factors and environmental conditions that drive the need for that nutrient. Nutritional needs differ when animals are not in good health but adequate data are generally not available to quantify a "health" requirement; therefore, preservation of good health is considered a component of maintenance. Cows, similar to most mammals, must mobilize body reserves to support lactation during the early postpartum period. Later in lactation, mobilized nutrients must be replenished, and those needs should be considered a requirement.

Conceptually, the term "requirement" suggests that there is a fixed amount of a nutrient required by an animal where no further increase in performance will occur when an animal is fed an additional amount of that nutrient. This principle is the basis for the use of breakpoint analysis to determine a nutrient requirement (Robbins et al., 2006; Pesti et al., 2009). However, animal performance responses to a nutrient seldom follow that pattern. Rather, the typical performance responses to increasing nutrient intake are curvilinear, where increases in animal performance occur at a diminishing rate to increasing nutrient intake (Bath, 1975; Pesti et al., 2009; Liu et al., 2017). In this case, the desired amount of nutrient intake would likely be based on the economic return to an increment in nutrient intake rather than a fixed requirement.

The requirements for individual nutrients provided in previous editions of the *Nutrient Requirements of Dairy Cattle* actually represent the average responses of individual dairy cattle or groups of dairy cattle that were fed varying amounts of nutrients. Within any given group of dairy cattle, inherent variability exists in the response of individual animals to a given increment in nutrient intake. Sources of this variation include measurement error, stage of lactation, milk production, body weight, and numerous other differences among individual cattle within a group, as well as true differences among individual cattle that are phenotypically similar. Because of the inherent variance in the requirements among dairy cattle, the use of the term "minimum requirement" has become obsolete.

To overcome some of the problems with the use of minimum requirements, the National Academies of Sciences, Engineering, and Medicine's Food and Nutrition Board within the Institute of Medicine (IOM)[1] has adopted a new set of nutrient standards (IOM, 2006) collectively referred to as Dietary Reference Intakes (DRIs). In that system, the following terms and definitions are used:

1. The estimated average requirement (EAR) is defined as the average daily nutrient intake estimated to meet the requirements of half of the healthy individuals in a particular life stage and gender group.
2. The Recommended Dietary Allowance (RDA) is the daily dietary nutrient intake sufficient to meet the requirements of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group.
3. Adequate Intake (AI) is the average daily nutrient intake that a panel of experts determined should meet or exceed the requirements of a specific group (or groups) based on limited experimental data; AI is used when an RDA cannot be determined.
4. Tolerable Upper Intake Level (UL) is the highest average daily nutrient intake that is likely not to pose a risk of adverse health effects to almost all individuals in the general population.

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[1]As of March 2016, the Health and Medicine Division continues the consensus studies and convening activities previously carried out by the Institute of Medicine (IOM).

In the DRI system, an RDA can only be determined when the EAR and the variability of the EAR (typically expressed as a coefficient of variation) can be determined. Determination of an EAR requires many feeding experiments in which varying nutrient intakes have been used. The RDA is the EAR plus 2 standard deviations, so that 97 to 98 percent of the individuals in a population that consume the RDA will have sufficient nutrient intake to meet their individual needs. By feeding to meet the needs of 97 to 98 percent of the population, the possibility that an individual will be underfed is almost nil, but the majority of individuals within the population will consume excess nutrient. In the DRI system, the only exception to feeding to meet 97 to 98 percent of the population is for energy requirements, in which even a moderate excess in energy intake over the long term can have severe negative impacts on health such as obesity and type 2 diabetes. For many nutrients, adequate data are available to establish an EAR, but because of factors such as different response measurements and different experimental designs among studies, inadequate data are available to obtain an accurate estimate of the standard deviation. In those situations, the RDA is equal to 1.2 times the EAR because the coefficient of variation in energy metabolism in similar humans is about 20 percent, and variation in metabolism of other nutrients was assumed to be similar to that of energy. In the DRI system, requirements expressed as AI are reserved for situations when an EAR cannot be determined. Typically, this is where insufficient numbers of feeding experiments have been conducted with the target species, and the variability of the response to a nutrient is so great that an EAR and hence the RDA cannot be determined. Finally, the UL is conceptually similar to maximum tolerable levels (MTLs) reported for minerals (NRC, 2005) and vitamins (NRC, 1987) in animal nutrition. However, UL includes an "uncertainty factor" so that the UL is below the level (sometimes much lower) at which an adverse effect may be observed. The MTL is the level at which an adverse (but not necessarily toxic) effect was observed.

The committee adopted an approach similar to the DRI system in establishing the nutrient requirements for dairy cattle. When the term "requirement" is used in this publication, it is equivalent to the EAR used in the DRI system and reserved for nutrients in which the average requirement is known with confidence. When possible, measures of variation were included in the text. The variation might be determined among treatment means, among animals within studies, or from a meta-analysis or by regression analysis where the mean predicted response and the standard error of the estimate of the predicted response have been determined. Similarly, in this report, when a suggested feeding amount of a nutrient is expressed as an "Adequate Intake," it means that insufficient data have been collected to determine an estimated average requirement. For some of the vitamins and trace elements, there are currently insufficient data available to determine an average requirement. In many cases, this is due to the wide variability in nutrient availability or the lack of sufficient stud-

ies with multiple feeding levels to determine an EAR and its variance. The committee does not specify an equivalent to an RDA (i.e., a safety factor of 2 standard deviations) for dairy cattle because of limited data and because of the economics of dairy production. For several nutrients, inadequate data are available to estimate the standard deviation of the response to nutrient supply. Furthermore, meeting the equivalent of an RDA (i.e., requirement plus 2 standard deviations) may cause the diet to exceed the MTL for some nutrients.

Feed is the largest single expense in raising and caring for dairy cattle. The ingredients used and the nutrient composition of diets have large effects on the economics of dairy production. For some nutrients that are relatively inexpensive to supplement, such as certain vitamins and trace minerals, the cost of feeding to meet 97 to 98 percent of the animals in a group would be low. However, for macronutrients such as energy, protein, and some of the macrominerals, the cost of such an approach would be high. Depending on the nutrient, feeding sufficient amounts to cover 97 to 98 percent of the cows within a group would likely be uneconomical, may cause environmental issues by excess excretion of the nutrient in the manure, and, in the case of energy, result in overconditioned cows.

REPORTING AND APPLICATION OF THE REQUIREMENTS

The majority of the requirements in this publication are reported as absorbed (minerals) or metabolizable (energy, protein, and amino acids) nutrient intakes. Examples include metabolizable energy, metabolizable protein and amino acids, and absorbed minerals. When nutrients are expressed this way, a reliable means to estimate nutrient supply in the same terms is required. For some feed ingredients and nutrient classes, the ability to predict nutrient availability is inadequate, and this is discussed in the chapters on individual nutrients.

Similar to previous reports (NRC, 1987, 1989, 2001), a factorial system has been used to express the requirements for most nutrients according to physiological function and the amount and composition of production. This is discussed in detail in various chapters, but using energy requirements as an example, maintenance energy requirements are based on an animal's body weight and include a fixed adjustment assumed to account for normal activity for cattle that are not grazing. An activity allowance based on topography and distance to the milking center is included if cows are grazing. Energy requirements for growth are based on an animal's growth rate and the composition of growth. Energy requirements for reproduction are based on the stage of gestation and size of the fetus and uterus. Finally, energy requirements for milk production are based on the amounts and composition of the milk produced. The animal's total energy requirements are the sum of the individual requirements for maintenance, growth, reproduction, and milk production.

By inclusion of both the average requirement and, when possible, measures of its variability, the committee has laid the groundwork for incorporating some of the principles identified in the human DRI system. This variability may or may not have been captured if the average requirement had been based on group means or pen feeding experiments reported in the literature as compared to using individual animal data. Even when experimental treatments were applied to individual animals, the experimental design impacts reported variation. Reported variation is greater from experiments using completely randomized designs as compared to designs in which individual animal variation is removed (e.g., Latin square designs or designs with covariance in the statistical model). This has the greatest impact when an index of variance is used in weighting study effects in the meta-analysis to determine the response to a nutrient. In addition, reported variation includes not only true animal-to-animal variation but also measurement variation, which for some responses can be quite high.

In addition to variation in requirements among individual animals, uncertainty with respect to diet composition needs to be considered before an RDA approach can be used. Recent publications have documented the uncertainty in the knowledge of feed ingredient composition. Some of the uncertainty in feed composition is due to the mislabeling of feeds being submitted for analysis to feed analytical laboratories that were used as a source of data for feed composition tables (see discussion in Chapter 19). The degree of variation in nutrient composition varies greatly among feed ingredients; some ingredients are consistent enough that sampling is not required, whereas composition of other feeds is so variable that frequent sampling is needed to ensure that the nutrient content of the diet can be verified (St-Pierre and Weiss, 2015). Day-to-day variation was the greatest source of variability in diet dry matter concentrations, whereas individual farm, month-to-month, and sampling was the greatest source of variation for other nutrients (St-Pierre and Weiss, 2015). Because of the expense of feed analysis, strategies for ingredient sampling and analysis have been proposed (St-Pierre and Cobanov, 2007) such that feeds and nutrients within feeds that have high inherent variation in composition are analyzed more frequently.

Estimates of variability in nutrient requirements and feed composition could be incorporated into multiobjective diet formulation procedures in the future so that users can set a specific probability that dietary nutrient constraints (requirements) are met (i.e., stochastic formulation). Diet formulation procedures based on the cost and uncertainty of ingredient nutrient composition have been identified (St-Pierre and Harvey, 1986a,b; Tozier and Stokes, 2001). In general, these approaches result in greater numbers of individual feed ingredients and selection of feeds with lower inherent variability in nutrient composition being incorporated into the diet (St-Pierre and Harvey, 1986a,b) and often increase the cost of the diet depending on the risk (or probability of meeting requirements) one is willing to accept. Some of the increased cost could be balanced against increased production because of reduced variability in diet nutrient concentrations. The optimal amounts of nutrients to be fed depend on the production responses to nutrient intakes in relation to their cost, the uncertainty in the knowledge of the actual nutrient concentrations of the feeds within a diet, and the variability in the requirements among dairy cattle within the group (St-Pierre and Harvey, 1986c; Cabrera and Kalantari, 2016).

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2

Dry Matter Intake

INTRODUCTION

The control of dry matter intake (DMI) is complex, with mechanisms to ensure an adequate supply of energy as well as to prevent its overconsumption. Various stimulatory and inhibitory signals are integrated in feeding centers of the brain to affect feeding behavior, which determines DMI. Stimulatory signals are related to energy status as well as various sensory, social, circadian, and habitual factors; inhibitory signals include those related to ruminal distension, rumen osmolarity, endocrine effects, and fuel sensing by tissues. Whereas DMI is related to energy required for milk production, maintenance, and change in body reserves, it is also affected by the interaction of diet and physiological state. Understanding factors affecting DMI is essential to optimal ration formulation.

Physiological state and nutrient requirements vary greatly as dairy cattle cycle through lactation. Their diets include forages for proper rumen function, and forage fiber digests and passes from the rumen more slowly than other dietary components. Therefore, DMI by dairy cattle can be limited by distention of undigested residues in the gastrointestinal tract. In the immediate postpartum period, cows are in negative energy balance, but neither the filling effect nor the energy content of rations can be altered to eliminate this. Distention likely begins to control DMI when milk yield (MY) and energy requirements increase in the weeks following parturition and likely limits DMI through peak lactation. As MY declines through lactation, distention usually becomes less limiting, and mechanisms related to specific fuels dominate control of DMI.

PHYSICAL LIMITATIONS

Early investigations of the control of DMI in dairy cattle focused on physical limitations as Lehman (1941) suggested that DMI is limited by the ballast of undigested feed residues in the gastrointestinal tract. Research related to physical limitations to DMI has focused on forage characteristics that affect gut fill, the site of distention, and the mechanism by which distention limits intake. Feed intake was positively related to forage digestibility with a decreased response as digestibility increased (Blaxter et al., 1961), suggesting that distention from gut fill limits DMI less as digestibility increases. The reticulorumen is generally regarded as the site within the gastrointestinal tract at which distention limits DMI (Campling, 1970; Baile and Forbes, 1974). Tension receptors that respond to distention are located primarily in the reticulum and cranial sac (Leek, 1986). The mechanism by which distention limits DMI is likely via transmission of signals from these tension receptors to brain feeding centers rather than a limitation by volume per se. This is supported by dose-dependent decreases in DMI from additions of inert fill (water- or air-filled bladders, plastic cubes, etc.) into the reticulorumen in several experiments, as reviewed by Allen (1996). In addition, distention in the reticulorumen is determined by both volume and weight of contents; DMI was reduced 112 g for each kilogram of weight and 157 g for each liter of volume that was added to the reticulorumen as inert fill (Schettini et al., 1999). Furthermore, Dado and Allen (1995) reported a reserve volume of 16 L in the reticulorumen of lactating cows consuming a fill-limiting diet, indicating that additional capacity for volume may exist even when distention in the reticulorumen limits DMI.

The abomasum might also be involved in the physical limitation of DMI. Abomasal infusion of methylcellulose decreased DMI by sheep (Grovum and Phillips, 1978), and distention of the abomasum of sheep with water-filled bladders resulted in a dose-dependent reduction in DMI (Grovum, 1979). In addition, increasing abomasal emptying rate by suppressing the pyloric gate by pylorectomy and pyloroplasty increased DMI by sheep (Malbert and Ruckebush, 1989). Abomasal distention might generate a satiety signal because mechanoreceptors in the abomasal epithelium have

been described that discharge with distention (Harding and Leek, 1972). Alternatively, restricted flow from the abomasum might limit DMI by decreasing flow from the rumen and increasing ruminal distention. Regardless of the site at which the signal is generated, it is clear that DMI can be limited by distention within the gastrointestinal tract.

The filling effect of forages was reported to be related to fiber mass (Balch and Campling, 1962), and intake of forages was more highly related to their neutral detergent fiber (NDF) concentration than to other chemical measures (Van Soest, 1965). Although NDF concentration has been used as the only ration characteristic to predict DMI by dairy cattle consuming mixed rations of forages and concentrates (Mertens, 1987, 1994), there is substantial evidence that NDF alone is inadequate. Forage NDF has a longer ruminal retention time than other dietary components, including NDF from other sources. Retention time is longer because of longer initial particle size and greater buoyancy in the rumen over time (Allen, 2000). Although most studies report a significant decrease in DMI as forage NDF increased, the DMI response was variable, likely depending on the degree to which intake was limited by undigested feed residues in the rumen and the filling effect of the forage NDF (Allen, 2000).

Increasing ration NDF content by substituting nonforage fiber sources (NFFS) for concentrate feeds has shown little effect on DMI (Allen, 2000). NFFS include by-product feeds such as soyhulls, beet pulp, and distillers grains. Fiber in NFFS is less filling than forage NDF because it is less filling both initially (smaller particle size) and over time because it passes from the rumen more quickly and is often more quickly digested. Therefore, the forage NDF content of rations containing both forages and concentrates is a more important measure related to DMI than total NDF content.

Digestibility of NDF varies among forage type (e.g., grasses versus legumes) and decreases as forages mature and become more lignified. Greater NDF digestibility of forages measured in vitro or in situ was related positively to DMI and MY of lactating cows; a one-unit increase in NDF digestibility corresponded to a 0.17-kg/d increase in DMI and 0.25-kg/d increase in fat-corrected milk (Oba and Allen, 1999b). Within a forage type, NDF that is more fermentable clears the rumen faster and is less filling, allowing greater DMI when limited by distention. However, this applies only within forage type; NDF from perennial cool-season grasses is generally much more digestible than NDF from legumes but is also more filling and more likely to limit DMI, as discussed below.

The filling effect of forage NDF is also affected by initial particle size; decreasing particle size of forages by grinding and pelleting generally increases DMI with a greater intake response by pelleting low-quality forages compared with high-quality forages (Minson, 1963). Long-forage particles are required to form the rumen mat that functions to selectively retain small fibrous particles in the reticuloru-

men, increasing their digestibility (Grant, 1997). However, experiments that have evaluated effects of forage particle size have generally shown only small effects on DMI of lactating cows (Allen, 2000), probably because initial particle size was sufficient to form a rumen mat.

The filling effect of forage NDF is affected by the fragility of forage particles, which affects the rate of reduction in particle size from chewing during eating and ruminating (Poppi et al., 1981). Faster particle size reduction will increase the mass of particles below the threshold size to pass from the reticulorumen as well as decrease the ability of the rumen to selectively retain those particles by decreasing the size of the rumen mat. Although NDF of perennial grasses is less lignified and more digestible than NDF of legumes, DMI is generally lower for perennial grasses than legumes (Oba and Allen, 1999b). Grasses are more filling because retention time in the rumen is greater (Voelker-Linton and Allen, 2008; Kammes and Allen, 2012a,c). Forage fragility is likely a more important factor affecting DMI than in vitro NDF digestibility when comparing legumes and grasses. However, within forage type, in vitro NDF digestibility is likely related to forage fragility. Increased alfalfa maturity decreased passage rate of indigestible NDF and increased ruminal NDF pool size, which became a greater limitation to DMI as MY of cows increased (Kammes and Allen, 2012b). Because in vitro NDF digestibility is not related to DMI across forage family (i.e., grasses, legumes), in vitro NDF digestibility should be used to compare within forage type only.

The extent to which ruminal distention limits DMI of lactating cows is related to their energy demands determined primarily by MY (Oba and Allen, 1999a; Voelker et al., 2002); DMI is also affected by factors unrelated to the filling effect of rations, as discussed below.

METABOLIC CONTROL

Conrad et al. (1964) proposed that as diet digestibility increases, there is a breakpoint in digestibility at which limitation of DMI by physical fill in the gastrointestinal tract is replaced by satisfaction of energy demand. The "energostatic" control of food intake proposed by Booth (1972) suggested that animals eat to balance energy consumed with energy required. Later, Weston (1996) proposed that the strength of hunger signals is related to the magnitude of the energy deficit. However, these theories fail to explain the negative energy balance in the peripartum period when distention is not a factor limiting DMI. The concept that DMI is limited by energy supplied in excess of energy demand has some merit, but the mechanism is complex and only loosely controlled when based on energy per se.

Mayer (1953) proposed the glucostatic theory of the control of food intake based on research with nonruminant species. However, glucose has little effect on DMI of ruminants; glucose infused intravenously in cows, intraperitoneally in

heifers, intracerebroventricularly in calves, abomasally in lactating cows, and intraportally in sheep had no effect on DMI (Allen, 2000). However, various fuels derived from the fermentation and digestion of feeds can affect DMI of ruminants. Hypophagic effects of propionate infusions have been documented extensively for ruminants (Allen, 2000). Propionate is more hypophagic than acetate or butyrate when infused into the portal vein of sheep (Anil and Forbes, 1980) or mesenteric vein of steers (Elliot et al., 1985). Although propionate might be expected to decrease DMI compared with acetate because it has a higher energy content, propionate linearly decreased total metabolizable energy (ME) intake (diet plus infusates) compared with acetate in lactating cows when infused intraruminally as iso-osmotic mixtures (Oba and Allen, 2003d). These studies suggest that animals probably do not consume feed to meet their energy requirements per se but rather have fuel-specific mechanisms affecting satiety and hunger.

Signal from the Liver

Russek (1963) introduced the idea that the liver is involved in the control of food intake. The liver is in a unique position to monitor changes in fuel metabolism to control eating behavior because of its central role in energy metabolism of animals (Friedman and Stricker, 1976). It is likely involved in regulation of intake because hepatic vagotomy eliminated hypophagic effects of propionate infusion in sheep (Anil and Forbes, 1988) and hypophagic effects of fatty acid (FA) oxidation in rats (Scharrer, 1999). Research with nonruminants suggests that meals can be terminated by a signal carried from the liver to the brain via afferents in the vagus nerve that are affected by hepatic oxidation of fuels and generation of adenosine triphosphate (ATP; Langhans and Scharrer, 1992; Friedman, 1995). The liver is likely to be the primary sensor of energy status because it is a key anabolic organ with the unique advantage of sensing energy supply relative to energy demand (Allen and Bradford, 2012). The signal from the liver can be both stimulatory and inhibitory, depending on the firing rate of vagal afferents; firing rate is increased as energy status decreases, stimulating feeding, and is decreased when energy status increases, inhibiting feeding (Friedman, 1997). Energy requirements of the liver vary over the long term of weeks to months to meet needs for growth and lactation. However, hepatic oxidation of fuels and production of ATP can vary greatly over minutes, affecting feeding behavior and DMI. Normal fluctuations in liver energy status within days might stimulate both hunger and satiety depending on the balance between energy production and utilization. Allen et al. (2009) applied this metabolic control mechanism to ruminant animals and called it the hepatic oxidation theory (HOT) of the control of DMI. Because the liver utilizes fuels from the diet as well as those mobilized from tissues, the control of DMI and energy partitioning are inextricably linked (Allen, 2014).

Acetyl-CoA is the metabolic crossroad that all fuels must be converted to for complete oxidation. However, some fuels are also anaplerotic and can stimulate oxidation of acetyl-CoA in the tricarboxylic acid (TCA) cycle. Whereas some are obligatorily anaplerotic (e.g., propionate, lactate, glutamate), alternate pathways exist for others (e.g., glycerol). Propionate flux to the liver increases greatly during meals (Benson et al., 2002) and is likely a primary satiety signal. Whereas propionate is extensively metabolized by the ruminant liver, there is little net metabolism of acetate (Reynolds, 1995). Ruminant liver has high activity of propionyl-CoA synthetase but not acetyl-CoA synthetase (Ricks and Cook, 1981; Demigne et al., 1986) necessary for activation and subsequent metabolism, thus explaining differences in hypophagic effects of infusions of propionate and acetate in ruminants. Glycerol, like propionate, is a 3-carbon glucose precursor but is less likely to enter the TCA cycle and stimulate oxidation because it enters the gluconeogenic pathway at glyceraldehyde-3-phosphate. Abomasal infusions into lactating cows of propionic acid reduced DMI compared with glycerol infusions (Gualdrón-Duarte and Allen, 2017).

Carbohydrates

Cereal grains that are highly fermentable in the rumen can depress DMI of lactating cows; DMI is reduced when less fermentable grains are replaced with more fermentable grain (Allen, 2000; Oba and Allen, 2003b). Depression of DMI by highly fermentable starch sources likely occurs because greater ruminal propionate flux to the liver stimulates hepatic oxidation and generation of ATP, reducing meal size. In contrast, when site of starch digestion is shifted postruminally, a positive response in DMI is likely. This is because the fuels absorbed do not stimulate hepatic oxidation to the same degree as propionate and because the transit time from the rumen to the intestines significantly delays fuel absorption. Starch escaping the rumen is digested to glucose, which is absorbed and partially metabolized to lactate. DMI was reduced by both propionic acid and lactic acid treatments but not by glucose, and only propionic acid decreased ME intake when isocaloric solutions were infused to the abomasum of cows in the postpartum period (Gualdrón-Duarte and Allen, 2018). Whereas both propionate and lactate are anaplerotic, liver uptake of lactate is lower than propionate (Reynolds et al., 2003), which might explain different hypophagic effects.

Sugars are highly fermentable in the rumen, but hypophagic effects have not been reported, and they often increase DMI (Oba, 2011). Sugars are generally fermented to butyric acid, which is preferentially oxidized by ruminal epithelia (Weigland et al., 1975). The pectin content of feeds is low, and its effect on DMI is unknown. Whereas pectin is highly fermented in the rumen, most strains of pectin degrading rumen bacteria produce acetic and formic acids and relatively little propionic acid (Dehority, 1969).

Fat

Three review papers were available to examine the effect of various fat sources on DMI (Allen, 2000; Rabiee et al., 2012; Weld and Armentano, 2017). These partially overlapping reviews are consistent in demonstrating that supplemental fats can decrease DMI, but the effects vary by source. A meta-analysis by Weld and Armentano (2017) indicated that saturated fats containing long-chain FAs (LCFAs) tended to increase daily DMI (0.7 kg/3 percent added fat), whereas most other fats significantly decreased DMI (−0.8 to −1.3 kg/3 percent added fat). Exceptions were saturated fats with high palmitic acid content, which did not affect DMI, and medium-chain FAs (MCFAs), which decreased DMI 3.5 kg/3 percent added fat. Increased DMI by saturated fats might be from decreased propionic acid if fats are substituted for cereal grains in rations. Greater decrease in DMI for unsaturated compared with saturated fats is consistent with previous meta-analyses (Allen, 2000; Rabiee et al., 2012). Infusion of FAs into the duodenum depresses DMI if oleic, linoleic, and linolenic acids are present in the mixture but not when palmitic and stearic acid predominate (Drackley et al., 1992); therefore, the effects of feeding unsaturated FA may be explained by postruminal effects (Allen, 2000). Intake depression by unsaturated FA might be related to greater release of cholecystokinin (CCK; Allen, 2000), greater extraction and oxidation in the liver (Allen, 2000), or greater oxidation in enterocytes (Langhans, 2008) compared with saturated FA. The gut peptide CCK may affect satiety centrally or by decreasing rumen emptying, increasing distention. Unsaturated FA increased plasma CCK concentration and decreased DMI compared with saturated FA (Relling and Reynolds, 2007; Bradford et al., 2008), and a CCK receptor antagonist prevented intake depression by a diet high in unsaturated FA (Choi et al., 2000). The large reduction in DMI by MCFAs go directly to the liver from the portal-drained viscera, whereas LCFAs are absorbed in the lymphatic system, and unlike LCFAs, MCFAs do not require protein-mediated transport to cross the mitochondrial membrane (Papamandjaris et al., 1998). However, MCFAs also reduce NDF digestion and could enhance rumen fill effects to limit DMI as well.

Protein

Effects of dietary crude protein (CP) concentrations on DMI are variable and likely from a combination of physical and metabolic mechanisms (Allen, 2000). Increased ration CP increased daily DMI an average of 0.6 kg per percentage unit of CP in 7 of 25 comparisons reported in the literature (Allen, 2000). Roffler et al. (1986) evaluated treatment means from the literature and reported that the marginal response in DMI declined as the CP content of the rations increased. Positive responses in DMI from increased ration CP content is likely partly due to effects of increased rumen degraded protein effects on ruminal digestibility of feeds (Oldham, 1984) and a reduction in distention as NDF and dry matter (DM) digestibility increase. Although the marginal response in diet DM digestibility from increased ration CP content decreased as the CP content of the ration exceeded 15 percent, it remained positive when ration CP content exceeded 20 percent (Oldham, 1984). Improvements in digestibility of NDF and DM are likely to have greater effects for cows in which control of DMI is dominated by distention. However, positive effects of ration CP content on DMI might also occur when DMI is limited by hepatic oxidation when protein is substituted for starch (Allen, 2000). In addition, if higher CP increases MY this will increase clearance of metabolic fuels from the blood, delaying hepatic oxidation, potentially increasing DMI (Allen, 2014).

Higher dietary CP can increase DMI; however, excessive nonprotein nitrogen can reduce DMI (Conrad et al., 1977). In addition, excess amino acid (AA) and AA imbalances will increase deamination of AA as well as anaplerosis, stimulating oxidation of acetyl-CoA, potentially suppressing DMI (Allen, 2014). Supporting this, a meta-analysis showed that abomasal infusions of casein reduced DMI of cows when supply of metabolizable protein (MP) balance was positive and increased DMI of cows when MP balance was negative (Martineau et al., 2016). Excess AA (when MP supply was positive) likely resulted in greater deamination of AA, stimulating hepatic oxidation and satiety, which was much less likely when MP supply was negative. Besides increased anaplerosis from the AA carbon skeletons, the ammonia from deamination requires detoxification by urea synthesis, which generates an additional carbon skeleton, further contributing to oxidative metabolism in the liver (Oba and Allen, 2003e). Consistent with this, ammonium, compared with sodium, increased the hypophagic effects of propionate when infused intraruminally in lactating cows (Oba and Allen, 2003e). Meta-analyses have shown that supplementation of rumen-protected methionine has had variable effects on DMI dependent on its chemical form and the main forage source of the diet (Patton, 2010; Zanton et al., 2014). Supplying limiting AA will likely decrease anaplerosis and potentially increase DMI depending on the next most limiting AA and the extent to which hepatic oxidation is limiting DMI (Allen, 2014).

Interaction with Physiological State

Feeding behavior response to diet is also affected by physiological state. Variation in the hypophagic effects of propionate might be related to the balance between flux of propionate to the liver and rate of utilization of propionate for gluconeogenesis, affected by glucose (i.e., lactose synthesis) demand (Allen, 2000). The maximum rate of gluconeogenesis at any point in time is regulated by hormones such as insulin and glucagon, and increased glucose demand is expected to increase gluconeogenic capacity (McGrane, 2000). According to the HOT, when glucose demand is

high, gluconeogenesis increases, and this should decrease TCA cycle activity and oxidation of acetyl-CoA, resulting in greater meal size. Conversely, when glucose demand is low, TCA activity is increased, generating ATP, resulting in satiety and smaller meal size. In support of this, the marginal depression in DMI from propionate infusion was positively related to plasma glucose concentration of cows (Oba and Allen, 2003c).

Hyperlipidemia in the periparturient period is common, and uptake of nonesterified FAs (NEFAs) by the liver increases greatly (Reynolds et al., 2003), resulting in increased FA oxidation, buildup of acetyl-CoA, and hepatic export of ketone bodies. Intake might be suppressed by hepatic FA oxidation and generation of ATP. The negative association between body condition score (BCS) at parturition and DMI in the postpartum period reported by most studies (Roche et al., 2009) is likely related to supply of acetyl-CoA. In addition, cows with greater fat mobilization during the postpartum period had a greater depression in DMI and greater negative energy balance compared with cows with less fat mobilization (Weber et al., 2013).

The primary source of acetyl-CoA is from mitochondrial β-oxidation of NEFA, but all fuels (lactate, glycerol, AA) that are completely oxidized in the mitochondria must enter the TCA cycle via metabolism to acetyl-CoA. Hypophagic effects of anaplerotic metabolites appear to be dependent on the availability of acetyl-CoA to be oxidized (Stocks and Allen, 2012, 2013). Consistent with this, lower doses of propionate were more hypophagic, reducing total ME intake for cows in the postpartum period that were in a lipolytic state compared with cows in mid-lactation (Oba and Allen, 2003a). The concentration of acetyl-CoA in hepatocytes varies with changes in physiological state over the long term as well as diurnally (Piantoni et al., 2015). The postprandial reduction in NEFA supply to the liver, affected by insulin secretion and sensitivity of adipose tissue, likely affects feeding behavior by reducing acetyl-CoA available for oxidation. Following the initiation of meals, plasma insulin concentration increases and plasma NEFA (Allen et al., 2005) and liver acetyl-CoA concentrations decrease (Piantoni et al., 2015). DMI by cows in the postprandial period was positively related with the extent to which plasma NEFA and hepatic acetyl-CoA concentrations decreased following the initiation of meals following feeding (Piantoni et al., 2015).

Cows in the postpartum period require glucose precursors, primarily from dietary starch, to satisfy glucose demand from the rapid increase in MY while DMI is depressed. However, ruminal fermentation of starch results in increased propionate production that can result in satiety. Several experiments have fed rations differing in starch content in the postpartum period (Andersen et al., 2003; Rabelo et al., 2005; Dann and Nelson, 2011; McCarthy et al., 2015; Albornoz and Allen, 2018). Increasing ration starch content increased DMI and MY in experiments reported by Andersen et al. (2003) and Rabelo et al. (2005), but in those experiments, cereal grains were substituted for forage, decreasing the forage NDF content of the ration and the contribution of ruminal distention to satiety, especially as lactation progressed and the lipolytic state diminished. Substitution of corn grain for NFFS that have much less filling effect than forage NDF had different effects in other experiments. Dann and Nelson (2011) substituted corn meal for NFFS to increase ration starch content from 21 percent to 25.5 percent, and the higher starch diet decreased DMI 1.5 kg/d, whereas McCarthy et al. (2015) substituted ground corn for NFFS to increase ration starch content from 21.5 percent to 26.2 percent, and the higher starch diet had no effect on DMI (kg/d) overall but interacted with time; treatment did not affect DMI during the first 2 weeks postpartum, but the higher starch diet began to increase DMI slightly by the third week postpartum. Albornoz and Allen (2018) reported that high moisture corn reduced DMI compared with dry ground corn with a greater reduction when fed in 28 percent starch rations compared with 22 percent starch rations. Increased ruminal fermentability of starch also decreased DMI of cows in the postpartum period when steam-flaked corn was substituted for cracked corn (Dann et al., 1999) and when barley was substituted for corn (Sadri et al., 2009) but did not affect DMI when high moisture corn was substituted for dry ground corn in a high (27 percent) forage NDF ration (Rockwell and Allen, 2016). It is likely that DMI by cows in the postpartum period is controlled by hepatic oxidation as well as ruminal distention and that the dominant mechanism is dependent on the availability of acetyl-CoA for oxidation, ruminal starch fermentability, and the filling effect of the diet. Feeding a moderately fermentable starch source (e.g., dry ground corn) will likely allow higher starch rations to be fed, restoring euglycemia sooner.

Decreased DMI from excessive ruminal fermentation might also be caused by an inflammatory response to lipopolysaccharide released from ruminal microbial lysis (Bradford et al., 2015). Insulin resistance is induced by inflammatory signals contributing to the lipolytic state (Bradford et al., 2015). The reduction in DMI might be from central effects of acute-phase proteins (Sartin et al., 2011) as well as increased anaplerosis from propionate and increased availability of acetyl-CoA for hepatic oxidation.

INTEGRATION OF SIGNALS

The signal from the liver and other peripheral signals are relayed to brain-feeding centers by sensory nerves and integrated to affect feeding behavior. The relative contribution of different signals likely varies temporally, within and across days. Intake is likely limited by distention of undigested feed residues in the gastrointestinal tract when energy requirements are high and by mechanisms related to specific fuels from the diet or body reserves in the immediate postpartum period and as MY declines through lactation. Signals related

to distension and metabolism have synergistic effects on DMI by ruminants. Additive effects have been reported for intraruminal infusion of short-chain FAs on DMI of lactating cows when rumens were distended by balloons (Mbanya et al., 1993) and forage NDF concentration of the diet (Choi and Allen, 1999). Therefore, mechanisms are not mutually exclusive, and different mechanisms can both contribute to, or dominate, control of feeding within days.

Homeorhetic mechanisms that affect DMI and energy partitioning over the long term of weeks or months, such as those related to insulin, growth hormone, and leptin, influence hepatic oxidation by affecting the supply of NEFA, glycerol, and AA to the liver and therefore short-term control of DMI by hepatic oxidation (Allen, 2014). Whereas many of leptin's effects on DMI are thought to be mediated centrally (Houseknecht et al., 1998), its lipolytic effects increase NEFA available for hepatic oxidation, likely contributing to satiety (Allen, 2014). Leptin concentration in blood was positively related to BCS of lactating cows (Bradford et al., 2006), and its effects on satiety are consistent with the lipostatic theory of body weight (BW) maintenance (Kennedy, 1953). Therefore, mechanisms controlling energy intake and partitioning are entwined and inseparable and are affected by both diet and physiological state of cows (Allen, 2020). The dominant mechanism affecting DMI will vary among cows fed the same ration, affecting their responses to changes in ration composition and providing an opportunity to optimize feeding strategies for cows fed individually or in groups (Allen and Piantoni, 2014).

Consistently accurate prediction of DMI in ruminants has been difficult to achieve because of an incomplete understanding and availability of data to describe the interactions among diet composition, physiological state, environment, and management factors affecting DMI. For additional discussions and reviews on intake, see Baile and McLaughlin (1987), NRC (1987), Forbes (2007), Roche et al. (2008), and Allen (2014, 2020).

EQUATIONS FOR PREDICTING DRY MATTER INTAKE

Lactating Cows

In lactating dairy cattle, milk production (energy expenditure) usually peaks 4 to 8 weeks postpartum, and peak DMI (energy intake) lags until about 10 weeks postpartum (NRC, 1989). Earlier editions of *Nutrient Requirements of Dairy Cattle* used various approaches to predict DMI. The last edition of this report (NRC, 2001) included an empirical equation to estimate DMI of lactating Holstein cows with the inclusion of only animal factors that could be easily measured or known. Several equations were evaluated using weekly data from both published and unpublished studies. The best overall prediction equation was the equation of Rayburn and Fox (1993), multiplied by an adjustment for week of lactation to account for depressed DMI during early

lactation developed by Roseler et al. (1997). Ration composition was not included because the model was evaluative, and it was assumed cows were consuming feed relative to their energy requirements (except in early lactation). Therefore, the estimated DMI represents the mean effects of diets used in the studies and their interaction with animal factors. No published DMI data were available for developing or modifying the equation for use with breeds other than Holstein, and readers were referred to Holter et al. (1996) for the prediction of DMI of Jersey cattle. The DMI prediction equations (NRC, 2001) for primiparous and multiparous cows were similar, so no adjustment was made for parity per se (BW and MY will account for most parity effects).

An empirical equation (Equation 2-1) derived by de Souza et al. (2019) from a data set composed of 31,635 weekly observations for 3,143 lactations (1,462 primiparous and 1,681 multiparous) on 2,791 cows (de Souza et al., 2019) is used to estimate DMI in the model. Data were collected from cows at 11 research stations across the United States from 2007 to 2016. The data set includes animal factors only for cows between 1 and 368 days in milk (DIM) fed a range of rations with mean $\pm$ standard deviation (SD) of 105 ± 50 days postpartum, 24.3 ± 4.55 kg DMI, 29.9 ± 6.23 Mcal/d milk net energy (MilkE), 624 ± 80.2 kg BW, 3.03 ± 0.459 BCS, 0.021 ± 1.22 kg/d BW change, and 149 ± 5.28 cm height. All cows were housed in confinement and fed a total mixed ration (TMR) once per day and milked two or three times per day. To predict DMI for lactating Holstein cows, Equation 2-1 includes MilkE, BW, BCS, an adjustment for parity, and a nonlinear adjustment to account for depressed DMI in early lactation:

$$\begin{aligned} \text{DMI (kg/d)} = &[3.7 + \text{Parity} \times 5.7) + 0.305 \\ &\times \text{MilkE (Mcal/d)} + 0.022 \times \text{BW (kg)} \\ &+ (-0.689 - 1.87 \times \text{Parity}) \times \text{BCS}] \times [1 - (0.212 \\ &+ \text{Parity} \times 0.136) \times e^{(-0.053 \times \text{DIM})}] \end{aligned}$$

(Equation 2-1)

where parity is an adjustment factor ranging from 0 (all primiparous) to 1 (all multiparous) and BCS is scaled from 1 (thin) to 5 (obese). The equation predicted DMI with a small mean bias and high accuracy and precision; the fit statistics for the cross-validation across studies of the equation using the modeling data set were root mean squared error of prediction (RMSEP) = 2.61 kg, mean bias = 0.008 kg, mean bias, percent of mean squared error (MSE) = 6.9, slope bias, % MSE = 2.28, and concordance correlation coefficient = 0.80.

Equation 2-1 was validated against the equation recommended in the previous report (NRC, 2001) using an independent data set comprising 9,050 weekly observations (de Souza et al., 2019). Equation 2-1 was superior for predicting DMI compared with the equation recommended in the previous report, especially for cows past peak lactation. Improved prediction of DMI in mid- to late lactation is likely because parity, BCS, and their interaction were included in Equa-

tion 2-1; as BCS increases, DMI is reduced by 0.70 and 2.6 kg/d per unit of BCS for primiparous and multiparous cows, respectively. In addition, the adjustment for the depression in DMI in early lactation was affected by parity in Equation 2-1 with a greater reduction for multiparous compared with primiparous cows. Compared with the previous equation (NRC, 2001), Equation 2-1 has a lower DMI per unit of milk energy output and a similar coefficient for the energy required for maintenance (de Souza et al., 2019). The lower DMI per unit of milk energy output is likely because of improvements in production and feeding over the two decades between the data sets used to develop the equations.

Equation 2-1 was developed using data exclusively from Holstein cows and is most appropriate to predict DMI for Holsteins. However, the equation predicted DMI reasonably well for Jersey and Holstein crossbred cows in a limited data set of treatment means reported in the literature (de Souza et al., 2019).

Ration Effects

Equation 2-1 predicts DMI of cows consuming rations with a wide range of composition. Accuracy of DMI prediction is limited by the potentially large effects of ration composition on DMI that are not accounted for when using animal factors only. Ration factors include those that affect DMI by their filling effects through distention as well as by metabolic effects. Including these factors should improve accuracy of DMI prediction, but accuracy is ultimately limited because of the many interactions among ration factors and physiological state. In addition, diet can limit or stimulate MY that can then affect DMI, increasing the complexity of DMI prediction. However, limited recommendations can be made to adjust predicted DMI for some ration characteristics.

This report includes an equation that combines factors related to the filling effect of rations and MY to help assess the effects of ration composition on DMI of lactating cows during ration formulation (Allen et al., 2019). The data set used includes 134 treatment means from 34 experiments reported in 32 peer-reviewed articles published from 1990 through 2015. It includes data for cows ranging from 60 to 309 days postpartum with a mean ± SD of 107 ± 48 days postpartum, 23.0 ± 2.8 kg DMI, 32.0 ± 7.5 kg/d MY within study, 643 ± 59 kg BW, and for ration concentrations (percent of DM) of 17.8 ± 1.6 for CP, 34.1 ± 4.6 for NDF, 20.5 ± 4.0 for acid detergent fiber (ADF), and 23.9 ± 5.7 for forage NDF (fNDF), as well as ration ADF/NDF of 0.600 ± 0.083 and a laboratory measure of NDF digestibility (in vitro or in situ, fNDFD) for the sole forage or major forage of 52.0 ± 12.3. The ratio of ADF to NDF was included as a proxy for forage fragility (Allen and Piantoni, 2014); legumes, with an ADF/NDF of ~0.8, are more susceptible to comminution than perennial grasses with an ADF/NDF typically ~0.6 or less (Voelker-Linton and Allen, 2008; Kammes

and Allen, 2012a). The full model included the linear and quadratic effects of ration CP, ADF, NDF, fNDF, ADF/NDF, and fNDFD, as well as the linear and quadratic interactions among ration factors, BW, and the mean MY for each study and its interaction with the ration factors. The mean MY for each study was included because distention likely becomes a more dominant mechanism limiting DMI as MY increases (Oba and Allen, 1999a; Voelker et al., 2002). The full model was reduced by backward stepwise regression to select the model with the lowest Bayesian information criterion and evaluated using a 5-fold cross-validation. The final equation was

$$\begin{aligned} DMI\,(kg/d) = {} & 12.0 - 0.107 \times fNDF + 8.17 \times ADF/NDF \\ & + 0.0253 \times fNDFD - 0.328 \times (ADF/NDF - 0.602) \\ & \times (fNDFD - 48.3) + 0.225 \times MY + 0.00390 \\ & \times (fNDFD - 48.3) \times (MY - 33.1) \end{aligned}$$

(Equation 2-2)

where DMI = dry matter intake, kg/d; fNDF = forage NDF content of diet, percentage; ADF/NDF = ADF as a fraction of NDF in the diet; fNDFD = digestibility of forage NDF measured in vitro or in situ, percentage; and MY = milk yield, kg/d. The observed DMI versus predicted DMI had an intercept of −0.05 kg and a slope close to unity (1.006), with a mean bias of 0.00 kg/d, a root mean square error (RMSE) of 1.55, and concordance correlation coefficient (CCC) of 0.83 (Allen et al., 2019).

DMI was positively related with MY and ADF/NDF and negatively related with fNDF. DMI and fNDFD were related positively for cows with high MY but related negatively for cows with low MY, diminishing the overall effect of fNDFD on DMI. In addition, DMI increased with MY to a greater extent for high fNDFD compared with low fNDFD. DMI and fNDFD were related positively for low-ration ADF/NDF but related negatively for high-ration ADF/NDF. Also, DMI and ration ADF/NDF were related positively when fNDFD was low, but DMI was not affected by ration ADF/NDF when fNDFD was high.

The primary equation to predict DMI in the present report includes animal factors only (Equation 2-1). Equation 2-2 is included to evaluate DMI once feeds and their dietary proportions are determined within the limitations mentioned by Allen et al. (2019). The equation should be limited to cows past 60 days postpartum because data from cows earlier in lactation were not included in the data set and because the control of DMI is likely dominated by metabolic mechanisms rather than distension for cows in the postpartum period. The data set included data from Holstein cows only. Because BW was not significant and not included in the final equation, the suitability of the equation for smaller breeds such as Jerseys and Holstein crossbreds is unknown. The data set did not include treatments with NFFS or ground or finely chopped forages. Whereas inclusion of NFFS in rations has been shown to have little effect on DMI of lactating cows (Allen,

2000), the ADF/NDF of NFFS varies widely, so evaluating effects of large changes in NFFS on DMI is not recommended. In addition, ground, pelleted, or very finely chopped forages should not be classified as forage for the purposes of predicting DMI with this equation. The rations included in the data set contained a single forage, or other forages contributed little to the ration. It is suggested that a weighted average be used for fNDFD when this equation is used for rations with multiple forages or that the mean fNDFD of the data set (52.0 percent) be used if a measure of fNDFD is not available. In addition, because it is a biological assay with expected variation across runs, fNDFD for all forage comparisons should be measured with the same method and incubation time and preferably within the same run or corrected using the same laboratory standard.

Besides adjusting for the filling effect of rations, fat sources containing unsaturated fats (e.g., oil, tallow, calcium salts palm, calcium salts LCFA) depressed DMI with a mean reduction of ~0.41 kg/1 percent added fat (Weld and Armentano, 2017). Whereas DMI can be increased by increasing the protein concentration of rations and reduced by propionic acid produced from ruminal starch fermentation, variation in response by physiological state and lack of sufficient data preclude recommendations to adjust DMI using these inputs. Diets also affect milk energy output and BW gain, so dietary adjustments to DMI should be used with caution.

Growing Heifers

The previous report (NRC, 2001) evaluated equations to predict DMI of growing heifers, including those developed by Stallings et al. (1985) using animal factors only, as well as equations that included both animal and ration factors developed by Quigley et al. (1986) and the calf equation from the *Nutrient Requirements of Beef Cattle* (NRC, 1996). After an initial evaluation using a small data set, the NRC equation for beef cattle was selected and validated with a larger data set of 2,727 individual observations on growing heifers ranging from 58 to 588 kg. However, validation statistics were not provided, and visual observation of the plot of observed versus predicted DMI indicates that the equation increasingly underpredicts DMI as DMI increases from 2 to 12 kg/d. Anele et al. (2014) reevaluated the equation from the previous edition of the *Nutrient Requirements of Beef Cattle* (NRC, 1996), with a much larger database of growing and finishing beef cattle and developed alternative equations to predict DMI. Based on the results of that evaluation, the Committee on Nutrient Requirements of Beef Cattle (NASEM, 2016) recommended continued use of the calf equation from the previous report to predict DMI of growing-finishing beef cattle, although they also presented alternative equations.

Hoffman et al. (2008) evaluated the equation recommended in the previous edition (NRC, 2001) and the equation developed by Quigley et al. (1986) using a database of daily pen DMI from 44 pens of Holsteins and 30 pens of crossbred heifers collected over 28 months (Maltecca et al., 2006). They determined that both equations were reasonably accurate but had significant DMI × BW prediction bias, over- or underpredicting DMI of light or heavy heifers. They developed alternative exponential or mixed models using BW only, or BW and ration NDF content, as well as equations using BW, ration NDF content, and temperature for Holstein and for crossbred heifers. The exponential model with a single BW (kg) term has similar precision compared with the equations reported by NRC (2001) and Quigley et al. (1986) but decreased DMI prediction bias. The equation for Holsteins from Hoffman et al. (2008) was modified to include mature BW (MatBW) so that it could be applied to all breeds (Equation 2-3). Holsteins were assumed to have a MatBW of 700 kg:

$$\begin{aligned} \text{DMI, kg/d} &= 0.022 \times \text{MatBW} \\ &\times (1 - e^{\{-1.54 \times (\text{BW/MatBW})\}}) \end{aligned} \quad \text{(Equation 2-3)}$$

where MatBW is the expected MatBW of the heifer.

When Hoffman et al. (2008) included ration NDF in the DMI equation, it modestly increased R^2, decreased standard errors of prediction, and eliminated bias compared to the equation that only used BW. The Holstein-based NDF equation was modified to include MatBW so that it could be applied to all breeds (Equation 2-4):

$$\begin{aligned} \text{DMI, kg/d} &= [0.0226 \times \text{MatBW} \times (1 - \exp\{-1.47 \\ &\times (\text{BW/MatBW})\})] - [0.082 \times (\text{NDF} - \{23.1 + 56 \\ &\times (\text{BW/MatBW}) - 30.6 \times (\text{BW/MatBW})^2\})] \end{aligned}$$
$$\text{(Equation 2-4)}$$

where NDF is the NDF concentration (DM basis) of the diet.

Whereas the temperature effect on DMI was significant, its effect was small and prediction bias was observed for both light and heavy heifers. Equation 2-3 is recommended to predict DMI for heifers when diet NDF concentrations are not known. Equation 2-4 is recommended to estimate DMI when ration NDF is known with confidence. However, because of the diversity in diets, environments, and numerous other factors, users are encouraged to measure actual DMI rather than relying on estimated values.

FEEDING MANAGEMENT, FEEDING BEHAVIOR, AND ENVIRONMENTAL FACTORS AFFECTING FEED INTAKE

The goal of any feeding system is to provide the opportunity for cows to consume the amount of feed specified in a formulated ration. Considerations in choosing a feeding system should include housing facilities, equipment necessities, herd size, labor availability, and cost. Nutrients can be effectively supplied by feeding either a TMR or individual ingredients. A TMR allows for the mixing of all feed ingredients together based on a prescribed amount of

each ingredient. When consumed as a TMR without sorting of ingredients, more even rumen fermentation and a better use of nutrients should occur than when feeding ingredients separately. Electronic feeders reduce the labor involved in individual-concentrate feeding and provide an opportunity to feed concentrates through several feedings of smaller amounts each day. Feeding forages and concentrates separately limit the accuracy of ration formulation when forages are provided free choice and the amount fed is usually unknown or individual cow amounts are calculated from a group average intake. A partially mixed ration provides some of the advantages of a TMR while allowing concentrates to be fed according to the needs of individual cows.

Eating Habits and Cow Behavior

Early lactation cows (63 DIM) producing 23 to 44 kg of milk per day fed a TMR ad libitum ate an average of 5 hours per day (Dado and Allen, 1994). Cows in that study were housed in tie-stalls, were fed twice per day, and had access to feed 22 hours per day. Meal frequency ranged from 9 to 13 (mean of 11) eating bouts per day that averaged 29 minutes per bout. Mean DMI at each eating bout was about 10 percent of the total daily DMI, which ranged from 15 to 27 kg/d. However, meal size was highly variable within a day, with larger meals following feeding and smaller meals at night. DMI and MY data from multiple studies were positively related to eating time and ruminating time per day as well as meal frequency (Johnson and Devries, 2018). Eating time can be affected by over an hour per day by differences in concentration of forage NDF, forage NDF digestibility, and particle size, which might limit DMI under competitive feeding situations (Grant and Ferraretto, 2018).

Behavior at the feed bunk is often affected by social dominance. Dominant cows, usually older and larger, tend to spend more time eating than do cows with a lower social rank in a competitive situation, such as when bunk space is restricted (Albright, 1993). Socially dominant animals, not necessarily the highest producers, tend to consume more feed at the bunk in these situations (Friend and Polan, 1974). Competition at the feed bunk increased meal size and length and decreased meal frequency, with no effect on DMI or total eating time in one study (Hosseinkhani et al., 2008), but reduced eating time and increased eating rate, with a slight increase in DMI in another study (Olofsson, 1999). Martinsson (1992) and Martinsson and Burstedt (1990) found that limiting the access to feed to 8 hours per day decreased milk production of cows averaging about 25 kg/d by 5 to 7 percent compared with cows that had free-choice access to feed.

Albright (1993) recommended at least 46 cm of bunk space per cow. However, the optimal or critical feed bunk space needed probably varies depending on competition among cows, the total number of cows having access to the feed space, and the availability of feed over a 24-hour period. Friend et al. (1977) evaluated bunk spaces of 50, 40, 30, 20,

and 10 cm per cow, for early lactation cows with mature equivalent productions of 7,700 to 10,000 kg/y. Average time spent at the feed bunk (3.7 hours/d) did not decrease until only 10 cm of space per cow was available. When there was 20 or 10 cm per cow, the correlation of dominance to duration of eating periods increased. For growing dairy heifers, feed-bunk space requirement varies with age. Longenbach et al. (1999) found that rapid growth in growing heifers fed a TMR could be maintained in young heifers (4 to 8 months old) with 15 cm of bunk space. But, by the age of 17 to 21 months, feed bunk space needed to be similar (47 cm) to that recommended for lactating cows.

Cattle prefer mangers that allow them to eat off a smooth surface in a natural grazing position. Albright (1993) cited evidence showing cows eating with their heads down produce 17 percent more saliva than cows eating with their heads in a horizontal position. Feed-wasting activities associated with elevated bunks, such as feed tossing, are eliminated when cows eat with their heads down (Albright, 1993).

Feeding Frequency

Whereas several studies have reported effects of feeding frequency on milk production and on feeding and sorting behavior, few studies have reported effects of feeding frequency on DMI. Potential benefits of increased feeding frequency might be from metabolic responses from a more consistent ruminal fermentation and supply of absorbed fuels. However, Hart et al. (2014) reported that frequency of feed delivery had no effect on DMI by primiparous cows and little effect on DMI by multiparous cows (30.6, 29.7, and 31.1 kg/d for one, two, and three times per day feeding, respectively) and did not affect MY for either group when housed in free stalls. In addition, DMI and MY were not affected by feeding twice per day compared with feeding once per day (Niu et al., 2014), and increasing frequency of concentrate feeding from two or six times per day had no effect on DMI, yield of milk and milk components, or ruminal fermentation characteristics (Macleod et al., 1994). Increased frequency of pushing up feed to cows did not affect lying time, yield of milk and milk components, or feed sorting for cows housed in tie-stalls (Miller Cushon and DeVries, 2017) and had little effect on feeding time or diurnal pattern of feed alley attendance for cows in free stalls (DeVries et al., 2003). Delivering feed once per day to cows ad libitum in the morning (0830 h) compared with the evening (2030 h) affected daily rhythms of feeding and lying behavior in both the winter (Niu et al., 2014) and summer months in Pennsylvania (Niu and Harvatine, 2018) but did not alter MY or composition in either study or DMI during the winter months. However, delivering feed in the evening decreased DMI and digestibility of DM and NDF compared with delivering feed in the morning during the summer months. Delivering feed to cows between milkings (twice per day) tended to decrease DMI compared with delivery at milking time ($P < 0.10$,

27.2 versus 26.5 kg/d) with no effect of milk or milk components, increasing efficiency of milk production (4 percent fat-corrected milk/DMI, 1.83 versus 1.67; King et al., 2016). Cows ate smaller meals more slowly and more frequently, possibly promoting a more stable rumen environment.

Sorting

Sorting of rations is affected by frequency of feeding and ration characteristics and varies among cows. Sorting was reduced by increasing frequency of feeding from once to twice per day (DeVries et al., 2005) and by adding a molasses-based liquid feed (DeVries and Gill, 2012).

Supplementing a molasses-based liquid feed in high-straw, dry cow diets improved DMI and reduced sorting against long particles but increased sorting against short particles (Havekes et al., 2020a). Addition of water to decrease the DM concentration of a ration from 81 percent to 64 percent reduced sorting and tended to increase NDF intake and milk fat percentage but did not affect DMI or MY (Leonardi et al., 2005). In addition, adding water to a high-straw diet improved DMI and reduced sorting against the longest ration particles by cows in the dry period (Havekes et al., 2020c). However, addition of water during summer months to decrease the DM concentration of a ration from 56 percent to 51 percent or 44 percent increased sorting and the temperature of feed in the hours after feeding and decreased DMI, suggesting that adding water during high ambient temperatures might have accelerated spoilage (Felton and DeVries, 2010). Feeding shorter chopped wheat straw in a high-straw diet improved DMI and reduced sorting by cows in the dry period (Havekes et al., 2020b). Adding long hay to rations increased selective consumption of fine particles but also increased intake of longer particles because of their greater concentration in the ration, but quality of hay had no effect on sorting (Leonardi and Armentano, 2003). Whereas cows consistently sorted against long particles in favor of fine particles, sorting was highly variable among cows (Leonardi and Armentano, 2003). Primiparous cows sorted more than multiparous cows, but sorting was not affected by stage of lactation (DeVries et al., 2011).

Grouping Cows

Grouping cows according to their nutrient requirements can help optimize MY and efficiency of milk production. Mechanisms controlling DMI and energy partitioning vary across a lactation, and ration composition should be altered to maximize energy intake in early lactation and partition energy to MY and limit gain in body condition later in lactation (Allen and Piantoni, 2014). Fresh cows should be grouped separately and offered a more filling ration with moderately fermentable starch sources until distention begins to limit DMI. The more filling ration will likely reduce risk of displaced abomasum and help maintain rumen pH by buffering rumen contents. However, control of DMI by cows in early to mid-lactation is dominated by ruminal distension, and they benefit from rations that are less filling with higher starch contents to achieve and maintain high MY. Cows in late lactation will likely gain excessive body condition if offered this ration, which might compromise health when the cows calve. Therefore, once cows replete body reserves past peak lactation, they should be offered a ration with less starch content to maintain body condition and MY (Allen and Piantoni, 2014). Primiparous cows do not peak in DMI as early in lactation, but they are more persistent in DMI after peak than are multiparous cows. If possible, primiparous and multiparous cows should be grouped separately because of differences in DMI and social hierarchy. Primiparous cows are usually more timid and of lower social rank in the herd initially, but they gradually rise in social rank as more cows enter the herd or as older cows leave (Wierenga, 1990). Phelps and Drew (1992) reported an increase of 725 kg in milk over a 305-day lactation for first-lactation animals when grouped separately instead of being mixed in with older cows. However, although primiparous cows grouped alone visited the robotic milking unit and feed troughs more frequently, they spent less time eating with no effect on DMI or MY than when housed in a group of 30 percent primiparous and 70 percent multiparous cows (Bach et al., 2005). Production response to grouping primiparous cows separately likely varies depending on group size and bunk space per cow.

Weather

The thermal neutral zone of dairy cattle is about 5 to 20°C but varies among animals. Temperatures below or above that zone alter intake and metabolic activity. Ruminants adapt to chronic cold stress conditions by increasing thermal insulation, basal metabolism, and DMI (Young, 1983). Rumination activity, reticulo-rumen motility, and rate of passage are also increased (Young, 1983). However, in extreme cold, increased DMI may not compensate for the increased metabolic rate, so animals may shift energy use from productive purposes to heat production. A rise in ambient temperature above the thermal neutral zone decreases DMI and milk production. Acute heat stress with average temperature humidity index (THI) of 72 decreased DMI of lactating cows 11.5 percent compared with when they were in a thermoneutral environment with an average THI of 57 (Collier et al., 2018). However, heat stress can decrease DMI by cows more than 50 percent compared with a thermoneutral environment (NRC, 1981) and the reduction in DMI to acute heat stress increases for cows with higher MY (Collier et al., 2018). Water consumption by cattle increases with ambient temperature, but acute heat stress can decrease water intake along with DMI and milk synthesis, and the reduction in water intake increases for cows with higher MY (Collier et al., 2018).

Collier et al. (1981) suggested that the effects of heat stress on MY had a lag of 24 to 48 hours, and West et al.

(2003) reported that DMI was most sensitive to mean air temperature 2 days before, declining 0.85 kg/d for each degree C increase in mean air temperature over the range of 25 to 32°C, whereas MY was most sensitive to THI, declining 0.88 kg/d for each unit increase in THI over the range in THI of 73 to 83°C for Holstein cows. Furthermore, the declines in MY and DMI were substantially less when evaluated with climatic measures on the same day compared with those measured 2 days earlier. Igono et al. (1992) reported that the diurnal pattern of temperature during the day affected MY and that a cool period of less than 21°C will minimize the decline in MY. The equations used for predicting DMI of lactating cows in this report do not include a temperature or humidity adjustment factor because of insufficient DMI data outside of the thermoneutral zone to validate equation modifiers. During periods of heat stress, actual DMI will likely be greater than predicted from the reduction in MY; the reduction in DMI accounted for only 50 percent of heat stress–induced decrease in MY (Wheelock et al., 2010).

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3

Energy

ENERGY UNITS

Energy requirements for maintenance and milk production are expressed in net energy for lactation (NEL) units. The NEL system has been used by the National Research Council (NRC) for dairy cattle nutrient requirements for several editions because it uses a single energy unit (NEL) for both maintenance and milk production. The classical energy flow system used in animal nutrition for decades is as follows: gross energy (GE), digestible energy (DE), metabolizable energy (ME), and finally net energy. In the current version, the DE, ME, and NEL values of feeds are all considered as the actual amount of energy that would be provided based on the animal and diet; in other words, DE and ME are not the potentially maximum digested or metabolizable energy but the DE and ME expected in a given situation (cow and diet). Thus, the model is for evaluation and should be used with caution for formulation.

Based on work from the USDA Energy Metabolism Unit at Beltsville 50 years ago, the efficiency of using ME for maintenance (0.62) and milk production (0.64) was considered essentially the same (Flatt et al., 1965; Tyrrell and Moe, 1972). Using only the last two decades of work at Beltsville, as reported in Moraes et al. (2015), the conversion of ME to milk is 0.66, and the conversion of ME to body reserves to milk is similar. Therefore, one feed energy value (NEL) is used to express the requirements for maintenance, pregnancy, milk production, frame gain (growth), and changes in body reserves (tissue that is lost and gained during times of nutrient excess or deficiency) of adult cows. The efficiencies of using NEL for pregnancy, frame gain, and changes in body reserves are adjusted to fit within this system for adult cows, as discussed later. The energy requirements for cattle before their first parturition are given on an ME basis (see Chapters 10 and 11). As discussed in the seventh revised edition, one nutrient can alter the digestibility of other nutrients, and the conversion of DE to ME is altered by the composition

of the diet; therefore, ME and NEL values are not accurate or valid for individual feeds and should only be calculated for total diets.

ENERGY VALUES OF FEEDS

The method used to estimate feed and dietary energy values in this edition is similar to that used by NRC (2001) but includes significant modifications. The seventh edition did not give fixed NEL values for a feed; rather, NEL values of diets were based on the composition of feeds and diets and level of intake. In this edition, modifications were made to improve accuracy and account for more sources of variation than in the seventh edition. Deficiencies with the seventh revised edition that were addressed include the following:

- The digestibility discount as intake increased was too great (Huhtanen et al., 2009; White et al., 2017; de Souza et al., 2018). In NRC (2001), the digestibility discount was larger in diets that had higher basal total digestible nutrient (TDN) concentrations; TDN was essentially a proxy for dietary starch content, and diets with more starch often are consumed at greater intakes. The structure of this equation resulted in exaggerated negative effects on digestibility as starch content and intake were both increased.
- The digestibility discount was applied to the entire diet; however, intake does not affect digestibility of all nutrient fractions similarly.
- Protein was appropriately given a higher DE value than starch (5.65 compared to 4.2 Mcal/kg), but the ME value of excess protein was not correct. When protein is used as a fuel source, its ME value is similar to starch. The previous version overestimated the energy value of protein when fed in excess of requirement.
- The equations used to convert DE at production intakes to ME and ME to NEL had negative intercepts, which

likely resulted in underestimating the conversion efficiency of lower-energy diets.

- Level of intake was calculated as a multiple of maintenance energy, which results in a circular argument. Intake of NEL altered the efficiency of converting DE at maintenance intake to NEL, which in turn altered NEL intake.

Other improvements made from the seventh revised edition include the following:

- Nonfiber carbohydrate and TDN are no longer used. Many of the factors that affect starch digestibility have been quantified; therefore, including starch in the equation and adjusting for those factors should improve accuracy. The negative associative effects of starch on fiber digestibility have been quantified, and these are used in place of TDN to estimate digestibility discounts and DE.
- The base for DE calculations was set as a cow consuming dry matter (DM) at 3.5 percent of body weight (BW) and fed a diet with 26 percent starch. These values are the averages in the data set used to generate digestibility values. In the previous edition, the base was a cow fed at maintenance (approximately 1.2 percent of BW), which required substantial extrapolation of digestibility values.
- Rather than using an essentially constant efficiency for converting DE to ME, energy lost via urine and methane is now calculated using diet and animal characteristics, resulting in more variable efficiencies, which should increase accuracy over a wider array of diets.
- The energy values for protein are calculated using values derived from the protein system (i.e., rumen degradable protein [RDP], rumen undegradable protein [RUP], and digestibility of RUP). In the previous version, the energy value of protein was calculated independently of the protein system.

OVERALL ENERGY SCHEME

The overall approach (see Figure 3-1) used to estimate diet energy values is as follows: (1) feed is separated into fractions that mostly approximate uniform fractions, (2) gross energy values are calculated based on these fractions, (3) base digestibilities for each feed fraction are calculated assuming dry matter intake (DMI) at 3.5 percent of BW and a dietary starch content at 26 percent, (4) adjustments are made to base digestibility values for level of intake on a DMI/BW basis and for dietary starch, and (5) estimates of urinary energy (UE) and gas energy output are calculated based on diet and animal characteristics. Feed NEL values are no longer provided, even in the tables, as diet NEL supply must be based on the whole diet, and thus NEL values for individual feeds are misleading. The same is true for ME and, to a lesser extent, DE. Base DE values (i.e., DMI = 3.5 percent of BW and diet contains 26 percent starch) for feeds are in Table 19-1; however, the DE of a diet formulated using table values likely will differ from one formulated using the model even if the composition of the ingredients is the same.

FEED FRACTIONS

The summative approach to estimating DE in the seventh revised edition was retained, but feed was separated into more fractions: neutral detergent fiber (NDF), starch, fatty acids (FAs), crude protein (CP) (N×6.25), ash, and residual organic matter (ROM). The FA fraction includes FAs with more than four carbons and specifically does not include the short-chain volatile FAs or lactic acid. The ROM fraction is DM not accounted for in the main feed fractions (Equation 3-1). This by-difference fraction contains water-soluble carbohydrates, ingested fermentation and other short-chain FA (e.g., acetic, butyric, and lactic acids), glycerol (both free and the glycerol moiety of triglycerides [TGs]), soluble fiber (pectins and gums), and any components not accounted for in the main feed fractions (e.g., tannins and waxes). The FA content of TGs includes an extra water molecule for the hydrolysis of each FA ester bond. Thus, the total mass of hydrolyzed FA and glycerol is 106 percent of the original TG mass for typical feed lipids. To estimate the amount of ROM (glycerol) in a TG, the mass of FA must be divided by 1.06. This correction may not account properly for the ROM content of some lipids, such as TGs with shorter-chain FA, phospholipids, and glycolipids, but these fractions are not generally measured, and any error would be small. In addition, the correction is not appropriate for FA from nonesterified sources as shown in Equation 3-1. The CP equivalent from supplemental nonprotein nitrogen (sNPNCPE) is separated from CP when estimating energy values. The concentration of ROM is also adjusted (i.e., 181 / 281 = 0.64) to correctly account for the mass of supplemental nonprotein nitrogen (NPN) (Equation 3-1). Without this correction, urea (281 percent CP and −181 percent ROM) would have a GE value of 8.6 kcal/g instead of 2.5 kcal/g. Detailed information regarding assays for these fractions is given in Chapter 18.

$$ROM = 100 - Ash - NDF - Starch$$
$$- (FA / FatFactor) - (CP - 0.64 \times sNPNCPE)$$

(Equation 3-1)

where FatFactor = 1 if Feedtype = fatty acid or FA soap and 1.06 for all other feeds, and values are a percentage of DM.

The NDF fraction is a heterogeneous mixture of carbohydrates, lignin, nitrogen-containing compounds, and ash. Because both ash and CP are included as unique fractions in the ROM equation, any ash and CP contained in the NDF fraction will be counted twice and will result in an underestimation of the ROM fraction with an equal overestimation of the NDF fraction. For most feeds, CP comprises <10 percent of NDF (but can approach 20 percent in some feeds), and ash comprises <2 percent of NDF but can be >7 percent in some feeds (Crocker et al., 1998). For mixed
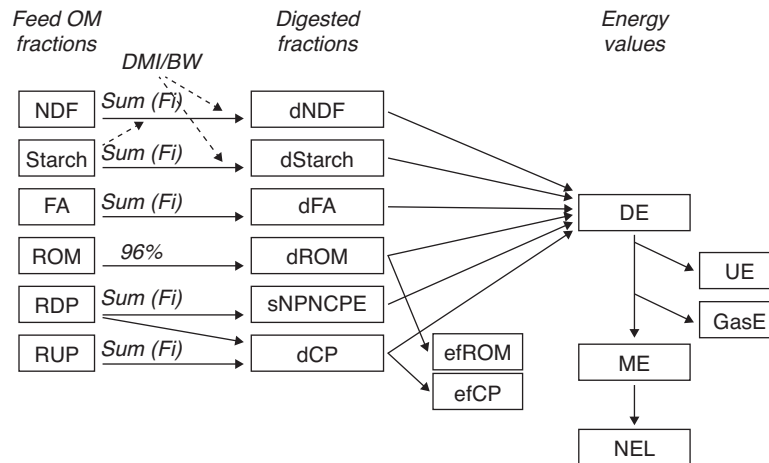
FIGURE 3-1   Feed energy supply system for dairy cattle. Each feed (Fi) is fractioned into NDF, starch, FAs (>4 carbons), ROM (mostly sugars, pectins, gums, the glycerol moiety of TGs, and fermentation acids), RDP, and RUP. These are converted to digested nutrients (dNDF, dStarch, dFA, dROM, and dCP) using base digestion coefficients for each individual feed. Starch and NDF digestion are adjusted for level of intake, and dNDF is further adjusted for the content of starch in the whole diet. The RDP that is from supplemental NPN on a CP-equivalent basis (sNPNCPE) is separated from dCP because it has a lower digestible energy (DE) value than dCP. The digested fractions are then converted to an apparent DE value by multiplying each digested fraction by its enthalpy of combustion and then subtracting endogenous fecal energy, which includes undigested microbial protein and endogenous fecal ROM (efCP, efROM). The amount of UE is estimated based on urinary nitrogen (UN), with UN a function of apparently digested CP and N captured. Gaseous energy (GasE) is a function of DMI and the content of FA and digested NDF in the diet. ME is calculated as DE minus UE, and GasE and is converted to NEL using a constant efficiency.

diets, the sum of ash and neutral detergent insoluble CP usually comprises 6 to 7 percent of the NDF (Tebbe et al., 2017), so that the ash and CP-free NDF in a diet average about 94 percent of the NDF value.

Although the double subtraction of neutral detergent insoluble ash and CP is incorrect, NDF, rather than ash- and CP-free NDF, is used in energy supply equations because:

1. Data on the concentrations of ash- and CP-free NDF for many feeds are limited.
2. Most publications that reported in vivo digestibility values for NDF did not subtract CP or ash from NDF; therefore, estimated NDF digestion coefficients of the current model can be compared directly to in vivo data.
3. Neutral detergent-insoluble CP and ash are usually quantitatively small fractions, and analytical precision is likely less for ash- and CP-free NDF than for NDF.
4. The true ROM digestibility and endogenous fecal ROM estimates are more precise when NDF is used rather than ash- and CP-free NDF (Tebbe et al., 2017).
5. The CP and ash corrections are not quantitatively important for estimating energy values for most diets because the errors largely cancel each other out. In Tebbe et al. (2017), diets with the greatest difference between ash- and protein-free NDF and NDF resulted in a difference of <0.1 percent in the sum of digested NDF and digested ROM.

Gross Energy Values of Feed Fractions

Estimated GE of a feed or diet is calculated by multiplying the proportion of each fraction by its respective GE value and summing (Equation 3-2). This GE value serves as a useful reference for animal experiments.

Starch: 4.23 Mcal/kg
FA: 9.4 Mcal/kg
NDF: 4.2 Mcal/kg
ROM: 4.0 Mcal/kg (committee estimate assuming this fraction is predominantly sugars, organic acids [mostly lactic and acetic], glycerol, and soluble fiber)
CP (excluding supplemental NPN): 5.65 Mcal/kg
sNPNCPE: 0.89 Mcal/kg (calculated from the heat of combustion of urea at 2.5 Mcal/kg or 0.89 Mcal/kg CP equivalent)

The GE of a diet or feed (Mcal/kg) is calculated as

$$\begin{aligned} \text{GE\_DM of feed} = {}& 0.042 \times \text{NDF\_DM} + 0.0423 \\ & \times \text{Starch\_DM} + 0.040 \times \text{ROM\_DM} \\ & + 0.094 \times \text{FA\_DM} + 0.0565 \times (\text{CP\_DM} \\ & - \text{sNPNCPE\_DM}) + 0.0089 \times \text{sNPNCPE\_DM} \end{aligned}$$

(Equation 3-2)

where feed fractions are expressed as a percentage of DM.

ESTIMATING THE DIGESTIBLE ENERGY VALUE FOR FEEDS AND DIETS

True Digestibility Coefficients of Feed Fractions for Base Conditions

In the previous edition (NRC, 2001), DE of diets was estimated for a cow fed at maintenance and then discounted as DMI increased and as the energy concentration of the diet (expressed as TDN) increased. In this version, the base condition is for an animal with a DMI of 3.5 percent of BW and fed a diet with 26 percent starch. All diets are assumed to have adequate RDP to meet microbial requirements and adequate forage NDF to promote proper rumen conditions. The DMI and starch concentration for base conditions reflect the mean of the database. Inadequate data are available to accurately estimate effects of RDP or forage on digestibility. Under practical conditions, RDP is usually adequate, but if diets do not supply adequate RDP (see Chapter 6), diet energy values may be overestimated. Diets with inadequate forage NDF (see Chapter 5) can cause ruminal acidosis, resulting in lower than estimated NDF and energy digestibilities. Some of the negative effects of inadequate forage NDF on digestibility should be accounted for by the starch adjustment. Although most of the digestibility data used to develop equations are from Holstein cows, digestibility is usually not different between Holstein and Jersey cows (Aikman et al., 2008; Knowlton et al., 2010; Uddin et al., 2020).

Neutral Detergent Fiber

Because there is no endogenous fecal NDF, apparent and true digestibility of NDF are the same. NDF can be expressed as NDF, NDF on a CP-free basis, on an ash-free basis, or on a CP-and ash-free basis. Based on limited data (Tebbe et al., 2017), the digestibility of CP- and ash-free NDF is slightly greater than the digestibility of NDF, but the NDF concentration in the feed is less, so concentrations of digested NDF and digested CP- and ash-free NDF are similar. Thus, digested NDF is used to estimate energy. In the model, two methods can be used to estimate base digestibility of NDF; one is the lignin-based equation (Equation 3-3a) from the seventh revised edition, and the other uses 48-hour in vitro NDF digestibility (IVNDFD; Lopes et al., 2015, Equation 3-3b). Incubations for 48 hours were more accurate at estimating in vivo NDF digestibility by lactating cows fed ad libitum (ca. 24 kg DMI/d) than were 30-hour incubations (Lopes et al., 2015). Inadequate IVNDFD data from published studies that measured in vivo digestibility were available to make a robust comparison of the two methods. The evaluations in Chapter 20 are based on the lignin equation, and the lignin method is the default method used in the model; however, the user has the option of using the IVNDFD equation or directly entering an NDF digestibility value.

$$\text{Digested proportion of NDF at base (dNDF\_NDF\_base)} = \{0.75 \times (\text{NDF\_DM} - \text{Lignin\_DM}) \times [1 - (\text{Lignin\_DM} / \text{NDF\_DM})^{0.667}]\} / \text{NDF\_DM}$$
(Equation 3-3a)

where nutrients are expressed as a percentage of DM.

$$\text{Digested proportion of NDF at base} \\ (\text{dNDF\_NDF\_base}) = 0.12 + 0.61 \times \text{IVNDFD}$$
(Equation 3-3b)

where IVNDFD is 48-hour in vitro digestion expressed as a proportion of NDF.

For the common macronutrients, the in vivo digestibility of NDF is the most variable, and more research is needed to improve its estimation using commercially applicable laboratory methods. This research must include comparisons of laboratory-based estimates to in vivo measurements in dairy cows fed typical diets (e.g., Kendall et al., 2009).

Starch

Starch digestibility is dependent on innate properties of starch granules in grains, on the timing of harvest, and on mechanical processing that occurs postharvest (see Chapter 5). Starch digestibilities of the major starch sources are shown in Table 3-1, and these values are used as base starch digestibilities in the electronic feed library.

Protein

The digestibility of protein is based on the protein model, so that the true total-tract digestibility of the protein in a feed is the sum of RDP and the digested portion of RUP (dRUP). The proportion of protein degraded in the rumen and proportion of RUP that is digested are not dependent on DMI (see Chapter 6); therefore, protein digestibility in the model is not affected by intake. The committee recognizes the possible error in this assumption. However, given that most potentially degraded protein that is undegraded because of a high passage rate will probably be digested in the total tract, this error is likely small when calculating DE. A recent meta-analysis by White et al. (2017) supports the idea that the total-tract digestibility of protein is less affected by intake than that of other nutrients.

$$\text{Proportion of digested CP (dCP\_CP)} = (\text{RDP\_DM} + \text{dRUP\_DM}) / \text{CP\_DM}$$
(Equation 3-4)

where RDP, dRUP, and CP are a percentage of DM.

Residual Organic Matter

Based on the Lucas test, ROM is a uniform feed fraction with a high true digestibility and an endogenous fecal frac-

TABLE 3-1   Proportion of Starch Digested at Base (dStarch_Starch_base) for Various Starch Sources[a]

Feed	dStarch_Starch_base
Default	0.91
Corn grain, dry, fine grind (<1,250 μm)[b]	0.92
Corn grain, dry, medium grind (1,500 to 3,250 μm)	0.89
Corn grain, dry, coarse grind (>3,500 μm)	0.77
Corn grain, high moisture, fine grind (<2,000 μm, mean = 1,450 μm)	0.96
Corn grain, high moisture, coarse grind (>2,500 μm, mean = 3,630 μm)	0.90
Corn grain, steam flaked	0.94
Sorghum grain, dry, ground	0.83
Sorghum grain, steam flaked	0.94
Corn silage <30 percent DM	0.91
Corn silage 32–37 percent DM	0.89
Corn silage >40 percent DM	0.85
Grain sorghum silage[c]	0.85
Barley, steam rolled	0.94
Barley, ground	0.91
Wheat	0.93

[a] Coefficients were derived from experiments, reviews, and meta-analyses using lactating dairy cows (Bal et al., 1997; Cammell et al., 2000a,b; Firkins et al., 2001; Ferraretto et al., 2013).

[b] Because of incomplete data, particle size classifications for corn grain are not continuous. For corn with particle sizes not listed, interpolation can be used.

[c] Based on data from beef cattle (Gutierrez et al., 1982; Hart, 1987).

tion whether calculated using the standard NDF value or ash- and CP-free NDF (Tebbe et al., 2017). The true digestibility of ROM calculated with NDF was 0.96 and was set as the base digestibility (dROM_ROM_base) in the model.

Fatty Acids

The base digestibility of FAs (dFA_FA_base) is set at 0.73 for most feeds; however, for supplemental fat sources, true digestibility is dependent on the source of FAs and was based on published digestion data (see Table 4-1; Chapter 4). Digestibility for FAs is affected by FA saturation and length and perhaps by interactions among different FAs and can be depressed by high-fat diets. Because the FA profile of total diets will vary less than the FA profile of feeds, the true digestibility of FAs among basal diets is also likely less variable. Therefore, the committee decided to assign all basal feeds (excluding fat supplements) the same true digestibility for FAs, which represents the average true FA digestibility of mixed diets. Digestibility of FAs from different fat sources and supplements is discussed in Chapter 4.

Adjustments to the Base Digestibilities for Intake and Diet Composition

The digestibilities of NDF and starch are adjusted for level of intake, and NDF digestibility is also adjusted for starch

content of the total diet. The depression in digestibility as intake increases has long been recognized (Tyrrell and Moe, 1975) and was included either implicitly (NRC, 1989) or explicitly in previous editions (NRC, 2001). In NRC (2001), the intake discount was greater with higher baseline digestibilities. The overall depression in DM digestibility with increasing intake was overestimated in NRC (2001) (Huhtanen et al., 2009; White et al., 2017; de Souza et al., 2018). One likely reason for this overestimation was that level of intake and baseline digestibilities are confounded.

Neutral Detergent Fiber

The committee considered several approaches but adopted the adjustments to NDF digestibility from a meta-analysis of individual cow data from multiple studies at multiple locations conducted by de Souza et al. (2018) with modifications. In de Souza et al. (2018), digestibility of NDF in response to intake was curvilinear with a maximum at 3.5 percent of BW. Decreased estimated digestibility at lower intakes is counter to discounts in previous NRC versions, and the data set was limited in that range of intake, and many of the low DMI in the data set were from an experiment that fed low-quality (low digestibility) diets. Their data set also did not include any observations with DMI greater than about 5.5 percent of BW. Therefore, the committee decided to modify the equation to remove this depression at lower intakes while retaining the depression at higher intakes. This was done by calculating the marginal slope (first derivative of the DMI curve of de Souza et al., 2018) from a DMI of 3.5 percent of BW to the limit of the data (5.5 percent of BW) and averaging those values. The resulting average slope was 1.1, which was used as the linear discount factor for NDF as DMI (percentage of BW) increased.

On the basis of meta-data, White et al. (2017) derived an equation with a 7 percentage unit decrease in NDF digestibility per unit increase of DMI/BW. However, in their derivation, starch had no effect on NDF digestibility. In contrast, a meta-analysis of literature means by Ferraretto et al. (2013) estimated that increasing starch by 1 percentage unit linearly decreased NDF digestibility by 0.5 percentage units, but they reported DMI did not significantly affect NDF digestibility. In contrast with those meta-analyses, the negative effects of DMI and starch have been demonstrated in experiments specifically designed to test for those effects. Therefore, the committee adopted modified equations of de Souza et al. (2018), which include both a starch and DMI term (Equation 3-5a). Although this equation likely should include a factor to account for the fermentability of the starch, data were insufficient to do so.

$$\text{Digested proportion of NDF (dNDF\_NDF)}$$
$$= \text{dNDF\_NDF\_base} - 0.0059 \times (\text{Starch\_DM} - 26)$$
$$- 1.1 \times (\text{DMI\_DM} - 0.035)$$
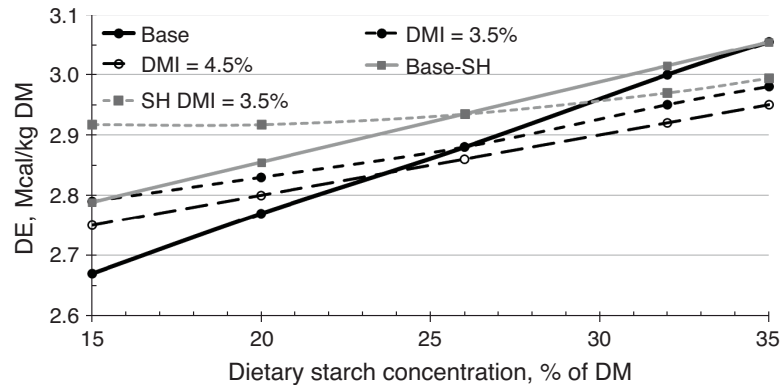
$$\text{(Equation 3-5a)}$$

FIGURE 3-2   Effects of increasing dietary starch content on the DE value of example diets with and without adjusting NDF and starch digestibility for dietary starch concentration and DMI (Equations 3-5a and 3-5b) where starch replaces either forage NDF or soyhulls (SH). In all diets, the concentration of NDF + starch was 62 percent, and the diets contained 16 percent CP, 3 percent FAs, and 14 percent ROM with apparent digestibilities of 0.65, 0.73, and 0.70, respectively, and 5 percent ash. In one set of diets (black lines), base NDF digestibility and starch digestibility at 26 percent dietary starch and DMI of 3.5 percent of BW were 0.46 and 0.91 (typical values for a forage-based diet). The solid line represents the dietary DE content if no adjustments in NDF or starch digestibility were made for intake or dietary starch concentration. The line with short dashes shows DE content after adjusting for dietary starch concentration but keeping DMI at 3.5 percent of BW. The line with long dashes shows DE content after adjusting for dietary starch with a constant DMI of 4.5 percent of BW. In the second set of diets (gray lines), all concentrations and digestibilities were the same as above, except changes in dietary starch were achieved by exchanging starch with NDF of SH. The diet with 35 percent starch had no NDF from SH, but the diet with 15 percent starch had 20 percent NDF from SH. Assumed digestibility of NDF (without discount) from SH was 0.60 so that total diet NDF digestibility (without discount) ranged from 0.46 (35 percent starch and no SH) to 0.52 with 15 percent starch and 20 percent SH NDF. In both set of diets, note that the increased DE content expected from replacing NDF with starch is diminished by the drop in NDF digestibility caused by starch. Thus, if voluntary feed intake is not altered by diet, the value of added starch is less than expected. Also note that intake has less impact on DE content than does starch.

where starch is as a percentage of diet DM and DMI_BW = DMI/BW (kg/kg).

The digestibility of NDF in diets with substantial NDF from fibrous by-product feeds often decreases at a faster rate with increasing DMI than NDF in diets where most of the NDF is from long-forage particles (Potts et al., 2017; White et al., 2017); however, exceptions exist (Edionwe and Owen, 1989). The concentration of long-forage NDF likely affects the digestibility of NDF from shorter particles. With adequate long particles, small-particle NDF may be trapped in the rumen mat and be digested, whereas with inadequate long particles, the small particles flow from the rumen quicker without extensive digestion. Inadequate data are available to model these effects; therefore, particle size of the NDF source is not included in the model. The committee also recognizes that NDF digestibility could be depressed if diets contained inadequate RDP; however, data were deemed inadequate to derive an equation, and estimates are based on the assumption that RDP is not limiting, which is usually the case in practical situations.

Starch

In their 2018 article, de Souza et al. reported that starch digestibility decreases by 1.0 percentage unit for every 1-unit increase in DMI as a percentage of BW. Ferraretto et al. (2013) also found a negative relationship between DMI and starch digestibility, but the effect was 0.24 percentage units

per kilogram of DMI (approximately equal to a ~1.3 percent decrease per unit increase in DMI_BW). The data for both studies are biased heavily toward dry ground corn grain, and it seems likely that digestibility of starch from more fermentable sources of starch (e.g., high-moisture corn, barley, and wheat) would be affected less by intake; however, inadequate data were available to quantify a source of starch effect. In the model, when DMI_BW >0.035 (i.e., 3.5 percent of BW), the proportion of starch that is digested decreases by 1.0 percentage units per unit DMI_BW, and when DMI_BW <0.035, the proportion of starch digested increases (Equation 3-5b). This adjustment may underestimate starch digestibility of highly fermentable starch sources when fed at high DMI.

$$\text{Digested proportion of Starch (dStarch\_Starch)} = \text{dStarch\_Starch\_base} - 1.0 \times (\text{DMI\_BW} - 0.035)$$
(Equation 3-5b)

Examples of the effect of altering starch and NDF digestibility based on DMI and dietary starch concentrations on dietary DE are shown in Figure 3-2.

Other Considerations for Changes to the Base Digestibility

Almost no data exist to determine whether ROM digestibility is depressed with greater intake, but most components

of ROM are likely not affected by intake. As discussed above, digestibility of protein is not affected by DMI. The committee recognizes that the method of predicting effects of intake on digestibility in the current version was based on the inherent differences in ad libitum intake among cows or groups of cows sometimes fed diets of varying composition (e.g., Huhtanen et al., 2009; White et al., 2017; de Souza et al., 2018) rather than on designed experiments where intake was a treatment such as in studies by Tyrrell and Moe (1975). However, the first approach was considered more relevant for commercial applications where cows are generally fed ad libitum. Thus, these equations may not be accurate in situations where animals, especially heifers, are fed at restricted intake, and digestibility of NDF, starch, FA, and organic matter (OM) may be greater in restricted-fed animals than predicted by the current equations. However, de Souza et al. (2018) reported that their equations were reasonably accurate for predicting the digestion of NDF and starch in restricted-fed dairy heifers.

Estimating Endogenous Fecal Material and Apparent Digestibilities

The mass of fecal matter from endogenous sources cannot be measured in ruminants; it can only be estimated using statistical methods. This endogenous (or metabolic) fecal matter is mainly bacteria and bacterial residue comprising mostly CP and ROM. Endogenous CP and ROM are substantial and must be considered when converting truly digested nutrients into DE, which is calculated from apparent digestibility. In addition, these values are necessary for estimating the apparent digestibility values of CP and ROM for comparison to digestibility data collected in experiments. Apparent and true digestibilities are considered the same for NDF, starch, and FAs.

Endogenous fecal CP (to be consistent with terminology in Chapter 6, this will be referred to as metabolic fecal CP or MFCP), which represents sloughed endogenous cells and secretions, and undigested microbial CP are described in detail in Chapter 6.

$$\text{MFCP, g/kg DMI} = 11.62 + 0.134 \times \text{NDF\_DMI}$$
(Equation 3-6a)

where dietary NDF is as a percentage of DM.

$$\text{Fecal microbial CP (fMCP), g/kg DMI} = (\text{Microbial CP (g/d)} \times 0.2) / \text{DMI}$$
(Equation 3-6b)

The endogenous masses were multiplied by the appropriate enthalpies (5.65 for CP and 4.0 Mcal/kg for ROM) to obtain endogenous fecal energy, which is subtracted from the sum of the DE from nutrients as discussed above. The DE values in the feed composition table (Chapter 19) were calculated with diet NDF set at 30 percent so that endogenous fecal CP = 15.6 g/kg DM or 0.088 Mcal/kg DMI. Undigested

bacterial CP was set as 16.5 g/kg DMI or 0.093 Mcal/kg (based on the average quantity of microbial protein synthesized and average DMI in the data set), and endogenous fecal ROM (efROM) was set at 34.3 g/kg DMI or 0.137 Mcal/kg DMI (Tebbe et al., 2017). Total endogenous fecal energy was $0.088 + 0.093 + 0.137 = 0.318$ Mcal/kg DMI.

To estimate apparent digestibilities of OM, CP, and ROM, which is useful in comparing model generated data to in vivo digestibility data, estimated endogenous output and estimated true digestibility of the fractions are used.

$$\text{Apparently Digested Proportion of ROM}$$
$$(\text{adROM\_ROM}) = [(\text{ROM} \times 0.96) - 3.43] / \text{ROM}$$
(Equation 3-7a)

where ROM is a percentage of DM.

$$\text{Apparently Digested Proportion of CP (adCP\_CP)}$$
$$= [(\text{RDP} + \text{dRUP}) - (\text{fMCP} + \text{MFCP})] / \text{CP}$$
(Equation 3-7b)

where all variables are kg/d.

$$\text{Apparently Digested Proportion of OM}$$
$$(\text{adOM\_OM}) = (\text{NDF} \times \text{dNDF\_NDF} + \text{Starch}$$
$$\times \text{dStarch\_Starch} + \text{FA} \times \text{dFA\_FA} + \text{RDP} + \text{dRUP}$$
$$+ 0.96 \times \text{ROM} - \text{MFCP} - \text{fMCP} - \text{efROM}) / \text{OM}$$
(Equation 3-7c)

where NDF, Starch, FA, RDP, dRUP, ROM, OM fMCP, MFCP, and efROM are in kg/d.

Estimating the Digestible Energy of Feeds and Diets

DE was calculated by multiplying the estimated truly digested nutrient concentrations for each feed by their respective heats of combustion and then subtracting the energy in the endogenous fecal excretions and undigested bacteria. To estimate DE from CP, the CP equivalent from supplemental NPN is subtracted from RDP. The NPN is assumed to have the energy value of urea, which is then added. As can be seen in Equation 3-1, ROM is corrected for supplemental NPN.

Digestible Energy

$$\text{Digestible Energy (DE\_DM; Mcal/kg of DM)}$$
$$= 0.042 \times \text{NDF\_DM} \times \text{dNDF\_NDF} + 0.0423$$
$$\times \text{Starch\_DM} \times \text{dStarch\_Starch} + 0.0940 \times \text{FA\_DM}$$
$$\times \text{dFA\_FA} + 0.0565 \times (\text{RDP\_DM}$$
$$- \text{sNPNCPE\_DM} + \text{dRUP\_DM}) + 0.0089$$
$$\times \text{sNPNCPE\_DM} + 0.040 \times \text{ROM\_DM} \times 0.96 - 0.00565$$
$$\times \text{MFCP} - 0.00565 \times \text{fMCP} - 0.0040 \times \text{efROM\_DM}$$
(Equation 3-8)

where feed fractions are a percentage of DM, endogenous fractions are g/kg, and digestibilities are expressed as proportions.

Estimating the Metabolizable Energy of Diets

Energy lost in urine and via methane is subtracted from DE to obtain ME. Gaseous energy (methane) is calculated as described in Chapter 14 as

$$\text{Gas Energy Loss (GasE\_DM; Mcal/kg DMI)} = (0.294 \times \text{DMI} - 0.347 \times \text{FA\_DM} + 0.0409 \times \text{dNDF\_DM}) / \text{DMI}$$
(Equation 3-9)

where DMI is kg/d and FA_DM and dNDF_DM are a percentage of diet DM.

UE is calculated from estimated urinary N (N excretion):

$$\text{UN (g/d)} = \text{g Urinary N per day} = (\text{DMI} \times \text{CP\_DM} \times \text{adCP\_CP} - \text{Milk CP} - \text{Body gain CP}) \times 1,000 / 6.25$$
(Equation 3-10a)

where DMI, milk CP, and body gain CP are in kg/d, and CP_DM and adCP_CP are proportions.

If the animal is not lactating and within 60 days of parturition, the "Milk CP" term in Equation 3-10a is replaced with "$0.00014 \times$ Mature BW." That term was derived by calculating the amount of protein retained (kg/d) in the gravid uterus at 250 days of gestation based on an average Holstein and Jersey calf birth weight (see gestation requirements in this chapter and in Chapter 6). For Equation 3-10a, body protein gain in lactating cows can be ignored because the effect is likely less than the imprecision associated with the equations. For example, a 100-g/d increase in body protein would change average estimated ME by <0.5 percent. For growing heifers, body protein gain is estimated as described in Chapter 11.

UE (Mcal/kg DMI) was estimated from urinary N excretion (g/d) as

$$\text{UE\_DM (Mcal/kg DMI)} = (0.0146 \times \text{UN}) / \text{DMI}$$
(Equation 3-10b)

The coefficient (0.0146 Mcal/g of urinary N) was calculated from recent experiments that measured urinary energy and urinary N excretion (Morris et al., 2021).

Metabolizable energy was then calculated by subtracting gas and urinary energy from DE:

$$\text{ME\_DM (Mcal/kg DMI)} = \text{DE\_DM} - \text{GasE\_DM} - \text{UE\_DM} \quad \text{(Equation 3-11)}$$

In previous NRC editions, the conversion of DE to ME was considered on an individual feed basis. The current equation uses whole-diet estimates of urinary energy and gas energy, and thus it is only valid for the total diet. In NRC (2001), a correction was added to increase the efficiency of converting DE to ME for diets with higher fat so that the ef-

fective conversion for the fat was 100 percent. In the current equation, dietary FAs are used to estimate methane losses; therefore, the efficiency of converting DE to ME is greater for high-fat diets.

Estimating the Net Energy Lactation of Diets

The conversion of ME to NEL (Equation 3-12) is predicted on a whole-diet basis based on the average efficiency measured between 1974 and 1995 (see Table 3-2) in studies at the Beltsville Energy Metabolism Unit as reassessed by Moraes et al. (2015). The mean was 0.66 with a 95 percent confidence interval of 0.64 to 0.69. No correction for concentration of dietary fat is used with this equation:

$$\text{Net Energy of Lactation per kg DM (NEL\_DM; Mcal/kg)} = 0.66 \times \text{ME\_DM}$$
(Equation 3-12)

ENERGY REQUIREMENTS

Changes from the seventh revised edition include the following:

- The maintenance requirement is increased from 0.08 to 0.10 Mcal per kg of metabolic BW.
- The efficiency of using ME for lactation is increased from 0.64 to 0.66.
- Growth requirements have been simplified and are now explicitly related to the size of an animal relative to its mature BW (MatBW). The composition of gain does not change with diet and growth rate at a given BW, with the assumption that animals will be fed for rates of gain that maintain body condition.
- The composition of body condition score (BCS) change is not dependent on the starting BCS, so the energy required per kilogram of BW for body condition gain (or available from loss) is a constant.
- Requirements for physical activity have been updated.

The basic unit of dietary energy for dairy cattle is NEL, and all energy requirements are adjusted to be equivalent to this unit. Estimates for conversions of ME and changes in retained energy (RE) in the seventh edition (and several previous versions) were based on data from Moe et al. (1971) at the USDA Energy Metabolism Unit at Beltsville, Maryland. Recently, Moraes et al. (2015) reanalyzed the data from that laboratory, and the NEL requirement for maintenance was increased partly in response to this reanalysis and is biased toward the latest decade of work at Beltsville. Thus, the efficiency of using ME for replenishing body reserves and the efficiency of using mobilized body reserves for milk production also are now biased toward the later years of this reevaluation of the Beltsville data, as shown in Table 3-2.

TABLE 3-2   Energetic Parameters from Reanalysis of Data Over Several Decades from Beltsville Energy Metabolism Unit (Moraes et al., 2015) and Values for Seventh (NRC, 2001) and Current Editions[a]

Parameter	1963–1995	1974–1995	Seventh Edition	Eighth Edition
ME for maintenance (Mcal/kg$^{0.75}$ BW/d)	0.14	0.16	0.13	0.15
NEL for maintenance (Mcal/kg$^{0.75}$ BW/d)	0.086	0.10	0.080	0.10
Conversion efficiencies				
ME to NEL	0.63	0.66	0.64	0.66
ME to RE during lactation	0.70	0.74	0.75	0.74
NEL to RE during lactation[b]	1.11	1.12	1.17	1.12
RE to NEL[b]	0.89	0.89	0.82	0.89
ME to RE when dry[b]			0.60	0.60
NEL to RE when dry[b]			0.94	0.91

[a] All energy requirements must be converted to diet NEL equivalents for use in the model. NEL for maintenance is calculated as ME × conversion of ME to NEL. RE is retained energy, or the energy of tissue gain or loss.

[b] The NEL required for RE during lactation or when dry is the conversion of ME to RE divided by the conversion of ME to NEL. Because ME is converted to tissue energy more efficiently than to milk energy, these values are greater than 1, so it takes less than 1 Mcal of feed NEL to store 1 Mcal of body tissue. The lower efficiency for dry cows likely is because dry cow diets are higher in fiber, resulting in greater heat of fermentation and diet-induced thermogenesis.

Maintenance Requirements

The NEL requirement for maintenance (NELmaint) of adult dairy cattle is

$$\text{NELmaint (Mcal/d)} = 0.10 \times \text{BW kg}^{0.75}$$
(Equation 3-13)

Based on Moraes et al. (2015), this value would have a 95 percent confidence interval of about ±0.06. This is a substantial increase from previous versions and adds about 2.5 Mcal of NEL to the energy requirement of the average Holstein cow. Given the intensive selection for milk production in dairy cattle over the past 50 years with average milk production now three times that of the 1960s, it seems reasonable that modern dairy cows have metabolic rates for maintenance that are greater than they were 50 years ago. Cows of similar size and breed and in similar conditions may vary as much as 10 percent in their maintenance requirements (Van Es, 1961). This is consistent with more contemporary data from studies of residual feed intake showing that the intake for cows of similar BW and production varies by 7 percent after accounting for parity, location, diet, and other environmental effects (Tempelman et al., 2015); some of this variation could be caused by genetic variation in maintenance. Measured fasting heat production (Flatt et al., 1965) in dry nonpregnant dairy cows averaged 0.073 Mcal/unit metabolic BW (MBW), and estimated fasting heat production of dairy cows using regression analysis suggested an identical value (NRC, 2001). Because these measurements were made with cows housed in tie-stalls in metabolic chambers, past committees added a 10 percent activity allowance to account for normal voluntary activity of cows that would be housed in drylot or free-stall systems, such that the NELmaint was set at 0.080 Mcal/kg MBW for mature dairy cows. This value has been used since NRC (1978). However, newer data and reevaluation of older

data all derived maintenance coefficients that were greater than 0.09, with some as high as 0.14. Moraes et al. (2015) reanalyzed the data from the Beltsville Energy Metabolism Unit and found that the apparent maintenance requirement for adult dairy cows increased with year of measurement. Maintenance requirements were 0.073, 0.087, and 0.122 Mcal/kg MBW for the years 1963 to 1973, 1974 to 1983, and 1984 to 1995, respectively, based on respective efficiencies of converting ME to NEL of 0.60, 0.62, and 0.69. Even with a lower efficiency of converting ME to NEL, the NELmaint of cows from 1984 to 1995 would be greater than 0.10 × MBW. As with all requirements, maintenance requirements are not known with certainty. For example, assuming conversions of ME to NEL of 0.66, other studies have yielded NELmaint (per kilogram of MBW) of 0.096 (Kirkland and Gordon, 1999), 0.09 (Birkelo et al., 2004), 0.11 (Xue et al., 2011), 0.11 (Dong et al., 2015), 0.14 (Foth et al., 2015), and 0.10 (Morris and Kononoff, 2021) for lactating cows; the NEm coefficient was 0.098 for fasted nonlactating cows (Birnie et al., 2000). The committee chose 0.10 × BW kg$^{0.75}$ because it is simple and within the bounds determined by Moraes et al. (2015) for the last two decades of data in their study.

The most recent revision of the *Nutrient Requirements for Beef Cattle* (NASEM, 2016) also supports the higher value for dairy cattle. Converting their equation to BW (instead of shrunk BW) and adjusting for dairy breeds results in a NELmaint of 0.095 × MBW. In addition, NASEM (2016) suggests that maintenance requirements per unit MBW do not decrease with age, they are 20 percent greater for lactating than nonlactating cows across beef breeds, and maintenance energy requirement is correlated positively with the genetic potential for milk production. Based on Table 19-1 of NASEM (2016), NELmaint should be 0.095 × MBW for nonlactating and lactating dairy cows. The current committee recognizes that maintenance requirements could be considered greater for lactating than nonlactating cows because

(1) lactating cows generally have a greater mass of liver and other internal organs as a proportion of BW, (2) these organs produce more heat per unit mass than skeletal muscle, and (3) high-producing cows seem to require more ME for maintenance than low-producing cows (Moe et al., 1970; Baldwin et al., 1985; Ellis et al., 2006). However, the current committee considers that the increased mass and heat production of internal organs in lactating cows is a cost of milk production and should be assigned as part of the incremental heat loss in the conversion of ME to NEL for an animal that is digesting and metabolizing more feed nutrients.

The Committee on Nutrient Requirements of Beef Cattle (NASEM, 2016) applied a breed adjustment factor for maintenance of 1.2 for Holsteins and Jerseys (compared to British beef cattle breeds). Whether dairy cattle breed alters maintenance requirements or energy metabolism is not clear. Tyrrell et al. (1991) compared nonlactating and lactating Holstein and Jersey cows. Although actual milk yields (MYs) were greater for Holstein cows than for Jersey cows, energy output in milk as a function of MBW was similar, and there was no evidence to suggest that energy requirements for maintenance or production differed between breeds once adjusted for MBW. The committee considered setting maintenance requirement based on BW adjusted to a standardized BCS and to a nonpregnant status. Such an adjustment has been used for maintenance requirements in dogs and cats (Hand et al., 2000) and is consistent with the idea of setting maintenance energy requirements as a proportion of body protein mass (Agnew and Yan, 2000). Birnie et al. (2000) examined fasting heat production of 12 dry cows that were fed to be either thin or fat (mean BCS 1.3 versus 4.7 with BW of 467 versus 692 kg) and determined that daily NELmaint per cow was essentially the same. The NELmaint per unit MBW was also the same if BW was adjusted to a BCS of 3.0, assuming 1 BCS was 10 percent of BW. The committee recommends that future research examine the relationship between condition score and maintenance requirements but did not make any adjustment in the current requirement because most of the chamber data with dairy cows did not include information on body condition.

Lactation Requirements

The NEL concentration in milk is equivalent to the sum of the heats of combustion of individual milk components. No changes have been made to NEL requirements except for minor changes to equations for composition to account for true protein and NPN fractions. As with the seventh edition, the heats of combustion of milk fat, true protein, NPN CP equivalent, and lactose are 9.29, 5.71, 2.21, and 3.95 kcal/g, respectively. Milk CP, when estimated as $6.38 \times N$, contains 5 to 6 percent NPN (DePeters and Ferguson, 1992). Assuming milk CP is 6 percent NPN and 94 percent true protein, then the NEL value of milk CP is 5.5 kcal/g. If the CP content of milk is known and the true protein content is not known, the NEL concentration of milk is calculated as

$$NEL\ (Mcal/kg) = 9.29 \times kg\ Fat/kg\ Milk + 5.5 \times kg\ Crude\ Protein/kg\ Milk + 3.95 \times kg\ Lactose/kg\ Milk$$
$$(Equation\ 3\text{-}14a)$$

If true protein is measured, the energy of true protein is adjusted up to account for the energy of NPN, which was assumed to equal 5.5 percent of milk CP and to have heat of combustion of urea (2.5 Mcal/kg) or $5.71 + 0.055 \times 2.5 = 5.85$, so the NEL concentration of milk is calculated as

$$NEL\ (Mcal/kg) = 9.29 \times kg\ Fat/kg\ Milk + 5.85 \times kg\ True\ Protein/kg\ Milk + 3.95 \times kg\ Lactose/kg\ Milk$$
$$(Equation\ 3\text{-}14b)$$

Milk lactose content is the least variable milk component and is generally about 4.85 percent of milk and varies only slightly with breed and milk protein concentration. If milk lactose is not measured, it should be set at 0.0485 kg/kg milk in the above equations.

When milk fat is the only milk constituent measured, NEL concentration can be calculated using the formula of Tyrrell and Reid (1965):

$$NEL\ (Mcal/kg\ of\ milk) = 0.360 + 0.0969 \times Fat\ (\%)$$
$$(Equation\ 3\text{-}14c)$$

The NEL system in this edition is based on yield of total energy in milk and does not account for many of the differences in metabolic transactions or the substrates required for synthesis of individual milk components. Attempts to assign differential efficiencies of converting feed ME to the NEL of individual milk components have been made (Baldwin, 1968; Dado et al., 1993); however, these calculations ignore energy losses in metabolic transactions outside of the mammary gland and thus are higher than those measured by calorimetry (Moraes et al., 2015). The measured calorimetric inefficiency of use of ME for milk includes losses associated with metabolic transactions for conversion of absorbed nutrients into milk components, the energy required for nutrient absorption, and increased rates of metabolism in visceral tissues required for support of increased milk production. Currently, data are lacking to confidently assign unique efficiencies of converting ME to the NEL of individual milk components.

Activity Requirements

The maintenance requirement is assumed to provide adequate energy for normal activity of cows in confinement. On many confinement farms, the distance between the housing area and milking center can be substantial, but this probably has little effect on overall energy expenditures

(see Chapter 13). Energy expended for walking over a level surface is approximately 0.35 kcal of NEL/kg of BW per kilometer walked (Brosh et al., 2006; Aharoni et al., 2009; Brosh et al., 2010). If the milking center was 200 m from the pen and a 650-kg cow was milked three times per day, the NEL expended for walking would be about 0.3 Mcal/d (about 2 percent of her maintenance requirement). Grazing cattle expend much more energy walking and gathering food; discussion and equations for grazing can be found in Chapter 13.

Environmental Effects

Equations for the energy requirements of thermal regulation have been developed and are used for beef cattle (NASEM, 2016). In general, lactating dairy cows typically operate at much higher metabolic activity level and produce more heat per day than do beef cattle. In addition, dairy cows are generally housed in environments that provide some shelter from cold conditions. Thus, cold stress is not as important for dairy cows as beef cattle. For lactating cows in cold environments, the change in energy requirement is probably minimal because of the normally high heat production of cows consuming large amounts of feed, and they likely require very little extra dietary energy to counteract cold environments if they are kept dry and are not exposed directly to wind. Young (1976) summarized experiments with ruminants in which an average reduction in DM digestibility of 1.8 percentage units was observed for each 10°C reduction in ambient temperature below 20°C. Much of this lowered digestibility under cold stress was related to an increased rate of passage through the digestive tract (Kennedy et al., 1976). Because of the effects of low temperature on digestibility, under extremely cold weather conditions, feed energy values could possibly be lower than expected.

Dairy cows are often heat-stressed, and lactating cows producing the most milk are the most likely to be heat-stressed. However, the committee determined that insufficient data were available to quantify these effects accurately in dairy cows and to account for all important factors such as ambient temperature, relative humidity, radiant energy exposure, night-cooling, air speed, level of production, and heat abatement programs. Heat stress may increase the maintenance requirement of dairy cattle by 7 to 25 percent (NRC, 1981), but these values are based on very little direct data. Measured by indirect calorimetry, fasting heat production increased about 5 percent and ME requirement for maintenance increased about 10 percent when dry cows were housed at 36°C compared with 18°C (Kurihara, 1996). Heat stress induces behavioral and metabolic changes in cattle (West, 1994; Wheelock et al., 2010). Some changes, such as increased respiration rate, panting, and immune activation, likely increase energy expenditures, but the most important responses to heat stress are the physiological responses that result in decreased milk production and feed consumption. When cows eating ad libitum in thermoneutrality (temperature humidity index of 75) were either heat-stressed (temperature humidity index of 65) or pair-fed at thermoneutrality, heat production per unit of MBW was decreased similarly, likely due to decreased feed intake, and no increase in the maintenance requirement was detected (Lamp et al., 2015). Heat-stressed animals employ novel homeorhetic strategies that decrease milk production and decrease feed intake without a change in lipid mobilization (Baumgard and Rhoads, 2013). The decrease in DMI induced by heat stress is greater than what would be expected based on the decrease in MY therefore, equations developed to estimate DMI under thermoneutral conditions will likely overestimate DMI under heat stress, and users may need to modify the DMI estimates. Ultimately, these changes decrease the need for heat dissipation and are important for survival. However, they are difficult to model. Because of limited data, adjustments for heat stress have not been included in the calculation of maintenance requirements; further research is needed.

Pregnancy Requirements

Energy requirements for gestation in NRC (2001) were calculated from a linear function of day of gestation starting at day 190 and scaled to calf birth weight based on serial slaughter data (Bell, 1995; Bell et al., 1995). However, over a longer gestation period, the gravid uterine growth is better described by a logistic or decaying exponential growth function (Koong et al., 1975; Ferrell, 1991). The function was rearranged so that birth weight of the calf was an input rather than an output. Gravid uterine weight at parturition (GrUter_Wt$_{(t\,=\,parturition)}$) and uterine weight immediately after calving (Uter_Wt$_{(t\,=\,Parturition)}$) were estimated from calf birth weight using data from Bell et al. (1995) and House and Bell (1993) data:

$$\text{GrUter\_Wt}_{(t\,=\,\text{parturition})} = \text{Calf birth weight} \times 1.825$$
$$\text{(Equation 3-15a)}$$

$$\text{Uter\_Wt}_{(t\,=\,\text{Parturition})} = \text{Calf birth weight} \times 0.2288$$
$$\text{(Equation 3-15b)}$$

Average birth weight (kg) of calves born from multiparous cows are 44 (Holstein), 26 (Jersey), 38 (Ayrshire), 48 (Brown Swiss), 36 (Guernsey), and 36 (milking shorthorns), and birth weight of calves born from heifers averages 91 percent of those weights (Legault and Touchberry, 1962; Olson et al., 2009; Dhakal et al., 2013; Kamal et al., 2014). Calf birth weight also can be estimated from MatBW: 0.063 times MatBW for a cow and 0.058 times MatBW for a heifer. For these calculations, full term is assumed to be 280 days of gestation, which is also used in the software.

Nonlinear regression (i.e., a logistic function) of the data from Bell et al. (1995) and House and Bell (1993) was used to derive Equation 3-16a. The model also predicts uterine

involution postpartum (Equation 3-16b) to maintain mass balance and predict release of tissue energy and amino acids for productive use in early lactation as described by Hanigan et al. (2009):

$$GrUter\_wt = (GrUter\_Wt_{(t = parturition)}$$
$$\times e^{-(0.0243 - (0.0000245 \times DayGest)) \times (280 - DayGest)}$$

(Equation 3-16a)

$$UterWt = ((Uter\_Wt_{(t = Parturition)} - 0.204)$$
$$\times e^{-0.2 \times DayLact}) + 0.204 \quad \text{(Equation 3-16b)}$$

where DayGest = day of gestation (day of gestation must be between 12 and 280), and Uter_Wt$_{(t = parturition)}$ = estimated weight (kg) of uterus immediately postcalving (Calf birthweight $\times$ 0.2288). The involution rate is not known with certainty, but the value of 0.2/d will result in essentially complete involution by day 21 of lactation.

Daily rates of wet tissue deposition (kg/d) are derived from Equations 3-16a and 3-16b as (variables defined above):

$$During\ gestation: GrUter\_Wt_{Gain} = (0.0243$$
$$- (0.0000245 \times DayGest)) \times GrUter\_Wt$$

(Equation 3-17a)

$$During\ involution: GrUter\_Wt_{Gain} =_t -0.2 \times DayLact$$
$$\times (Uter\_Wt - 0.204) \quad \text{(Equation 3-17b)}$$

The NEL gestational (Gest) requirements were calculated from the rate of change in gravid uterine tissue mass and by assuming tissue contained 0.882 Mcal of energy/kg (House and Bell, 1993; Bell et al., 1995), an ME to gestation energy efficiency of 0.14 (Ferrell et al., 1976; NRC, 2001), and an ME to NEL efficiency of 0.66.

$$Gest\_NEL\ (Mcal/d) = GrUter\_Wt_{gain} \times (0.882/0.14)$$
$$\times 0.66 = GrUter\_Wt_{gain} \times 4.16$$

(Equation 3-18)

Over a 60-day dry period, NEL requirements are essentially the same whether calculated using NRC (2001) or the new model; however, calculated NEL requirements will be lower for far-off dry cows and greater in prefresh cows using the new model as compared to the previous model (see Table 3-3). Protein is discussed in Chapter 6.

Changes in Body Weight and Composition During Growth and Lactation

In the seventh edition, body composition equations were based largely on data from beef cattle with the standard reference animal having a MatBW of 500 kg. Modern Holsteins have a MatBW of ~700 kg (Tempelman et al., 2015), and dairy breeds are generally less muscular than beef breeds. Several publications have reported the composition of growing and mature Holsteins in the past 20 years, and the committee deemed that sufficient data were available to develop equations for Holsteins. Details on the data set and models can be found in de Souza et al. (2018).

In this edition, body energy change is partitioned as (1) body frame gain (i.e., true growth), (2) body reserves or condition gain (or loss), and (3) pregnancy-associated gain (considered a pregnancy requirement). Frame gain is normal skeletal growth, the normal body gain that occurs as animals mature from birth to adult, and includes the normal gains in skeletal muscle, adipose tissue, bone, organs, intestinal tract, and gut contents. Frame gain is the gain in BW without overnight fasting, assuming an animal maintains a constant BCS and is not pregnant. All requirements in the current model related to growth assume BW gain is frame gain. The tissue that is lost and gained during times of nutrient excess or deficiency in the life of an animal is body reserves. Changes in body reserves are generally, but not always, observed as changes in BCS. Condition score changes are expected during a normal lactation cycle but can also occur in growing heifers if fed more or less than needed for normal growth. Pregnancy-associated gain includes the growing fetus and associated tissues, including placenta and mammary gland that increase as gestation progresses and are considered a pregnancy requirement.

BW can be divided into empty BW (EBW, the actual tissues of the animal) and gut fill. Based on data of lactating dairy cows, gut fill is about 5.2 times DMI (Gibb et al., 1992;

TABLE 3-3   Comparison of Gestation Energy and Protein Requirements[a] Calculated Using NRC (2001) and Current Model (Assumed Birth Weight of Calf = 44 kg)

Day of Gestation	Gestation NEL, Mcal/d		Gestation MP, g/d	
	NRC (2001)	Current	NRC (2001)	Current
50	0	0.04	0	3
100	0	0.1	0	13
150	0	0.5	0	43
200	2.7	1.4	199	125
220	3.0	2.0	245	185
250	3.4	3.5	306	320
275	3.8	5.4	357	489

[a] See Chapter 6 for the details on protein.

Andrew et al., 1994). For the typical cow, eating at 3.5 percent of BW, gut fill would be 18 percent (5.2×3.5 percent) of BW similar to NRC (2001) and NASEM (2016). Thus, 1 kg of frame gain for a cow includes 0.82 kg of EBW and 0.18 kg of gut fill. The committee recognizes that gut fill may not be 18 percent of BW gain in all cases, especially if animals are fed at restricted intake or fed diets of mostly poor-quality forage.

Energy of Tissue Mobilization and Repletion

The tissue that is lost and gained during a lactation cycle or during other times of nutrient deficiency or excess in the life of a cow is mostly lipid and considered body energy reserves. Like most mammals, a dairy cow typically mobilizes body reserves during early lactation and repletes them during later lactation and the dry period. Optimum management of body reserves improves the health and profitability of dairy cows. Overly fat cows, especially those around the time of calving, have lower feed intake and increased risk for dystocia and health problems. Conversely, overly thin cows have insufficient reserves for maximum milk production and often do not conceive in a timely manner.

Changes in body energy reserves are usually observed as changes in BCS. Although evaluation of BCS is subjective in nature, it is the only practical method to evaluate body energy stores of dairy cows on most farms. In the United States, the most common systems of BCS use a 5-point scale originally proposed by Wildman et al. (1982) with a BCS of 1 being extremely thin and a score of 5 being extremely fat. Edmonson et al. (1989) developed a BCS system using a 5-point scale based on visual appraisal of eight separate body locations (see Figure 3-3). Analysis of variation due to cows and to individuals assessing BCS suggested that visual appraisal of just two locations (between the hooks and between the hooks and pins) had the smallest error due to assessor and accounted for the greatest proportion of variation due to individual cows.

Despite the emphasis on measuring BCS over that past 30 years, data are surprisingly lacking on the mathematical relationships between BCS, BW change, gut fill, and body composition changes of dairy cows. Much of the available data are from transition cows during which time BCS and feed intake are changing in opposite directions so that actual BW loss is masked by increases in gut fill as feed intake increases during early lactation. Studies are needed in this area.

Body Weight Change per Body Condition Score

In NRC (2001), each BCS unit was associated with a change in BW of ~14 percent, or about 80 kg for a typical Holstein cow, and the weight gain or loss associated with changes in BCS was considered to be 18 percent gut fill. Using deuterium oxide dilution, Komaragiri and Erdman (1997) observed a change of 63 kg per unit BCS in cows with an average BW of 667 kg, and Komaragiri and Erdman (1998) observed a change of 59 kg per unit BCS in cows with an average BW of 634 kg. Other studies found BW per BCS values of 56 kg for 640-kg cows (Chillard et al., 1991), 56 kg for 558-kg cows (Otto et al., 1991, which was incorrectly interpreted in the seventh edition), and 56 kg for 597-kg cows
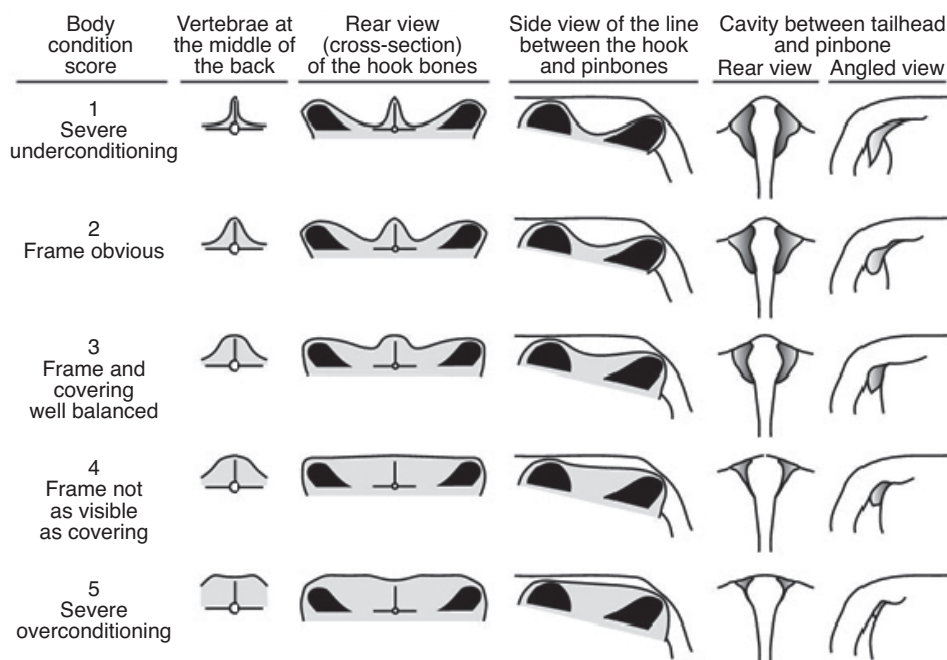
FIGURE 3-3   Body condition scoring chart.
SOURCES: M'hamdi et al. (2012); adapted from Edmonson et al. (1989).

(Waltner et al., 1994). A summation of the data across these studies suggests a mean BW change per unit of BCS of 9.4 percent of BW. This BW change is assumed to be entirely EBW change; in other words, changes in body mass that are due to gains or losses in BCS are not associated with changes in gut fill. In the seventh edition, gut fill was set at 18 percent of BW, and therefore, the mass of gut fill changed with BW as BCS was lost or gained in lactating cows. In growing animals, gut fill increases proportionally as an animal matures, but this is not true for cows, in which gut fill varies with the changes in DMI (Andrew et al., 1994) during the lactation cycle. Therefore, changes in BW associated with changes in BCS are assumed to be all body tissue (EBW) with no change in gut fill per unit BCS; hence, 1 kg of live body gain is 1 kg of empty body gain for changes in BCS. Cows do not eat more as they gain BCS; in fact, BCS is inversely associated with DMI (Garnsworthy, 2006; de Souza et al., 2019). Assuming gut fill is 18 percent of BW, a change in 1 BCS unit would be equal to 11.5 percent of BW for a cow at a BCS of 3. This value should be slightly higher for a thin cow and slightly lower for a fat cow.

Composition and Energy Content of Changes in Body Reserves

In the seventh edition, the composition of changes in body reserves was dependent on the starting and ending BCSs, with a greater proportion of fat in the change as average BCS increased. With that system, the RE of empty body changes associated with reserves varied from 5.1 Mcal/kg in very thin cows to 9.6 Mcal/kg in very fat cows. However, based on the constant fat content per unit BW change cited earlier, this is not supported by evidence. The current committee deemed the difference too small to warrant the increased complexity of differential energy values for BCS changes for cows with BCS ranging from 2 to 4. Most cows on farms are within these bounds; hence, the composition of BW change for BCS changes is considered a constant.

The energy value of a kilogram of true body tissue that is lost or gained is dependent on the relative proportions of fat and protein in the tissue and their respective heat of combustion. As in the seventh edition, the committee chose 9.4 and 5.55 Mcal/kg for retained body fat and protein. The current committee estimates that gain or loss of empty body in lactating cows between BCS of 2 and 4 contains 62.2 percent fat, 27.6 percent water, 8.1 percent protein, and 2.1 percent ash and has an energy value of 6.3 Mcal/kg. These values are based on the fat content of EBW from Chillard et al. (1991), Komaragiri and Erdman (1997, 1998), Otto et al. (1991), and Waltner et al. (1994), as well as the protein and ash content of fat-free mass of Waldo et al. (1997). Because gut fill does not change with BCS, the composition and energy value of BCS gain or loss is the same on a BW as an EBW basis. Assuming that 1 BCS unit equals 9.4 percent of BW, a 1-unit change in BCS for a 650-kg cow equals 61 kg of body mass containing 385 Mcal of energy and 5.0 kg of

protein. Because ME is used more efficiently for gain of reserves than for production of milk during lactation (0.74 versus 0.66), a 1-unit gain in BCS (385 Mcal of reserve RE) requires 520 Mcal of ME, and this equates to only 343 Mcal of feed NEL. Conversely, a loss of 1 BCS unit for a 650-kg cow would equal a loss of 385 Mcal of RE and provide 343 Mcal of NEL (equivalent to the energy in 490 kg of milk with 3.5 percent fat).

Based on the values from Table 3-2, the energy requirement in NEL units for body reserves gain is as follows:

If lactating:

$$NEL\ (Mcal/kg\ gain) = 6.3\ Mcal\ RE/kg \times 0.89$$
$$= 5.6\ Mcal\ NEL/kg\ BW\ gain$$
$$\text{(Equation 3-19a)}$$

If not lactating:

$$NEL\ (Mcal/kg\ gain) = 6.3\ Mcal\ RE/kg \times 1.10$$
$$= 6.9\ Mcal\ NEL/kg\ BW\ gain$$
$$\text{(Equation 3-19b)}$$

The NEL available from mobilization of body tissue and thus not needed in the diet is

$$NEL\ available\ (Mcal/kg\ loss) = 6.3\ Mcal\ RE/kg$$
$$\times 0.89 = 5.6\ Mcal\ NEL/kg\ BW\ loss$$
$$\text{(Equation 3-19c)}$$

Mobilization of body tissue is normal during early lactation to support the energy needs for lactation, as it is in many mammalian species. A loss of 0.5 BCS units typically occurs during the first 60 days postpartum in dairy cows.

Energy Requirements for Frame Growth

In NRC (2001), energy requirements for growth were developed for heifers using the beef NRC (1996) system. The RE associated with gain was dependent on where the animal was in its growth curve relative to a standard reference animal with 498 kg MatBW and on the animal's average daily gain (ADG). The effect of ADG on the composition of gain was very small, with ADG taken to a power of 1.097. Published reports in the past 20 years with widely divergent nutritionally induced changes in rate of gain of Holstein heifers show that the composition of gain can change much more than that previous equation estimated (Radcliff et al., 1997; Brown et al., 2005; Meyer, 2005; Davis Rincker et al., 2008). In studies where BCS was measured, diets that cause different rates of gain can cause large differences in BCS (Radcliff et al., 1997); however, in studies with younger heifers, diets that cause divergent rates of gain resulted in very little change in the composition of gain (Meyer, 2005). Ideally, the changes in body composition due to fast or slow daily gain from dietary manipulation of heifers should be assigned to changes in body

reserves and not to frame gain, but data on the effects of diet on gain and BCS are lacking. Thus, due to insufficient data, no allowance was made for rate of gain to alter the fat content of growing heifers in the current model. For growing heifers, BCS is not used, but the assumption is that the heifers will be fed to maintain moderate body condition. The committee recommends that further studies be conducted so that body gain of heifers can eventually be partitioned into frame gain and condition change, using a system to assess change in body fatness such as body condition scoring or ultrasonic fat depth.

The committee reemphasizes that frame gain assumes appropriate gains of lean and fat tissues for an animal maintaining a BCS of 3. An animal can gain frame mass while losing body condition. The seventh edition allowed for cows in their first and second lactations to gain frame mass and change condition simultaneously, but the growth equations for cows were not included in the computer model. The current version supports both frame gain and body condition changes for cows but includes only frame growth for heifers.

In the current version, both BW and the gain in BW for frame growth in heifers are 85 percent tissue and 15 percent gut fill. For immature cows, gut fill is calculated as 18 percent of BW or BW gain. Requirements for frame growth are described and justified in Chapter 11. The equations are as follows:

$$\text{Fat in Frame ADG (Fat\_ADG), g/g} = (0.067 + 0.375 \times (\text{BW/MatBW})) \times \text{EBG/ADG}$$
$$\text{(Equation 3-20a)}$$

$$\text{Protein in Frame ADG (Protein\_ADG), g/g}$$
$$= (0.201 - 0.081 \times (\text{BW/MatBW})) \times \text{EBG/ADG}$$
$$\text{(Equation 3-20b)}$$

$$\text{RE of Frame ADG (RE\_FADG), Mcal/kg} = 9.4$$
$$\times \text{Fat\_ADG} + 5.55 \times \text{Protein\_ADG}$$
$$\text{(Equation 3-20c)}$$

The efficiency of converting feed ME to net energy for gain (NEg) using NRC (2001) equations averaged about 0.40. The efficiency of converting NEL to NEg is based on conversions of 0.40 for ME to NEg and 0.66 for ME to NEL and is thus $0.40/0.66 = 0.61$; therefore,

$$\text{ME for Frame ADG (ME\_FADG, Mcal/kg)}$$
$$= \text{RE\_FADG/0.4} \quad \text{(Equation 3-20d)}$$

$$\text{NEL for Frame ADG (NEL\_FADG, Mcal/kg)}$$
$$= \text{RE\_FADG/0.61} \quad \text{(Equation 3-20e)}$$

Comparison of New Energy System to the 2001 System

A variety of diets that differed in forage quality and concentrations of starch, forage NDF, total NDF, fat, and CP fed at DMIs of 3.5 and 4.8 percent of BW were evaluated using the NRC (2001) equations and equations in this version. Two dry cow diets were also evaluated. The concentration of NEL (Mcal/kg) in lactating cow diets averaged about 8 percent higher (range, 6 to 12 percent) using the new model, and dry cow diets were about 10 percent higher. Using the 2001 model, energy concentrations in the lactating cow diets ranged from 1.53 to 1.63 Mcal/kg and from 1.65 to 1.77 Mcal/kg for the new model. Concentrations of NEL in dry cow diets increased from about 1.4 to 1.55 Mcal/kg. The greatest difference for lactating cow diets was observed at the high intake (about 10 percent higher). Generally, the NEL concentration of high-starch diets increased more than low-starch diets when comparing the new model to the old model. Energy requirements on average increased about 8 percent with the greatest relative increase for high-BW, low-producing cows. Energy requirements using the new system increased about 6 percent for a 650-kg cow producing 55 kg of milk but by about 11 percent for the same cow producing 30 kg of milk compared to NRC (2001). The greatest relative effect was on dry cows. The energy requirement of a 700-kg dry cow 20 days before calving increased by about 30 percent. Because this is an energy system, the comparison that is most important is energy balance (NEL intake − [NEL for maintenance + lactation + gestation + growth]). For lactating cows, NEL balance averaged about 0.6 Mcal/d (about 1.5 percent of NEL requirement) more with the new system compared to NRC (2001). For dry cows, NEL balance was about 2.8 Mcal less with the new system compared to the old one. For lactating cows, the difference between the new system and the old system was related to milk production. Net energy balance was less with the new system for lower-producing cows than the old system. Conversely, NEL balance was greater with the new system than the old system for higher-producing cows. This means that a higher-energy diet fed to a high-producing cow (also high DMI) will support greater milk production using the current system than the same diet would using the NRC (2001) system. Conversely, using the current system a cow would need to consume a slightly higher-energy diet to obtain the same production and body condition than would the NRC (2001) system. For dry cows, a diet formulated to exactly meet requirements using NRC (2001) would not meet the energy requirements using the current system even though energy density of the diet would be greater with the current system than with NRC (2001).

Energy Partitioning

Production response to increased energy intake is dependent on how energy is partitioned between MY and body energy reserves. Energy partitioning is mostly affected by stage of lactation but also by the interaction between diet and the physiological state of cows as they progress through lactation. Cows that produce more milk need more glucogenic fuels, so increasing the starch content of rations results in a more positive milk response for cows that produce more milk

(Voelker et al., 2002). MY response to dry ground corn substituted for soyhulls at 30 percent of the diet DM increased linearly with MY as it increased from 28 to 62 kg/d, with no response for cows at the lower end of the range in MY (Boerman et al., 2015). As lactation proceeds, insulin concentration and sensitivity of tissues increase. Increasing glucose supply beyond that required for milk production increases plasma concentrations of glucose and insulin and partitioning of energy to body reserves. Intravenous glucose infusion of up to 30 percent of NEL requirement in mid-lactation cows linearly increased plasma insulin concentration, energy balance, BW, and back fat thickness, without affecting DMI or MY (Al-Trad et al., 2009).

Decreasing diet starch content by substitution of high-fiber by-products, or even fat, for cereal grains increases energy partitioning to milk (Boerman et al., 2015; Potts et al., 2017). For instance, substitution of soyhulls for dry ground corn in diets of mid-lactation cows increased yield of milk fat linearly with a subsequent linear decrease in BW with no effect on MY (Ipharraguerre et al., 2002). In addition, substitution of beet pulp for barley grain in rations fed to cows in late lactation linearly decreased plasma concentrations of glucose and insulin, BCS, and back fat thickness; increased ruminal pH linearly; and tended to linearly increase milk fat yield and milk energy output (Mahjoubi et al., 2009).

Increasing dietary starch can increase the risk of milk fat depression by altering ruminal biohydrogenation of long-chain unsaturated FAs (Bauman et al., 2011), as discussed in Chapter 4. Certain conjugated linoleic acid (CLA) isomers, including *trans*-10, *cis*-12 C18:2, are produced in the rumen when biohydrogenation is altered by highly fermentable diets. This CLA isomer downregulates several genes involved in lipogenesis, decreasing de novo FA synthesis in the mammary gland (Baumgard et al., 2002) while having opposite effects on expression of genes involved in lipogenesis in adipose tissue (Harvatine et al., 2009; Jenkins and Harvatine, 2014). Thus, this CLA isomer has a role in energy partitioning by reducing milk energy output sparing energy for lipid synthesis in adipose tissue.

Feeding high-starch diets to high-producing cows in early lactation may support maximal production of milk with minimal loss of body reserves; however, in later lactation, once cows have adequate body reserves, replacing starch with other energy sources such as digestible fiber can help prevent overfattening while still maintaining high milk production. Although a quantitative prediction of effects of diet on energy partitioning is not currently feasible, the effects of diet on partitioning should be considered when formulating diets and are useful when combined with observation of cow responses to diets on farms.

Feed Efficiency

Feed efficiency is a complex trait for which no single definition is adequate. For simplicity, dairy feed efficiency is usually defined as milk output per unit of feed input, with the units generally being mass, energy, protein, or economic value. Although the major product for a dairy cow is milk, changes in body tissue can result in misleading values for feed efficiency and should not be ignored. When evaluating feed efficiency over an animal's lifetime, all feed used as a calf, heifer, and cow and all products produced, including milk, meat, and newborn calves, should be considered. When evaluating feed efficiency of lactating cows for portions of a lactation, corrections should be made for changes in body tissue as

$$\text{Feed efficiency} = (\text{Milk energy} + \text{Change in body energy}) / \text{Feed energy input} \quad \text{(Equation 3-21)}$$

Feed efficiency could also account for feed that is wasted by the cow and losses that occur during harvesting, storing, mixing, or feeding. To define efficiency on a global scale, consideration should be give to human-consumable inputs versus other foods, fossil fuels, water, and land, as well as outputs of greenhouse gasses, pollutants, fertilizers, and other products not used for human consumption. How dairy cattle are fed also impacts the broader ecosystem rural sociology, food quality, animal well-being, the need for oil, and the beef industry (fewer dairy cows will increase the need for beef cows). These considerations have been discussed (Oltjen and Beckett, 1996; Arriaga et al., 2009; Capper and Bauman, 2013; Connor, 2015; VandeHaar et al., 2016). Improvements in feed efficiency generally translate into improvements in environmental sustainability, as illustrated by Capper et al. (2009).

Feed efficiency, no matter which metric is used, is generally greater with greater milk production per cow (Vande-Haar et al., 2016). The first portion of feed eaten by a cow is used for maintenance; feed consumed above maintenance requirement is captured in milk or tissue. If milk energy output is considered in units needed for maintenance, then a cow producing milk at 3× her maintenance requirement uses only 25 percent of her NEL intake for maintenance and can use 75 percent for milk, assuming no change of body tissue. At 4× maintenance, she uses 80 percent of her NEL intake for milk. The dairy industry in North America has increased feed efficiency considerably over the past 100 years as milk production has increased. Currently, the average cow operates at ~3× maintenance intake, so there is still room for improvement. VandeHaar (1998) estimated that Holsteins with a MatBW of 625 kg would attain nearly maximal lifetime efficiency at 21,000 to 24,000 kg of milk per year. At one time, this seemed an unlikely level of productivity for the average farm, but with current technologies, it now seems possible. However, because maintenance requirements per unit of MBW have increased, higher levels of production will be needed to achieve maximal efficiency. If maintenance is 25 percent greater (0.10 versus. 0.08), then the milk production to achieve maximal efficiency also will be 25 percent

greater, assuming no change in MatBW. To continue to improve efficiency in dairy cattle, the industry may need to focus more on efficiency as a goal than as the by-product of focusing on productivity. Breeding programs have started to focus on efficiency by selecting against larger cows and by selecting for a more negative residual feed intake, which is a measure of actual versus predicted intake for an individual cow. Residual feed intake is not very useful in making nutrition and management decisions on farms, but it shows promise as a tool for genetic selection (Veerkamp et al., 1995; Connor, 2015; Pryce et al., 2015; Tempelman et al., 2015).

When feeding and managing cows, maximizing feed efficiency, as defined by milk output per unit feed input, is seldom a worthy goal. Diets high in fat, starch, and protein and low in fiber will almost always increase milk to feed ratio, but these types of diets are not always conducive to optimal profit, health, and sustainability. As described earlier in this chapter and elsewhere, high-grain (starch) diets are more digestible and can increase feed intake and milk solids output during peak lactation. However, high starch decreases digestibility of fiber, and high starch and fat can decrease feed intake in some cases. Monitoring responses to diets is a key part of managing for efficient milk production. Moreover, one of the important contributions of ruminants is their ability to digest foods that humans cannot effectively use or will not consume. Cattle can make use of fiber and thus enable humans to indirectly derive nutrients from fiber. Ruminants can convert the myriad of high-fiber by-product feeds that are available across most of the world into human food.

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4

Fat

INTRODUCTION

From a nutritional perspective, "fat" is a generic term to describe dietary compounds that are predominantly fatty acids (FAs), which include triglycerides (TGs), phospholipids, galactolipids, nonesterified FAs, and salts of FAs. The glycerol backbone in TGs and some phospholipids is nutritionally equivalent to a carbohydrate, as are the sugar moieties in galactolipids. These FA compounds are soluble in nonpolar solvents, and traditionally, crude fat (CF) or ether extract has been defined based on gravimetric determination of lipid-extractable components of feeds. Because the solvent used is not always truly an ether, and various modifications of the chemical procedure exist, the term "crude fat" is used in this publication, rather than "ether extract," to define lipid mass in feeds determined gravimetrically. This solvent-extracted mass includes the non-FA portion of the crude fat, including moieties covalently bound to FA as well as non-FA lipids. Fat is typically fed to increase the energy density of the diet, and long-chain FAs are the major energy-rich moiety of fats. Therefore, specifying dietary FA content of feeds is preferable to extraction methodology, especially because different extraction methodologies, such as ethyl-ether extraction, hexane or "petroleum ether" extraction, or acid hydrolysis ether extraction, yield different values. Additionally, determination of total FA in feeds using quantitative recovery and subsequent analysis by gas–liquid chromatography gives useful information on the specific FA fed. Quantitative extraction of lipids prior to FA analysis is critical, and internal standards of FA that do not occur in feeds should be added to adjust for loss of FA during the analytical process. However, when extraction of lipids is followed by subsequent FA analysis, there is no concern about using solvent systems that also extract non-FA components, provided these compounds do not subsequently manifest as unidentified FA in the chromatogram and mistakenly contribute to total FA. See Chapter 18 for additional details on methodology.

The primary dietary FAs are the 16-carbon and 18-carbon saturated FAs, palmitic acid (C16:0) and stearic acid

(C18:0), and the unsaturated 18-carbon FAs with *cis* double bonds: either a single double bond at the 9 carbon (oleic acid, C18:1 *cis*-9), double bonds at the 9 and 12 positions (linoleic acid, C18:2 *cis*-9, 12), or double bonds at the 9, 12, and 15 positions (the "alpha" form of linolenic acid, C18:3 *cis*-9, 12, 15). Other "*cis*" unsaturated FAs, such as palmitoleic (C16:1 *cis*-9) and gamma-linolenic acid (C18:3 *cis*-6, 9, 12), generally are not major components of mixed diets but may occur at significant levels in certain feeds. Elaidic acid (C18:1 *trans*-9) and other *trans*-C18:1 FAs may also be present in diets. Vaccenic acid (C18:1 *trans*-11) is present naturally in unprocessed tallow, and elaidic acid and other *trans*-C18:1 FAs are prevalent in partially hydrogenated fat supplements and may be present in other feeds. Also, FAs of 20 carbons or more can be found in feeds such as rapeseed and marine products. The list is not so vast as to preclude individual FA analysis, especially because all are easily quantified by the same gas–liquid chromatography method. As more feeds are analyzed by chromatography, near-infrared spectroscopy prediction equations might be able to provide information on FA content. If FA concentration is not known, it can be estimated from crude fat data (Daley et al., 2020; see Chapter 19). Within, but not across, a specified feed source, the fraction of crude fat that is FA, and the proportion of specific FA within total FAs, often is reasonably constant. However, many domestic plant species have distinct strains that vary markedly in FA proportions as a result of selective breeding or by direct genetic modification.

The term "fat" can refer to TGs in a solid form, but "oil" usually refers to TGs containing more unsaturated FAs and are liquid at 25°C. Oleic, palmitic, and stearic acids have melting points of 7°C to 16°C, 63°C, and 71°C. Salts of FAs have higher melting points that are dependent on the cation. Adding liquid oils or molten fats to feeds has the additional value of reducing dustiness of feeds. High melting point fats manufactured into dry, granular products can be handled easily in conventional feed systems, and this can be a significant advantage provided they remain nutritionally

available. When typical forages and grain concentrates are fed to cattle, dietary FA concentration is near 3 percent of diet dry matter (DM), but all-forage diets and early growth pasture can have significantly higher levels of FAs (Boufaied et al., 2003). Various forms of supplemental fat can be fed, including oilseeds, rendered animal fats, extracted plant oils, and processed dry, free-flowing fats. In addition to increasing energy density of the diet, fat supplementation can also increase absorption of fat-soluble nutrients and provide essential FAs (Jenkins and Harvatine, 2014). In general, diets with greater than 7 percent total dietary FAs are not recommended (NRC, 2001).

RUMEN METABOLISM, DIGESTION, AND ABSORPTION

For general reviews of lipid digestion and absorption in ruminants, see Noble (1981) and Jenkins (1993), as well as the quantitative reviews of FA digestion of Glasser et al. (2008b), Schmidely et al. (2008), and Boerman et al. (2015a). Dietary esterified FAs are rapidly hydrolyzed by lipolytic microorganisms within the rumen to yield free FAs. Following hydrolysis, individual unsaturated FAs can be hydrogenated by ruminal bacteria. Complete hydrogenation of an unsaturated FA involves several steps that may be performed by different microbial species (Dewanckele et al., 2020); therefore, the extent of biohydrogenation and the products of hydrogenation vary. Stearic acid formed by complete biohydrogenation of unsaturated dietary C18 FA is produced by ruminal microbes in intimate contact with an unsaturated FA molecule that is not buried inside other FA molecules. These exposed FAs are dispersed on feed particles or are part of the microbial flow and are not thought to physically reaggregate. This distributed stearic acid may have a different digestive fate than stearic acid or stearate salts aggregated into larger particles in concentrated fat supplements. Biohydrogenation of linoleic or linolenic acid reduces their postabsorptive ability to meet the animal's requirement for these two essential FAs.

Incomplete biohydrogenation of linoleic and linolenic acid can result in a variety of FAs, including various conjugated linoleic acids (CLAs) and *trans*-monoenoic FAs (Bauman and Griinari, 2003; Bauman et al., 2011). *Trans*-monoenoic FA can also arise from isomerization of oleic acid. Some bacteria hydroxylate FAs, but these routes probably represent a minority of FA transformations in the rumen (Fulco, 1974; McKain et al., 2010). Microbial production of *trans*-10 FA is increased in diets with higher starch fermentability and ruminal lactate concentration. Several of these microbially produced FAs have bioactivity and health implications in both the animal and humans eating the fat derived from these animals (Bauman and Lock, 2006). Most dramatically, *trans*-10, *cis*-12 CLA directly reduces mammary gland fat secretion in lactating cows. Other CLAs have also been shown to be active, but *cis*-9, *trans*-11 CLA, which is formed largely from mammary gland desaturation

of absorbed *trans*-11 C18:1, does not depress milk fat secretion (Bauman and Lock, 2006). Elevated milk *trans*-10 C18:1 has a strong statistical association with milk fat depression (Matamoros et al., 2020) and may (Shingfield et al., 2009) or may not (Lock et al., 2007) have a direct causal effect on mammary fat secretion. If *trans*-10 FA is bioactive, it is much less potent and required at higher concentrations than the active CLA isomers. However, *trans*-10 FA also frequently occurs in much greater quantities in milk fat than those CLAs that depress milk fat secretion.

Estimates for net ruminal disappearance of polyunsaturated FAs (PUFAs), presumably via biohydrogenation, range from 60 to 90 percent (Bickerstaffe et al., 1972; Mattos and Palmquist, 1977; Jenkins and Bridges, 2007). Because of hydrogenation in the rumen, C18:0 and C18:1 are the major FAs leaving the rumen. Some of the 18:1 leaving the rumen is *cis*-9 (oleic) but also includes *trans*-10, *trans*-11, and other monoenoic 18-carbon FAs derived from isomerization of dietary oleic or partial hydrogenation of linoleic and linolenic acids (Glasser et al., 2008b). Loss of oleic, linoleic, and linolenic acids in "unprotected" feed fats was 86, 82, and 86 percent, respectively (Jenkins and Bridges, 2007). This extensive ruminal loss of PUFA was true for oilseeds and for calcium (Ca) salts, but duodenal oleic flow was often increased with oilseeds or "protection." Data showing formaldehyde treatment effects to increase duodenal passage of unsaturated FA to the duodenum are very limited, but formaldehyde treatment does appear to increase transfer of dietary linoleic and linolenic, as well as oleic, into milk and body fat (Jenkins and Bridges, 2007). Because monoenoic FAs are an important amphiphile, this increased duodenal flow of oleic acid may be beneficial for FA digestion.

The ruminal escape of linoleic acid averages 20 percent with a range of 5 to 30 percent; for linolenic acid, corresponding values are 8 percent and 0 to 15 percent (Doreau and Ferlay, 1994). Noble (1984) reported estimates of pyloric flow of linoleic acid as 0.3 to 0.5 percent of diet intake on an energetic basis. Glasser et al. (2008b) reported a mean flow to the duodenum of total C18:2 of 2.3 g/kg dry matter intake (DMI), with a range of 0 to 12.7. In addition, they reported that linoleic acid ranged from 5.4 to 98.4 percent of total 18:2, with means of 65.3 percent and 80.3 percent in basal and lipid-supplemented diets, respectively. In this same review, 18:3 flow ranged from 0 to 3.5 g/kg DMI, with an average flow of 0.5. The linoleic acid requirement for growing and reproductive swine is set at a 1 g/kg intake (NRC, 2012). A dairy diet could easily contain 1 percent linoleic acid, so 10 percent apparent escape would just meet this requirement. Ca salts of rapeseed oil FAs did not protect these PUFAs compared to free rapeseed oil TG (Ferlay et al., 1993). Similar results were reported for soybean oil and Ca salts of soybean oil (Lundy et al., 2004). Small changes in mass flow of these essential FAs may have important effects on animal health, but these responses may be difficult to evaluate and the changes in FA flow difficult to produce or

predict with current technology. The postabsorptive fate of PUFA following absorption is discussed in Lanier and Corl (2015).

In addition to their role as sources of essential FAs and duodenal conjugated linoleic and *trans*-monoenoic FAs, plant oils rich in unsaturated FAs have previously been purported to have a marked negative effect on fiber digestibility (Jenkins, 1993). Based on analysis of literature of fat effects on total-tract neutral detergent fiber (NDF) digestibility in lactating dairy cattle, Weld and Armentano (2017) found a marked negative effect of supplemental C12 and C14 saturated FAs (lauric and myristic) on total-tract NDF digestibility, a modest negative effect of vegetable oils, and no significant depressing effect of other FA sources. At 3 percent supplemental FA from vegetable oils, the long-chain FA oil effect would correspond to a decrease of about 1.3 percentage units of digestible NDF. The response appears linear, so feeding a very high level of oils should be avoided. In the few studies that reported rumen NDF digestion, oil supplements did not appear to decrease rumen digestion. At typical fat supplementation levels, the negative effect of oils on digestible energy (DE) intake through depression of NDF digestion is minor compared to their positive effects on diet energy density and their potentially negative effects on DMI (Weld and Armentano, 2017). Although there was no significant effect associated with C:16 in Weld and Armentano (2017), subsequent studies have shown increased NDF digestibility when fats composed mostly of C16:0 are added to the diet (de Souza and Lock, 2019; Western et al., 2020).

Microbial oxidation of long-chain FAs is limited, although disappearance of FAs of 14 carbons and shorter occurs in the rumen in vivo (Wu et al., 1991), although in vitro, loss is in FAs shorter than C14 (Wu and Palmquist, 1991). Net FA balance across the rumen is also affected by FA synthesis by rumen microorganisms, which is obvious at least for odd-chain and branched-chain FAs both in vitro (Wu and Palmquist, 1991) and in vivo (Vlaeminck et al., 2006). Regression of FA flow at the duodenum versus intake revealed a slope of only 0.8 (g duodenal FA/g intake FA) and an intercept of 9.3 (g duodenal FA/kg DMI) with a common regression for "protected" and other lipids (Doreau and Ferlay, 1994). The intercept gives an estimate of net endogenous rumen synthesis of FA, and the slope of 0.8 indicates 20 percent true disappearance of FA in the rumen, assuming a constant endogenous FA synthesis as fat intake increases. Disappearance could be due to microbial catabolism, or catabolism or absorption by forestomach epithelium. A similar result was obtained when plotting FA duodenal flow (g/d) against intake of FA (g/d), with an intercept of 93 g/d and a slope of 0.84, corresponding to 16 percent true digestibility in the rumen (Boerman et al., 2015a). If endogenous FA is set at 15 g/kg lipid free organic matter digested in the rumen and assumed to decrease linearly as FA intake increases, then the slope of estimated dietary FA passage relative to intake could be interpreted as a lower true ruminal digestibility of 8 percent (Jenkins, 1993). The

quantitative relationship based on constant endogenous FA synthesis of 9.3 g/kg DMI and 20 percent true rumen disappearance of FAs would yield 0 percent FA apparent ruminal digestibility at 4.6 percent dietary FA, negative values (net ruminal synthesis of FAs with duodenal flow exceeding intake) below that, and about 7 percent apparent digestibility in the rumen at 7 percent FA in diet DM.

Most FAs synthesized by rumen microbes are incorporated into phospholipids. Approximately 85 to 90 percent of the FA leaving the rumen are free FAs, and 10 to 15 percent are microbial phospholipids. Because FAs are hydrophobic, they associate with particulate matter and pass to the lower gut with those particles.

Bile and pancreatic lipase are required for duodenal TG digestion and absorption. If TGs are fed at moderate levels in a form that protects them from rumen microbial hydrolysis (e.g., formaldehyde-protected casein–fat emulsion), sufficient lipase activity is present for triglyceride hydrolysis (Noble, 1981). However, pancreatic lipase does not appear to be inducible (Johnson et al., 1974) and may become limiting if large quantities of TG are presented to the small intestine. In the absence of substantial amounts of monoglyceride reaching or being formed in the small intestine, the mechanism for FA emulsification in ruminant is unclear but may involve lysolecithin and monounsaturated FAs (MUFAs) in addition to bile acids. Oleic acid and mono-olein are considered important intestinal amphiphiles with a critical micellar concentration of 0.55 and 0.60 mM and saturation ratios of 1.04 and 1.70, but *trans*-9 18:1 and *trans*-11 18:1 FAs have critical micellar concentrations of 1.20 and 0.70 mM, as well as saturation ratios of 0.51 and 1.39, which makes them less nonpolar than palmitic and stearic acids, which have a critical micellar concentration of 1.80 and 1.40 mM and saturation ratios of 0.16 and 0.07 (Freeman, 1969). The *trans*-monoenoic FAs are present in duodenal chyme as a result of ruminal microbial action on dietary unsaturated FAs (Glasser et al., 2008b). Comparable values for linoleic acid are 0.35 mM and 1.04. Lysolecithin is formed by pancreatic phospholipase activity on lecithin from microbial or hepatic origin. MUFAs are predominantly from digesta leaving the rumen; therefore, increasing the flow of unsaturated FAs to the duodenum may improve FA digestibility. Intestinal infusion of an emulsifier can improve digestion of FA (de Souza et al., 2020). FA emulsification and micelle formation in the small intestine are essential for the efficient absorption of fat.

MODEL USED FOR FATTY ACID DIGESTION

The mathematical model of total-tract FA digestibility applied in this revision is the simplest possible (other than fixed FA digestibility across all feeds) and likely oversimplifies the biology inherent in the digestion process while allowing different FA sources to be assigned different inherent digestibility. Total-tract digestibility, as opposed to intestinal, is estimated to be consistent with the energy model and to allow access to

TABLE 4-1 Calculated Total-Tract Digestibility Coefficients of FA

Fatty Acid Source Class	Total-Tract Digestibility Coefficients[a]	Standard Error	Fatty Acid Composition (% of FA)[b]
Common feeds	0.73	0.026	—
Oil seeds	0.73	0.041	—
Oil	0.70	0.033	PUFA[c] >20%, UFA[d] >65%
Blended triglyceride	0.63	0.027	PUFA <20%, UFA >56%
Tallow triglyceride	0.68	0.029	MUFA[e] >36%, UFA <56%
Saturated fatty acid enriched triglycerides	0.61	0.037	MUFA >25%, UFA <36%
Extensively saturated triglycerides	0.44	0.030	MUFA <20%, UFA <25%
Calcium salts palm fatty acid	0.76	0.027	MUFA >30%
Saturated fatty acid enriched nonesterified fatty acid	0.69	0.022	MUFA <15%, UFA <20%
Palmitic acid, ~85%	0.73	0.077	—
Palmitic or stearic acid >90%	0.31	0.046	UFA <2%

[a] Regression of apparently digested FA on FA intake yields a true digestion coefficient, but because the regression intercept for apparently digested FA is set to 0, true and apparent digestion are equal (Daley et al., 2020).

[b] Classifications provide unique classes among the triglyceride supplements reported in this database by assigning the fat source to the first row it satisfies. MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; UFA = unsaturated fatty acid.
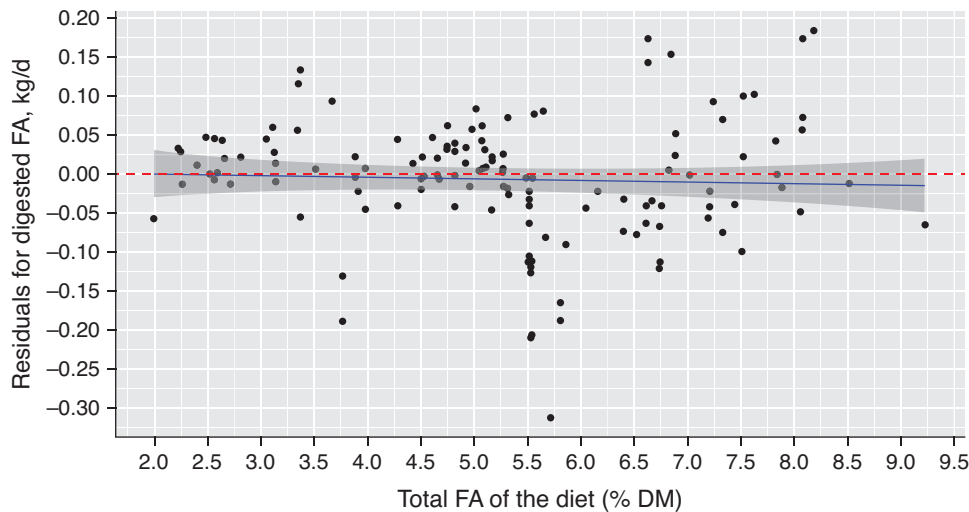
FIGURE 4-1 Residuals for final prediction model used for FA digestion. Concentration of dietary FA had a positive but nonsignificant ($P = 0.65$) estimate, indicating no depression in FA digestion with increased dietary FA concentrations. The intercept was 0.005, and slope was 0.002.

SOURCE: Figure and statistical information courtesy of V. L. Daley based on regression analysis from Daley et al. (2020).

the most data possible. The model applies constant digestibility of FA for a given feed independent of the amount of DM or FA fed. It assigns digestion coefficients to classes of feeds as estimated by multiple linear regression analysis (Daley et al., 2020). In this simple model, diet digestibilities are therefore assumed to be additive, with the digestibility of total diet FAs the weighted average of the individual feeds as is commonly done with static nutrients in ration balancing. This model used a meta-regression of apparently absorbed FA (across the entire tract) as a linear function of FA intake (i.e., a "Lucas" test) from distinct classes of FA sources as defined in Table 4-1. The slope parameter determined for each class is the estimated true digestibility for total FA for the feeds in that class. The intercept (FA absorbed at 0 FA intake) was small, statistically not different from 0, and positive (implying negative endog-

enous fecal FA, which is impossible). Therefore, the intercept (and endogenous FA secretion) was set to 0 in the final model. Inclusion of DMI, or total diet FA concentration as additional independent terms, was tested and found to be nonsignificant (V. L. Daley, National Animal Nutrition Program, personal communication, January 4, 2021). The residuals for the chosen equation showed no bias due to dietary FA concentration (see Figure 4-1). Because endogenous fecal FA has been set to zero, true and apparent digestibility are the same. Based on the regression equation, the default true digestibility of FA from most feeds was set at 0.73 (see Table 4-1). Digestibility of FA for individual feeds can be changed at the discretion of the user.

Because FAs are synthesized in the rumen and cell sloughing occurs along the digestive tract, some endogenous fecal FAs should be excreted. Therefore, setting endogenous FA

secretion to zero is probably not biologically correct but was adopted based on regression fitting and parameter bounds within the realm of possibility. Fitting fecal FA (White et al., 2017, sup. 3) to a simple linear digestion model of FA resulted in a similarly impossible negative intercept for fecal FA at zero FA intake. Only by fitting an increasing slope function of fecal FA to FA intake could a positive value for endogenous fecal FA be obtained statistically. Depending on the equation, estimated endogenous fecal FA was 1.7 or 2.0 g/kg DMI (White et al., 2017). The alternative approach of including an endogenous fecal FA term combined with true FA digestibility decreasing as FA concentration increased (White et al., 2017) was considered, but adding this complexity to the model was not necessary when the FA source classes used in the final model were included.

Assuming constant true digestibility of FA over typical FA intakes allows the digestibility of FA from an added fat supplement to be estimated from the difference in apparent FA digestibility of the basal and supplemented diet. Although the original concept of the Lucas plot was to apply to a nutrient that was consistent across all feedstuffs, the regression of Daley et al. (2020) extends this concept to summing FA according to fat supplement classes. The true digestion coefficient is constant with DMI and diet FA concentration within a class but can differ among classes. If apparent digestibility is plotted against dietary FA with no consideration of different fat classes, there are at least two reasons for this curve to have a diminishing slope. One is that the true digestibility of FA decreases with increased FA intake due to increased FA diet concentration or increased DMI. Second, if FA supplements with lower than average digestibility are incorporated more commonly into the higher FA diets, then the slope will also decrease with increased FA intake. The model used in the current revision can explicitly account for the latter effect only. If there is an undetected general decrease in FA digestion as FA in the diet increases and if FA supplements are present in greater amount in higher FA diets, then the inherent digestibilities (see Table 4-1) of these supplements are underestimated. However, in practice, if adding the supplement increases FA in the diet and this decreases the overall FA digestibility, that effect is implicitly incorporated into the digestibility estimates in Table 4-1.

The previous NRC (2001) fat digestibility model was found to provide poor fit to the experimental data available even after fitting new parameter estimates to the existing model and allowing for an effect of DMI (White et al., 2017). White et al. (2017) derived new models to estimate fat digestibility, and all were superior to the equation used in NRC (2001) and included parameter estimates that allowed for effects of diet FA concentration and DMI on predicted FA digestibility in addition to other independent variables. However, the committee chose to use the Daley et al. (2020) model because of its biological basis, its simplicity, and its overall accuracy.

Total intestinal (postruminal) apparent digestibility decreases with increased duodenal FA flow in basal and supple-
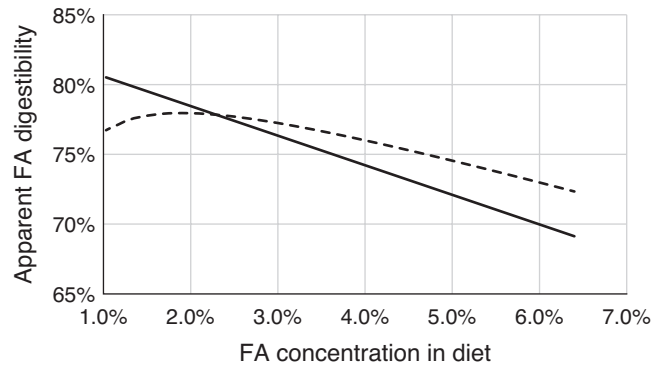
FIGURE 4-2 Predicted intestinal (postruminal) apparent digestibility as FA intake increases (solid line) compared to calculated total-tract apparent digestibility (dashed line).
SOURCE: From linear regression equations for duodenal flow and intestinal digestibility in Boerman et al. (2015a).

mented diets; intestinal digestibility, $\% = 82.5 - 0.0088 \times$ duodenal FA flow, g/d (Boerman et al., 2015a). Within that data set, duodenal FA flow ranged from 100 to 1,800 g/d, and predicted intestinal digestibility decreased from 81 to 67 percent over that range. Plotting FA digestibility versus dietary FA should not be confused with a Lucas plot, although they use the same information. Under conditions of nonzero endogenous fecal nutrient secretion and constant true digestibility, plotting apparent digestibility against intake would result in a curvilinear function rising to a horizontal asymptote as apparent digestibility increases with intake, approaching true digestibility at infinite intake (Palmquist, 1991). Nonzero (positive) endogenous fecal FA in conjunction with decreasing true digestibility could yield a curve of similar shape to the dashed line in Figure 4-2. Duodenal flow can change because of changes in DMI, FA concentration in the diet, and possible alterations in ruminal net appearance or disappearance of FA. As duodenal flow exceeds intake at low diet FA concentrations and is less than intake at higher FA concentrations, the total-tract digestibility versus intake of FA will not follow the same function as intestinal digestibility. Using the linear regressions for intestinal digestibility and for duodenal flow versus intake (Boerman et al., 2015a), a calculated curvilinear function for total-tract apparent digestibility is obtained (see Figure 4-2). This total-tract function supports the effect of total dietary FA greater than 2 percent of diet DM continuously decreasing digestibility. However, the calculated relationship resulting from combining linear ruminal and intestinal regressions gives a relationship that is nonlinear and a smaller range of digestibility, especially for basal-type diets. This presumably explains why White et al. (2017, sup. 3) did not observe a significant quadratic response when total-tract fecal FA excretion was regressed against diet FA in low-FA diets.

Previous discussion of FA intake effects on FA digestibility was based on meta-regression approaches and includes

TABLE 4-2   Effect of Amount of Supplemental Fat on Digestibility of FA in Supplemental Fat

Fatty Acid Supplement	Low, % of Diet DM		High, % of Diet DM			Reference
	First Concentration of Added Fat Supplement	Fatty Acid Digestibility of Fat Supplement	Concentration of Total Fat Supplementation at Second Level of Supplement	Fatty Acid Digestibility of Fat Supplement	Fatty Acid Digestibility of Second Increment of Fat Supplement	
Tallow[a]	1.80	90.3	5.00	72.9	60.7	Weisbjerg et al., 1992
Mildly hydrogenated tallow	1.80	39.7	5.7	52.8	58.8	Drackley and Elliott, 1993
Palm calcium salt	1.60	93.0	3.40	87.9	82.8	Weiss and Wyatt, 2004
Palm calcium salt	2.70	88.0	4.80	77.5	63.4	Wu et al., 1991
Crushed rapeseed	2.30	74.7	4.70	69.7	65.0	Murphy et al., 1987
Hydrogenated palm	1.7	38.4	3.4	36.5	34.5	Weiss and Wyatt, 2004
Blended fat	2.00	80.6	3.90	70.1	75.2	Wu et al., 1991

[a]This study measured intestinal digestibility (not total tract).

effects both across and within studies. However, when multiple levels of the same fat supplement are added to a basal diet in the same study, digestibility of the supplemental fat decreases with increased addition (Palmquist, 1991). This was observed in five of the seven comparisons in Table 4-2. As the model of FA digestion implemented in this version ignores this effect, the user is cautioned that at very high levels of FA supplementation, overall FA digestibility and energy content of the diet may be overestimated.

The discussion and literature reviews above consider total-tract digestibility of FAs as a group. Total-tract digestibility of individual 18-carbon FAs is misleading, as biohydrogenation of unsaturated C18 FAs can lead to an overestimate of their digestibility and an underestimate for dietary C18:0 (Glasser et al., 2008b). Measuring total-tract digestibility of total C16 and total C18 FAs each as a unique group of FA, in addition to total FA, is possible and desirable. Changes in apparent digestibility of diet FA with added FA supplements can provide an estimate of the true digestibility of the specific chain length of FA added. Drackley and Elliott (1993) observed slightly lower total-tract digestibility for C16 versus C18 in basal diets and with supplemental partially hydrogenated tallow. In an analysis of literature data, apparent intestinal digestibility ranged from 65.3 to 83.8 percent for long-chain FA, with a mean of 77.1 percent for C16:0, 72.8 percent for C18:0, and 74.5 percent for all FAs combined (Boerman et al., 2015a). In low-fat diets, intestinal digestibility was similar at 76.7 percent for C16 and 77.8 percent for total FA. Intestinal digestibility of C18:0 was significantly lower than other C18 FAs when intestinal digestibility was determined with fecal samples, although this difference appeared not to occur in diets without supplemental fat, suggesting some confounding with FA source. Intestinal FA digestibility declined with increasing FA intake across studies; the increased FA intake could be a result of increased diet DMI or increased FA concentration. Glasser et al. (2008b) plotted C18:0 absorption from the small intestine versus C18:0 duodenal flow (both per kg DMI) across ruminant

species and found a decreasing absorption rate with higher duodenal flow. This negative quadratic effect did not occur for unsaturated C18 FA. Using the same data set, Schmidely et al. (2008) showed intestinal disappearance of C16 total FA as a constant 73 percent of duodenal flow, while total C18 total decreased with duodenal flow with a linear coefficient of 0.85 times duodenal flow and a quadratic coefficient of −0.0017. Based on this and other regressions in that study, when C18 total intake exceeds 3.8 percent of dietary DM, C18 intestinal digestibility fell below that of total C16. Intestinal digestibility of C18:0 drops across even low-FA diets (i.e., less than 800 g/d FA and less than 500 g/d duodenal C18:0) as FA intake increases (Boerman et al., 2015a). Addition of fat sources with 33.1 percent C16:0, 53.3 percent C18:0, and 5.2 percent *cis*-9 C18:1, or 84.3 percent C16:0, 4.1 percent C18:0, and 8.7 percent *cis*-9 C18:1, altered the FA digestibility of the basal diet from 76.7 to 67.6 percent and 76.3 percent (Western et al., 2020). Authors calculated the digestibilities of the supplements as 55 percent and 77 percent, and most of the difference was attributed to digestion of 18-carbon FA.

Intestinal digestibility must be coupled with ruminal disappearance to predict total-tract digestibility, and in the case of the regressions from Schmidely et al. (2008), this virtually eliminates the drop in total C18 digestibility with increased dietary concentration. One additional advantage of using FA rather than CF occurs when determining digestibility of a FA-rich feed added to a basal diet. Generally, the non-FA CF in the basal diets is less digestible than the FA, and supplements contain most of their CF as FA. Therefore, CF digestibility may be increased with fat supplement addition, even though FA digestibility may remain the same or decrease.

Digestibility of supplemental fat sources varies, so a common measure that helps discern differences among fat sources would be useful. Iodine value (IV) is directly proportional to the degree of unsaturation: the higher the IV, the more unsaturated the fat. Low-IV feeds will usually contain considerable

quantities of C16:0 or C18:0 or both. Unlike rumen biohydrogenation, which requires a free FA, industrial hydrogenation (partial or extensive) can be done on fatty acyl groups in intact TGs. The low IV of hydrogenated palm oil and tallow and other common fat sources is primarily the process of converting unsaturated C18 FAs to C18:0. Hydrogenated TGs from palm or animal fat, containing predominantly C16:0 and C18:0 with less than 15 percent C18:1, can have digestibility below 50 percent (Jenkins and Jenny, 1989), and increasing the degree of hydrogenation for tallow TGs (e.g., 40 to 15 percent C18:1 with corresponding IV of 45.0 to 16.4) is directly related to the decreasing digestibility (Pantoja et al., 1996). Hydrogenated tallow is less digestible than conventional tallow (Pantoja et al., 1995). Digestibility of FA in supplemental fat can be low when the IV is below 40 (Firkins and Eastridge, 1994). Data in that review suggested some benefit of increased C16 to C18, but interaction with IV and FA level made this difficult to interpret across studies. Boerman et al. (2015a) showed C18:0 intestinal digestibility to be negatively correlated to C18:0 duodenal flow, but the effect was not significant for C16:0 digestibility versus C16:0 duodenal flow.

Supplements containing mostly saturated free FAs with IV of 14 and 12 percent C18:1 were much more digestible than hydrogenated tallow triglyceride with an IV of 8 and C18:1 of 9 percent (Elliott et al., 1994), but there was no difference in FA digestibility between hydrogenated tallow triglyceride with 15.6 percent C18:1 and saturated tallow FA with 11.2 percent C18:1 (Eastridge and Firkins, 1991). In addition, supplements containing almost pure C16:0 FA can have true digestibility below 50 percent (Piantoni et al., 2013), and supplements that are primarily stearic can have digestibilities below 30 percent (Piantoni et al., 2015; Boerman et al., 2017). Given the sensitivity of digestibility to IV in this range, as well as uncertainty as to what specifically limits FA digestibility at elevated levels of FAs in the diet, it is not reasonable to conclude feeding saturated fats as hydrolyzed FA or salts always removes concerns about intestinal digestibility of these fats. Ca salts of palm FA are probably the most highly digestible sources of supplemental fat and were much more digestible than strongly hydrogenated palm oil (Weiss and Wyatt, 2004). The effect is due to some combination of a relatively high IV of palm FA versus the hydrogenated palm oil (47 versus 7) due to conversion of C18:1 to C18:0 during hydrogenation; the fact that C18:1 in Ca salts of palm oil FAs are partially protected by Ca saponification, thereby providing an intestinal amphiphile (Jenkins and Bridges, 2007); and the fact that the hydrogenated palm oil was a triglyceride. Predicting the digestibility of a fat source from its FA content and esterification state is risky, and having empirical estimates of digestibility, especially for extensively saturated FA sources, seems prudent.

Decreasing particle size of dry granular fats may increase digestibility, but responses have tended to be small and not statistically significant. A summary of trials (Firkins and Eastridge, 1994) indicated that mean FA digestibility of prilled (n = 8) and flaked (n = 5) hydrogenated tallow was 77 and 69 percent, respectively. Fat structure, the form in which FAs are fed, may have modest effects on digestibility. A review of the literature (Firkins and Eastridge, 1994) indicated that FA digestibility of diets containing triglyceride prills or FA prills was 77 or 73 percent of control diets without added fat. However, effects of fat structure might have been confounded: mean IV and C16:18 ratios were 20.7 and 0.41 for triglyceride prills and 11.2 and 0.45 for FA prills. Mean prill sizes between 284 and 325 microns had no effect on FA digestibility, but 600-micron prill size increased total-tract digestibility of total, C16, and C18 FAs compared to smaller prills when an 85 percent palmitic acid fat supplement was fed (de Souza et al., 2017).

Direct estimates of total C16 and C18 FA digestibility in basal and supplemented diets to derive an empirical estimate of the digestibility of an FA supplement should be obtained when manufactured supplements containing mostly fat are used. Ideally, the fat supplements should be tested at two levels above basal to quantify any digestion depression. The same is true of full fat seeds in various mechanically processed or whole forms.

Effects of fat sources on DMI must be considered when assessing the value of supplementation on energy intake. In addition, if these dietary fats contain unsaturated FAs that induce milk fat depression and reduce milk fat secretion, retained tissue energy balance may be positive even if energy intake is not increased (Harvatine and Allen, 2006). Adding a fat with less than half the digestibility of the carbohydrate it replaces will not increase the DE density of the diet much, but it does decrease the fermentation load in the rumen, which may be an advantage. Replacement of starch with fiber and a palmitic acid–based fat supplement resulted in equal energy intake with enhanced milk energy output; this effect could be useful to reduce body condition gain in later lactation cows (Boerman et al., 2015b).

Oilseeds are rich in unsaturated FAs (Glasser et al., 2008a); therefore, FA profile as fed is not a major source of variation for FA digestibility. Digestibility of FA in oilseeds is probably more a function of the size and physical nature of the specific oilseed and subsequent processing. If oilseeds contain FAs, especially linoleic acid, that are precursors for bioactive FAs, fine processing should be avoided to reduce milk fat–depressing effects, and this lack of processing might then reduce FA digestibility. In lactating cows, intact oilseeds have lower intestinal digestibility of FA than other FA sources, but this can be improved by grinding (Boerman et al., 2015a). Delinting of cottonseed reduces its FA digestibility, but mechanical processing can enhance biohydrogenation intermediates that depress milk fat (Reveneau et al., 2005). In steers, FAs in whole canola seed are poorly digested, but this is improved by processing (Aldrich et al.,

1997). However, even crushed rapeseed supplementation reduced FA digestibility compared to the basal diet (Murphy et al., 1987). Reducing the particle size of roasted soybeans did not affect total-tract FA digestibility (Tice et al., 1993).

Final assignment of digestibility coefficients to FA supplied by different feeds was based on regression analysis of 30 published studies that provided total-tract apparent digestibility of FA and diet FA information (Daley et al., 2020). Study was considered a random effect, and observations were weighted using reported standard errors. Intakes of apparently digested FA were regressed on FA intakes provided from 11 classes of feeds using feed library information and reported dietary FA information to yield true digestibility coefficients (see Table 4-1). Several studies included fat supplements that were poorly digested, and these were either TGs highly enriched in saturated FA or almost pure palmitic or stearic FA supplements. Not all fat supplements included in the regressions have example feeds included in the printed or electronic feed tables included in this report; however, their presence in the regression model is required to better estimate FA digestibility coefficients for other FA sources included in the library. The FA composition was used to provide nonoverlapping classes of diverse fat sources while having at least some replication within a fat class across multiple papers. These classifications do not necessarily extend beyond that database. The one example of very highly enriched palmitic acid was grouped with two examples of highly enriched stearic acid primarily to exclude them from other classes as all three had digestibilities that would probably preclude their use as commercial supplements. The slightly greater digestibility of oils over tallow can be expected based on FA profile, but for reasons that are not clear, the value for blended animal/vegetable fats did not fall in this continuum. This total-tract FA digestibility data set includes papers reporting intestinal digestibility plus those reporting only total-tract apparent digestibility. In this larger data set, neither diet FA concentration nor DMI significantly improved model fit of total-tract apparent FA digestibility, and these terms were not included in the final model used to derive FA digestibility of the FA classes. This regression also included a fixed zero intercept. Not fixing the intercept to zero resulted in a nonsignificant positive digestible FA intake at zero FA intake, corresponding to an impossible negative endogenous secretion. This set of conditions provided optimal fit and was consistent with zero endogenous FA secretion, true digestibility, and apparent digestibility being equal and constant true digestibility as FA intake increased.

MILK FAT COMPOSITION

Milk fat has a much more complex FA composition than dietary fat. Milk contains more than 400 different FAs, but only about 14 are present above 1 g/100 g milk fat (Jensen, 2002). Milk FAs can be derived from de novo synthesis in the mammary gland, from FAs synthesized in the adipose tissue and subsequently mobilized or absorbed FAs of dietary or microbial origin (Glasser et al., 2008a; Shingfield et al., 2010). Milk FAs synthesized in the mammary gland are primarily C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0. The six FAs shorter than C16 form about one-quarter of milk FAs by weight, but a larger fraction of milk FA if expressed on a molar basis. Mammary gland synthesis of C16:0 is augmented by dietary supply as many feeds and fat supplements contain C16:0, so milk C16:0 is a combination of synthesized and absorbed FAs. Milk C16:0 yield, which is usually about one-third of milk FA yield by weight, can approach or even exceed 50 percent of milk fat by weight when supplementary C16:0 is supplied (Loften et al., 2014). Milk yield of C16:0 is therefore dependent on the overall rate of mammary gland C16:0 synthesis, blood supply of dietary C16:0 and C16:0 supplied by the adipose tissue (minus any desaturation into C16:1), and the efficiency of their incorporation into milk triglyceride. Most of the rest of the fat in milk are 18-carbon FAs derived directly from the diet but can come from body tissue. Most C18 in milk is oleic acid (20 to 30 percent of total milk FA by weight) followed by C18:0 (9 to 14 percent; Jensen, 2002). As explained previously, most of the absorbed C18 will be C18:0 and C18:1. Milk 18:1 can be taken up directly from blood or produced from blood-derived C18:0 by activity of delta-9-desaturase in the mammary gland. Oleic acid content of milk is increased by body fat mobilization (Jorjong et al., 2014). For reasons that are not entirely clear, very little C16:0 or shorter FAs are desaturated, so milk has only small amounts of monoenoic FAs shorter than C18:1 (about 3 percent of total milk FA by weight). Milk TGs contain a mixture of FAs within a molecule. Based on carbon number (total number of FA carbons per triglyceride molecule), milk TGs range from 28 (e.g., this could be a triglyceride with C4, C10, and C14 or one with C4, C8, and C16 acyl groups esterified to the glycerol) to about 54 (which would be a TG with three 18-carbon fatty acyl groups). Physical characteristics of milk TGs may be impacted by total FA proportions and by the distribution of FAs with the various TGs. TGs with different FA residues on the noncentral carbons of glycerol have an anomeric center at the glycerol number 2 carbon, and therefore the 1 and 3 positions are distinguished as sn1 and sn3. Many milk TGs contain at least one short-chain FA, preferentially bound to the *sn*-3 position (Parodi, 1982). Although milk FA composition is generally reported on a weight basis, TGs composed of one butyryl, one palmityl, and one oleiyl moiety (carbon number 38) are equimolar in these FAs (33.3 percent each); however, on hydrolysis, this example TG would yield 14 percent butyric, 41 percent palmitic, and 45 percent oleic by FA weight. For the major FAs present in milk, coefficients of variation may be up to 15 percent,

while minor FAs have a greater variation relative to their absolute amount.

LACTATING COW RESPONSES TO DIETARY FAT

Production responses to supplemental fat are dependent on the nature of the fat and lactation stage. Milk yield response to supplemental fat is influenced by several dietary factors, including basal diet, fat composition of the supplement, and amount of supplemental fat, as well as animal factors such as stage of lactation, energy balance, and level of production. Milk fat yield response is best divided into secretion of milk FA that can be synthesized in the mammary gland (C16 and shorter, primarily saturated) and those that reflect incorporation of mobilized adipose or dietary FA into milk (C16 and C18, with the largest proportion of the C18 secreted as oleic). Comparing yields of these milk FAs to determine shifts in milk FA precursors is preferable to comparing the proportion of each in total FA, as any increase in the proportion of one group necessitates a decrease in the proportion of another, even if yields of the FA with lowered proportion are not decreased. Adding dietary C16:0 increases milk fat yield due to greater secretion of milk C16:0, although the apparent marginal efficiency of transfer is approximately only 15 to 35 percent (Lock et al., 2013; de Souza et al., 2016; Dorea and Armentano, 2017). If C16:0 is fed versus C18:0, yield of C16 and C18 FA in milk responds in kind (Rico et al., 2014). Actual transfer of dietary C16:0 to milk C16:0 may be greater than apparent efficient transfer if the added dietary C16:0 depresses mammary de novo C16:0 synthesis.

Formation of FAs that are bioactive and milk fat depressing is enhanced by rapid carbohydrate fermentation and corresponding low ruminal pH and requires a source of unsaturated FA precursors (Griinari et al., 1998). Both these conditions can occur in lactating cows consuming large amounts of typical mixed rations with low dietary FA concentration (Stoffel et al., 2015). At these high levels of DE intake, even the low, native unsaturated FA content of common dairy forages and concentrates may be adequate to produce enough bioactive FAs to cause inhibition of de novo synthesis of milk FA. In addition, dietary cation–anion balance of the diet also modifies the potential for milk fat depression (see Chapter 7).

Dietary C16:0 presumably produces no bioactive FA to reduce secretion of de novo milk FA and has little effect on total mass secretion of FA with 14 carbons or less or secretion of total milk 18-carbon FA (Lock et al., 2013; Dorea and Armentano, 2017). Added dietary C16:0 does, however, shift de novo milk FA (including de novo C16) to shorter chain lengths and lower molecular weight (Enjalbert et al., 1998), so unchanged mass secretion (g/d) implies increased secretion on a mol/d basis. Feeding palmitic in place of stearic acid also caused a linear reduction in secretion of milk FAs as carbon chain length increased from C8 to C14 (Rico et al., 2014). Similarly, dietary C18:0 should increase milk C18:0

and C18:1 without reducing secretion of shorter milk FAs through generation of bioactive FAs. Eighteen-carbon unsaturated FAs can decrease secretion of de novo FAs through generation of bioactive FAs but may elevate secretion of milk C18 FAs, mostly as oleic and *trans*-monoenoic FAs (Stoffel et al., 2015). The net result on total milk fat secretion when adding unsaturated C18 FAs is dependent on the balance of these opposing changes. Typically, milk with an FA profile favoring C18:1 and C18:0 is increased at the expense of shorter saturated FAs synthesized exclusively in the mammary gland (Glasser et al., 2008a). High levels of added linoleic acid decrease total yield of milk 18-carbon FAs (He et al., 2012), presumably due to synthesis of active milk fat–depressing FAs such as *trans*-10, *cis*-12 CLA (Jenkins and Harvatine, 2014). Oleic acid can reduce milk fat synthesis but is less potent than linoleic (He et al., 2012). This negative effect of oleic may be due to synthesis of bioactive FAs in the rumen, but oleic is not known to be converted to any CLA. The active FA may be *trans*-10 18:1 or some other bioactive monoenoic FA, or oleic acid could indirectly increase CLA formation from PUFA. When compared directly, linolenic acid has not proven more deleterious than oleic (Kelly et al., 1998; AbuGhazaleh et al., 2003; Rego et al., 2009), and in some studies, it is less milk fat depressing than linoleic (He and Armentano, 2011). A review of studies on feeding oils and oilseeds concluded that both linoleic and linolenic FAs reduced shorter-chain milk FAs compared to milk 18-carbon FA (Glasser et al., 2008a). A regression analysis across dietary FA concentrations confirms that unsaturated dietary FAs cause a linear depression of de novo milk FAs (Dorea and Armentano, 2017). Palm oil (not to be confused with palm kernel oil) and tallow are both mixtures of C16:0 and C18:1, although tallow has more C18:0 and less C16:0 than palm oil. These fats behave as expected, with increased C16:0 secretion from absorbed C16:0, decreased secretion of C14:0, and shorter FAs due to the mild inhibitory effects of dietary oleic acid and small amounts of PUFA, as well as small increases in secreted milk C18 due to absorbed C18 FAs. This would explain the generally positive effects of tallow and palm oil on milk fat yield whether fed as TGs, FAs, or Ca salts.

Infusion of FAs or TGs into the abomasum or duodenum allows the effect of FAs to be measured independently of ruminal microbial alteration and ruminal synthesis of bioactive FAs. In these experiments, the range of infusion has been approximately 200 to 600 g/d. If the typical flow of linoleic acid at the duodenum is about 100 g/d and around 15 g/d for linolenic acid, then for some of the more unsaturated fat sources used, the levels of C18:2 or C18:3 infused may be quite high compared to normal. Results have been mixed. No decrease for milk short-chain FAs was seen when fat low in PUFA was infused (Enjalbert et al., 2000; Kadegowda et al., 2008). Studies using TGs or FA blends richer in PUFA have produced variable results. Infusion of linseed oil resulted in no change in C16 and shorter milk FAs secretion but increased longer-chain milk FA yield (Moallem et al.,

2012). Christensen et al. (1974), Drackley et al. (1992), and Litherland et al. (2005) all reported some decrease in various milk short-chain FAs with fat infusion. The three latter studies also decreased DMI with infusion of fat, which may be an important causative factor in the decreased milk FA synthesis. Nevertheless, the potential for some postruminal inhibitory effect of the unsaturated FAs on milk fat secretion cannot be totally discounted.

If fat supplementation is started during the early postpartum period, a lag may occur before a positive milk response is observed (Jerred et al., 1990; Schingoethe and Casper, 1991). An extensive summary by Chilliard (1993) indicated that the average fat-corrected milk response to fat supplementation (average increase of 4.5 percentage units in dietary CF) during early lactation (beginning before 4 weeks postpartum and ending before 11 weeks postpartum) was 0.31 kg/d and not significantly different from controls. Average 4 percent fat-corrected milk response to fat supplementation during peak lactation (beginning before 8 weeks and ending by 24 weeks postpartum; average increase of 3.6 percentage units of dietary CF) was 0.72 kg/d and significantly different from controls. In middle to late lactation (average increase of 3.4 percentage units of dietary CF), the response was 0.65 kg/d, which was not significantly different from control. Average milk production of cows in this summary was less than 35 kg/d. Milk-yield responses to supplemental fat in cows that produce more than 40 kg/d are not well defined. Using cows with prior production ranging from 32.2 to 64.4 kg/d, response of milk production and DMI to added stearic acid fat source was greater for the more productive cows (Piantoni et al., 2015). Some of the variation in response of milk protein and fat yields to added fat may be due to varying depression of feed intake when feeding different supplemental fats.

Nutritionists often apply a maximum to the amount of unsaturated FAs in lactating cow diets to prevent the onset of milk fat depression. There are three reasons to question this concept. First, bioactive FAs have their greatest incremental effect at the lowest level, and the effect diminishes as the concentration of these FAs increases further in milk (Bauman and Lock, 2006). Second, adding fat to the diet clearly depresses de novo FA formation while raising secretion of preformed dietary FAs in milk. The effect of depression of FA synthesis in the mammary gland by dietary unsaturated FA may be masked (but still present) at low levels of FA addition when only total milk fat concentration is measured. This could be detected by milk FA analysis while it would be missed by measuring only total milk fat concentration. Finally, the effect of unsaturated FAs on reducing milk fat synthesis in the mammary gland happens at total diet FA below 3 percent, which can be achieved by adding 1.7 percent oil to basal diets containing only 1.2 percent FA (Stoffel et al., 2015) with a linear affect across dietary FA concentrations (Dorea and Armentano, 2017). The effect at these very low levels of FA may or may not apply to FA in basal feedstuffs,

but that is difficult to determine experimentally without introducing confounding factors. Grasses differ in FA content and composition based on species and maturity, with total unsaturated FA content more than 2.5 percent possible (Mir et al., 2006). Pasture with more than 4 percent FA is not uncommon (Roche et al., 2011). Based on a summary of FA concentrations (Kalac and Samkova, 2010), perennial ryegrass can range from 2.2 to 4.4 percent total FA with 1.1 to 3.2 percent linolenic acid, and corn silage can have 1.2 to 4.0 percent FA. Following linolenic acid, linoleic and palmitic acids are the next most prominent FAs in leafy forages, but forages containing grains and seed may also contain oleic acid. Even in diets not containing exogenous fat supplements, variations in feed FA concentration and type of FA may alter milk fat secretion. Increased concentration of unsaturated diet FAs may lower the ratio of milk FAs with carbon chains of 16 or less to milk FAs with C18, and shifts from PUFA to saturated FAs in feeds may increase total milk fat yield, although both of these effects are likely to be small.

Milk fat and total milk yields often increase in response to fat feeding, but protein concentration decreases. Milk protein concentration can decrease due to decreased secretion of protein or because of dilution by increased lactose secretion and volumetric milk yield. The effect of dietary fat to reduce milk protein concentration diminishes slightly as the amount of supplemental fat increases: for example, $y = 101.1 - 0.638x + 0.0141x^2$, where y = milk protein concentration [(treated/control, percent) × 100] and x = percentage of total dietary fat (Wu and Huber, 1994). Casein is the milk nitrogen fraction that is most depressed (DePeters and Cant, 1992). Although milk protein percentage is usually depressed, protein yield usually remains constant or is increased. Of 83 comparisons (fat supplementation versus control) summarized by Wu and Huber (1994), milk protein yield was unchanged or increased in 57 comparisons and decreased in 26. However, in 15 of the 26 comparisons in which protein production was decreased, milk production also was decreased. A meta-analysis restricted to continuous lactation trials showed decreased protein percentage, increased milk yield, and no change in milk protein production even though DMI declined with fat addition to the diet (Rabiee et al., 2012). Adding fat supplements containing mostly C16:0 and C18:0, Hu et al. (2017) reported increased yield of milk, milk fat, and milk protein, while protein percentage usually declined. Why milk protein production does not increase at a similar rate to milk volume with fat supplementation has not been determined.

DIETARY FAT INTERVENTIONS AND REPRODUCTION

Fat supplementation can positively influence reproductive performance of dairy cows. A summary of 20 studies indicated that first-service conception rate or overall conception rate was increased in 11 of the studies (Staples et al., 1998). Over all studies, the mean increase was 17 percentage units. Three studies found a negative influence of supplemental fat

on reproduction, but the effects were confounded by substantial increases in milk production. Feeding fat increases follicle numbers and the size of the dominant follicle. Whether those changes in follicular dynamics have a positive effect on reproductive performance is unknown. Potential mechanisms by which fat influences reproduction include amelioration of negative energy balance, enhancement of follicular development via changes in insulin status, stimulation of progesterone synthesis, and modification of the production and release of prostaglandin F2α (Staples et al., 1998). In the studies reviewed by Staples et al. (1998), change in energy status was not related to change in conception rate. Likewise, the effects of fat on circulating insulin have not been consistent, although the trend is toward a reduction. How a reduction in plasma insulin could benefit reproduction has not been determined, but the impact of glucose balance during lactation and its interaction with reproductive processes has been reviewed (Lucy et al., 2014). Fat supplementation consistently increases plasma progesterone concentration, but the change might be because of depressed clearance rather than increased production (Hawkins et al., 1995). Staples et al. (1998) proposed that feeding fats that are rich in linoleic acid suppresses prostaglandin F2α and prevents regression of the corpus luteum. The importance of considering omega-3 and omega-6 FA was reviewed by Santos et al. (2008). Thatcher et al. (2011) proposed mechanisms whereby the proper ratio of duodenal linoleic and linolenic acids could benefit reproductive performance. Effects of supplementing omega-3–rich FA sources in the diet to favorably influence various reproductive processes and overall reproductive success are reviewed in Moallem (2018). A more recent meta-analysis also concluded that changes in fat nutrition improved gross reproductive performance (Rodney et al., 2015). However, the manipulations summarized as treatments in this analysis included changes in total dietary FA from various sources, addition of CLA, and feeding isolipid diets where the pattern of FA changed, so the nature of the treatment is not clear except that it involved changes in some aspect of fat feeding. For example, one treatment was substitution of flaxseed for sunflower seed (increased linolenic acid), and another treatment was substitution of Ca salts of palm oil for flaxseed (decreasing linolenic). One of the most common treatments was mixed CLA (de Veth et al., 2009), which improved reproduction when supplemented up to 10 g/d. Interestingly, this effect was not related to improved energy balance caused by reduced milk FA yield. Given the different patterns of bioactive FAs formed when different unsaturated C18 FAs are fed, this effect of CLA supplements supports the need for measuring FA effects independently as opposed to total dietary FA content.

The difficulty of summarizing these data into a clear and actionable effect is obvious. An extensive review is available (Roche et al., 2011) of the effects on reproductive function in lactating cows of dietary interventions, including modifying FA amounts and FA profile. These authors stated that the effect of dietary fat on reproductive outcomes "is difficult to interpret," and the most impactful nutritional management should be directed toward achieving optimal body condition in freshening cows. Berry et al. (2016) indicated that while evidence for nutritional effects on reproduction existed, the scale of the effect was probably commonly overstated. In addition, many studies of nutrition and reproduction fail to properly identify the experimental unit and may incorrectly interpret replication. Large numbers of animals are required for accurate estimates of gross reproductive performance, and recommendations for effective designs have been made (Lean et al., 2016).

DAIRY FAT AND HUMAN HEALTH AND POSSIBLE MODIFICATION OF MILK FAT

Dairy products are processed and can have fat removed or concentrated relative to raw milk. In addition, feeding methods and genetics of dairy animals can alter raw milk fat concentration and FA composition. Most often, dairy fat is eaten as part of dairy foods that contain nonfat components that may also impact human health, positively or negatively, which readers need to recognize. The most recent U.S. government guidelines for human diets identified Ca and vitamin D as shortfall nutrients and saturated fat as being overconsumed (HHS and USDA, 2015). Studies that directly measure human health and longevity are epidemiological in nature and, therefore, can never conclude cause and effect, only association. When human studies utilize classical dietary intervention, the end point is almost always measurement of a biomarker that, in turn, has reputed association with subsequent long-term health results. Studies may specifically study the effect of milk fat on human atherogenic disease (or a biomarker) or be more general and study consumption of the "typical" range of dairy products and overall human health. A recent study of this latter sort suggests that dairy food consumption is not associated with all-cause mortality and has positive associations with several important health measures (Thorning et al., 2016). Considerable disparity exists in epidemiological reviews and summaries regarding the impact of dairy products on human health, and it is beyond the scope of this chapter to review them. However, even if dairy foods are in fact beneficial, this does not preclude the possibility that milk products, and specifically milk fat, could be altered to make milk products more beneficial for human health.

Human health could potentially be improved by modifying milk fat via feeding by (1) increasing the content of various beneficial bioactive FAs present in milk in trace, but potentially effective, concentrations; (2) altering the major FA composition of milk to reduce saturated FA and elevate oleic or PUFA while maintaining or increasing milk fat concentration; (3) reducing total milk fat yield relative to fluid and protein, which in turn could be used to produce lower-fat dairy products (or human diets); and (4) enriching the content of milk omega-6 or omega-3 FAs.

Milk contains over 400 FAs; many of these are in trace amounts. Some of these FAs can have potent biological effects (Lock and Bauman, 2004). This bioactivity could plausibly result in various positive and negative effects on human health, many of which may not be related to plasma cholesterol. The branched-chain FAs in milk mostly derived from microbial sources were deemed underexplored for human health (Taormina et al., 2020). Epidemiological studies of milk products have indicated positive health associations with dairy products, but the role of the specific dairy foods and dairy fat component is not clear (Elwood et al., 2010). The actions of different CLAs on human health are complex. Changes in the diet of cows can cause diverse changes in the trace FAs present in milk, and often many of the trace FAs are not measured accurately. These complexities prevent any clear road to enhancing potential health benefits by using nutrition to alter the trace bioactive FAs present in milk. However, the potential positive effects of these FAs should not be discarded when considering the recommendations for the level of milk fat in the human diet.

The effects of dairy products and dairy fat on human health are not resolved, but it is clear that the general advice for extremely low-fat, high-carbohydrate diets (no more than 20 percent of calories from fat) was not well justified at its inception and is being abandoned by experts in human nutrition (German and Dillard, 2004). Broad, but not unanimous, concerns remain over too large a proportion of human dietary fat coming from saturated fat (Burlingame et al., 2009; USDA, 2015). The current trend in human dietary recommendations is maintaining fat levels at about 30 percent of calories but increasing MUFAs and PUFAs, while reducing medium-chain saturated FAs and industrially hydrogenated fats due to their content of various *trans*-FAs. In addition, common guidelines still often call for consumption of "low-fat" dairy products because of beneficial effects of dairy products but concerns that dairy fats do not help achieve the desired FA profile for the human diet. Most evidence suggests health benefits of milk for children, with limited evidence to support decreasing milk fat (O'Sullivan et al., 2020) or milk fat globule membrane components (Ortega-Anaya and Jiménez-Flores, 2019). Consuming milk or milk products, regardless of their saturated FA content, was associated with positive outcomes on cardiovascular health (Mena-Sánchez et al., 2019; Rietsema et al., 2019; Companys et al., 2020; Hirahatake et al., 2020) and metabolic syndrome (Drehmer et al., 2016; Mena-Sánchez et al., 2019).

The broad terms "saturated fat" and "animal fat" persist in relation to human consumption and labeling of foods but are of limited utility in describing milk fat, which contains saturated FAs ranging from 4 to 22 carbons in length. Saturated FAs of different chain lengths have markedly different effects on typical biomarkers used to assess potential atherogenic risk. Consumption by humans of lauric, myristic, and palmitic acids (C12:0, C14:0, and C16:0) is related to elevated low-density lipoprotein (LDL) cholesterol, a bio-

marker for negative coronary health impacts (Kris-Etherton and Yu, 1997). Shorter (C10:0 and lighter) saturated FAs, as well as C18:0, stearic acid, appear neutral relative to consuming carbohydrate, while C18:1 has positive (cholesterol-lowering) effects (Kris-Etherton and Yu, 1997). Controlled substitution of these medium-chain saturated FAs for carbohydrate clearly elevates LDL cholesterol, but C12:0 simultaneously reduces the total to high-density lipoprotein (HDL) cholesterol ratio, which is a marker associated with better cardiovascular health (Mensink et al., 2003). Therefore, the primary putative negative human health claim associated with dairy fat is most likely limited to the effect of medium-chain saturated FAs on human LDL cholesterol, which seems to ignore the neutral or beneficial effects of these same FAs on total to HDL cholesterol and ignores differences among lauric, myristic, and palmitic acids. Whether these changes in LDL cholesterol from these FAs translate into higher risk for human cardiovascular disease and decreased longevity when consuming dairy products containing fat is far from established. Generalizations are complicated by the form of dairy fat consumed and the possible positive impacts of dairy products on HDL, blood pressure, and type 2 diabetes (German et al., 2009).

The FAs fed to dairy cattle affect the FA proportions in dairy food fats. Feeding C16:0 fats to cows increases total milk fat secretion mostly by increasing C16:0 with only slight absolute decreases in C14:0 and C12:0, with predictable increases in the molar proportion of C16:0 in milk relative to other FAs. As described earlier, increased C18 unsaturated FAs in lactating cow diets will reduce C16 and shorter chain saturated FAs in milk while usually elevating C18:0 and C18:1 in milk fat. Therefore, a simple strategy to alter milk FA composition to the suggested healthier balance of more C18:0 (neutral) and C18:1 (beneficial) and less medium-chain (C12:0 through C16:0) FAs is to include oils that contain oleic, linoleic, and linolenic acids in lactating cow diets, which reduces yield of milk short-chain FAs while increasing milk 18-carbon FA secretion. Added stearic acid in the dairy diet may not always decrease secretion of short-chain milk FAs but can increase secretion of milk 18-carbon FA, so it too can alter the milk FA proportion in this same direction. Feeding more unsaturated FAs to cows, however, can also increase *trans*-FAs in milk. The effect of feeding cows to alter dairy fats in this manner and then subsequently feeding this modified fat to humans has been reviewed by Livingstone et al. (2012). Careful evaluations of the data present in the original studies reveal that three of five studies found statistically significant reductions of total blood cholesterol; three showed significant reduction in LDL cholesterol, and only one study showed a significant change in HDL cholesterol, which was increased by the modified milk fat (Noakes et al., 1996; Tholstrup et al., 1998, 2006; Poppitt et al., 2002; Seidel et al., 2005). Overall, these data suggest a tendency for LDL and the total cholesterol to HDL cholesterol ratio to be lowered by these fats, a potentially

beneficial change in milk fat relative to human atherogenic health, although the data are neither extensive nor completely consistent.

There are several caveats to feeding vegetable oils to lactating animals to modify dairy fat. Excess oil feeding to cows can reduce total milk fat secretion and concentration, reducing the value of milk under current marketing schemes. Although milk-testing labs can estimate the FA profiles of milk fat at the farm level to use in payment schemes and incentives, there is currently no widespread economic incentive in the United States to alter milk FA profile, but there is for elevated milk fat concentration and yield. Feeding vegetable oils not only increases milk oleic acid while reducing medium-chain FAs in milk but also may increase flow of 18-carbon *trans*-monoenoic FAs into the duodenum followed by absorption and secretion in milk. Even if *trans*-FAs from ruminants may be less of a health concern than the *trans*-FAs from industrially hydrogenated TGs (Gebauer et al., 2011; Oteng and Kersten, 2019), current food labeling does not distinguish these and simply reports total *trans*-FAs. This type of oil feeding can elevate *trans*-FAs above 5 percent of milk fat, which could cause *trans*-FAs to exceed 0.5 g in a 10-g serving of dairy fat. The Food and Drug Administration in the United States requires labeling *trans*-FA content when the 0.5-g per serving level is exceeded. Recommendations from FAO/WHO called for less than 1 percent of human energy consumption from *trans* fats from all sources, ruminant and industrial, combined (Burlingame et al., 2009). Dietary unsaturated FAs, especially linoleic acid, even at low levels present in basal diets, will often shift the milk FA profile to a higher content of C18:1 (including *trans*-C18:1) and lower medium-chain saturated FAs without noticeably changing milk fat yield. Because measurement of the milk FA shift is practical with existing milk analysis procedures, an economic incentive to alter milk FA profile to achieve this shift is possible. However, the benefit of such a shift in milk FA profile to human health is not proven, and labeling requirements for *trans*-FAs must be considered.

Lactating cow diets that promote lower milk fat through highly fermentable carbohydrate and less effective fiber can yield low-fat milk products that can be left low fat or fortified with nondairy fat with different FA profiles. These diets tend to reduce milk FAs of all lengths. Use of bioactive milk fat synthesis inhibitors, such as *trans*-10, *cis*-12 CLA, can have the same effect and potentially avoid the negative animal health effects and reduction in fiber digestion associated with subacute rumen acidosis due to rapidly fermentable carbohydrate and shorter fiber. Direct feeding of these potent bioactive CLAs to cows inhibits milk FA secretion and lowers short-chain saturated FA relative to C18:1 and total C18 (Bauman et al., 2011) without elevating *trans*-FA content of milk that occurs when increased amounts of unsaturated FAs are fed as a source for synthesis of the milk fat–depressing CLA (Perfield et al., 2007). However, the demand for milk

fat and the economic incentives for milk fat production through milk fat pricing have not moved dairy production in this direction.

Slight increases in PUFA in milk are possible by altered feeding of cows with current technologies but will not greatly increase human dietary intake of these PUFAs. Content of linoleic and linolenic acid in milk is minor and difficult to change in unprocessed milk by feeding methods for reasons discussed above and reviewed by Lanier and Corl (2015) and Lock and Bauman (2004). For example, milk fat on average has about 0.6 percent of FA as α-linolenic acid. If a human diet derived 30 percent of calories from fat and all that fat was dairy fat, then 0.18 percent of calories would be from linolenic acid. Doubling this linolenic milk content, which has been accomplished by linolenic supplementation of cow diets, would deliver about 0.36 percent of human calories from linolenic acid. This would still be below the lower end of the recommended range of 0.5 to 2 percent of calories from linolenic acid. In contrast, ubiquitous soybean oil contains 8 percent linolenic acid, so the same increase (0.18 percent to 0.36 percent human calories from linolenic acid) could be obtained by human consumption of 27.5 percent calories from dairy fat and 2.5 percent calories from soy oil. In addition, many rich sources of linolenic acid also contain higher ratios of desirable eicosapentaenoic acid and docosahexaenoic acid to linolenic. Dairy processing can easily remove fat from dairy products to yield low-fat products. When high-fat "dairy" products are desired, dairy fats can be combined with other oils or specific FA supplements to deliver the desired FA in significant amounts, while avoiding the biological inefficiencies inherent in the lactating cow. These changes affect melting point and other important properties of the fats and can be much more precisely standardized in food processing than by animal feeding.

SUMMARY

For maximum performance, cows generally should not be fed diets with more than 7 percent of the DM as FA, and the economic optima may be considerably less. Feeding higher concentrations of fat can result in reduced DMI, even if the fat has minimal effects on ruminal fermentation and fiber digestion (Schauff and Clark, 1989; Weld and Armentano, 2017). A reduction in DMI may negate part or all of the advantage of using fat to increase dietary energy density and can limit milk-production responses. Optimal amounts of fat to include in diets depend on numerous factors, including the FA composition of the fat, physical and chemical form of fat, the basal diet, stage of lactation, environment, level of milk production, and feeding management. Limiting dietary FA concentration to less than 5 percent might be prudent during early lactation, when feed-intake depression due to fat supplementation has been observed (Jerred et al., 1990; Chilliard, 1993). Cereal grains and forages can contain about

3 percent FA, but FA profiles can vary significantly. Oilseeds and animal or animal–vegetable blends are acceptable fat supplements; however, at high levels of supplementation, choice of FA supplements needs to be carefully balanced to prevent milk fat depression or reduced DMI.

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5

Carbohydrates

INTRODUCTION

Carbohydrates are the primary source of energy in diets fed to dairy cattle and usually comprise 60 to 70 percent of the diet. Besides being the primary energy source to the animal, carbohydrates provide substrate for growth of ruminal microbes and production of microbial protein, and fibrous carbohydrates help maintain proper gastrointestinal function. The carbohydrate fraction of feeds is a diverse mixture ranging from polymers to simple sugars that are usually partitioned according to their digestion characteristics in the animal. Carbohydrates are broadly classified by their solubility in neutral detergent, including the insoluble neutral detergent fiber (NDF) fraction and the neutral detergent soluble fraction (see Figure 5-1). The NDF carbohydrates are further categorized as forage NDF (fNDF) or nonforage NDF (nfNDF), each containing hemicellulose, cellulose, and lignin. The neutral detergent-soluble carbohydrates (NDSC) are divided into starch, water-soluble carbohydrates (WSC; e.g., fructans, sugars), and neutral detergent-soluble fiber (NDSF; e.g., pectic substances).

Availability to the animal varies by carbohydrate fraction and source of the carbohydrate (e.g., type of feed, processing, growing environment). Cattle have the capacity to digest starch, lactose, microbial glycogen, and trehalose (disaccharide) based on enzymes present in the pancreatic secretions and small intestinal mucosa (Kreikemeier et al., 1990). All other carbohydrates, including sucrose and those in NDF, must be degraded and utilized by gastrointestinal microbes for them to provide nutrients to the animal. Total-tract digestibility of the carbohydrate fractions affects total nutrient supply to the animal, whereas ruminal fermentation affects the type and temporal supply of fuels, which affect microbial protein production as well as energy intake and partitioning by the animal (Allen, 2014). The type and temporal supply of fuels are affected by the type and source of carbohydrates, as well as interactions with other diet and animal factors. Physically effective NDF (peNDF) is the fraction of NDF

in the diet with particles of a size adequate to form a rumen mat, which entraps small, potentially degradable particles in the rumen and enhances rumination. The increased retention time of digesta in the rumen increases total-tract digestibility and provides additional buffering capacity, likely reducing risk of low ruminal pH and a depression in NDF digestibility.

NEUTRAL DETERGENT FIBER

NDF is the most common measure of fiber used for routine feed analysis. It is a simple and inexpensive method that measures most, but not all, of the chemical compounds that comprise the fiber fractions that are indigestible by mammalian enzymes. NDSF components such as pectins, β-glucans, fructans, and gums are not included in the NDF fraction. NDF has largely replaced other measures of fiber. Crude fiber does not quantitatively recover hemicellulose and lignin, and acid detergent fiber (ADF) does not include hemicellulose but includes some soluble fiber (e.g., pectin) unless ADF is done sequentially on NDF residue (Van Soest, 1994). Within a specific feedstuff, concentrations of NDF and ADF are highly correlated, but the correlation is lower for mixed diets that contain different fiber sources.

The concentration of NDF in feeds or diets is correlated negatively with energy concentration because NDF is generally less degradable than the NDS fractions of feeds. However, digestibility of NDF is highly variable depending on source, ruminal retention time, and the ruminal environment. The acid detergent lignin (ADL) fraction and some of the hemicellulose and cellulose associated with lignin are essentially indigestible by bacterial and mammalian enzymes (Van Soest, 1994). Accordingly, NDF digestibility is negatively associated with lignin concentration within feed type, although the relationship is not consistent. Lignin does not affect the digestibility of the NDS fraction of feeds. Therefore, it is most useful to express ADL on an NDF basis. The ADL content of forages increases with maturity and is
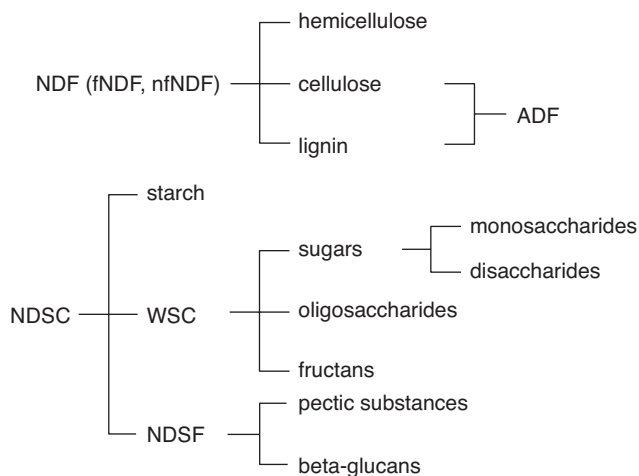
FIGURE 5-1   Carbohydrate fractions in feeds include NDF and NDSC. ADF is a component of NDF.

greater for legumes than grasses at similar stages of maturity (Smith et al., 1972), and warm-season grasses generally have greater ADL concentration than cool-season grasses (Mandebvu et al., 1999).

NEUTRAL DETERGENT-SOLUBLE CARBOHYDRATES

NDSC are diverse and include water-soluble carbohydrates, starch, and NDSF. Except for the monosaccharides, lactose, malto-oligosaccharides, glycogen, and starch, all other NDSC must be degraded to monosaccharides or fermented by gut microbes to be of nutritional value to the animal. The concentration of NDSC in feeds has been estimated by difference as nonfiber carbohydrates (NFC), which is calculated as 100 percent of dry matter (DM) minus the sum of NDF, crude protein (CP), crude fat (CF), and ash; sometimes the CP and ash in NDF are subtracted from NDF to prevent redundant correction for ash and CP (NRC, 2001). Although NFC has been used as an estimate of NDSC, its use is not recommended for diet formulation. It aggregates analytical errors of each of the component assays. Furthermore, it incorrectly conveys the idea that a single pool of NDSC is uniform in its digestion and fermentation characteristics.

WSC include mono-, di-, oligosaccharides, and some polysaccharides. Sugars include monosaccharides (glucose, fructose, etc.) and disaccharides (sucrose, lactose). Lactose is found specifically in milk products, whereas other sugars are found in many feeds, including cane and beet molasses (50 to 60 percent of DM), fresh forages (2 to 10 percent of DM), vegetable pulps (12 to 40 percent of DM), candy or bakery by-products (variable), and other processed human food by-products. Oligosaccharides such as stachyose (2 to 7 percent of DM) and raffinose (1 to 2 percent of DM) are

found in soybeans (Kumar et al., 2010). Cool-season grasses are the primary source of fructans (0 to 30 percent of DM), a storage carbohydrate consisting of varying chain lengths of fructose sometimes with a single glucose at the reducing end of the chain.

Starch contains polymers (amylose and amylopectin) of glucose units linked by bonds that can be cleaved by mammalian enzymes. It comprises the majority of the NDSC in feeds derived from grain crops and tubers that are generally increased in the diet to meet the energy demands of lactating dairy cows. The primary sources of starch fed to cows are corn, barley, wheat, oats, sorghum, millet, silages made from the associated plants, and tubers such as cull potatoes. Concentrations of starch and NDF in corn and sorghum silages are inversely related because starch production during kernel filling dilutes the fibrous stover fraction of the plant (see feed tables, Chapter 19). Forages are supplemented with cereal grains to increase energy density, provide glucose precursors, and decrease the filling effects of rations. The starch concentration of lactating cow diets ranges from less than 20 percent to more than 30 percent of DM.

The most common NDSF components encountered in ruminant feeds are pectins and mixed-linkage β-glucans. Pectins are composed of a backbone molecule made primarily of galacturonic acid and varying amounts of side chains made of arabinose, galactose, and other sugars. Primary sources of pectin in dairy cattle diets include legume forages, soybean hulls, and citrus and sugar beet pulps. Mixed-linkage β-glucans have the same structure as cellulose, except for a periodic bend in the linear glucose chain caused by a β-(1,3) linkage. These β-glucans are found in some small grains such as barley. In some feeds such as citrus pulp, NDSF concentrations can reach 40 percent of DM. Currently, NDSF is calculated by subtracting the concentrations of NDF and starch from the concentration of 80 percent ethanol insoluble residue where NDF and ethanol residues are express on an ash- and CP-free basis (Hall et al., 1999). Using 80 percent ethanol rather than water as a solvent removes some shorter-chain fructans from NDSF.

RUMINAL AND TOTAL-TRACT DIGESTION

Soluble fiber and WSC have the potential to be nearly completely degraded in the rumen, whereas ruminal digestibilities of NDF and starch are lower and highly variable by source and processing. WSC are very rapidly utilized by mixed ruminal microbes, and few of these carbohydrates likely escape ruminal utilization. Reported in vivo rates of ruminal disappearance of glucose, sucrose, and lactose were greater than 250 percent/h, and no residual glucose, fructose, or sucrose was detected in duodenal digesta 1 hour postdosing into the rumen (Weisbjerg et al., 1998). Although sugars are rapidly fermented in the rumen, there is little evidence that moderate substitution of sugars for starch in diets decreases ruminal pH. Inclusion of sugars in diets by replacing

starch generally either increases ruminal pH or has no effect for several possible reasons, including less acid produced per mole of hexose fermented and storage of glucose as microbial glycogen (Oba, 2011). The WSC may be partially converted to microbial glycogen that can be fermented in the rumen or pass to the small intestine. Rate of disappearance of grass fructans and raffinose is also rapid in mixed batch culture (Thomas, 1960), and orchardgrass fructan disappeared at 62 percent/h in vitro (Hall and Weimer, 2016). The disappearance rates of NDSF from pectin-rich citrus, sugar beet pulp, and alfalfa ranged from 20 to 40 percent/h in vitro (Hall et al., 1998), and pectin isolated from alfalfa showed similar rates of fermentation in vitro (Hatfield and Weimer, 1995). Unlike WSC, the digestibility of NDSF is reduced at low pH (Strobel and Russell, 1986). The availability and ruminal digestion of WSC and NDSF are not as markedly affected by processing as is starch.

Ruminal digestibilities of starch and NDF are typically lower than soluble fiber and WSC and are highly variable. Ruminal fermentability of starch from various cereal grains ranges from less than 30 percent to more than 90 percent (Nocek and Tamminga, 1991; Firkins et al., 2001). It is affected by many factors, including grain type, endosperm vitreousness, processing (e.g., rolling, grinding, steam-flaking), conservation method (e.g., dry, ensiled), ration composition, and animal characteristics. Wheat, barley, and oats have starch that is more readily degraded than corn starch, and sorghum starch is most resistant to degradation in the rumen and digestion by the animal (Huntington, 1997). Based on a meta-analysis (Ferraretto et al., 2013), ruminal starch digestibility was greater for wheat (79 percent) and barley (71 percent) than for corn (54 percent), whereas total-tract starch digestibility did not differ (93 to 94 percent). Better descriptions of grain particle size and other discriminating variables will help characterize ruminal digestibility differences within categories. The basis for these differences is related to the characteristics of protein in the endosperm of each grain. Starch granules in the endosperm of seeds are embedded in a protein matrix, and endosperm proteins vary in solubility and resistance to digestion (Kotarski et al., 1992). Vitreous endosperm in some grains contains prolamin proteins (e.g., zein in corn and kafirin in sorghum) that are insoluble and resistant to digestion, decreasing enzyme access to starch granules; in contrast, the proteins in floury endosperm are readily solubilized, allowing greater access to starch (Hoffman and Shaver, 2011). The vitreousness of corn grain endosperm is a function of genetics and maturity. Corn grain vitreousness increases with maturity (Phillipeau and Michalet-Doreau, 1997) and ranges from 0 percent to more than 75 percent at full maturity (Hoffman et al., 2010). Because corn silage and high-moisture corn are harvested before physiological maturity, their degree of vitreousness is less than that of dry shelled corn.

When grains are ensiled, ruminal fermentability of starch can be affected by both grain moisture concentration (af-fected by maturity at harvest) and storage time. In vitro starch digestibility (IVSD) of high-moisture corn samples increased by 9 percentage units from October to August of the following year (Ferraretto et al., 2014). Furthermore, IVSD was negatively related to DM concentration and positively related to concentration of ammonia-N, likely because endosperm proteins are solubilized over time, increasing starch fermentability. The increase in protein solubility and IVSD over time is greatest for grains with higher moisture concentration, and changes were greatest over the first months of storage (Allen et al., 2003). The likely increase in ruminal starch digestibility of ensiled feeds over time should be considered when formulating diets.

To a greater degree than has been noted for other NDSC, the rate of starch hydrolysis is increased by more extensive processing, with greater response for grains with more vitreous endosperm, such as sorghum and corn (Huntington, 1997). Processing increases access of enzymes to starch granules by reducing particle size, which increases surface area, or by swelling and disrupting kernel texture by steam-flaking. Reducing particle size by cracking and grinding significantly increases rate of starch degradation (Galyean et al., 1981; McAllister et al., 1993; Ferraretto et al., 2013), and the effect is greater with unprocessed than with heat-processed grains. Ruminal starch digestibility of ensiled (64 percent) and steam-flaked or steam-rolled (59 percent) corn was numerically greater than dry corn (54 percent) in a meta-analysis (Ferraretto et al., 2013). However, the number of treatment means for ensiled and steam-flaked/rolled corn was small compared with dry shelled corn, and treatment effects were not significant. In that study, total-tract starch digestibility was positively related to ruminal starch digestibility across starch sources. Total-tract starch digestibility was also positively related to postruminal starch digestibility (percentage of duodenal flow), but the wide range in total-tract starch digestibility from less than 84 percent to greater than 98 percent indicated that digestion postruminally does not completely compensate for starch that escapes ruminal degradation. Ruminal and total-tract starch digestibilities were linearly related with each unit increase in total-tract starch digestibility, corresponding to a 3.4 percentage unit increase in ruminal starch digestibility.

Ruminal digestibility of starch is also affected by the starch concentration of diets. In lactating cows, the fractional rate of starch digestion as well as ruminal digestibility of starch increased when corn grain was substituted for fNDF (Oba and Allen, 2003a) or nonforage fiber sources (NFFS; beet pulp; Voelker and Allen, 2003). Ruminal amylolytic enzyme activity for low-starch (21 percent) diets was calculated as 68 percent of that for high-starch (32 percent) diets (Oba and Allen, 2003a). The increases in starch digestibility and calculation of higher amylolytic enzyme activity with higher levels of starch feeding could be caused by an increased number of amylolytic bacteria in the rumen (Mackie and Gilchrist, 1979). Furthermore, 6-hour starch digestibility in vitro of

various feeds averaged 32 percent when using inoculum from cows fed a 50:50 concentrate/hay diet but only 7 percent with inoculum from a strictly hay-fed cow (Cone et al., 1989). Greater starch digestibility with higher-starch diets indicates that starch degradability in the rumen does not follow first-order kinetics and is a function of both the source as well as characteristics of the microbial population in the rumen.

NDF is degraded primarily in the reticulorumen, with additional degradation occurring in the large intestine. Across studies reported in the literature, ruminal digestibility of NDF accounts for over 90 percent of total-tract NDF digestibility (Huhtanen et al., 2010; Gressley et al., 2011). However, significant compensatory digestion can occur postruminally when ruminal NDF degradation is suppressed (Oba and Allen, 2000b). Increasing starch in the diet typically depresses ruminal (Firkins et al., 2001; White et al., 2016) and total-tract (Ferraretto et al., 2013) NDF digestibility. The digestibility of NDF depends on characteristics intrinsic to the NDF source that affect the maximal rate and extent of digestion, retention time in the fermentation compartments, and concentrations and activity of microbial enzymes (Allen and Mertens, 1988). Ruminal retention time is a function of characteristics of the animal (e.g., dry matter intake [DMI]) and feed (e.g., particle size, fragility, digestion characteristics).

The chemical composition of NDF varies greatly, which affects its susceptibility to enzymatic degradation. Lignification of NDF varies among forages and NFFS and is negatively related to digestibility (Van Soest, 1994). The indigestible NDF (iNDF) fraction is positively related to lignin concentration, and potentially digestible NDF (pdNDF) is the fraction remaining (NDF − iNDF); both can be expressed on a DM or NDF basis. The rate of pdNDF degradation in vitro is adversely affected by low pH and inclusion of starch (Grant and Mertens, 1992). Oba and Allen (2003b) reported a positive linear relationship between mean ruminal pH and degradation rate of pdNDF; degradation rate declined from ~4 percent/h at pH 6.5 to ~1 percent/h at pH 5.7.

The actual digestibility of pdNDF is positively related to rate of degradation and the length of time microbial enzymes have to cleave the bonds. The NDF in plant tissues (e.g., mesophyll, xylem, phloem) degrades at different rates (Akin, 1989; Wilson, 1991), and their maximal rate of NDF degradation depends on chemical composition and anatomical structure that affects accessibility of substrates to enzymes (Jung and Allen, 1995). Thus, surface area likely limits rate of NDF degradation unless other factors such as ruminal pH inhibit fibrolytic activity (Russell et al., 2009). Perennial C3 grasses generally have greater NDF concentration than legumes, as well as lower concentrations of lignin and iNDF. Although the rate of digestion of pdNDF is generally slower for grasses compared with legumes (Smith et al., 1972), ruminal digestibility is usually greater because of the greater pdNDF concentration and longer retention time in the rumen (Voelker Linton and Allen, 2008; Kammes and Allen, 2012a). Ruminal digestibility of NFFS is highly variable, depending

on composition, rumen pH, and the peNDF of the diet, which affects their retention in the rumen (Firkins, 1997). Ruminal degradation in vivo proceeds at less than the maximal rate because concentrations and activity of enzymes are limited by environmental effects (pH, nutrient availability) on microbial populations. Availability of rumen-degradable protein (RDP) can limit NDF digestibility in continuous culture (Griswold et al., 2003), and the limitation of diet RDP on ruminal NDF digestibility appears to vary among feeds (Soliva et al., 2015). Decreasing RDP decreased DMI quadratically (especially below 9.2 percent of DM) and tended to decrease ruminal ADF digestibility linearly (Reynal and Broderick, 2005). The direct effect of RDP on ruminal NDF digestibility has not been well studied in lactating dairy cows. Decreasing metabolizable protein (both RDP and rumen-undegradable protein [RUP]) significantly decreased total-tract NDF digestibility (Lee et al., 2011, 2012), most likely as a result of decreased RDP limiting ruminal NDF digestibility.

Greater DMI is associated with decreased rumen retention time and digestibility of NDF (Riewe and Lippke, 1970); total-tract NDF digestibility decreased 4.4 percentage units as DMI increased from 2.5 to 5.0 percent of body weight in a meta-analysis with lactating Holstein cows (de Souza et al., 2018). Oba and Allen (1999a) reported a negative linear relationship between responses in DMI and total-tract NDF digestibility among 32 cows when they were fed diets containing brown midrib corn silage (bm3) or its near-isogenic control corn silage.

Digestibility of dietary NDF can be depressed when ruminal retention time is reduced such as when high proportions of finely chopped or pelleted forages (Allen, 1997) or when NFFSs (Grant, 1997) are included in diets. Forage fragility varies greatly and affects the rate of reduction in particle size from chewing during eating and ruminating (Poppi et al., 1981). Faster particle size reduction will increase the mass of particles below the threshold size to pass from the reticulorumen and decrease the ability of the rumen to selectively retain those particles by decreasing the mass of large fibrous particles in the rumen (Kammes and Allen, 2012d). Diets with more digestible fiber within a forage type (e.g., brown midrib corn silage and less mature grasses and alfalfa) tend to have shorter ruminal retention times (Oba and Allen, 2000a; Kammes and Allen, 2012b; Kammes et al., 2012a) and grasses are retained longer than alfalfa (Voelker Linton and Allen, 2008; Kammes and Allen, 2012a,d). Length of cut did not affect the pool size or retention time of NDF in the rumen for orchardgrass (Kammes et al., 2012b) or alfalfa-based diets (Kammes and Allen, 2012c). This might be because peNDF entering the rumen after chewing was either similar or above a critical threshold for ruminal retention of particulate matter between diets.

Absorbed nutrients produced from ruminal degradation and fermentation or intestinal digestion of carbohydrates in the gastrointestinal tract have different effects on DMI and energy partitioning. The primary nutrients that ruminal

degradation provides to the animal are volatile fatty acids (VFAs) and microbial protein. The VFA can provide up to 70 percent of the energy required by cattle (Bergman, 1990), with the types and amounts produced affecting how the animal's glucogenic or ketogenic needs are met. Ruminal concentrations of the VFA do not indicate amounts of production because information on rumen liquid volume, passage, and differences in rates of absorption for the VFA is lacking (Sutton et al., 2003). Increasing the starch concentration of diets at the expense of NDF can greatly increase propionic acid production without affecting the production of acetic or butyric acids (Sutton et al., 2003), but starch passing from the rumen may be digested to glucose that is absorbed or metabolized to lactate in the small intestine (Reynolds et al., 2003). Ruminal fermentation of sugars generally increases the production of butyric acid (Oba, 2011), but ruminal fermentation of pectin yields mainly acetic acid and relatively little propionic acid (Dehority, 1969). Although the VFA produced can differ by carbohydrate type (Murphy et al., 1982; Weimer, 2011), they are also affected by pH (Strobel and Russell, 1986), dietary forage inclusion (Murphy et al., 1982), concentration of RDP (Malestein et al., 1984), amount of organic matter (OM) degraded, and likely other undefined factors. Propionate production from starch has been reported to differ between diets with higher or lower amounts of forage (Murphy et al., 1982). As dietary starch is increased and pH declines, populations of amylolytic/lactate-producing and lactate-utilizing bacteria both increase (Mackie and Gilchrist, 1979). As starch supplementation increases, the lactate utilizers likely convert a greater proportion of lactate to propionate (Baldwin et al., 1962; Aschenbach et al., 2011).

PREDICTION OF RUMINAL CARBOHYDRATE DIGESTIBILITY

Ruminal fermentation of carbohydrates affects productivity and is a critical consideration for diet formulation. Whereas efforts to predict ruminal carbohydrate digestibility have been abundant, there is considerable uncertainty remaining. Chemical analyses (e.g., NDF, starch) have improved, but a better understanding and description of carbohydrate degradation and digestion will likely improve future predictability. Ruminal digestibility values used in diet formulation are usually derived from feed dictionaries or values reported in the literature, single time-point incubations, rumen models based on rates of digestion and passage, or empirical equations based on diet factors and DMI.

Feed Libraries

Ruminal digestibility of NDF and starch in vivo is highly variable. Whereas some of this variation can be accounted for by feed type, and means can be included in tables of nutrient composition, table values do not account for the large variation resulting from the effects of growing environment, genetics, conservation method, and processing on the feeds, limiting their usefulness, especially for feeds with highly variable composition such as forages and some by-product feeds.

Single Time-Point Incubations

Digestibility of NDF and starch in vitro or in situ by ruminal microbes is often measured at single time points. These methods can provide important relative information to compare feeds, but they are less useful to predict ruminal digestibility in vivo. Ruminal digestibility is affected by ruminal pH and enzyme activity, which vary with the diet and its interaction with animal factors, so rates of digestion are affected by method of determination (e.g., in vitro, in situ; Krizsan et al., 2012). Total-tract NDF digestibility was related to in vitro NDF digestibility with an incubation time of 48 hours ($r = 0.55$, $P = 0.01$) but not 30 hours in a small data set of 21 diets from seven experiments, but total-tract NDF digestibility was overestimated by ~7 percentage units, and the bias was greater as digestibility increased, indicating that equations will be needed to convert in vitro digestibility into in vivo digestibility (Lopes et al., 2015). Although in vitro NDF digestibility at single time points may not be acceptable for prediction of in vivo digestibility, they have been positively related to response in DMI and milk yield (Oba and Allen, 1999b) and are best used to compare feeds for allocation to different groups of cows, troubleshooting, or purchasing considerations.

Mechanistic and Empirical Rumen Models

Mechanistic models have been developed and have evolved over the past several decades to predict ruminal carbohydrate digestibility to more accurately predict metabolizable energy and metabolizable protein in diet formulation programs. The basic concept of mechanistic rumen models is that digestion of OM in the rumen is a competition between the rates of degradation and passage (Waldo et al., 1972). The major problem with this approach is the lack of accurate data for rates of degradation and passage of individual feeds or nutrient fractions in vivo. Whereas feeds can be fractionated and rates of degradation of fractions can be measured, the rates obtained do not represent actual rates in vivo because of differences in particle size (surface area), enzyme activity, and pH between measurement conditions and in the rumen of cows (Firkins et al., 1998; Krizsan et al., 2012). Also, accurate passage rates for each nutrient fraction within specific feeds that correspond to its rate of digestion are nonexistent. The use of the same overall passage rate for all fractions within feeds will overestimate ruminal digestibility of soluble fractions and small particles that have faster rates of passage and will underestimate ruminal digestibility of large particles that have much slower rates of passage.

Models of digestion in, and passage from, the rumen that have been developed over the past several decades such

as Molly (Baldwin et al., 1987; Hanigan et al., 2013) and the Cornell Net Carbohydrate and Protein System (Russell et al., 1992; Sniffen et al., 1992; Van Amburgh et al., 2015) and its derivatives and have made valuable contributions. They have helped students understand the complex interactions of the diet, microbes, and the animal; codify research to understand rumen function; stimulate and prioritize ideas for new research; and stimulate ideas to solve problems during diet formulation. However, whereas they have these advantages over empirical models, they are also less accurate because of incomplete knowledge, numerous required inputs, and lack of accurate data for parameterization (France et al., 2000).

Prediction of carbohydrate digestion with empirical equations gives up some of the potential advantages of mechanistic models based on rates of digestion and passage in favor of increased accuracy. Empirical equations have been developed to predict ruminally degraded starch and NDF based on diet composition and DMI (White et al., 2016) and are used to predict microbial protein production (see Chapter 6).

PHYSICALLY EFFECTIVE NEUTRAL DETERGENT FIBER

A sufficient mass of long, fibrous particles is required to optimize digestive efficiency and proper rumen function. The particles form a mat in the rumen that acts as a filtration bed to increase retention of potentially escapable particles containing pdNDF, increasing NDF digestibility as well as digesta mass in the rumen to buffer fermentation acids. A greater mass of digesta increases rumen buffering both directly, by cation exchange, and indirectly, by stimulating rumen movements that enhance mixing and absorption of fermentation acids, as well as by stimulation of rumination and salivary buffer secretion (Allen, 1997). Other benefits include reduced risk of abomasal displacement and a greater ability to maintain euglycemia and reduce mobilization of body reserves when feed intake decreases in the short term (Allen and Piantoni, 2014).

peNDF is that fraction of the diet that contributes to the formation of this retention mechanism. Whereas increased peNDF concentration of the diet can increase buffering and NDF digestibility, the increased digesta mass can also reduce DMI when limited by ruminal distension. Effectiveness of NDF can be determined multiple ways, but the most accepted definition is the ability of feeds to stimulate chewing (Sudweeks et al., 1981; Allen, 1997). Researchers have assessed whether milk fat can be used as an index of "effective" NDF (e.g., Grant, 1997; Caccamo et al., 2014), which includes the "physically effective" (pe) component that stimulates chewing plus the chemical component of NDF that reduces ruminal acid production when substituted for starch (Armentano and Pereira, 1997). However, because other factors such as bioactive fatty acids (Lock et al., 2006) and feeding of sugars (Oba, 2011) that are not associated with peNDF affect milk fat, use of milk fat production to assess sufficiency of peNDF should be done with caution.

Within the NDF fraction, fNDF that has not been finely processed is the primary contributor to peNDF of diets, and rumen pH is positively related to fNDF but not total NDF concentration of diets (Allen, 1997). Forages that are long or coarsely chopped provide NDF in a form that is more physically effective than NDF in NFFS such as soyhulls or corn gluten feed, or NDF in cereal grains. Many NFFS have a large fraction of potentially digestible NDF, small particle size, and relatively high specific gravity (Batajoo and Shaver, 1994). Furthermore, they have similar or faster passage rates than forages (Bhatti and Firkins, 1995) with similar or slower degradation rates (Firkins, 1997). Based on chewing, NFFS NDF was 30 to 80 percent as effective as fNDF across studies (Mertens, 1997). However, peNDF values of feeds are determined relative to the forage used in each experiment and can vary from one experiment to another. For instance, physical effectiveness of whole cottonseed was 50 percent compared with long-theoretical length of cut (9.5 mm) but 127 percent compared with short (4.8 mm) alfalfa silage (Mooney and Allen, 1997).

Evaluation of treatment means from experiments that compared particle length within the same forage indicated a breakpoint for effect of forage particle length on total chewing time per day at a mean sieve aperture size of 0.3 cm (Allen, 1997). Therefore, a threshold above which little additional response in chewing time to increased peNDF concentration of the diet is likely, and further increases might limit DMI (Zebeli et al., 2012). Using peNDF of individual feeds in ration formulation followed by evaluation of the peNDF of the total mixed ration (TMR) is useful because of the potential for particle size reduction during mixing, especially with vertical mixers. Mertens (1997) suggested that peNDF of rations be measured by determining the proportion of TMR particles retained on a 1.18-mm screen and multiplying by the total NDF concentration of the diet. However, this assumes that diet DM and NDF are equally distributed across all screens if done on an as-fed basis, which is rarely the case. Another limitation to using particle size measurements is the lack of standard methodology used across experiments with use of both wet- and dry-sieving with different sieve designs. The Penn State Particle Separator (PSPS) was introduced as an inexpensive and rapid method to characterize particle size (Lammers et al., 1996) and is a common method of determining particle size of forages and TMRs on farms.

A meta-analysis approach detected associations between peNDF retained on and above a screen with an 8-mm aperture (peNDF >8) and rumen pH, time < pH 5.8 (h/d), and rumination time (min/d) with a breakpoint at 18.5 percent peNDF >8 (Zebeli et al., 2012). That analysis further demonstrated that the requirement for peNDF must be balanced with effects of peNDF on DMI because DMI was decreased when peNDF >8 exceeded 14.9 percent, suggesting diets should contain between 14.9 and 18.5 percent peNDF >8 depending on whether maximizing intake or rumen pH was the goal. Although the relationships were reasonably strong, the database used for the meta-analysis did not include many

diets with NFFS, which have been used to lessen both fNDF and starch concentrations while maintaining DMI and milk production (Dann et al., 2015). When peNDF is calculated by multiplying the fraction of the diet over a threshold particle size by the NDF of the entire diet, the resulting value will increase by adding any source of NDF, even if its physical effectiveness is zero. This problem could be solved by calculation of peNDF as the NDF concentration of the fractions above a threshold size (e.g., what is retained on an 8-mm screen), as suggested by Zebeli et al. (2012). Data have not been adequately reported in the literature at this time to evaluate this, and it could not be rapidly and routinely measured on farms. The peNDF of the DM consumed is also affected by sorting, which increases with excessive large particles, particularly in dry rations (Leonardi and Armentano, 2003; Kmicikewycz et al., 2015). For these reasons, peNDF should be considered as an optimal range to target rather than a minimum that can be exceeded without potential negative consequences.

RECOMMENDATIONS

The previous committee (NRC, 2001) concluded that the application of the effective fiber concept was limited because of a lack of standard, validated methods to measure effective fiber of feeds and to establish requirements. Progress has been made since that publication but is still limited by the availability of published research reporting the particle size distribution of dietary NDF needed for a robust implementation. Because of this, the committee recommends using fNDF as one option for estimating physical form adequacy of diet in combination with other factors.

Forage Neutral Detergent Fiber

fNDF is recommended as a primary consideration for diet formulation rather than total NDF because of a greater positive relationship with ruminal pH (Allen, 1997) and a greater negative relationship with DMI (Allen, 2000). The minimum fNDF likely varies from 15 to 19 percent of diet DM and depends on the proportion of total NDF, starch, and perhaps other NDSC in the diet. The average effective value of NDF from nonforage sources was set to 50 percent of that for NDF from forage. For every 1 percentage unit decrease in NDF from forage (as a percentage of dietary DM) below 19 percent, the recommended concentration of total NDF was increased 2 percentage units, and maximum starch was reduced 2 percentage units (see Table 5-1). Data are needed to determine whether concentrations of WSC and NDSF affect NDF requirements. The minimum total NDF was set at 25 percent based on studies cited in the previous edition (NRC, 2001) and comes with caveats (i.e., the forage was assumed to have adequate particle size, dry ground corn was the predominant starch source, and cows were fed a TMR).

The optimal fNDF concentration of diets to maximize energy intake is higher than the minimum to reduce risk of

TABLE 5-1   Recommended Minimum Forage and Total NDF and Maximum Starch Concentration of Diets for Lactating Cows When a Diet Is Fed as a TMR, the Forage Has Adequate Particle Size, and Dry Ground Corn Is the Predominant Starch Source

Minimum fNDF	Minimum Total NDF	Maximum Starch
19	25	30
18	27	28
17	29	26
16	31	24
15	33	22

Optimal diet forage NDF concentration

17   27

<- Higher dry matter intake

Faster ruminal clearance rate of forage NDF ->

Finely chopped forages ->

Higher diet starch, lower NFFS concentrations ->

Higher diet starch degradability ->

<- Supplemental buffers

Grain fed separately, infrequently ->

Limited feed bunk space, slug feeding ->

Greater daily variation in diet composition ->

FIGURE 5-2   Factors affecting the optimal forage NDF concentration of diets for lactating cows. Clearance rate of forage NDF from the rumen is affected by rate of degradation, forage fragility, and rate of passage.
SOURCE: Adapted from Allen (1995).

acidosis. Optimal fNDF for lactating cows likely ranges from 17 to 27 percent of diet DM and is a function of milk yield and the cows' drive to eat as well as other factors shown in Figure 5-2. Less-filling diets will likely benefit cows with high milk yield with DMI limited by ruminal distention by allowing greater feed intake while maintaining rumen fill. However, the greater energy concentration of the diet might result in less rumen fill for cows with lower milk yield and DMI limited by metabolic mechanisms (Allen, 2000). The filling effect of fNDF is not constant but is affected by the initial size and fragility of forage particles, which affect ruminal retention time and formation of the rumen mat (Allen, 2000). The optimal fNDF concentration of diets also depends on diet fermentability (Allen, 1997), which is highly dependent on the concentration and fermentability of starch. At a given fNDF concentration, diet fermentability can be decreased by substituting grains (i.e., starch) with NFFS or by substituting sources of starch that are less fermentable such as dry ground corn for high-moisture corn, wheat, or barley. Diet fNDF concentration must increase when high percentages of highly fermentable starch

sources (e.g., wheat, barley, high moisture corn) are included in diets, whereas supplemental buffers will allow lower fNDF concentration. The optimal fNDF is also affected by feeding method; TMR decreases variation in rumen pH over time compared with feeding grains separately (Robinson, 1989), allowing lower fNDF diets with higher starch concentration. For component feeding systems, when concentrate is offered more than twice daily, fewer effects on production, milk composition, and ruminal conditions have been reported (Cassel et al., 1984; Robinson, 1989; Maltz et al., 1992). Eating rate is decreased with increased fNDF concentration (Oba and Allen, 2000a) or forage particle size (Kammes et al., 2012b), and optimal fNDF will be greater when competition for feed bunk space encourages some cows to slug feed. fNDF concentration should be increased when greater variation in carbohydrate fractions is expected. Variation might be from differences in forage DM or NDF that are not adjusted for by diet formulation. Although intentional variation in fNDF concentration of diets did not affect production responses of early to mid-lactation cows, treatment diets averaged 23.2 percent fNDF and always exceeded 21 percent fNDF, and cows were not fed diets with lower fNDF diets for more than a couple of days (Yoder et al., 2013). Variation not accounted for by reformulation of diets will likely result in negative effects (e.g., milk fat depression, decreased NDF digestion) when diets are closer to the recommended minimum fNDF concentration.

Physically Adjusted Neutral Detergent Fiber

A potential option for assessing adequacy of the physical form of diets is the physically adjusted NDF (paNDF) system (White et al., 2017a,b), which was developed to provide guidance on diet particle size requirements to attain a given rumen pH and its interaction with other diet components, including diet NDF, fNDF, starch, and percent forage. However, this system is new and has not undergone rigorous field testing. The new system was termed "paNDF" to avoid confusion with peNDF, which is based on stimulation of total chewing. Particle size recommendations are based on the PSPS (Lammers et al., 1996) because of the available data and its wide use on farms. In the paNDF system, many variables describing particle size, composition of the diet, predictions of ruminally degraded carbohydrates, and more are inputs to predictions for DMI, rumination time, and ruminal pH. Although the effect of high paNDF on limiting DMI is important, the focus of the system is only on the role of paNDF to maintain the mean ruminal pH selected. The ruminal pH goals within the paNDF system are proxies for describing a desirable rumen environment and are not intended to be predictive of ruminal pH or recommendations for optimal ruminal pH. Ruminal pH reflects the net production, absorption, metabolism, and neutralization of fermentation acids plus other factors (Allen et al., 2006). Two mean ruminal pH targets (6.0 and 6.1) are represented in Table 5-2 to illustrate the interactions among the model inputs. At the database's average ADF/NDF (0.63), the discrete values

of 3, 9, and 15 percent of DM in the TMR on the 19-mm sieve were chosen to reflect the range in the model's predictiveness. At each of those discrete cutoffs, the minimum percentage of DM on the PSPS 8-mm screen to maintain the mean ruminal pH shown is predicted. Particle size characteristics can be interpolated in diets with varying model terms (forage, starch, NDF, and fNDF percentages). As in Table 5-1, there is a consistent relationship in which less fNDF is needed to maintain any categorical mean pH as starch declines.

Assuming limited sorting of feed by the animals, an example of how the paNDF system works would be if a new forage source is coarser, then the TMR would be expected to have higher percentage of DM on the 19- and 8-mm screens; in such a case, the percentage of fNDF could be decreased without an expected negative impact on ruminal pH. In contrast, if a coarser forage is replaced by more finely ground forage, the fNDF inclusion in the diet should be increased. Similarly, a more conservative (i.e., higher) percentage of particles on the 19-mm sieve could be selected when ruminal starch digestibility is expected to be greater than average because of more extensive grain processing.

The impact of ADF/NDF is demonstrated in Figure 5-3, when other terms are held constant; as dietary fNDF decreases below 20 percent, a greater percentage of particles is needed on the 8-mm sieve with increasing ADF/NDF, which is partly affected by grass/legume (Allen and Piantoni, 2014) but also affected by other factors such as percent cereal grains in the diet.

Caveats for the Physically Adjusted Neutral Detergent Fiber System

The paNDF system was not designed to predict ruminal pH but, rather, to demonstrate how chemical composition and physical form of the diet affect rumen pH. In turn, improved understanding should ultimately guide ration formulation and TMR evaluation. Although a variety of pH targets can be chosen within this system, results appear reliable only between pH 6.0 and 6.1, which is the recommended range in usage. The range among individual studies in the derivation data set was 5.44 to 6.83 (White et al., 2017a), but the model included random effects that reduce variation among studies and is therefore sensitive to small changes in mean ruminal pH. In addition, derived solutions are sensitive to BW and CP because these factors affect DMI, which is used to predict total time ruminating. When evaluating paNDF it is recommended to assume a diet of 17 percent CP and BW of 630 kg so that changes in diet factors related to paNDF remain within conditions included in the data from which the models were derived.

The paNDF system was based on studies that primarily were from individually fed dairy cattle and therefore do not reflect the greater potential to sort against long or dry forage or toward grain (Kmicikewycz et al., 2015; Miller-Cushon and DeVries, 2017) compared with commercial settings or overcrowding conditions that limit feed bunk space and encourage slug feeding. Factors shown in Figure 5-2 must be considered

TABLE 5-2 Predicted Minimum Dietary DM on the 8-mm Sieve of the PSPS with Varying DM on the 19-mm Sieve Predicted to Maintain Mean Ruminal pH ≥6.0 or ≥6.1 for Lactating Dairy Cattle Fed TMR Varying in Dietary Composition[a]

TMR composition, % (DM Basis)[b]				Maintenance of pH 6.0 % TMR on 19-mm Sieve			Maintenance of pH 6.1 % TMR on 19-mm Sieve		
				3	9	15	3	9	15
Forage	Starch	NDF	fNDF	Minimum % on 8-mm Sieve[c]			Minimum % on 8-mm Sieve[c]		
40	35	25	19	20	13	12	53	43	33
40	30	28	19	26	17	14	53	42	33
40	25	30	22				17	10	11
40	25	30	19	19	12	11	36	26	19
40	25	30	17	32	23	17	50	40	31
40	20	33	17	11	10	10	19	12	11
40	20	33	15	24	15	12	32	22	16
40	20	33	14	30	21	15	39	29	21
40	15	35	13				17	10	10
50	35	25	20	25	17	13	40	30	22
50	35	25	18	40	31	23	59	48	38
50	30	28	22	12	10	10	23	14	12
50	30	28	20	26	17	13	38	28	20
50	30	28	18	41	31	23	54	44	34
50	25	30	22				17	10	10
50	25	30	20				31	22	15
50	25	30	18	24	15	12	46	36	27
50	20	33	19				18	10	10
50	20	33	17				32	22	16
60	30	28	23				42	32	23
60	30	28	22				51	41	31
60	25	30	24				22	13	10
60	25	30	23				30	20	14
60	25	30	22				38	28	19
60	20	33	20				26	17	12

[a] Total mixed rations (TMRs) from the Penn State Particle Separator (PSPS) with only two sieves and a pan (White et al., 2017a,b). These two pH targets are provided as examples, and this approach is not intended to be used to predict ruminal pH.

[b] fNDF = forage NDF. Other variables in the model were set to their means (White et al., 2017a). The mean ADF/NDF was 0.63 (SD = 0.26) in the overall data set (used for this table) and 0.56 (0.07), 0.63 (0.14), and 0.63 (0.08) for 40, 50, and 60 percent forage, respectively.

[c] When a row is blank, pH 6.0 is predicted to be achieved at all possible combinations of particle size percentages; that is, there is no minimum on the 8-mm sieve to predict a mean ruminal pH of 6.0.

in addition to the paNDF system with the nutrition advisor's experience and judgment. Ruminal buffers and other minerals might influence mean ruminal pH independent of any of the diet variables shown, particularly for diets that have high amounts of rumen-degraded starch. For situations in which high-forage diets are fed (such as typical diets fed to dry cows), the paNDF system is not necessarily useful and should not be used to predict rumination time or DMI (outside of the data range). In the meantime, the committee recommends further research on PSPS fractions, including differences in moisture and NDF concentrations among diets and on other parameters that could improve the paNDF model or lead to the development of other particle size-based systems.

Feed Analysis

Forages should be tested routinely for concentrations of DM and NDF because of the large variation among and within sources. Starch concentration should also be tested routinely for corn silage, small grain silages, and other variable starch sources. Frequent sampling and testing will help determine more accurate values because of other sources of variation, including sampling and laboratory errors. Frequency of testing depends on inclusion rate, the variation expected, and the length of time the forage will be fed. Abrupt changes in quality are more likely to occur in upright silos compared with horizontal silos, with small fields of forage, and when weather during harvest is less than optimal. Each lot of NFFS that is typically variable (e.g., distillers grains) should be tested before inclusion in rations, especially if inclusion rate is high unless feeding rates on the farm make this impractical.

Testing forages for in vitro NDF digestibility is recommended for allocating multiple forages to different groups of cows, troubleshooting, or purchasing considerations. In vitro digestibility is a biological measure and more variable across runs than chemical measures. Therefore, comparisons should be made within a run or results corrected to a standard that is replicated within runs. Because perennial grasses are
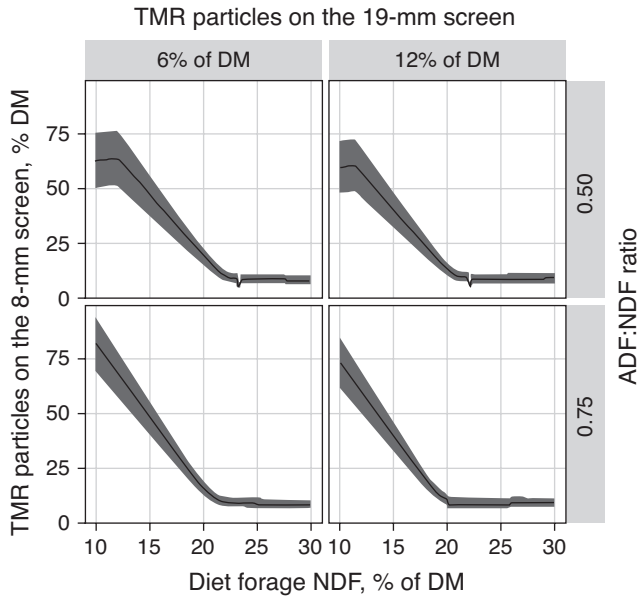
FIGURE 5-3   Example of how the dietary ratio of ADF/NDF (0.50 for the top two plots or 0.75 for the bottom two plots) influences the paNDF model of White et al. (2017b). A ratio of 0.5 is typical of a grass-based diet or a diet with substantial amounts of by-products, and a ratio of 0.75 is more typical of a diet based on corn silage and alfalfa. The line represents the ensemble prediction, and the shaded area represents the minimum to maximum range of predictions from individual ensemble members as an estimated confidence range. The mean ADF/NDF in that database was 0.63. When the total mixed ration (TMR) particles on the 19-mm sieve of the PSPS are fixed at 6 percent (left two plots) or 12 percent (right two plots) of DM, the TMR recovered on the 8-mm sieve (left axis, percentage of DM) is the minimum needed to achieve a mean ruminal pH of 6.1 with varying dietary forage NDF (percentage of DM; bottom axis). Other variables in the model are held constant to their means. SOURCE: White et al. (2017b).

generally more filling than legumes (Oba and Allen, 1999b), mixed grass–legume forages should be tested for ADF to help determine the fraction of grass and legume in the forage; ADF/NDF is ~0.8 for legumes and ~0.6 for grasses (Allen and Piantoni, 2014).

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6

Protein

INTRODUCTION

Dietary crude protein (CP) is defined as nitrogen (N)×6.25, assuming feedstuffs average 16 g of N per 100 g of true protein (TP). Chemistry of feed CP is detailed in NRC (2001) and Chapter 18. Feedstuffs vary widely in their relative proportions of protein and nonprotein N (NPN), in the rate and extent of ruminal degradation of protein, and in the intestinal digestibility and amino acid (AA) composition of rumen-undegradable protein (RUP). Metabolizable protein (MP) supply, defined as TP digested postruminally, should be used to describe and assess protein nutrition. However, intestinally absorbed AAs, and not protein per se, are metabolized. Since the NRC (2001) derivation of optimal lysine (Lys) and methionine (Met) concentrations in MP, an effort has been made to develop models to balance dairy rations for AA, as done for swine and poultry. Absorbed AAs are vital to the maintenance, growth, reproduction, and lactation of dairy cattle. Although AAs are used principally as building blocks of proteins, they are also involved in many other metabolic functions. For example, AAs other than leucine (Leu) and Lys can serve as precursors for gluconeogenesis, and all can be converted to fatty acids (FAs) or serve as sources of metabolic energy when oxidized to CO_2; Met is converted to S-adenosylmethionine, which is directly involved in hundreds of reactions. Because of the central role of proteins in cellular function, protein synthesis is regulated, and AAs have been shown to exert control through intracellular signaling pathways, including the mammalian target of rapamycin (mTOR) and integrated stress response pathways (Arriola Apelo et al., 2014b).

The main goals of protein and AA nutrition are to characterize feeds for rumen-degradable protein (RDP) and digestible RUP, predict the conversion of RDP into ruminal microbial CP (MCP), and account for how efficiently the resultant MP and metabolizable AA are used under different animal and dietary conditions. Responses to individual AAs can differ from a weighted mean response (i.e., protein); further research

is needed to fully replace MP as a primary nutrient with metabolizable AA. When RDP or metabolizable RUP is in excess or when metabolizable AAs are not properly balanced, their carbon skeletons are metabolized by the microbes or the animal for energy; however, the excess N poses an environmental challenge (see Chapter 14). Therefore, improved prediction equations are needed to relate dietary inputs of AA and N to ruminal and animal responses so that animal performance can be optimized while minimizing environmental impact.

MAJOR DIFFERENCES FROM THE PREVIOUS VERSION

Metabolizable Protein

Many factors influencing ruminal degradation of different dietary protein sources and the importance of ruminal degradability on the efficiency of microbial protein synthesis (EMPS) were discussed in the previous version (NRC, 2001). In particular, the 2001 update highlighted an improved library to estimate RDP and the intestinal digestibility of RUP, and it included an improved prediction equation to estimate MCP flow from the rumen. The current committee started with the NRC (2001) components and updated the database to include more studies using exclusively dairy cattle that reported duodenal or omasal flows of microbial N, nonammonia nonmicrobial N (NANMN), and AA. The updated data were used to evaluate NRC (2001) and, when compared to observed data, they compared favorably with other models, but all models evaluated by Pacheco et al. (2012) exhibited room for improved accuracy or precision. Predictions of both NANMN flows based on duodenal (Bateman et al., 2005) or omasal (Broderick et al., 2010) sampling were biased when the NRC (2001) model was used. Therefore, the committee deemed that protein supply should start with a fresh appraisal of data reporting measured ruminal outflow of N from dairy cattle. Because most flow studies did not independently derive RUP of feeds consistent with

NRC (2001) and did not measure intestinal digestibility, the current feed library must be used to extend microbial N and NANMN flows to an MP basis.

NRC (2001) estimated RDP based on kinetics of N disappearance of feedstuffs in situ. The N that washes out of bags prior to incubation and the N that remains in bags regardless of length of incubation are the A and C fractions, respectively. The B fraction is computed by difference of A and C from the total N. The degradation rate (k_d) of the potentially degraded B fraction is derived from in situ incubations. The in situ information can subsequently be used with rate of passage (k_p) in a first-order model to predict the extent of ruminal degradation. Because of limited N kinetic data, some feeds were poorly represented in the feed library (NRC, 2001). The NRC (2001) feed library also had limited estimates of intestinal digestibility for RUP (dRUP) for many feeds, prompting the use of increments of 5 percentage units to document data limitations. The current publication has a much stronger representation among feeds for the components used to derive RUP and dRUP.

In contrast with improved feed library values for N disappearance and for dRUP, the k_p of feed components has received little research attention since the NRC (2001) publication. One of the three k_p equations in NRC (2001) had a coefficient error corrected by Seo et al. (2006), and those authors noted a reasonably unbiased fit of the equations to the data, which were primarily derived from the use of rare earth markers. The committee considered whether ruminal in situ kinetics and k_p equations should be retained based on a complete reassessment of equations compared to a database with postruminal flows (White et al., 2017b). In that report, neither k_d nor k_p accounted for significant variation in ruminal NANMN outflows. However, the equations from that paper were for classes of feeds, and distinguishing RDP based on the A, B, and C fractions within feed classes was deemed insufficient by the committee to yield accurate differences in RUP among feeds processed to increase RUP. Consequently, a system was devised to retain elements of the NRC (2001) system (A, B, C, and k_d) but to re-derive static k_p values for forages and concentrates to remove the bias compared to NANMN flows (see subsequent section).

The NRC (2001) model estimated duodenal supply of MCP using intake of total digestible nutrients (TDN), which was discounted for negative associative effects. The equation was biased (St-Pierre, 2003; White et al., 2017b) and incorrect mechanistically because TDN includes postruminal digestion, which would not influence ruminally synthesized MCP. Therefore, the committee assessed a more mechanistic prediction of MCP that integrated responses to RDP and rumen-degraded starch and neutral detergent fiber (NDF).

NRC (1989) utilized an efficiency of RDP transfer to microbial N of 0.9, which would yield an RDP requirement of $1.11 \times MCP$ (i.e., $1/0.9 = 1.11$). Because the mean RDP/MCP was 1.18 in their data set, NRC (2001) revised this efficiency to 1.18; that is, if RDP intake was <118 percent of MCP, then MCP was limited to 85 percent (i.e., 100 percent /

118 percent $\times$ 100) of RDP intake. The beef NASEM (2016) maintained its previous conversion efficiency of 100 percent, assuming blood urea nitrogen (BUN) transfer into the rumen would provide the shortfall needed for N assimilation into rumen MCP. Dairy cattle have different feeding conditions than grazing or feedlot beef cattle, and completely relying on BUN would not compensate for incomplete efficiency of transfer of RDP to MCP. Li et al. (2019) observed that the efficiency of transfer of ruminal ammonia N to microbial N was less than 70 percent under normal dairy feeding conditions. However, this efficiency is variable and declines as RDP and CP intake increases. Thus, the committee did not attempt to capture the potential use of BUN in support of microbial N synthesis directly and adopted an approach to predict MCP as a function of both RDP and rumen-degraded carbohydrate (starch and NDF), which is similar to that described by White et al. (2017b). While not direct, this approach inherently accommodated BUN transfer as it was derived from in vivo observations where such transfer is occurring.

In the current version, the committee retained the definition of net MP supply as the sum of MP from RUP and MCP. Duodenal flow of endogenous protein is not included in the net supply because it is synthesized from previously absorbed AA and, as such, does not represent a new source of MP and AA to the animal. To predict the requirements, estimations of the endogenous urinary loss and metabolic fecal output have been revisited. The current version also corrects the erroneous assumption that the transfer coefficient for MP to milk protein and other protein secretions is constant under different dietary and physiological conditions (Hanigan et al., 1998; Arriola Apelo et al., 2014b; Patton et al., 2014). Gestation requirements have been adjusted to utilize the model of Koong et al. (1975) fitted to the data of Bell et al. (1995), using estimated calf birth weight as an input. The efficiency of utilization of MP to proteins exported and protein accretion during growth is assumed to vary except for endogenous urinary loss. The urinary loss being a nonprotein fraction of end products of metabolic pathways, its efficiency is assumed to be 100 percent. The requirements for growth were derived as net protein (NP) gain, which was calculated as a function of user-specified live weight gain. The efficiency of conversion of MP to NP for growth is now calculated as a function of body weight (BW) relative to mature weight (see Chapter 11). Although requirements and associated recommendations of MP vary with the physiological status of the cows and the supplies of MP and energy, recommendations of MP are presented, assuming that energy is supplied to meet requirements. An efficiency of utilization of MP estimated to maximize export proteins has been defined as the "target" efficiency and is used to calculate recommendations.

Metabolizable Amino Acids

Of the 20 primary AAs that occur in proteins, 9 are usually classified as being "essential" AAs (EAAs) and 1 (arginine

[Arg]) as conditionally essential. AAs are termed "essential" if their carbon skeletons cannot be synthesized by animal cells or are synthesized at a rate insufficient to meet needs. The EAAs include histidine (His), isoleucine (Ile), Leu, Lys, Met, phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val). In contrast, AAs classified as "nonessential" (NEAAs) are those that can be synthesized de novo in adequate quantity given adequate supplies of N; these are alanine (Ala), asparagine (Asn), aspartate (Asp), cysteine (Cys), glutamate (Glu), glutamine (Gln), glycine (Gly), proline (Pro), serine (Ser) and tyrosine (Tyr). Although Arg is often classified as an NEAA in other animals (e.g., Hou et al., 2015), it has been traditionally classified as an EAA in dairy nutrition. Even if its de novo rate of synthesis is significant, it would be insufficient in high-producing dairy cows and is therefore usually considered part of the "10 EAAs." However, jugular or abomasal infusion of Arg (Vicini et al., 1988) or deletion from a mixture of AA infused postruminally (Schwab et al., 1976; Doepel and Lapierre, 2011) did not affect milk protein yield. De novo synthesis of arginine in dairy cows was estimated at approximately 30 g/d (Doepel et al., 2004; Martineau et al., 2014). In NRC (2001), the duodenal flows of EAA were estimated semiempirically by adjustment of the factorially determined flows using regression models based mainly on the RUP fraction. In the current edition, postruminal flow of AA is estimated using a factorial method based on predictions of MCP, RUP, and endogenous duodenal CP. The flow of individual metabolizable AA, considered the net supply of AA, is calculated using the corresponding AA composition of feed factored to RUP and MCP and their respective digestibilities. Although endogenous duodenal proteins contribute to the duodenal protein flow, it is considered that these proteins are synthesized mainly from arterial supply and as such do not constitute a new net supply and therefore do not contribute to the net flow of metabolizable AA.

The committee investigated the use of variable ruminal degradabilities and intestinal digestibilities of individual EAAs among protein sources. Because White et al. (2017a) noted inconsistencies in methods used among feeds, the committee maintained the approach of factoring feed AA composition through RUP and dRUP. However, the committee recognizes that some, but not all, individual studies reported variable AA disappearance. For the AA composition of MCP, the committee adopted the approach derived from Sok et al. (2017). Although still retaining a constant AA profile, this approach modified the AA profile by accounting for protozoal contribution to MCP supply. It also addressed differences in AA profiles among fluid- and particulate-phase bacteria. The conversion of MCP to a TP basis was derived using AA recoveries accounting for hydrolysis losses rather than assuming 80 percent TP in the MCP, as done in NRC (2001).

Recommendations for AA are based on a factorial approach, rather than the proportional approach used in NRC (2001): this means that the recommendations of each EAA represent a quantitative assessment based on the sum of each EAA needed to fulfill each designated metabolic function. Recommendations are given for all EAAs, except Arg. The approach used for AA recommendations follows that used for the MP, and a target combined efficiency is used for each AA, assuming that energy requirements are adequately met. However, because of variable efficiency of AA use for several postabsorptive processes, MP and AA requirements are not constant and related to the energy supply and the physiological status of the cows. The Trp supply data are very limited, and thus predictions of Trp supply and recommendations need to be interpreted with caution.

METABOLIZABLE PROTEIN AND AMINO ACID SUPPLY

Rumen-Degraded and Undegraded Protein

Rationale for a Revised System

The NRC (2001) estimation of RDP and RUP resulted in mean (19 percent of the mean squared prediction error [MSPE]) and slope (22 percent of MSPE) biases when compared to observed NANMN flows (White et al., 2017b). Subsequent editing of the metadata by the committee to correct data entry errors reduced those biases to 7 percent of mean square error (MSE) each with a mean bias of 120 g/d and a slope bias of −0.31 g/g. An empirical prediction of ruminal digestibility based only on the A, B, and C fractions without use of k_d and k_p was considered by the committee; however, that failed to capture adequate variation among feeds. The addition of more feed categories for the B fraction degradation coefficient did not solve the sensitivity problem. For example, soybean meal, expeller-processed soybean meal, and nonenzymatically browned soybean meal had essentially equal calculated RUP values: 0.28, 0.30, and 0.28 g/g of CP. The committee assumed that a k_d would be required to match expectations of increased RUP with certain processing (Schwab and Broderick, 2017). Although k_d requires multiple time points and has procedural issues (Broderick et al., 2010), recommendations are discussed to improve its accuracy (see Chapter 18).

Considerations for Kinetics of Degradation

The current committee assessed the NRC (2001) technique for estimating kinetics of ruminal N degradation in situ for feedstuffs and recommends its continued use (see Chapter 18). The current database has a few notable considerations for users. The literature contains two types of fishmeal: ruminant and nonruminant grades (England et al., 1997), whereas users might be more likely to be using ruminant grade. Many of the earlier studies assessing fishmeal used a 24-hour in situ incubation; these values were included in NRC (2001) but removed from the current database because 24 hours is not long enough to properly estimate the C fraction (Liebe et al., 2018). In contrast, because of analytical issues, corn

gluten meal has N disappearance in situ that seems to be underestimated (Murphy and Kennelly, 1987), which has been addressed (Stern et al., 1997).

As in the previous report, the current committee had to retain studies without correction for bacterial contamination to provide a robust database. Lack of correction for bacterial N contamination can underestimate the k_d of the B fraction (Wanderley et al., 1993). Bacterial N contamination represents up to 9 percent of the residual feed dry matter (DM) at 72 hours of incubation (Yang et al., 1999). Adding starch to the diet decreased the k_d of N in grasses (De Visser et al., 1998). In addition, the C fraction would be overestimated (and B fraction underestimated) by the degree to which bacterial N contamination is a proportion of residual N. The effect on predicted RDP would therefore depend on a culmination of factors, including errors associated with deriving k_d and A, B, and C fractions; that is, estimates of k_d are correlated with predicted A, B, and C fractions (Woods et al., 2003). Bacterial contamination can decrease the estimation of RDP by up to 5 percentage units (Alexandrov, 1998). However, this error is likely to partially (but not necessarily consistently among feeds) compensate for error from particle washout from bags.

Although in situ approaches have problems, various other approaches also have limitations or need further validation against in vivo measurements (Stern et al., 1997). Derivation of k_d presents a logistical challenge for feed analyses labs because more time points are needed. Based on omasal flow estimates, Reynal and Broderick (2005) suggested that NRC (2001) underestimated k_d, but this effect depended on the protein source (Brito et al., 2007b). In a meta-analysis of omasal N flows, similar conclusions were reached (Broderick et al., 2010). Despite these issues, the committee recommends further research to improve the accuracy of in situ kinetics used in calculation of RUP in protein sources. Numerous literature sources (Liebe et al., 2018) have become available since NRC (2001) to improve the feed library for nearly all common feeds. White et al. (2017b) and Liebe et al. (2018) explained exclusion criteria and corrections made when $A + B + C$ did not sum to 100 percent.

Considerations for Ruminal Passage Rate

The committee considered using k_p data from the scientific literature or deriving static k_p empirically. Much of the k_p data used by NRC (2001) were derived from feed particles marked with rare earths, which can migrate to small particles and bacteria (Bernard and Doreau, 2000). Therefore, the B fraction may not pass at the same rate as the rare earths marking the particulate fraction. Predictions varied considerably from passage rates of undegradable NDF (Krizsan et al., 2010). Likewise, undegradable NDF does not necessarily pass at the same rate as potentially degradable NDF (Firkins et al., 1998). Soluble protein (fraction A) passes at rates much faster than particulate protein and can contribute significantly to RUP (Broderick et al., 2010; Huhtanen et al., 2014). Mechanisti-

cally, actual ruminal passage kinetics probably are even more complex than either of these approaches (Gregorini et al., 2015). The foundational principle that the marker should pass with the nutrient of interest (B protein in this case), not the particles or adherent bacteria, remains an elusive problem. Because k_d was required and measured k_p from markers were deemed problematic, the committee empirically derived a static k_p that allowed RUP to be predicted using a first-order model without bias as compared to NANMN flows (corrected for endogenous N) and converted to a CP basis.

An existing database containing postruminal N flows and diet descriptions from publications (Hanigan, 2006; Bateman et al., 2008) was updated (Roman-Garcia et al., 2016) and used to evaluate and update the model. For each feed in each diet, the mean CP, A, B, C, and k_d values were imported from the current feed library (see Chapter 19). Standard regression techniques yielded unrealistic parameter estimates for static k_p. The committee initially attempted to address this by increasing the number of feed categories; however, the static k_p values remained unrealistic and extremely variable for some feed categories. The NANMN calculation is derived by difference of nonammonia N (NAN) and microbial N flows and therefore aggregates error of both measurements. In addition to the limited number of observations for some feeds, fitting to NANMN is a challenge as it represents an aggregate of all feeds contributing RUP and is subject to potential variation introduced from endogenous N flows. The combination of large variation in observed NANMN flows, the aggregation of many ingredient contributions to NANMN, and the unbalanced nature of the data likely contributed to the unreasonable results with a mixed model. However, Bayesian hierarchical models allow study-specific parameters to follow a distribution for which the mean vector is the set of parameters common to all studies (Moraes et al., 2018b). Using this approach, the static k_p were estimated to be closer to expectations while accounting for random effects of studies.

Revised Estimates of Static k_p

The committee assumed that the k_p from wet and dry forages and concentrates underpinning the Seo et al. (2006) model provided information that could be leveraged to derive a set of static k_p values that would remove the bias in overpredicting RUP. The model used was of the following form:

$$Dt\_RUP = \sum_{c=1}^{N_c} \left(\begin{array}{c} \left(1 - DCa_c\right) \times \left(Dt\_CPAIn_c - Dt\_CPAIn_{NPN,c}\right) \\ + \dfrac{Kp_c}{Kp_c + Kd_c} \times Dt\_CPBIn_c + Dt\_CPCIn_c \end{array} \right)$$

(Equation 6-1)

where

$Dt\_$ = total diet concentration of the specified nutrient,
subscript c represents the class of feed the ingredient belongs to,

DCa_c = ruminal degradability of fraction A in feed class c,

$CPAIn_c$ = CP intake of fraction A within feed class c,

$CPAIn_{NPN,c}$ = intake of ruminally degraded nonprotein N × 6.25 (CP equivalents) within feed class c,

$CPBIn_c$ = intake of crude protein of fraction B within feed class c,

$CPCIn_c$ = CP intake of fraction C within feed class c,

Kp_c = static k_p for feed class c (e.g., forage or concentrate),

and Kd_c is the weighted average of the in situ determined k_d for each feed in class c.

A Bayesian approach was used to derive estimates for DCa_c and Kp_c. The observations of K_p from Seo et al. (2006) were used as priors. DCa_c was included to allow for potential escape of the A fraction as reported by Broderick et al. (2010). DCa_c was initialized with a noninformative prior. The equation was fitted to observed postruminal flows of NANMN minus estimated endogenous N flows (see the subsequent section), and N was multiplied by 6.25 to convert to CP.

No gains in precision were realized for models with greater complexity than a single static k_p for all forages and another static k_p for concentrates. The solution using only a k_p for forage and another for concentrates yielded an estimate of 6.4 ± 4 percent escape of protein in the A fraction, a static k_p for the B fraction of 5.28 ± 0.63 percent/h, and 4.87 ± 0.33 percent/h for concentrates and forages, respectively. The concordance correlation coefficient (CCC) was 0.54, root mean square error (RMSE) was 40.9 percent of the mean, and mean and slope biases were 0.1 and 3.8 percent of MSE. These static k_p are not affected by dietary factors, whereas the k_p from NRC (2001) all increased modestly with increasing dry matter intake (DMI), and two k_p were affected by NDF and concentrate percentages. The committee concluded that the model with static k_p for forage and concentrates with some passage of the A fraction represented the best compromise to yield unbiased RUP values (versus NANMN × 6.25).

Metabolizable Protein Supply

Endogenous Protein Flow to the Duodenum

Because endogenous proteins arriving at the duodenum have been synthesized in gastric and pregastric compartments, the AA used for their synthesis is mainly, if not totally, from arterial origin. This means that these AAs have been previously absorbed and then used by gut tissues to synthesize secreted proteins. As such, they are not a new input of AA to the cow and need to be removed from the duodenal flow to estimate the net MP and AA supply (Lapierre et al., 2006). Due to the technical challenge of assessing endogenous N (EN) flow in ruminants, duodenal EN measurements are scarce. Nevertheless, duodenal EN flow has been determined in growing and mature cattle but by different methods. A limited database (see Table 6-1) was constructed to develop a regression between EN and DMI. Ørskov et al. (1986) reported an EN flow from the rumen and abomasum when cattle received no feed and were only infused into the rumen with volatile fatty acids (VFAs). The data from steers fed at low intakes indicated a disproportionate ratio of duodenal EN relative to DMI when compared to cows fed at higher intakes. These data support the inclusion of an intercept in the regression, which was not included in the NRC (2001) estimation. The physiological status (growing versus mature) was not significant when included in the regression. Therefore, duodenal EN (g N/d) can be estimated as

$$\text{Du\_EndN (g/d)} = 15.4 \pm 2.6 + 1.21 \pm 0.24 \times \text{DMI}$$
(Equation 6-2)

where DMI is kg/d.

Both the slope and intercept are significant ($P \leq 0.01$). Compared with this equation, the estimation of duodenal EN in NRC (2001) of 1.9 g N/kg DMI would underestimate EN in cattle with a DMI lower than 22.3 and overestimate when DMI is higher than 22.3 kg/d.

TABLE 6-1   Duodenal EN (g N/d) Flows from Cattle as Reported

Animal	DMI, kg/d[a]	BW, kg	EN	Method[b]	Reference
2 dairy cows	8.34	500	18.8	[15]N	Brandt et al. (1980)
2 dairy cows	0 (3.4)	675	15.4	Rumen VFA inf	Ørskov et al. (1986)
4 dairy cows	14.4	625	34.0	^{15}N-Leu dilution	Ouellet et al. (2002)
4 dairy cows	17.6	607	40.0	^{15}N-Leu dilution	Ouellet et al. (2010)
Dairy cows	18.0	600	40.9	Meta-analysis	Marini et al. (2008)
Dairy cows	17.9	597	29.5	Meta-analysis	Sauvant et al. (2013)
3 steers	2.86	300	23.0	RDP-free diet	Hart et al. (1990)
2 steers	0 (2.2)	278	11.6	Rumen VFA inf	Ørskov et al. (1986)
4 steers	0 (1.8)	278	13.3	Rumen VFA inf	Ørskov et al. (1986)
4 steers	3.14	424	24.4	RDP-free diet	Hannah et al. (1991)
4 steers	3.37	372	26.2	RDP-free diet	Lintzenich et al. (1995)
Growing cattle	6.82	359	24.2	Meta-analysis	Marini et al. (2008)

[a] When VFA were infused, DMI = 0 and value in parentheses = kg of DM infused in the rumen.

[b] RDP = rumen degraded protein; VFA inf = volatile fatty acid infusion.

Microbial Protein Supply

Adequate RDP is needed to maximize MCP production, NDF digestibility, and DMI (Schwab and Broderick, 2017). Microbial protein is the major supplier of MP, but its prediction also is important for estimating NANMN and therefore requirements of RUP. If the MCP supply is predicted to decrease, then RUP supply would need to increase, and vice versa, to maintain MP supply. Calculations of microbial N flow and ruminal protein balance (calculated as RDP − MCP − endogenous CP) are not independent. The committee did not use apparent ruminal N balance as a variable to assess RDP requirements for microbes because, although relevant biologically, its quantification propagates nonrandom error (i.e., bias) to an unknown degree. Rather than using RDP derived from flow data, Galyean and Tedeschi (2014) assessed the effect of total CP to influence EMPS in beef studies. Few studies intentionally limit CP for dairy cattle, and often total CP is maintained while exchanging RDP for RUP. Decreasing RDP in dairy studies is associated with decreasing MCP (Ipharraguerre and Clark, 2014).

Microbial N was predicted from RDP and predicted rumen-degraded carbohydrate (starch plus NDF) using an asymptotic integrated form of the Michaelis–Menten equation (Thornley and France, 2007). Previously, this equation form was used for microbial growth (Russell et al., 1992). The following equation was derived using the same data set as used for RDP estimates and derivations for rumen-degraded starch (RDS; kg/d) and rumen-degraded NDF (RDNDF, kg/d) as in White et al. (2017b). The same studies from White et al. (2017b) were used in the current derivation of microbial N flow with the major difference being that RDP (kg/d) was modified to reflect the current k_d/k_p approach and was fit using a Bayesian approach (Moraes et al., 2018a).

$$\text{Microbial N (g/d)} = [\beta_0 + (\beta_1 \times \text{RDP})] / [(1 + \beta_2/\text{RDNDF}) \times (1 + \beta_3/\text{RDS})]$$

(Equation 6-3)

$$\text{Where RDNDF} = [-31.9 + (0.721 \times \text{NDF}) - (0.247 \times \text{St}) + (6.63 \times \text{CP}) - (0.211 \times \text{CP}^2) - (38.7 \times \text{ADF}/\text{NDF}) - (0.121 \times \text{ForWet}) + (1.51 \times \text{DMI}) \times ((\text{NDF}/100) \times \text{DMI})]/100$$

(Equation 6-4a)

$$\text{RStDig} = [(71.2 - (1.45 \times \text{DMI}) + (0.424 \times \text{fNDF}) + (1.39 \times \text{St}) - (0.0219 \times \text{St}^2) - (0.154 \times \text{ForWet})) \times (\text{St} / 100) \times \text{DMI}]/100$$

(Equation 6-4b)

ForWet is concentration of wet forage (greater than 20 percent DM) in the diet (percentage of DM), DMI is in kg/d and NDF, and starch (St), CP, acid detergent fiber (ADF), and forage NDF (fNDF) are a percentage of diet DM. The RDNDF prediction had a negative slope for dietary starch percentage, accounting for negative associative effects (White et al., 2016).

Predicted values should be multiplied by 6.25 to convert to CP. The numerator contains an intercept ($\beta_0 = 101 \pm 11$) and a coefficient for RDP intake ($\beta_1 = 82.6 \pm 4.2$), where RDP intake is kg/d. The denominator represents the main ruminally degraded carbohydrates, NDF ($\beta_2 = 0.094 \pm 0.028$) and starch ($\beta_3 = 0.027 \pm 0.010$), both of which are kg/d. The RMSE for the fit and root mean squared error of prediction from cross-validation were 29.7 and 29.8 percent of the mean (278 g/d), respectively. The CCC was 0.52 and 0.50 for the fit and cross-validation, respectively. Because Equation 6-3 has an intercept (i.e., 101), the maximum predicted MCP is equal to RDP supply. Research is needed to improve estimation of MCP for heifers and dry cows, which have much lower intakes of RDP than lactating cows.

To limit unrealistic estimated requirements for RDP, observed microbial N flow was fitted against predicted RDP (percent DM) and ruminal NDF and starch degradabilities (percentage of respective intakes using the generalized additive mixed model from the mcgv R package) (Wood et al., 2016). In the resulting smoothed response, microbial N flow was visualized to maximize at 12.0 percent RDP and to decline linearly as RDP decreased below 12.0, but the confidence interval widened, particularly as RDP declined below 10.0 percent. Because deficient RDP also depresses DMI (Firkins et al., 2006), which is the major driver of MCP production, the committee recommends a minimum of 10.0 percent RDP (derived with the current approach, which is generally higher than NRC, 2001) and no more than 12.0 percent RDP to optimize MCP supply.

A limited number of studies in dairy cows where diet RDP varied and RUP was held constant have been conducted. Kalscheur et al. (2006) reported a linear increase in milk protein yield and a curvilinear increase in DMI as diet RDP increased from 6.8 percent to 11.0 percent in diets containing 5.8 percent RUP. Substitution of urea N for 0, 1.2, 2.4, and 3.7 percent of dietary RDP resulted in a linear decrease in DMI, milk protein, and MCP in diets containing 5.6 percent RUP (Broderick and Reynal, 2009). Maximal DMI and milk protein yield occurred at 12.2 percent and 12.3 percent RDP, respectively, in diets that varied in 10.6 to 13.2 percent RDP (Reynal and Broderick, 2005). These results are consistent with the committee's general recommendations of 10 to a maximum of 12 percent RDP.

Equations 6-4a and 6-4b (see Figure 6-1) were deemed a more biological representation than the NRC (2001) approach to predict MCP, but there are some important caveats. The database is underrepresented with studies using small dairy breeds and with DMI greater than 30 kg/d. However, because the equations predicted percentage (not absolute) rumen degradabilities of NDF and starch (White et al., 2016), the predicted MCP flow should scale with DMI. From standard Michaelis–Menten form, the substrates for microbial protein synthesis in Equations 6-4a and 6-4b are rumen-degraded starch and NDF, implying an EMPS relationship (g microbial N/kg degraded NDF + starch) with maximal rates set by the RDP supply. Attempts to directly predict EMPS (g microbial
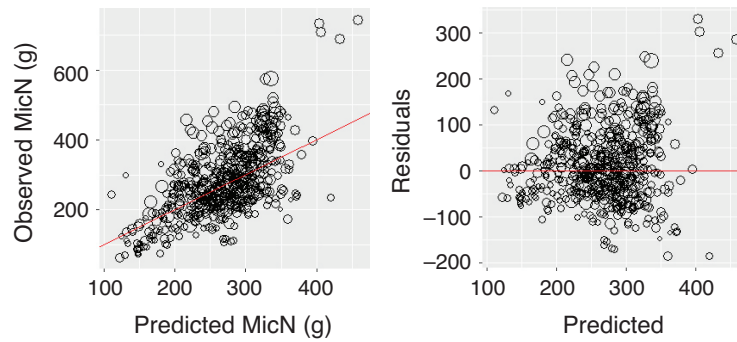
FIGURE 6-1   Observed, predicted, and residual (observed minus predicted) microbial N (MicN) flows (g/d). Symbols are sized according to 1/SE of the respective study. Number of treatment means = 580.

N/kg organic matter [OM] truly degraded in the rumen) from the source data had evidence for heteroscedasticity, and the model for ruminal OM digestibility had several coefficients that were $0.05 \leq P \leq 0.10$ (White et al., 2016). In addition, future refinements in rumen-degraded starch models (i.e., inclusion of adjustments for processing) lend themselves more directly to the equation than to equations predicting EMPS from OM degradation.

Bacteria can make AA from carbon skeletons derived from central metabolism, and the integration of metabolic pathways influences EMPS (Hackmann and Firkins, 2015b). Although some currently used models assume that preformed AAs only affect yield of amylolytic bacteria, this assumption ignores the complexity of the mixed community that includes fibrolytic and amylolytic bacteria (Firkins, 2010). NRC (2001) summarized reports for lactating cows fed diets with differing rumen-degraded starch and RDP, concluding that there was no consistent benefit in synchronization that could be distinguished from other potential explanations. Despite the clear indication of such synchrony from various in vitro studies, the current committee agrees with NRC (2001) in vivo–based conclusion. In theory, synchronization should be important and could be modeled in formulation software. However, synchrony of rumen-degraded carbohydrate and RDP is hard to distinguish in practice (Hall and Huntington, 2007). For example, total mixed rations allow disparate rates of RDP and carbohydrate degradation to cross over multiple meals.

Certainly, BUN contributes to microbial N assimilation, but a distinctive qualification must be made for high-producing dairy cattle compared with beef cattle and sheep. With low CP diets often fed to beef and sheep, ruminal ammonia is buffered by BUN transfer to the rumen, particularly when ruminal VFA concentration is increased as would happen with higher-concentrate diets (Lu et al., 2014; Patra and Aschenbach, 2018). Urea synthesized by the liver and transferred back to the gut is an important short-term reservoir of N for growing and lactating cattle resulting from intermittent eating patterns (Reynolds and Kristensen, 2008). However, the response became incrementally more important as dietary CP decreased below 15 percent. Moreover, most of

these studies were short-term experiments from which labile body protein reserves are more plentiful. Calsamiglia et al. (2010) concluded that "urea-N salvaging mechanism, despite its obvious theoretical benefit to ruminant N efficiency, is poorly utilized under common applied dietary conditions of dairy cattle." They concluded that increasing dietary CP percentage increased the urea excreted in urine rather than cycling to the gut. However, this is inconsistent with analyses of bovine studies utilizing double-labeled urea (meta-analysis by Li et al., 2019). In the latter work, urinary output and gut entry increased throughout the range of dietary CP intake, although the fractional proportion of urea released by the liver excreted in urine increased and that entering the gut decreased as dietary CP increased.

The committee recognizes the need for research to improve efficiency of N capture into milk protein; however, few data from lactating dairy cattle support relying on BUN to buffer against insufficient RDP intake. The microbial N derived from urinary urea-N (MNU; product/precursor ratio of enrichments of bacterial ^{15}N/urinary urea-^{15}N) was 21 percent of the urea-N recycled to the gastrointestinal tract when dietary CP was 15 percent and declined with increasing CP (Batista et al., 2017). Those authors argued that the urea-N used for anabolism computation should not be used as a representation of microbial assimilation of urea-N. A direct evaluation of MNU avoids all misrepresentations in ^{15}N-urea kinetics plus errors in postruminal flows of microbial N. In four studies with dairy cattle (see Figure 6-2), the MNU averaged 14.6 percent when dietary CP ranged between 12.5 and 18.6 percent. Use of the product/precursor ratio in this case may result in biased estimates due to label dilution in the ruminal ammonia pool, that is, urea is not the direct precursor for microbial protein synthesis (Li et al., 2019), suggesting BUN could be a significant source of microbial N in the rumen under conditions when RDP is limiting.

Amino-N probably enhances EMPS when degraded carbohydrate is available (Hackmann and Firkins, 2015b), as would be expected in lactating dairy cattle. Dietary urea replacement of amino-N decreased MCP in dairy cattle (Brito
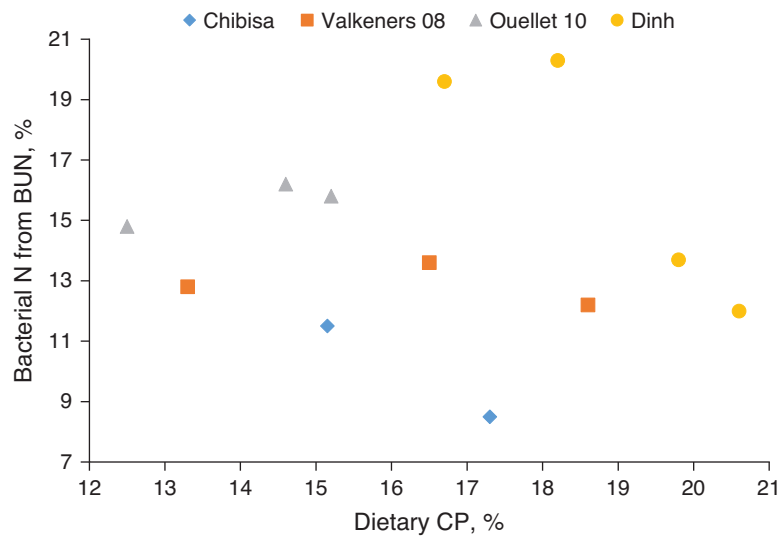
FIGURE 6-2   The product/precursor ratio of ^{15}N enrichment in ruminal bacteria divided by the ^{15}N enrichment in urinary urea-N (assumed in equilibrium with BUN) converted to a percentage and expressed against dietary CP percentage. Accounting for the random effect of study and excluding dietary CP >19.0 percent resulted in an average of 14.6 percent with a standard error of mean (SEM) = 2.1 percent. The plotted data from the studies (Dinh, 2007; Valkeners et al., 2008; Ouellet et al., 2010; Chibisa and Mutsvangwa, 2013) are unadjusted for the random effect of study.

et al., 2007; Broderick and Reynal, 2009). Hence, BUN recycling to the rumen was deemed to provide an important N source to buffer intermittent CP consumption, but these factors were considered when choosing not to include BUN transfer in the MCP model. Additionally, because the microbial equations were derived from in vivo observations, those factors were intrinsic to the derivation to the extent they can be captured in the carbohydrate and RDP terms. This contrasts with the approach taken by the beef NASEM (2016), which assumed 100 percent efficiency in converting RDP to MCP, implying that the balance of ammonia absorption from the rumen and BUN transfer into the rumen could compensate completely for RDP deficiencies.

Beet and citrus pulp (high in soluble fiber) and sugars are extensively degraded in the rumen. A moderate amount of beet pulp replacing high-moisture corn supported similar MCP production in dairy cattle (Voelker and Allen, 2003). Soluble fiber is highly degradable (Miron et al., 2002). The enzymatic assay reported as "starch" in nearly all of the source data used to derive the prediction equations included sugars. The responses to supplemental sugars have been mixed (Oba, 2011). Because of limited data, the equation might underpredict MCP when feeding high amounts of soluble fiber or sugars, but no evidence was available to make an adjustment.

Supply of MCP was not adjusted for supplemental fat for various reasons. One equation in the beef NASEM (2016) predicts rumen MCP supply as a function of fat-free TDN. Fat has not had consistent effects on MCP production (Oldick et al., 1999). Hanigan et al. (2013) observed a positive effect of dietary fat on microbial protein outflow using a data set with 227 treatment means. Schmidely et al. (2008) did not

observe an effect of fat on EMPS unless OM digestibility was depressed. In that case, EMPS is predicted to increase because the denominator decreased, not that the numerator (MCP outflow) increased. Those authors suggested that the improved EMPS from supplemental fat was from suppressed protozoa and their resultant predation of bacteria. Hanigan et al. (2013) suggested that uptake of preformed FAs could spare carbon for synthesis of other cellular components and for adenosine triphosphate (ATP) production by fermentation. However, FAs might be internalized as droplets rather than be directly used for membranes (Bauchart et al., 1990). The sparing effect was not well supported in one of the few studies that addressed bacterial growth directly (Maczulak et al., 1981). Improved efficiency of MCP synthesis from feeding fat has been attributed to k_p or other factors (Nagaraja et al., 1997). Moreover, high amounts of fat can be toxic to bacteria (Hackmann and Firkins, 2015a). Bacteria take up exogenous fatty acids to a lesser extent than do protozoa (Karnati et al., 2009). Thus, decreasing protozoal outflow from supplementing fats could increase usage of preformed FAs because of greater bacterial outflow resulting from less predation. Because of high DMI, bacterial predation should affect lactating dairy cows less than nonlactating ruminants (Firkins et al., 2007). For all of these reasons, the current committee chose to not account for fat.

Intestinal Digestibility of Rumen-Undegradable Protein

The current feed library has a much more robust inclusion of dRUP values compared with NRC (2001). The values are derived from a mix of in vitro and mobile bag studies. Proper analytical procedures must be followed to obtain accept-

able results (Liebe et al., 2018; see Chapter 18). Intestinal digestibility of RUP remains a critical research area, including standardization, prevention of analytical artifacts, and a better understanding of differential AA digestibility within RUP. Validation of all analytical methods against in vivo observations is essential to ensure they are representative across a broad range of feeds. An in vivo, isotope-based approach demonstrated the inaccuracy of the three-step procedures for assessing dRUP for several important ingredients and the extent of variation in digestibility of individual AA within the RUP fraction (Estes et al., 2018; Huang et al., 2019). That method is not adaptable to commercial settings, but it can be used to evaluate in vitro and in situ approaches.

Metabolizable Amino Acid Supply

Correction of Amino Acid Composition of Proteins Obtained from Hydrolysis

Because the committee moved toward a factorial AA approach, special attention was devoted to accurately defining the AA composition of each protein fraction. Correction factors for incomplete recovery of AA from a 24-hour acid hydrolysis have been published (Lapierre et al., 2019) and are detailed in Chapter 18. Those recovery factors were used to correct all reported AA composition obtained from protein hydrolysis and are thus intrinsic to all protein equations in the final model. The abbreviation AA_{corr} will be used when referring to AA concentrations corrected for incomplete recovery from 24-hour hydrolysis. Users of the derived equations must keep in mind that the equations were built from corrected AA flows (i.e., estimations of the flows of metabolizable AA, secretions, and accretions as well as efficiency calculations were all done using true corrected AA flows). However, predictions of duodenal AA flows were converted to uncorrected flows for presentation purposes and to allow direct comparisons with published data.

Metabolizable Amino Acids from Microbial Crude Protein

Assessing metabolizable AA supply from MCP in ruminal outflow requires knowing (1) the TP/CP ratio of MCP, (2) the AA composition of microbial TP, and (3) the digestibility of microbial TP. Assumptions of 80 percent TP in MCP and 80 percent digestibility of TP in the small intestine (NRC, 2001) have been used since NRC (1985). Despite the importance of these constants in predicting metabolizable AA supply, few studies have evaluated them, particularly for dairy cattle (Patton et al., 2014). To evaluate the TP/CP ratio, the recovery of total AA after hydrolysis of bacteria was investigated. Formalin used as a preservative for bacteria decreased the recovery of some AA and should be avoided (Volden et al., 1999); therefore, data from these studies have been excluded. Accounting for the incomplete recovery of AA after a 24-hour hydrolysis, hydration upon hydrolysis (1 g of TP

would yield approximately 1.15 g of free AA), and that Gln and Asn are transformed into Glu and Asp, respectively, TP/CP averaged 82.4 percent in a broad survey of published AA composition of fluid- and particle-associated bacteria (Sok et al., 2017). The remainder of the CP that is not TP is mostly nucleic acids. The current edition assumes this proportion, 17.6 percent, which is slightly lower than the 20 percent used in NRC (2001). Bacterial RNA-N/N plus DNA-N/N is typically less than 15 percent (McAllan, 1982), which is consistent with other measurements (Czerkawski, 1976) or theoretical calculations (Hespell and Bryant, 1979). RNA-N ranged from 9 to 13 percent of total N in bacteria (Susmel et al., 1993). In another survey, the total nucleic acid–N (including DNA) ranged from 11 to 24 percent of total N (mean of 14 percent) in bacteria and was lower in protozoa (Fujihara and Shem, 2011). Glucosamine is ignored in these studies but comprises about 2 percent of polysaccharide-free bacterial cells (Czerkawski, 1976); it is 7.8 percent N (about half of the 16 percent N in CP), so it should contribute about 1 percent CP. Increasing ruminal k_p and therefore growth rate of bacteria could increase the nucleic acid–N contribution to total CP (Bach et al., 2005), although the nucleic acid–N/N ratio is probably less variable in the ruminal bacteria of dairy cows (Firkins et al., 2006). Taking all this into consideration, the committee modified 80 percent TP to 82.4 percent TP in MCP. Undegraded nucleic acids in RUP should be negligible (Calsamiglia et al., 1996).

The AA composition differs between fluid- and particle-associated bacteria and protozoa, but insufficient data were available to determine whether AA profile of microbes is affected by feeding condition (Sok et al., 2017). For the model, a constant contribution of fluid- and particle-associated bacteria and protozoa to ruminal outflow was derived (33.4, 50.1, and 16.5 percent, respectively). After weighting for these proportions and accounting for differential recoveries of AA after hydrolysis, a AA_{corr} composition of the TP of microbial protein was derived (see the section "Secretion and Accretion") and used with the factorial method to assess the contribution of MCP to metabolizable AA flow. The updated AA profiles reflect more current measurements and expectations but still retain limitations associated with dietary influence on bacteria and protozoa AA composition.

The inclusion of the protozoa contribution to MCP (16.5 percent) becomes important because protozoa contain much more Lys than bacteria (Jensen et al., 2006), with values up to twice as high (Reynal et al., 2003; Fessenden et al., 2017). Because of the potential for bacterial contamination of protozoal samples (Sylvester et al., 2005), the difference in Lys might even be greater. The high Lys concentration in protozoal protein is likely due to diaminopimelic acid metabolism (Williams and Coleman, 1992; Martin et al., 1996). Merchen and Titgemeyer (1992) did not note a difference in supply of Lys resulting from defaunation of growing ruminants. The greater Lys concentration of protozoa can make a

significant difference if protozoal outflow is enhanced by faster k_p (Jouany, 1996), as expected for dairy versus beef cattle. Unfortunately, protozoal Lys outflow has rarely been studied in dairy cattle. The Ile concentration was also higher for protozoa, whereas Met, Thr, and Val concentrations were lower than in bacteria; particulate-phase bacteria have increased Arg and Phe but decreased Thr compared with fluid-phase bacteria (Sok et al., 2017).

NRC (2001) assumed that the TP in MCP reaching the duodenum was 80 percent digestible. Results using isotopes or intragastric infusion of bacterial protein suggest digestibility greater than 80 percent, and the digestibility of both fungal and protozoal protein is probably higher than that of bacterial protein (Jouany, 1996). Tas et al. (1981) estimated true digestibility of microbial protein at 87 percent, compared to 81 percent from intragastric infusion (Storm et al., 1983). Apparent absorption of nucleic acid–free bacterial ^{15}N in steers was 74 percent (Salter and Smith, 1984), which was higher than apparent absorption of nonammonia ^{15}N in growing steers (Firkins et al., 1987). In that study, ^{15}N absorption between duodenum and ileum was similar to total N (including feed N). Low intakes by these nonlactating ruminants make large differences between apparent and true digestibility; for example, from 69 (apparent) to 86 (true) percent of N entering the duodenum (Tas et al., 1981). Fonseca et al. (2014) reported that fluid- and particle-associated bacteria had standardized digestibilities of 76.8 and 75.5 percent, respectively, for total AA in cecectomized roosters. These results are near the average of 76 percent from their literature search.

Limited data on intestinal digestibility of MCP in dairy cattle are available. The estimated mean digestibility of bacterial AA-N in dairy cattle ranged from 75 to 77 percent (Larsen et al., 2001). Those authors derived bacterial N and AA from duodenal and ileal samples for this calculation, although the digestibility could be underestimated because of bacterial cells produced in the ileum. The digestibility of AA in bacterial cell walls (especially diaminopimelic acid) is lower than the rest of the TP-AA, particularly Gram-positive bacteria. Thus, EAA digestibilities were all higher than the mean for digestibility of total AA-N. There is insufficient support to have differential digestibilities for individual AA in microbial protein. In fluid-associated bacteria, His and Met had higher digestibilities than total AA, and Val was lower; in particle-associated bacteria, Arg was higher, and Val was lower in cecectomized roosters (Fonseca et al., 2014). Based on this limited information, the committee extended the constant 80 percent true digestibility for all microbial EAA. Consequently, the conversion of MCP to MP is assumed to be 82.4 percent TP in CP at 80 percent digestibility = 65.9 percent (slightly higher than 64 percent in NRC, 2001).

Metabolizable Amino Acids from Rumen-Undegradable Protein

White et al. (2017a) evaluated the ruminal degradability and intestinal digestibility of EAA as affected by feed category. Only 10 published studies (53 treatment means) reported ruminal AA disappearance in situ (at 12 or 16 hours) that met inclusion criteria. The committee deemed that a broader database would be needed before differential EAA ruminal degradabilities or intestinal digestibilities could be predicted with confidence. Therefore, the AA profile of the RUP fraction of feedstuffs is assumed to be the same as in the original feedstuff, which is consistent with NRC (2001), the French PDI (Rulquin et al., 1998), the Dutch DVE/OEB2010 system (van Duinkerken et al., 2011), and the Cornell Net Carbohydrate and Protein System (CNCPS) (Van Amburgh et al., 2015). Changes in AA profiles probably are greater for feeds with higher RDP (Boucher et al., 2009a). Ratios of AA profile after/before exposure to rumen fluid changed only modestly (Mupeta et al., 1997; Harstad and Prestløkken, 2001) or greatly, depending on the protein source (Cozzi et al., 1995; Paz et al., 2014). Met, Ile, Leu, and Phe increased and His and Lys decreased after incubation of extruded peas and soybeans (Walhain et al., 1992). No statistics were done, but EAA ratios depended on protein source (Piepenbrink and Schingoethe, 1998). In canola meal, Thr, the branched-chain AAs, and aromatic AA concentrations increased after incubation (Boila and Ingalls, 1992). Excessive heating can convert L- to D-racemers or crosslink AA, which decreases digestibility (Vrese et al., 2000), and intentional heating confirmed lower Lys intestinal digestibility for some feed sources (Boucher et al., 2009b).

The intestinal digestibility of AA within the RUP fraction was assumed to be the same as the CP digestibility for all AAs. Recent in vivo work suggests that assumption is likely incorrect (Estes et al., 2018; Huang et al., 2019). However, the literature data were inadequate to derive individual AA digestibilities for the range of ingredients used in dairy diets. Future research is needed to improve accuracy and consistency of predicting digestible RUP and AA. In addition, researchers should acknowledge the bias caused by underestimating AA recovery during AA hydrolysis, particularly Met, if not appropriately protected prior to acid hydrolysis (Higgs et al., 2015), and report values for Trp. To develop the models of this revised version, the AA composition of feed ingredients as reported in Table 19-2 was corrected to account for incomplete recovery using correction factors (Lapierre et al., 2019). Ruminal degradability and intestinal digestibility of total AA, not just individual AA, need to be reported so total AA can be used for standardization among studies (White et al., 2017a).

Amino Acid Composition of Postruminal Endogenous Protein

The AA composition assigned to the postruminal endogenous duodenal flow, which was removed from the observed flow to assess the net supply, was derived from Ørskov et al. (1986). The AA composition of rumen and abomasal fluids from cattle nourished with N-free intragastric infusion was

TABLE 6-2   AA Composition of the CP and TP Fractions Involved in the Estimation of AA Supply and Recommendations

| AA | g AA$_{corr}$/100 g CP | g AA$_{corr}$/100 g TP[a] | | | | g AA$_{calc}$/100 g TP[b] |
	Duodenal Endogenous	Microbial[c]	Scurf	Whole Empty Body	Metabolic Fecal	Milk
Ala	4.69	7.38	9.17	8.59	6.32	3.59
Arg	4.61	5.47	9.60	8.20	5.90	3.74
Asx	4.75	13.39	8.39	9.61	7.56	8.14
Cys	2.58	2.09	2.70	1.74	3.31	0.93
Glx	11.31	14.98	14.69	15.76	15.67	22.55
Gly	5.11	6.26	21.08	14.46	8.45	2.04
His	2.90	2.21	1.75	3.04	3.54	2.92
Ile	4.09	6.99	2.96	3.69	5.39	6.18
Leu	7.67	9.23	6.93	8.27	9.19	10.56
Lys	6.23	9.44	5.64	7.90	7.61	8.82
Met	1.26	2.63	1.40	2.37	1.73	3.03
Phe	3.98	6.30	3.61	4.41	5.28	5.26
Pro	4.64	4.27	12.35	9.80	8.43	10.33
Ser	5.24	5.40	6.45	5.73	7.72	6.71
Thr	5.18	6.23	4.01	4.84	7.36	4.62
Trp	1.29	1.37	0.73	1.05	1.79	1.65
Tyr	3.62	5.94	2.62	3.08	4.65	5.83
Val	5.29	6.88	4.66	5.15	7.01	6.90

[a] g AA$_{corr}$: AA composition corrected to account for the incomplete recovery of AA with 24-hour hydrolyses, expressed in hydrated form, and therefore sum to more than 100 for a given protein. Table modified from Lapierre et al. (2020).

[b] g AA$_{calc}$: AA composition calculated from the primary structure of the reference protein of each family, expressed in hydrated form, and therefore sum to more than 100.

[c] Adapted from Sok et al. (2017) using the correction factors proposed by Lapierre et al. (2019).

averaged; for Leu, only the rumen value was retained. The AA composition of endogenous protein contributing to the duodenal flow, once corrected for incomplete recovery with 24-hour hydrolysis, is presented in Table 6-2.

POSTABSORPTIVE USE OF METABOLIZABLE PROTEIN AND AMINO ACID

In NRC (2001), MP requirements included metabolic fecal, endogenous urinary, scurf, growth, gestation, and lactation. Those functions were retained in this version, although the approaches to estimate use of MP and AA changed. The greatest amount of available data was for milk protein yield, which allowed development of more comprehensive models for that function.

Estimation of Milk Protein Yield

NRC (2001) predicted the MP required to support a specific level of milk and milk protein yield (MPY). Additionally, a conceptual framework for the effects of individual AA on MPY was provided, and recommendations for Met and Lys were suggested. Shifting from an MP system to one based on AA implies that it is more accurate at predicting MPY. Therefore, the performance of existing MP-based equations was used as a benchmark. Data for all work on milk protein were collected from the literature by several teams (NRC, 2001; Hanigan et al., 2002; Bateman et al., 2008; Roman-Garcia et al., 2016) and additional data col-

lected from studies describing the effects of infused AA and AA fed in rumen-protected (RP) form. The complete data set contained 1,149 treatment means from 275 experiments. Of these, 898 treatments from 216 studies reporting milk production and milk protein content or output were used.

Evaluation of the National Research Council 2001 Model, Other Metabolizable Protein-Based Models, and the First Limiting AA Concept

The overall relationship between MP supply and MPY is quadratic, reflecting the decreased marginal return in MPY as protein supply increases (Hanigan et al., 1998; Huhtanen and Hristov, 2009; Lapierre et al., 2012a). However, in the NRC (2001) model as in many other models, MPY was linearly related to MP supply. The NRC (2001) MP system was evaluated using revised ingredient composition data (see Chapter 19) and revised energy supply equations (see Chapter 3). Estimation of MP allowable MPY by the 2001 model had an RMSE of 24.9 percent with significant slope bias. Overall, the NRC (2001) model underpredicted MPY by 28 g/d and overpredicted responses to changing MP supply, indicating that the partial efficiency of MP use (fixed at 0.67) in response to increased MP supply was too high. Net energy allowable MPY had an RMSE of 21.3 percent with significant slope bias. The model overpredicted MPY by 32 g/d on average ($P < 0.01$) and overpredicted the response to varying net energy for lactation (NEL), indicating the partial efficiency of NEL use may be overpredicted. Protein

TABLE 6-3   Residuals Analyses for NRC (2001)–Based Predictions of Milk Protein Production as Compared to Predictions from the Revised Model[a]

| | NRC (2001)[b] | | | |
	NEL	MP	Minimum of NEL and MP	Equation 6-6
N	922	926	922	926
Observed mean, g/d	922	921	922	921
Predicted mean, g/d	953	890	831	924
CCC	0.77	0.65	0.71	0.75
RMSE, g/d	194	228	210	133
RMSE, percent mean	21.0	24.9	22.8	14.4
Mean bias, percent MSE	3	2	19	0.0
Slope bias, percent MSE	38	32	21	3.1
Mean bias, g/d	−31	31	91	−2.7
Slope bias, g/g	−0.38	−0.44	−0.34	0.156
$P_{Mean\ Bias}$	0.0001	0.0002	0.0001	0.54
$P_{Slope\ Bias}$	0.0001	0.0001	0.0001	0.0001

[a] Predictions were not adjusted for random study effects.
[b] Predicted milk protein (g/d) = allowable milk (kg/d) × observed milk protein (%) × 10.

synthesis is an integrated process responding to both net energy and protein supply. Historically, the combination has been considered using a first limiting nutrient concept, which was modeled as the minimum of MPY available from MP or from NEL. Predictions based on that concept resulted in an RMSE of 22.9 percent with both mean and slope bias (see Table 6-3). Those biases and errors indicated the 2001 NRC models required revision.

Predicting Milk Protein from Essential Amino Acids and Energy Supply

Initial regression work indicated that digested energy intake (DEI) and AA supply were strong determinants of MPY. DEI was used to represent the effects of energy on milk protein synthesis as MEI cannot be calculated until the amount of catabolized protein is determined. Given that DEI includes DE from MP, there is an inherent correlation among the inputs that was addressed by removal of the DE associated with MP, resulting in a nonprotein DEI (DEInp). Additionally, this approach resolves the issue of MP having a greater DE value than carbohydrate, which can lead to optimizers based on DE (but not ME or NEL) to use MP rather than carbohydrates for energy. Thus, DEInp was used for the following work. Substitution of DE concentration for DEI or DEInp resulted in much poorer predictions.

Because several EAA and energy-yielding substrates regulate rates of protein synthesis (Dos et al., 2004; Gan et al., 2011; Appuhamy et al., 2014) and all of the EAA are substrates for protein synthesis, one may expect several of these to be important drivers of production. This is a potential challenge as many of the dietary nutrients are correlated, in particular among the EAAs. Met supply had the lowest correlations with other EAAs, ranging from 0.74 to 0.85 due to the

large number of studies that utilized independent additions of Met through infusions and feeding RP-Met. Hanigan et al. (2000) demonstrated that representations of milk protein synthesis and milk yield as a function of the most limiting nutrient (an EAA or energy) were inadequate. An additive representation with independent effects of three or more EAAs and energy supply resulted in significantly better fits to the data and very large increases in variation explained by the models. This concept is well supported at the tissue level, where independent protein synthesis and cell signaling responses to at least Met, Leu, Ile, Thr, insulin, and acetate concentrations have been observed (Appuhamy et al., 2012, 2014; Arriola Apelo et al., 2014c), and responses in MPY have been verified (Schwab et al., 1976; Yoder et al., 2020). The relative supplies of each AA also interact to regulate mammary (Bequette et al., 2000; Hanigan et al., 2000) and liver AA transport activity (Myers et al., 2017), resulting in variable efficiency of transfer from the gut to the mammary cells, which explains the diminishing returns response to MP supply (Whitelaw et al., 1986; Lapierre et al., 2007a).

The large number of factors controlling synthesis results in a complicated response surface and hampers experimental and modeling progress. Because it is almost impossible to isolate and control concentrations of all of the factors, future experimentation must report measurements of all controlling factors and include treatments that independently manipulate those factors to ensure that derived responses are truly representative of each factor. Additionally, experiments using multilevel treatments are needed to better define the response surfaces that can be expected to be nonlinear. There is a critical need for larger central composite designs to assess and quantify potential interactions among the factors. In the absence of those types of experiments, the surfaces must be derived across experiments, which is subject to much greater

variation and covariation as many factors are not controlled or fully reported across experiments. Such work should include genomic characterization of the animals so that variation among animals can eventually be at least partially described based on genetic potential.

Given the biological underpinnings and despite the challenges with data sufficiency, the committee was able to develop a multiple regression equation to predict MPY using an "all-models" approach (Burnham and Anderson, 2002) to evaluate all possible combinations of the absorbed supply of individual EAA supply; DEInp; digestible starch (dSt), digestible NDF (dNDF), and digestible FAs (dFAs); and BW and parity. Dietary macronutrients other than these were initially screened and found to be unrelated to MPY. Three different global equation forms were evaluated. The first utilized metabolizable EAA and DEInp as the primary driving variables:

$$\text{Milk Protein, g/d} = \text{Arg} + \text{His} + \text{Ile} + \text{Leu} + \text{Lys} + \text{Met} + \text{Phe}$$
$$+ \text{Thr} + \text{Trp} + \text{Val} + \text{NEAA} + \sum(\text{EAA}^2)$$
$$+ \text{DEInp} + \text{dFA} + \text{dNDF} + \text{BW} + \text{Parity}$$
$$+ \text{PubID} \qquad \text{(Equation 6-5)}$$

where DEInp (Mcal/d) represented DE intake minus the energy contribution from MP assuming 5.65 kcal/g of MP; the individual AAs represented metabolizable supplies (g/d) of each; NEAA (g/d) represented the absorbed supply of NEAA; the $\sum(\text{EAA}^2)$ term represented the sum of squared individual EAA supplies; dFA and dNDF are expressed as percentage of dietary DM; BW represented the reported BW (kg); Parity was a continuous variable ranging from 0 to 1 based on the reported number of animals in each parity, with 0 and 1 representing first parity and multiparous animals, respectively; and PubID represented the random effect of study. The second and third equations contained the same terms as the first equation, but the individual EAAs were expressed as a percentage of the total EAA or as a ratio to DEInp, respectively.

The top 5,000 models based on the Akaike information criterion (AIC) were extracted from each set of solutions for further consideration and summarization. The equations based on EAA as a ratio to DEInp proved statistically inferior and were abandoned. The model using AA, represented as a ratio to EAA, had very similar performance; however, the distinct disadvantage of this form is the inconsistency in responses to a given EAA caused by the division of each EAA by the total EAA. With this form, the addition of one EAA to the diet results in a greater denominator, resulting in a reduction in the calculated ratios for all other EAA. This results in nonadditive responses to individual AAs when they are added to the total versus substituted for another AA, which is not biologically consistent with the known mechanisms (Arriola Apelo et al., 2014b,c,d). Thus, equations expressing AA as a ratio to MP or total EAA supply were excluded from

further consideration, and all efforts focused on the general form represented by Equation 6-5.

Many of the top equations derived from Equation 6-5 performed equally well, and thus the choice of an equation for use was partially dependent on the objectives, how well the equation of choice matched the biological underpinnings, and the stability of the derived coefficients and model predictions. Of the EAAs, Trp and Val were each present in half of the top 5,000 equations, but the slope estimates were generally negative and deemed nonbiological, and neither performed well in cross-evaluations having unstable solution estimates (slope estimates varied by more than 100 percent using a bootstrap approach with resampling; Simon, 2007). Phe and Thr were also present in 48 percent and 47 percent of the solutions, respectively, but did not perform well in cross-evaluations. Arg was present in 68 percent of the top solutions and was stable on cross-evaluation but was deemed suspect due to its conditional essentiality. The NEAA term was also present in 68 percent of the top solutions and was stable on cross-evaluations, indicating some apparent effects of total N supply. Parity and dFA were also present in about half of the solutions but unstable during cross-evaluations. The remaining terms were present in more than 70 percent of the models (excepting Ile and Leu at 59 percent and 57 percent, respectively), and all were stable during cross-evaluations. Based on these statistical and biological arguments, models containing individual linear terms for Arg, Phe, Thr, Trp, Val, dFA, and Parity were not considered. Filtering to remove equations containing those terms and conducting further testing of the combination of the excluded EAA and the NEAA (OthAA) resulted in the following solution that was selected for use in the model:

$$\text{Milk Protein} (\text{g/d}) = -97.0 + 1.68 \times \text{His} + 0.885 \times \text{Ile} + 0.466$$
$$\times \text{Leu} + 1.15 \times \text{Lys} + 1.84 \times \text{Met} + 0.077$$
$$\times \text{OthAA} - 0.00215 \times \sum_{i=1}^{N_{\text{EAA}}} \text{EAAb}_a^2 + 10.8$$
$$\times \text{DEInp} - 4.60 \times (\text{dNDF} - 17.06) - 0.420$$
$$\times (\text{BW} - 612) \qquad \text{(Equation 6-6)}$$

where the individual EAA terms are expressed as g absorbed/d, DEInp is Mcal/d, dNDF is percent of DM, BW is in kg, and EAAb^2 represents the squared supply of each of the EAAs present in the equation (His, Ile, Leu, Lys, and Met) denoted by the subscript a. OthAA represents the absorbed supply of the NEAA plus Arg, Phe, Thr, Trp, and Val. dNDF and BW are centered to the mean of the data for use in the equation, and thus their mean values (17.06 percent and 612 kg, respectively) were included as subtractions in the equation. The selected model also reflects refitting after the squared EAA term was reduced to a summation of the squares of His, Ile, Leu, Lys, and Met. All terms in Equation 6-6 were stable under cross-evaluation, and the equation had
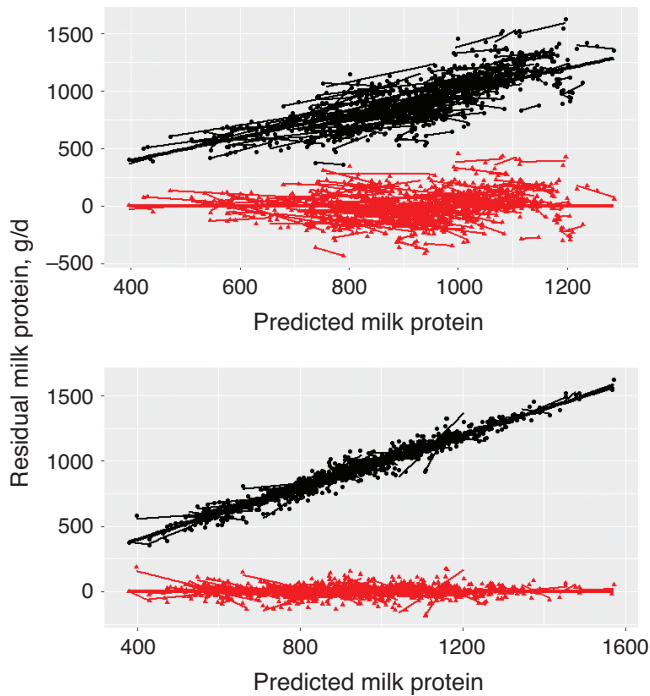
FIGURE 6-3   Residual errors for predictions of true milk protein by Equation 6-6. Data in the top panel were not corrected for the random effects of study, and data in the bottom panel were corrected for those effects. Data colored black represent observed versus predicted, and the data colored red represent residuals versus predicted. The lines connecting points (i.e., splines) are the linear regressions within study.

stable RMSE and CCC estimates across the evaluations. Protein yield predictions from this equation represent the predicted milk NP supported by a given diet (NP-milk).

The RMSE for Equation 6-6 was 14.4 percent with 0 percent mean bias and 3.1 percent of the MSE as slope bias, and the CCC was 0.75 and represents a substantial improvement in fit over the NRC (2001) equation (see Table 6-3). The residual errors for the predictions are displayed in Figure 6-3.

Correlations among parameter estimates should be low if the data are adequate to uniquely define those parameters, and this was the case for all of the linear coefficients in Equation 6-6 with the exception of a correlation of 0.67 between the coefficients for Leu and Lys, −0.51 for OthAA and Ile coefficients, and −0.43 for Lys and His coefficients. The remainder were all below 0.32 in absolute terms.

Residual errors for MPY were plotted and regressed against the supplies of each individual metabolizable EAA and of total metabolizable EAA to appraise performance relative to each of the primary inputs. If the effects of these inputs are appropriately captured in the prediction equations, there should be no observable slope or mean deviation in the residual errors. Conversely, if the residuals are correlated with an input that is represented in the prediction equations,

that is an indication that the representation is inadequate. If the residuals are correlated with inputs not represented in the prediction equations, that is an indication that those inputs should be added to the scheme. As expected, no evidence of biased responses to any of the EAA supplies was observed, indicating that the effects of those inputs are appropriately captured in the model. The residual errors were centered on the line of unity, and there were no systematic deviations from that line throughout the range of metabolizable AA supplies. The responses to individual EAAs were further evaluated in a similar manner using only studies that utilized infused protein or AA sources. The results were the same as for evaluations using all of the data. When studies using RP-AA were isolated in the same manner, the responses were also predicted without bias. Thus, the committee concluded that the responses to individual EAAs were unbiased.

Residual errors from Equation 6-6 were also plotted and regressed against other dietary and animal descriptors (see Figure 6-4). As above, if the descriptor was represented in the model, then the effects should be captured and there should be no pattern to the residuals. If the effect was not in the model, there should also be no pattern to the residuals if the exclusion of that effect was warranted. If there is a pattern to the residuals, this suggests the factor does have an effect and thus should be added to the model. The committee observed no clear pattern to the residuals versus EAA divided by DEInp, indicating that the independent effects of these two terms were properly captured in the model. No obvious patterns to the residuals were observed across or within studies when plotted against dietary concentrations of dFA, dNDF, or dSt, supporting the selection of a model that included dNDF but excluded dFA and dSt. The residuals were not significantly related to any other variables except for days in milk (DIM) (−0.79 g/d per DIM) and milk fat content (−0.96 g/d per milk fat percent unit). The DIM effect appeared to be associated with three studies in which DIM changed by a few days within study and thus was deemed an unreliable estimate. The committee felt the addition of milk fat may be problematic for high milk fat breeds, which were very poorly represented in the data used for model derivation. The potential effects of DIM and milk fat content should be examined further in the future.

Although the revised equation cannot be directly compared to the NRC (2001) model as the latter only provided predictions of milk production, those predictions can be converted to milk protein predictions by multiplying the predicted milk production times the reported milk protein concentration for each treatment in the meta-data.

Because the EAA coefficients of Equation 6-6 represent a reference set of parameters reflecting a group of animals producing on average 920 g of milk protein per day, additional work was undertaken to address the limitation of using a quadratic equation with a fixed maximum. Indeed, many herds today have pens of cows that produce more milk protein than can be achieved at the apex predicted by Equation 6-6. The
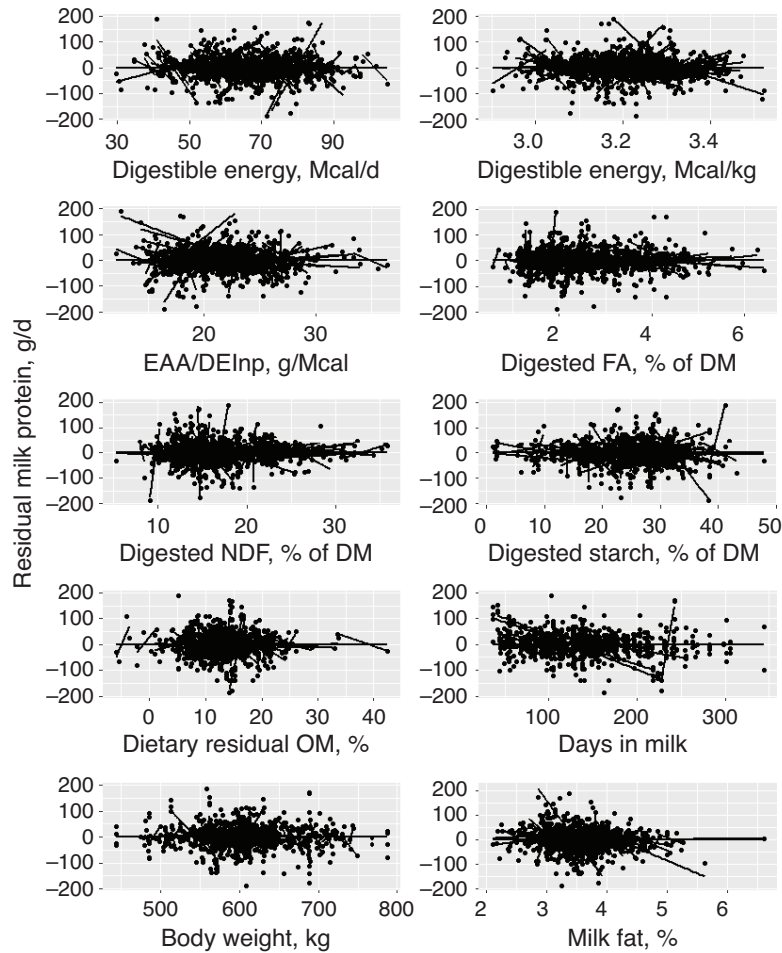
FIGURE 6-4   Residual errors from Equation 6-6 corrected for study effects versus various diet and animal factors.

form of the equation remained the same, but a set of algebraic equations was devised to allow scaling of the linear and quadratic coefficients for the EAA based on rolling herd average for milk protein (see Chapter 20).

Metabolizable Protein and Amino Acid Recommendations

The challenge in moving from a linear representation of the relationship between MP or AA supply and MPY to a multifactorial, nonlinear function with additive responses to independent variables is that one cannot define a set requirement for any of the driving variables. Indeed, an infinite combination of inputs would achieve a common level of production, and this prevents identification of a single set of requirements. Because of all of the possible combinations of EAA yielding a given level of production that can vary without bounds according to the above model, establishing reasonable biological bounds is critical. These bounds or suggested guidelines are provided for both MP and EAA based on net EAA use calculated in a factorial manner and respective target efficiencies. The factorial approach first requires the identification

and quantification of the functions that create a direct net demand on the EAA supply, either being protein secretion or protein accretion, often referred to as NP requirement. These functions are directly using EAAs that are removed, on a net basis, from the available pool of EAAs and therefore must be replenished by at least an equivalent amount on a timely basis. The second step is to assign an efficiency of utilization of the MP or EAA supply to support these different functions. The recommendations are calculated as the NP requirement divided by an efficiency. Estimation of target efficiency for MP and each EAA is detailed below.

Although the committee developed an equation to predict MPY, described in the section above, in parallel, the committee also defined MP and EAA recommendations to serve as general guidelines when MPY is entered rather than predicted, because an equation with multiple independent variables could still hide EAA imbalance. For example, a diet providing extremely low Lys but high Leu supplies (as in corn-based diets) could still predict high, but unachievable, MPY due to EAA imbalance. The first part of this section will describe the NP secretion and accretion. In the second

part, the basis of the calculation for the target efficiencies of utilization of MP and EAA is presented.

Secretion and Accretion

The quantification of protein secretion and accretion, the basis for the estimation of the MP and EAA recommendation, is detailed below. To be consistent among the different functions, each function is quantified as TP (as opposed to CP), and the secretion or accretion of AA is calculated as TP secretion or accretion multiplied by its respective AA composition. Furthermore, secretion and accretion represent NP or net AA demand; hence, those values need to be divided by efficiency to obtain total MP (or metabolizable AA) requirement. Rather than using the word "maintenance" to refer to requirements related to scurf, endogenous urinary loss, and metabolic fecal protein (MFP), reference will be made to nonproductive functions (INRA, 2018) in this edition. Indeed, MFP represents the largest contribution to so-called maintenance, but its magnitude is driven by losses associated with the high DMI of the lactating dairy cow and, as such, cannot truly be considered a maintenance requirement necessary to sustain cow basal metabolic function. Once the net TP or AA export or accretion is determined for each function, recommendations are calculated as the sum of export and accretion, each divided by its respectively assigned (endogenous urinary loss and gestation) or target (scurf + MFP + MPY + growth + body reserves) efficiencies.

Scurf

NRC (2001) based the estimation of scurf requirements on Swanson (1977). After examination of more than 2,400 individual calorimetry observations on cattle collected in the U.S. Department of Agriculture-Beltsville from the mid-1960s to the mid-1990s, the equation for scurf protein from Swanson (1977) has been retained in the current revision, but the committee adjusted the equation from a CP basis to a TP basis:

$$\text{NP-scurf (g/d)} = 0.20 \times BW^{0.60} \times 0.85 = 0.17 \times BW^{0.60}$$
$$\text{(Equation 6-7a)}$$

where 0.85 represents the TP/CP ratio of scurf, based on its AA composition and total N content.

To assess the net AA demand for scurf secretion, NP-scurf is multiplied by its AA composition. The AA composition of scurf was estimated using the head, hide, feet, and tail composition reported by Williams (1978) and van Amburgh et al. (2015). The mean of these studies, corrected to account for incomplete recovery of AA with 24-hour hydrolysis (Lapierre et al., 2019), is reported on a TP basis in Table 6-2.

$$\text{NetAA-scurf (g/d)} = \text{NP-scurf} \times [AA_{\text{corr-Scurf}}] / 100$$
$$\text{(Equation 6-7b)}$$

where $[AA_{\text{corr-Scurf}}]$ is in g AA/100 g TP.

Endogenous Urinary Excretion

The estimate of endogenous urinary NP requirement used by most models is 2.75 g CP/kg $BW^{0.50}$ based on Swanson (1977). However, urinary N excretion is derived from the catabolism of N metabolites and is not a protein secretion per se as for the other nonproductive functions. To better define the AA composition of this secretion, a literature review was conducted to quantify the composition of endogenous urinary-N (Lapierre et al., 2020). Briefly, the major N-metabolites in urine contributing to endogenous urinary losses are endogenous urea, endogenous purine derivatives (PD), creatinine and creatine, hippuric acid, and 3-methyl-His. From this review, daily excretion (per kg of BW) of endogenous urea has been quantified as 10 mg N, creatinine excretion as 9.46 mg N (25.5 mg creatinine), and creatine excretion estimated as 0.37 that of creatinine (i.e., 3.5 mg N). Daily urinary excretion of endogenous PD was estimated to average 27.1 mg N/$BW^{0.75}$. Urinary excretion of His (mg/d) as 3-methyl-His equals $7.82 + 0.55 \times BW$. Using a database from Spek et al. (2013), the remainder of the "measured" endogenous urinary-N excretion, representing 46 percent of this fraction, was assumed to be hippuric acid, formed in the liver to detoxify benzoic acid originating from rumen fermentation of dietary phenolic compounds. Although hippuric acid cannot be purely defined as "endogenous," it has probably been included in previous estimates of endogenous urinary excretion. Beside hippuric acid, most of the estimations of urinary excretion were based on BW, and therefore the endogenous urinary-N excretion was expressed relative to BW and averaged 53 mg N/kg BW. Using a different approach, the INRA (2018) estimated a daily endogenous urinary-N loss averaging 50 mg N/kg BW. Compounds constituting endogenous urinary-N excretion are not protein per se but N-metabolites that have AA as their origin; therefore, the standard N to CP conversion is assumed, meaning that the TP/CP ratio was set as 1.0.

$$\text{NP-endogenous urinary (g/d)} = 53 \times 6.25 \times BW \times 0.001$$
$$\text{(Equation 6-8a)}$$

The initial reason to revisit the endogenous urinary excretion was to identify which AAs were upstream of urinary endogenous losses. After examination of the synthesis process of the N-compounds detailed above, only the endogenous urea and 3-methyl-His excretions create a direct demand on EAA if Arg is considered as a conditionally essential AA. Endogenous PD are synthesized from Asp, Gln, and Gly; creatine and creatinine from Arg and Gly; and hippuric acid from Gly. Therefore, for all EAAs except His, endogenous urinary urea excretion (0.010 g N/BW per day) is used to estimate the AA endogenous urinary loss, assuming that this loss has the AA composition of the whole empty body reviewed in Lapierre et al. (2020; see Table 6-2). Therefore, for all EAAs except His:

NetAA-endogenous urinary (g/d) $= 0.010$
$\times 6.25 \times BW \times [AA_{corr\text{-}WholeEmptyBody}] / 100$

(Equation 6-8b)

where BW is in kg, and $[AA_{corr\text{-}WholeEmptyBody}]$ is in g AA / 100 g TP.

For His, the loss of urinary 3-methyl His must be added:

NetHis-endogenous urinary (g/d) His, g/d $= (0.010$
$\times 6.25 \times BW \times [His_{corr\text{-}WholeEmptyBody}] / 100) + (7.82$
$+ 0.55 \times BW) / 1,000$ (Equation 6-8c)

where $[His_{corr\text{-}WholeEmptyBody}]$ is in g His/100 g TP.

Metabolic Fecal Protein

The MFP secretion should represent the endogenous proteins secreted or sloughed in the gut lumen and not digested in the small intestine. They create a net demand on the digestible flow of AA: indeed, except for Phe, more than 80 percent of the EAAs used by the gut are derived from circulating (i.e., from previously absorbed) AA (MacRae et al., 1997), whereas the remainder would be from intestinal supply but is still derived from estimated metabolizable AA. NRC (2001) estimated MFP from DMI. However, rumen microbial synthesis from urea does not create a demand on metabolizable AA and should not be included in MFP. Accordingly, because this MFP value consists of "bacteria and bacterial debris synthesized in the cecum and large intestine, keratinized cells, and a host of other compounds" (Swanson, 1982), NRC (2001) corrected the initial calculation for the amount of undigested ruminal bacterial CP appearing in the feces of dairy cattle, and MFP (g MP/d) was estimated as [(DMI (kg) $\times 30$) $- 0.50$((bacterial MP/0.80) $-$ bacterial MP)]. This estimate was used as is for MP requirements, with no conversion of CP secretion to TP and no efficiency factor assigned to MFP excretion.

Using an isotopic dilution approach, Ouellet et al. (2002, 2010) developed a model to estimate MFP, allowing quantification of undigested endogenous proteins. Also, in a meta-analysis using 65 growing-finishing cattle studies (291 treatment means) and 43 dairy cow studies (164 treatment means), Marini et al. (2008) developed an equation regressing intake of digestible CP on CP intake. The equation had an intercept of 30 g CP/kg DMI, which represented MFP plus undigested bacteria synthesized from urea. This equation, including the NDF concentration of the rations, was adapted to fit the measured fecal MFP originating only from undigested endogenous proteins and adjusted to represent the ileal endogenous flow observed by Ouellet et al. (2002, 2007, 2010). In addition, endogenous secretions occurring across the hindgut were included based on observations in sheep (Sandek et al., 2001) and using the ileal flow of small intestinal endogenous protein secretion of 5.1 g CP/kg DMI

(Ouellet et al., 2007). However, due to the scarcity of data on the exact origin of this N, half of this input was assumed to originate from endogenous proteins and the other half from urea. Therefore, the daily loss of CP as MFP was estimated from Lapierre et al. (2020) as

CP-MFP (g/d) $= (11.62 + 0.134 \times NDF_{\%DM}) \times DMI$

(Equation 6-9a)

Note that the term MFP is kept, although the forestomach and small intestinal loss was truly measured at the ileum. Assuming 73 percent of TP in MFP, based on its AA composition and total N content,

NP-MFP (g/d) $= CP\text{-}MFP \times 0.73$

(Equation 6-9b)

The AA composition of the MFP is based on the AA composition of ruminal and abomasal isolates from Ørskov et al. (1986), except for Leu, for which only the rumen isolates were used, and the endogenous flow at the ileum in pigs (Jansman et al., 2002). The committee assumed that 70 percent of the MFP is from undigested duodenal flow and the remaining 30 percent from the intestine (Ouellet et al., 2002, 2010). The averaged composition is detailed in Table 6-2. Therefore, individual AA secretion in MFP would be

NetAA-MFP (g/d) $= NP\text{-}MFP \times [AA_{corr\text{-}MFP}] / 100$

(Equation 6-9c)

where $[AA_{corr\text{-}MFP}]$ is in g AA/100 g TP.

Milk

Milk TP secretion is the easiest export protein to measure. The factor to convert the N concentration into CP in milk should be 6.34, related to the AA composition of milk (Karman and van Boekel, 1986), rather than 6.38. As for the other secreted proteins, it is expressed as TP. If the TP/CP ratio is not known, the NPN content of the milk CP is assumed to be 4.9 percent (DePeters and Cant, 1992), and a fixed ratio of TP/CP of 0.951 will be used by the model.

Because early studies reported less than a 3 percent difference in the EAA composition of milk protein produced from cows fed solely NPN sources and milk protein from control cows (Syväoja and Virtanen, 1965) and no effect of forage/grain ratio on the AA composition of milk (Featherston et al., 1964), the AA composition of milk protein has been assumed to be constant. This may prove to be untrue under more severe dietary manipulations such as experienced during AA deletion studies. However, additional data are required to further test that hypothesis. Milk AA composition has therefore been calculated based on the primary structure of the reference protein of each family, as detailed by

Farrell et al. (2004) using the approach of Swaisgood (1995). Milk TP fractions reported in 15 manuscripts published between 1980 and 2012 (Lapierre et al., 2020) averaged 82.4 percent casein (percentage of total protein: 35.2 percent αs1-casein, 7.6 percent αs2-casein, 30.9 percent β-casein, and 8.7 percent κ-casein) and 17.6 percent whey (percentage of total protein: 3.7 percent α-lactalbumin, 10.5 percent β-lactoglobulin, 1.04 percent albumin, 1.64 percent IgG1, 0.18 percent IgG2, 0.04 percent IgA, 0.33 percent IgM, and 0.21 percent lactoferrin). The AA composition of milk TP calculated using this procedure is presented in Table 6-2. With this approach, there is no need to correct for incomplete recovery from 24-hour hydrolyses.

$$\text{NetAA-Milk (g/d)} = \text{NP-Milk (g/d)} \times [\text{AA}_{\text{calc-Milk}}] / 100$$
$$\text{(Equation 6-10)}$$

where NP-milk = milk TP yield and $[\text{AA}_{\text{calc-Milk}}]$ is in g AA/100 g TP.

Pregnancy

Knowledge of the rates of nutrient accretion in conceptus tissues (fetus, placenta, fetal fluids, and uterus) is limited for dairy cattle. Pregnancy requirements were calculated as described for energy in Chapter 3. The size of the gravid uterus at a given gestation day was estimated based on calf birth weight (see Chapter 3). From that function, the daily gain in mass (kg/d) of the gravid uterus ($\text{Gain}_{\text{GrUter}}$) was calculated (see Chapter 3), and that gain was assumed to contain 125 g of protein/kg of wet weight (Bell et al., 1995).

$$\text{NP-Gestation (g/d)} = \text{Gain}_{\text{GrUter}} \times 125$$
$$\text{(Equation 6-11a)}$$

Use of a constant fraction of protein for gravid uterine gain based on that derived at parturition will introduce some bias at time points prior to parturition, but the errors are likely small and will cancel out by parturition. The model includes involution of the uterus postpartum. The rate of involution of uterine tissue postpartum and the fate of the AA from the involuting tissue are unknown. Because of the lack of data, the committee assumed complete involution required 4 weeks (see Chapter 3), and related AA would contribute to NP supply.

The AA composition of protein accretion associated with pregnancy is based on the AA composition of the whole empty body (see Table 6-2) because direct data are unavailable. Individual AA accretion for pregnancy is

$$\text{NetAA-Gestation (g/d)} = \text{NP-gestation}$$
$$\times [\text{AA}_{\text{corr-WholeEmptyBody}}] / 100$$
$$\text{(Equation 6-11b)}$$

where $[\text{AA}_{\text{corr-WholeEmptyBody}}]$ is in g AA/100 g TP.

Based on the equations above and assuming typical DMI of dry cows, diet DM would need to contain 829 g of MP (9.6 percent CP) at 60 days prepartum and 956 g of MP (13.5 percent CP) at 5 days prepartum, if the efficiency of converting MP to NP-Gestation is 0.33 as used by NRC (2001) and 60 percent of dietary CP is retained in MP. Data are very limited on AA metabolism in gestating, nonlactating dairy cows. In cows at this physiological status, liver removal of group 1 AA (His, Met, Phe + Tyr, and Trp) relative to net portal absorption is approximately twice as large as the ratio observed in lactating dairy cows (Wray-Cahen et al., 1997; Larsen et al., 2015). Although it is not clear if high liver removal of group 1 AA prepartum is related to excess protein feeding or difference in physiological status, data are not sufficient to change the efficiency used in NRC (2001).

Using milk yield in the subsequent lactation as the response variable, dry cows' diets with as little as 11 percent CP appear adequate (see Chapter 12). However, few individual studies included dry cows fed diets with <10 percent CP. Low-protein diets may reduce DMI and fiber digestion, resulting in less NEL than predicted. Furthermore, based on a meta-analysis, diets with at least 14.5 percent CP yielded positive responses when fed to late-gestation nulliparous animals (see Chapter 12). To provide adequate RDP (~10 percent of DM, discussed above) for DMI and digestibility, dry cows' diets would need to contain about 12 percent CP (greater concentrations would likely benefit late-gestation heifers), and the committee recommends maintaining that concentration of CP. Another justification for recommending higher concentrations of CP is because the protein required to produce colostrum is not included in requirement calculations. Holstein cows may secrete more than 1 kg of protein in first milking colostrum. Although colostrum synthesis only occurs over a few days, it still represents a significant demand for AA. Estimated MP requirements for gestation by a dry cow producing a calf with a birth weight of 44 kg (see Table 3-3, Chapter 3) are about 25 percent less at 60 days prepartum and 37 percent greater at 5 days prepartum compared to NRC (2001).

Growth

Target frame growth rates for an average Holstein cow (mature birth weight of 700 kg) during first and second lactations are 0.19 and 0.15 kg/d, respectively, assuming that postpartum BW at first and second calvings is 82 percent and 92 percent of mature birth weight. For Jersey cows (with mature birth weight of 520 kg), targets are 0.14 and 0.11 kg/d for first and second lactations. These target rates assume that frame growth occurs consistently over the lactation, regardless of changes in intake, milk production, and body condition. The protein concentration of empty gain is a function of BW relative to mature weight, and empty BW gain associated with frame growth is considered 85 percent of live BW gain in cows. Using equations from Chapter 11 for cows at 82 percent and 92 percent of mature BW, the protein content of BW gain

associated with frame growth would be 11.2 and 10.6 percent for first- and second-lactation cows. Because values are so similar, growth during lactation was considered to contain 11.0 percent protein. Therefore, during lactation:

$$\text{NP-growth (g/d)} = \text{Frame weight gain (g/d)} \times 0.11 \times 0.86$$
$$\text{(Equation 6-12a)}$$

Additional details are in Chapter 11. However, if the change in BW is not frame growth but rather a change in body reserves, the protein content is assumed to be 8.0 percent protein. In the text, NP-growth is the sum of the NP for frame growth plus NP for body reserves.

The default values for frame gain during lactation can be altered by users. The 0.86 is the ratio of TP/CP derived from the AA_{corr} composition of the CP. The AA composition of growth is based on the AA composition of the whole empty body (see Table 6-2). Individual AA accretion for growth is

$$\text{NetAA-growth (g/d)} = \text{NP-growth}$$
$$\times [\text{AA}_{\text{corr-WholeEmptyBody}}] / 100$$
$$\text{(Equation 6-12b)}$$

where $[\text{AA}_{\text{corr-WholeEmptyBody}}]$ is in g AA/100 g TP.

Under most conditions, the amount of TP and AA required for growth during lactation will be extremely low (at target rates of growth, TP will equal 13 to 18 g/d).

Efficiency of Utilization of Metabolizable Protein and Amino Acids

The approach used to estimate MPY acknowledges that the efficiency of utilization of MP or AA is variable. European models (e.g., NorFor, 2011; Van Duinkerken et al., 2011; INRA, 2018) have adopted a variable efficiency of utilization of MP. Because the efficiency of utilization of MP and AA is variable, the concept of a "single," fixed MP or AA requirement is no longer tenable. One must consider the problem from a marginal return basis where the system is optimized when the marginal return from the last unit of input nutrient becomes 0. However, only response function for milk protein could be derived; therefore, the overall NP response to nutrient inputs is not reflected in the model system. Despite this limitation, if one assumes that nonproductive NP requirements are met before milk protein output is maximized, a target efficiency for use of AA and MP within the model system can likely be identified as an approximation of the point where the marginal return becomes 0.

The use of a combined efficiency for use of AA and MP for the secretion functions of scurf, MFP, and milk has been suggested (Lapierre et al., 2007a). The efficiency of use for gestation is not known, and different efficiencies (e.g., 0.50, 0.33, and 0.65) have been proposed by different committees (NRC, 1989, 2001; NASEM, 2016) with little or no supporting data. Because of lack of data, the committee retained the

efficiency (0.33) used by the previous committee (NRC, 2001) for nonlactating animals. The efficiency of AA for growth is also lower (0.40; NASEM, 2016). However, the NP needed to support growth and gestation by lactating cows is very small relative to other NP uses. For simplicity, growth was assumed to have the same efficiency as other functions for lactating cows but not for growing heifers or dry cows. An efficiency of 1.0 was used for endogenous urinary losses, because, contrary to the other secretions that are proteins, these losses are end products of N-metabolite metabolism, as in INRA (2018). For lactating cows that are not pregnant, efficiency of utilization of MP (Eff_MP) and of individual AA (Eff_AA) for the other functions is assumed variable and is calculated as

$$\text{Eff\_MP} = (\text{NP-scurf} + \text{NP-MFP} + \text{NP-milk}$$
$$+ \text{NP-growth}) / (\text{MP supply} - \text{NP-endogenous urinary})$$
$$\text{(Equation 6-13a)}$$

and

$$\text{Eff\_AA} = (\text{NetAA-scurf} + \text{NetAA-MFP}$$
$$+ \text{NetAA-Milk} + \text{Net AA growth})$$
$$/ (\text{metabolizable AA} - \text{NetAA-endogenous urinary})$$
$$\text{(Equation 6-13b)}$$

Using a combined efficiency for scurf, MFP, and lactation, and 100 percent efficiency for endogenous urinary resulted in improved predictions of the efficiency of MP use compared to fixed efficiencies for the nonproductive functions and a variable efficiency solely for MPY (Sauvant et al., 2015). The CNCPS–Version 6.5 (van Amburgh et al., 2015) also opted for a combined efficiency of utilization of AA, which included scurf, MFP, lactation, and endogenous urinary loss.

The efficiency of utilization of MP and individual AAs was calculated as detailed above for each treatment included in the database described in the section "Estimation of MPY." The committee then, a priori, removed some studies for uncertainties regarding the true availability of AA supply (e.g., dietary Met analogs) and studies involving intravenous infusions to end up with a final database of 921 treatment means. As expected, the calculated efficiencies were highly variable (see Table 6-4).

Target Efficiencies of Utilization of Metabolizable Protein and Amino Acids

Although the efficiency of utilization of MP and AA to support protein export plus accretion is variable, guidelines (not requirements) for adequate supplies of MP and individual EAAs are presented, based on the assumption that energy requirements are met. Target efficiencies were estimated as follows. First, studies were coded to look specifically at the increment of MP supply. Because sufficient details were not included to estimate protein accretion, an average growth of 0.19 kg/d (equivalent to 18 g TP/d as $0.19 \times 0.11 \times 0.86$, where

TABLE 6-4   Proposed Target Efficiencies of Converting MP and EAA to Export Proteins and Body Gain and the Associated Descriptive Statistics for the Database

MP or AA	Target	Mean	SD	Minimum	Maximum
MP	0.69	0.66	0.102	0.35	1.00
His	0.75	0.78	0.141	0.34	1.21
Ile	0.71	0.61	0.088	0.36	0.93
Leu	0.73	0.67	0.120	0.24	1.01
Lys	0.72	0.67	0.106	0.35	1.05
Met	0.73	0.71	0.120	0.36	1.13
Phe	0.60	0.54	0.087	0.23	0.82
Thr	0.64	0.58	0.078	0.35	0.86
Trp	0.86	0.77	0.125	0.42	1.20
Val	0.74	0.66	0.099	0.37	1.01

0.11 = protein/empty BW gain and 0.86 = empty BW/BW) was assumed for first-lactation Holstein cows and 0.14 kg/d (13 g TP/d) for first-lactation Jersey cows, as established in the previous section, whereas gestation requirements were set to 0. Equations relating the sum of export plus accretion of NP or individual NetAA to their respective efficiency of utilization (dependent variable) were developed using the rma.mv function from the metafor package in R (Viechtbauer, 2010; R Core Team, 2013, version 3.4.1, 2017-06-30). The hierarchy of the studies was considered, and data were weighted by √n. Unbiased estimates of fixed effects and valid estimates of SE were obtained using the robust function in the metafor package. The linear and quadratic terms were significant ($P < 0.02$) for MP and all EAAs. The target efficiencies (i.e., the efficiency when the sum of NP or EAA export plus accretion was maximal) were obtained using the first derivative of these equations relating the sum of export plus accreted NP or individual EAA to the efficiency of utilization of MP or individual EAA. Combining these results with careful examination of efficiencies observed in deletion studies, the target efficiency of Phe was increased from 0.57 to 0.60 to better reflect observations reported when only Phe supply was modified. These target efficiencies, as well as statistics of observed efficiencies, are in Table 6-4. The target efficiency of Phe and Thr may be slightly underestimated, but due to the lack of data, an underestimate of efficiency was preferred to a resultant underestimate of recommended supply.

Target efficiencies can be used to assess diets and identify AAs that may be limited or in excess. Efficiencies greater than the target efficiencies are an indication that for those EAAs, supply may be short and negatively impact MPY. On the other hand, EAAs with efficiencies less than the target are more likely to be in excess, which may not be harmful to the animal (unless there is a severe imbalance) but has economic and environmental costs. Under certain situations, efficiencies greater than target can be achieved without necessarily adversely affecting MPY, as evidenced by the number of observations above the target values. Unfortunately, despite their crucial biological roles, involvement of EAAs in functions like loss or regain of body tissue, reproduction, and

immune response is less well described in the literature than the functions used in the current version to estimate the efficiency of EAAs, especially when it comes to quantification; hence, they could not be included in the current calculations. However, even if not accounted in the current estimations of the efficiencies, a ranking could be done and the EAA with the largest difference between the observed and the target efficiencies would be the EAA with the shortest supply. So even though the true efficiency of each EAA might not be exactly right, the ranking among EAAs might be a useful indication of their relative supply. Also, the current framework based on biological functions could be adapted as more knowledge is gained on these different functions, and they could be included in the equations of estimations of the efficiency of utilization of EAAs. Additional work is required to more clearly identify the potential optimal efficiencies that could be achieved with rations balanced for all of the EAAs as well as defining confidence intervals surrounding these efficiencies.

Recommendations of Metabolizable Protein and Essential Amino Acids

After characterization of the daily NP and NetAA secretion or accretion and defining target efficiencies, recommendations for adequate MP and AA supply can be determined by dividing NP or NetAA by the target efficiencies. Efficiencies of using MP for growth (approximately 0.40) and gestation (0.33) are substantially lower than the efficiency for other uses. In lactating cows, growth is a very minor use of MP, and because of the assumption needed, when deriving recommendations for MP and EAA, it was given the same target efficiency as milk. The NP accreted for gestation in late-lactation, lower milk-yielding cows can be substantial; hence, its efficiency was kept at 0.33. The NP supply equations discussed above are used to estimate NetAA requirements for the various functions and thus are based on protein content calculated from AA composition rather than CP (i.e., nitrogen × 6.25). The base data for those equations, however, were CP; therefore, MP requirements (but not AA requirements) are calculated as

TABLE 6-5   Example of Adequate EAA Supplies for a Mature Nonpregnant Cow (650 kg BW) Consuming 26 kg/d of a Diet with 34 Percent NDF with Graded MPY, Based on Target Efficiencies in Table 6-4

MPY, g/d	g/d									
	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	MP
1,000	55	112	187	159	50	117	110	26	125	2,122
1,200	63	130	216	183	58	134	124	30	144	2,411
1,400	71	147	245	208	67	152	139	34	162	2,701
	% MP									
1,000	2.60	5.29	8.83	7.48	2.35	5.51	5.19	1.23	5.89	—
1,200	2.61	5.37	8.97	7.60	2.42	5.57	5.16	1.24	5.96	—
1,400	2.62	5.44	9.08	7.69	2.46	5.63	5.14	1.25	6.01	—

$$\text{Recommended MP supply} = [(\text{NP-scurf} + \text{NP-MFP} + \text{NP-milk} + \text{NP-growth}) / \text{Target\_Eff\_MP}] + (\text{NP-gestation} / 0.33) + \text{NP-endogenous urinary}$$
(Equation 6-14a)

$$\text{Recommendation for individual EAA digestible flow} = [(\text{NetAA-scurf} + \text{NetAA-MFP} + \text{NetAA-Milk} + \text{NetAA-growth}) / \text{Target\_Eff\_AA}] + (\text{NetAA-gestation} / 0.33) + \text{AA-endogenous urinary}$$
(Equation 6-14b)

For late-gestation cows and heifers, equations are

$$\text{Recommendation for MP supply} = (\text{NP-scurf} + \text{NP-MFP}) / \text{Target\_Eff\_MP} + (\text{NP-gestation} / 0.33) + (\text{NP-growth} / 0.40) + \text{NP-endogenous urinary}$$
(Equation 6-14c)

$$\text{Recommendation for individual EAA digestible flow} = [(\text{NetAA-scurf} + \text{NetAA-MFP}) / \text{Target\_Eff\_AA}] + (\text{NetAA-gestation} / 0.33) + (\text{NetAA-growth} / 0.40) + \text{AA-endogenous urinary}$$
(Equation 6-14d)

Emphasis should be placed on meeting the EAA rather than MP recommendations. Indeed, in studies where EAA but not MP recommendations were met, cows fed low MP diets and infused with mixtures of EAA (meeting EAA but below MP recommendation) had similar MPY than cows receiving an infusion of EAA + NEAA (at EAA and MP recommendations) (e.g., Schwab et al., 1976; Metcalf et al., 1996; Doepel and Lapierre, 2010). Recommendations for MP are given mainly for general comparison and are, on average, lower than the NRC (2001) recommendations. The current recommendations do not include any requirement for endogenous duodenal flow, as it is not included in the supply. Also, in the current edition: (1) the relative proportion of EAA to MP is slightly higher than in the previous edition because the digestible flows of AA are corrected for incom-plete recovery of AA in 24-hour hydrolyses and because MP recommendations are lower than in the previous edition, and (2) a recommendation for a single ratio of AA to MP is not given because this ratio changes as the proportion of the net output of milk increases relative to total output (see Table 6-5). However, when calculated in g/d for an average cow, the recommendations of Lys and Met are in a similar range compared with the estimations obtained with the proportional approach in NRC (2001).

MEETING THE RECOMMENDATIONS FOR METABOLIZABLE PROTEIN AND AMINO ACIDS

Balancing for Metabolizable Protein

The relationship between MPY and protein supply is better when supply was expressed as MP compared with CP, with a slight improvement of the relationship when the quadratic term of MP was included (e.g., Huhtanen and Hristov, 2009; Lapierre et al., 2009). Because MP supply is the sum of MP from MCP and from RUP, economics have generally favored achieving maximum or near-maximum MCP because of the relatively low cost of RDP and the good AA profile of MCP. Santos et al. (1998) published a comprehensive review of the effects of replacing soybean meal with various sources of RUP on protein metabolism and production, and in 76 percent of the metabolism studies, higher RUP (i.e., lower RDP) decreased MCP flow to the small intestine. Similarly, Ipharraguerre and Clark (2005) reported that the mean milk production responses to replacing soybean meal with RUP supplements varied from −2.5 to +2.75 percent. In addition, inadequate RDP supply can reduce digestibility and energy supply (Lee et al., 2012b; Luo et al., 2018). Therefore, RDP and fermentable energy provisions should be complementary to support efficient MCP synthesis. Although balancing diets for MP can be used as a general guide, balancing for individual EAAs derived from the MP, assuming that RDP supply is sufficient to maintain an efficient rumen fermentation,

should allow achieving similar animal performance but at a lower total MP and CP supply. Improved balance or supply of certain metabolizable EAAs may also increase DMI (see later section).

Balancing for Individual Amino Acids

AAs are the nutrients used as building blocks for the synthesis of all proteins. Metabolizable AAs are also vital to support a multitude of metabolic pathways, but to a lesser quantitative extent. For example, Met contributes to multiple pathways, including provision of a methyl group for many transmethylation reactions (Manjarin et al., 2014). Also, AAs except Leu and Lys can serve as precursors for gluconeogenesis; all AAs can be converted to FAs or serve as immediate sources of energy when oxidized to CO_2 (e.g., Bequette et al., 1996; Lapierre et al., 2002). Whether these needs are fully met when needs for protein synthesis are met is not clear. Some of these reactions and molecules can influence longer-term animal health, and thus the effect of a deficiency may not manifest within the time frame used for many production studies. In addition, some AAs play an active role in intracellular signaling, especially involving the mechanistic target of mTOR (Arriola Apelo et al., 2014b). The effects of AA, energy supply, insulin, and other factors on these signaling pathways appear to explain why the concept of the barrel and stave with a single limiting AA does not fully explain observed lactational responses, whereas the additive model represented by Equation 6-6 captures more of the observed variation. Current knowledge, however, limits the estimation of the EAA recommendations to their effect on protein secretion and accretion and the associated efficiency of utilization. Further research is required to explore interactions among EAAs and energy (Arriola Apelo et al., 2014a). Defining recommendations based only on such productive functions, however, does not mean that the roles of AAs in functions other than direct incorporation into protein are not important but rather reflects the lack of knowledge on the adequate assessment of these roles and the quantification of these demands.

Recommendations for MP are mainly given as a general guideline and to maintain historical perspective. As pointed out by NRC (2001), diets that provide sufficient amounts of MP may still be deficient in one or more EAAs. Conversely, diets that are apparently deficient in MP may also be completely sufficient in EAAs, and thus the emphasis should be put on balancing for individual EAAs. Indeed, from experiments where the supply of a single AA or a group of AAs has been changed, total MP supply per se is not the best predictor of MPY. For example, deletion of His, Lys, or Met from a total AA mixture infused postruminally did not change total MP supply significantly but decreased MPY by 20 percent (Weekes et al., 2006). Deletion of His or Phe from an EAA mixture decreased MPY by 23 percent (Doelman et al., 2015), whereas deletion of Phe from a complete AA

mixture decreased MPY by 16 percent (Doepel et al., 2016) with a minimal impact on MP supply. Infusion of NEAA did not affect MPY, despite a significant increase in MP supply (Doepel and Lapierre, 2010). Admittedly, the variation of the proportion of NEAA to MP supply is often beyond the expected physiological range in infusion studies, but variations due to dietary alterations can also be substantial. For example, from the database used by Doepel et al. (2004), with digestive flows of EAA estimated with NRC (2001), the proportion of total EAA relative to MP supply in control treatments varied from 42 to 48 percent (mean 45.4 percent): so, a ration providing 3,000 g/d of MP could supply between 1,260 and 1,440 g of EAA. In Lee et al. (2012a) and Giallongo et al. (2016), inclusion of RP-His, -Lys, and -Met allowed MP to decrease by 450 g/d while maintaining MPY. In Haque et al. (2015), substituting EAA to maintain the EAA supply while decreasing MP supply by 375 g/d maintained MPY. Together, these results indicate that the supply of individual EAAs and not total MP drives MPY. These studies show that one AA per se cannot be declared "limiting." The limitation occurs when the supply is sufficiently low to affect protein synthesis, which can be encountered for all EAAs, as shown with deletion studies (e.g., Weekes et al., 2006; Doelman et al., 2015; Doepel and Lapierre, 2016) and as reflected in Equation 6-6.

Earlier work suggested that His might be limiting in grass silage–based diets (Vanhatalo et al., 1999; Korhonen et al., 2000; Huhtanen et al., 2002). However, diets fed in these studies were also low in CP (between 13.2 and 14.7 percent CP). Similarly, in corn silage–based diets with low protein concentration (14 percent CP), RP-Met plus RP-Lys were unsuccessful in restoring MPY to the level observed with adequate MP diets (Lee et al., 2012b,c). The addition of RP-His to RP-Met and RP-Lys to a deficient MP diet restored MPY to the level of the adequate MP diet. Inclusion of single AA as RP-His or RP-Lys or RP-Met to a deficient MP diet was insufficient to restore MPY to that of the adequate MP diet, but the combination of the three RP-AAs was effective in maintaining MPY similar to the adequate MP diet (Giallongo et al., 2016). The overall conclusions were as follows: (1) at low CP concentrations, the proportion of MP originating from microbial protein increases; (2) although MCP is considered to have a good AA profile, it is low in His (see Table 6-2) relative to the composition of proteins involved in the determination of requirements (milk, MFP, and endogenous urinary); and (3) when the proportion of MCP relative to total MP supply increases, His supply decreases more rapidly than the supply of the other AAs and may create a deficiency. Low-protein diets may have multiple EAA deficiencies, which will be apparent when metabolizable EAA supply is evaluated.

With sufficient knowledge, rations can be balanced for individual EAAs, and once the recommendation for each EAA is met, there should be no need to balance for MP. The modeling work undertaken herein and by others represents a significant step on that path. The relationships among His,

Lys, and Met and MPY are well defined by the literature, but other EAAs are not as well studied. Whether those EAAs are generally sufficient in most diets is uncertain, and the lack of clean comparisons presumably contributed to greater uncertainties associated with their coefficients in Equation 6-6. Clearly, all EAAs are important, and it is possible that some NEAAs are important (Luo et al., 2018). More research is needed to better characterize these additional AAs. The current work represents a first step and provides a framework that is analogous to the approach taken in swine and poultry nutrition, where diets are prepared with large proportions of individual EAA ingredients. This practice has even led to a shortage of total N for de novo synthesis of NEAA, which can be alleviated through supply of NPN in the diet (e.g., Mansilla et al., 2017). Such a shortage of NPN is less likely to happen in dairy rations because of the significant recycling of BUN to the rumen and the need for adequate RDP to support maximal microbial growth.

Amino Acid Metabolism

Essential Amino Acids

Essential AAs were initially classified into two functional groups, groups 1 and 2, based on their pattern of utilization by the mammary gland of dairy cows (Mepham, 1982): AAs from group 1 have a stoichiometric transfer of mammary uptake to milk protein, and group 2 AAs have a mammary uptake greater than secretion in milk protein. Further measurements of AA net flux across the portal-drained viscera (PDV), the liver, and the mammary gland confirmed these classes. This grouping does not mean that the EAAs in the same group share common metabolic pathways. Group 1 AAs, including His, Met, Phe+Tyr, and Trp are, on a net basis, substantially extracted by the liver, barely removed by peripheral tissues, and extracted by the mammary gland on a net basis nearly equal to that secreted in milk protein (Lapierre et al., 2012a). In contrast, group 2 AAs, including Ile, Leu, Lys, and Val, are catabolized by the PDV, barely removed by the liver, catabolized by peripheral tissues, and extracted by the mammary gland at a higher rate than secreted into milk protein. The excess uptake of group 2 AAs relative to milk protein output increases with increased AA supply. Although their metabolism pattern differs from the branched-chain AAs and Lys, Arg and Thr could also be categorized in group 2. Mammary uptake of Arg and perhaps Thr is larger than secretion in milk protein; it did not vary with their supply (Lapierre et al., 2012a). The excess mammary uptake of group 2 AAs, relative to milk supply, can be used for mammary synthesis of NEAAs. For example, the carbons from Leu were incorporated into Glu (Wohlt et al., 1977). The N of excess Leu in goat (Rubert-Aléman et al., 1999) and of Lys in cows (Lapierre et al., 2009) was transferred to many NEAAs, either used for milk protein synthesis or released into the mammary vein. Similarly, the large excess of Arg uptake

is used to support mainly Pro but also Ala, Asp, Glu, Gly, and Ser (Clark et al., 1975; Roets et al., 1979). Some AAs such as Leu and Lys can provide ketogenic products that are used for fat synthesis and ATP or NADPH production. A balance model summarizing these interchanges, including metabolism of nonnitrogenous compounds, was found to largely be in balance for carbon and nitrogen, suggesting that knowledge regarding inputs and outputs for the mammary gland is mostly complete (Hanigan et al., 2001).

Nonessential Amino Acids

The classification of AA as being EAA or NEAA originates from research with nonruminant animals (Hou et al., 2015). Research with ruminants, especially dairy cattle, is extremely limited but indicates the classification is similar to nonruminants (Black et al., 1952, 1955, 1957; Black and Kleiber, 1958). In these former studies, however, Arg was included in the NEAA group. NRC (2001) termed Arg as essential because even if Arg can be synthesized by animal tissues, it would be at rates insufficient to meet requirements, particularly for high levels of production. However, deletion of Arg from an AA mixture did not affect MPY (Doepel and Lapierre, 2011), but Arg was significant in the model selection process above. It was removed from the model due to the conditional-essentiality designation rather than for statistical reasons. Given its presence in the best models, further work to define its role in MPY is warranted.

Other studies supported the general distribution of AAs into EAAs and NEAAs in a more indirect way. Postruminal infusions of a mixture of the 9 EAAs plus Arg was sufficient to increase MPY, and addition of the NEAA to the infused mixture did not further increase MPY (Schwab et al., 1976; Metcalf et al., 1996; Doepel and Lapierre, 2010). These observations indicate that under normal feeding conditions, individual NEAAs absorbed in amounts less than required for metabolic need can be synthesized at a sufficient rate such that MPY is not affected. Although there is no evidence that NEAAs as a group can limit MPY when dairy cattle are fed conventional diets, research is too limited to totally rule out the potential importance of specific NEAAs. Indeed, regulation of the rate of protein synthesis may be dominant over direct substrate effects (Luo et al., 2018).

There might also be an impact of low NEAA supply because EAAs are required to synthesize several of the NEAAs. For example, in nonruminants such as swine and poultry, the EAAs, Met and Phe, are precursors of the NEAAs, Cys and Tyr, respectively. Jorgensen and Larson (1968) reported that liver and mammary tissue from cows was able to synthesize Tyr from Phe. In lactating goats, 5.3 percent of the net Phe taken up by mammary glands was converted to Tyr (Hanigan et al., 2009). In dairy cows, the conversion of Phe to Tyr within the mammary gland represented 7 percent of the Tyr secreted into milk protein (Lemosquet et al., 2010). If the digestive flows of Cys or Tyr are limited,

their de novo synthesis from their EAA-precursor might be insufficient. No clear studies involving dairy cattle have measured the extent that these two NEAAs, Cys and Tyr, can "spare" Met and Phe. Pruekvimolphan and Grummer (2001) concluded from an experiment with lactating dairy cows fed a Met-deficient diet that Cys in feather meal probably cannot substitute for Met in MP. In fact, Cys concentration is very low in milk protein, which is the largest net demand on metabolizable AA. Therefore, the ratio of Cys to Cys + Met is much lower in milk (0.23) compared with that in microbial protein (0.46) or in most of the feed ingredients, especially the plant proteins, usually averaging greater than 0.40 (see Table 19-2). Therefore, the likelihood that digestive flow of Cys would be low enough to decrease Met availability due to its de novo synthesis is low in lactating cows fed typical diets. However, the ratio of Tyr to Tyr + Phe in milk (0.53) is higher than in MCP (0.48) and in most feed ingredients (usually less than 0.45). Increased MPY has been observed when formaldehyde-treated canola meal was supplemented with dietary Tyr (Rae and Ingalls, 1984), but substantial amounts of Tyr were destroyed or rendered unavailable by formaldehyde treatment (Rae et al., 1983). The MPY response in the former study may have occurred because of deficiency in Tyr or in Phe if used to synthesize Tyr; however, because no differences were observed between treatments on Phe and Tyr plasma concentrations, determining which mechanism was responsible for the lower MPY is not possible. Deletion of Phe from a total AA mixture infused postruminally in cows fed a protein-deficient diet decreased MPY and plasma Phe concentrations but had no effect on plasma Tyr concentrations (Doepel et al., 2016), whereas when Phe was deleted from an EAA mixture, plasma Tyr concentrations decreased (Doelman et al., 2015). Overall, no clear evidence suggests that for lactating cows, Cys and Tyr are of concern. However, more research is needed to clearly determine whether under some circumstances, de novo synthesis of these two NEAAs would occur to an extent that would decrease the availability of their EAA-precursor and directly limit protein synthesis.

Two other NEAAs have received some attention. Pro and Gln (including its intermediate precursor Glu) are similar in that (1) concentrations of both are considerably higher in milk protein (10.3 and 22.5 percent of TP, respectively) than in MCP (4.3 and 15.0 percent of TP, respectively) or in most feedstuffs, (2) extraction by the lactating dairy mammary gland is considerably less than the quantities secreted in milk protein (Clark et al., 1978; Illg et al., 1987), and (3) both can be synthesized in the mammary gland from Arg, either through transfer of the C skeleton for Pro or through transamination of the excess N from Arg for Gln. Duodenal infusions of Pro were only effective in increasing MPY in mid-lactation cows but not in early lactation (Bruckental et al., 1991) and had no effect in another study (Alumot et al., 1983); however, mammary uptake of Arg decreased and milk fat concentration increased in all three studies. Gln has been hypothesized to limit milk protein synthesis in cows during early lactation (Meijer et al., 1995). The reasons for the hypothesis were low plasma Gln concentrations after calving and slower recovery than for the other AAs (Meijer et al., 1995; Doepel et al., 2002), as well as the multiple functions involving Gln, including the immune system, purine and pyrimidine synthesis, and as an energy source (Doepel et al., 2006), all of which are very demanding in early lactation. However, postruminal infusion of 300 g/d of Gln during the first 3 weeks postcalving had only a limited effect on milk production, metabolic parameters, and immune function (Doepel et al., 2006).

The above milk protein meta-analysis demonstrated that collectively the NEAAs plus Arg, Phe, Thr, Trp, and Val had a small but highly significant impact on milk protein synthesis (Equation 6-6). Consideration of only the NEAA was also significant, and thus the effect is not driven solely by Arg, Phe, Thr, Trp, and Val. Because this effect is small and not clearly demonstrated in controlled trials, the committee recommends that the focus for balancing AAs should be placed on the EAAs, excluding Arg.

Rumen-Protected Amino Acids

Because ingested free AAs are readily catabolized by the microbes in the rumen, AAs need to be fed in a protected form to avoid or limit degradation by rumen microbes; however, this protection should not significantly interfere with absorption from the intestine. Without direct AA supplementation, minimal concentrations of free AAs are present in rumen fluid (Lewis and Emery, 1962; Velle et al., 1997; Volden et al., 2001) because AAs arising from hydrolysis of RDP are rapidly used for microbial protein synthesis or deaminated (Lewis and Emery, 1962). Even with diets averaging 66 percent of the CP as RDP, the contribution of free AAs to the total duodenal flux of AAs was less than 2 percent (Volden et al., 2001).

When unprotected forms of Lys, Met, and Thr were given in amounts similar to those recommended for protected forms (9 to 20 g), apparent ruminal degradation over an 8-hour period averaged 88 and 90 percent (Velle et al., 1997; Volden et al., 1998). Ruminal escape of 10 to 12 percent would be unlikely to elicit a detectable response at such low feeding levels. However, at higher doses (48 to 120 g), ruminal degradation decreased to 73 to 78 percent (Velle et al., 1997; Volden et al., 1998), suggesting that the need for rumen protection could be eliminated with high oral doses of free AAs. Such high doses, however, can be costly and may have detrimental or toxic effects on the cow. For example, with the high dose of unprotected Met, it was noted that "after administration of Met at the 120 g dosage, an unpleasant odor emanated from the cows. Mucous membranes were discolored, and feed intake was transiently depressed" (Velle et al., 1997), suggesting that the animals experienced some level of sulfur toxicity.

In addition, rumen populations may adapt to these high levels and become more efficient at degrading the AAs over a longer period but may be overwhelmed in the short term.

Types of Protection

Because Lys and Met often limit MPY, considerable effort has been made to develop technologies that would allow them to escape ruminal degradation without compromising their absorption in the small intestine. The bioavailability of a RP-AA is the combination of its rumen escape and intestinal digestibility. At the time of this writing, only these two AAs are commercially available in RP form. For research projects, however, other RP-AAs have been produced and have been effective (e.g., His [Lee et al., 2012a]; Leu [Arriola Apelo et al., 2014a]; Ile and Val [Leal-Yepes et al., 2019]). Commercial products differ in the technology used to protect the AAs from ruminal degradation. Details are not given for specific commercial products, but briefly, the matrix used for encapsulation is a combination of pH-sensitive polymer and lipid, lipid, a combination of fiber and lipid, or calcium salts of long-chain FAs. The pH-sensitive coating depends on the differences in pH between the rumen (encapsulated at rumen pH) and abomasum (released at acid pH). Physical protection systems (e.g., lipid coating) must provide a reasonable degree of protection against ruminal degradation while providing a reasonable degree of intestinal release. Physical handling of the RP-AA prior to ingestion might alter the bioavailability as some coatings are susceptible to damage during feed manufacturing (e.g., pelleting) and diet preparation (Wu and Papas, 1997). Methods to assess bioavailability of RP-AA are detailed in Chapter 18.

D- and L-Isomers of an Amino Acid

The D- and L-isomers of an AA are chemically identical, but one is the mirror image of the other, with the amino group being on one side or the other of the carbon chain. Despite this small difference, mammals can only incorporate the L-isomer of AAs into proteins. Small amounts of D-AAs exist in bacterial cell walls and in free form in some plants. The AAs produced industrially in pure form by fermentation (e.g., Lys, Thr, and Trp) are L-isomers. In contrast, AAs produced from chemical synthesis (e.g., Met) are a DL-racemic mixture. Animals need to convert the D-form into the L-form before it can be utilized for protein synthesis. Such conversion in mammals involves D-AA oxidase. The D form of Met is deaminated to yield the keto acid, 2-oxo-4-methylthiobutanoate. This can then be reaminated to the L-form of Met (Friedman and Gumbmann, 1989). The efficiency of conversion of D-Met to L-Met is species dependent: rats, chicks, pigs, rabbits, and dogs all demonstrate conversion to L-Met when D-Met is administered by either oral or intravenous routes, although this is not the case with primates (Lewis and Baker, 1995). In growing cattle, abomasal infusions of D- and L-Met produced similar increases in N retention (Campbell et al., 1996), although it tended to be less with DL-Met infusion compared with an equimolar dose of L-Met (Titgemeyer and Merchen, 1990). In dairy cows, a minimum of 75 percent of a bolus dose D-[1-^{13}C]Met was transformed into L-[1-^{13}C]Met (Lapierre et al., 2012b). The behavior of the two isomers was, however, totally different: the half-life of D-Met was much greater than the half-life of L-Met (52 versus 8 minutes). The mammary gland did not extract any D-Met, and the fractional hepatic removal of D-Met was numerically lower than the fractional extraction of L-Met, leading to an accumulation of the D-isomer in plasma (Lapierre et al., 2012b). Therefore, L-Met synthesized from the D-isomer elsewhere in the body, not in the liver or the mammary gland, could be used to support milk protein synthesis. No detrimental effects of the D-isomer over the L-isomer of Met are evident. The longer half-life of D-Met could offer the opportunity to delay clearance of the absorbed Met and act as a potential reservoir for L-Met synthesis (Lapierre et al., 2012b).

Met Analogs

Feeding an analog of AA may be an alternative to coated or encapsulated forms of AA because it would not be affected by handling such as pelleting and may be less expensive. Many analogs have been tested (Schwab, 1995), but the most studied is an analog of Met, DL-2-hydroxy-4-methylthiobutanoate (HMTBA), often referred to as HMB. The analog HMTBA has long been proposed as a means to provide Met and increase milk and protein yields of dairy cows fed rations limited in Met (Polan et al., 1970). The Ca salt of HMTBA has also been studied as a supplement for increasing milk and milk fat production (e.g., Loerch and Oke, 1989). Liquid HMTBA is available and extensively used in the poultry and swine industry as a substitute for Met.

There is no consensus on the "Met bioavailability" of HMTBA (ruminal escape × intestinal absorption × conversion to Met), but HMTBA is more resistant to rumen microbial degradation than free Met (Belasco, 1972, 1980; Patterson and Kung, 1988). It can be absorbed across the ruminal and omasal epithelium (McCollum et al., 2000), and ruminants possess the enzymes to convert HMTBA to Met (Belasco, 1972, 1980). Blood concentrations of Met increased linearly to HMTBA dose (Feng et al., 2018). Estimations of postrumen availability of HMTBA vary greatly. In dairy cows, up to 50 percent (Koenig et al., 1999, 2002) of the ingested dose was reported to flow from the rumen. Also, in dairy cows, net portal absorption of HMTBA averaged 13 percent of the ingested dose (Lapierre et al., 2007b), similar to the 12.5 percent observed in sheep (Lobley et al., 2006). Total bioavailability needs to account for HMTBA conversion to Met and metabolism in the gut tissues (McCollum et al.,

2000), and such consideration resulted in an estimate of 18 percent of the dose ingested being available for postabsorptive use (Lobley et al., 2006).

The isopropyl ester of HMTBA (HMBi) has also been studied. Rumen escape is greater than HMTBA because it is rapidly absorbed across the rumen wall (Graulet et al., 2004). This would explain why a positive response in milk protein concentration has been observed with HMBi supplementation despite only 2.3 percent of the HMBi recovered as HMTBA in omasal digesta (Noftsger et al., 2005). Absorbed HMBi is converted to HMTBA after or during absorption and subsequently converted to the keto acid of Met and then transaminated to L-Met (Baker, 1994). The HMTBA supplement is a racemic mixture of D- and L-isomers that can both be converted to 2-oxo-4-methylthiobutanoate followed by amination to L-Met. The oxidase for L-HMTBA exists predominantly in the peroxisomes within liver and kidney, whereas the dehydrogenase for D-HMTBA is a mitochondrial enzyme found in most tissues (Dibner and Knight, 1984; McCollum et al., 2000; Dibner, 2003). In vitro (McCollum et al., 2000) and ovine data (Lobley et al., 2006) indicate that HMTBA conversion to L-Met occurs in many tissues. In dairy cattle, intravenous infused HMTBA provided 15 percent of the Met used for milk protein secretion, of which 85 percent was produced in other tissues and transported to the mammary gland through blood. The remaining 15 percent was synthesized directly in the mammary gland. The liver removed 38 percent of the infused dose of HMTBA, but that removed was not apparently converted to Met as net Met hepatic release declined (Lapierre et al., 2011). Altogether, this would explain why measuring Met plasma concentration is not an appropriate way of estimating HMTBA availability and its potential value in supplying Met.

Effect of Rumen-Protected Amino Acid Supplementation

Since the publication of the last NRC (2001), numerous studies have been published on effects of balancing dairy rations for individual AAs, especially Lys and Met, with most of them based on the proportional approach (expressing the supply of each AA as a percentage of MP). Based on a meta-analysis of data from experiments involving postruminal AA supplementation, Vyas and Erdman (2009) reported a response in MPY to AA supplementation with a marginal efficiency decreasing from 39 to 25 percent for Lys and from 44 to 22 percent for Met over the range of the predicted AA supply. A review by Robinson (2010) concluded that RP-Met increased milk energy yield and the efficiency of N utilization for milk (N milk/N intake), RP-Lys decreased DMI but increased the milk/DMI ratio, whereas the combination of RP-Met and RP-Lys increased milk and milk energy yield, milk protein percentage, the efficiency of N utilization, and milk/DMI. However, these improvements were small and unpredictable based on dietary characteristics. A meta-analysis (Patton, 2010) performed on two commercial RP-Met sources

concluded that overall RP-Met increased milk protein percentage (+0.07 percent) and yield (+27 g/d). However, milk protein response to RP-Met could not be related to dietary characteristics. A meta-analysis performed on the effect of supplementing RP-Met plus RP-Lys to diets with ≤15 percent CP reported a small improvement in MPY when RP-AAs were fed (Sinclair et al., 2014). The authors concluded that cows fed low CP diets could respond to RP-Met and RP-Lys supplementation if DMI and other AAs were not limiting. Finally, a meta-analysis compared responses to Met supplementation via postruminal DL-Met infusion, HMTBA, or an RP-Met product (Zanton et al., 2014). In that analysis, MPY increased 2.23 g of protein/g of metabolizable Met, irrespective of the Met source, until reaching a breakpoint. Milk protein concentration increased for all modes of supplementation except for HMTBA, in which only milk yield tended to increase in response to the Met analog. The observed MPY responses observed by Zanton et al. (2014) are similar to the slope coefficient for Met in Equation 6-6. The above authors also observed a positive milk fat yield response that did not differ among DL-Met supplements (1.9 g fat/g Met).

Studies have been conducted to evaluate the use of RP-Met during the transition period because of its multiple roles during this critical period. Feeding RP-Met to cows starting a few weeks prepartum and continuing a few weeks postpartum has increased early lactation milk and milk component yields (Osorio et al., 2013; Batistel et al., 2017) and improved neutrophil function (Osorio et al., 2013; Batistel et al., 2018). Feeding RP-Met during transition has also reduced various measures of oxidative stress (Batistel et al., 2018), which may partially be responsible for improved immune function. Production responses may in part be caused by Met's role as a methyl donor and its role in transmethylation reactions via activation of S-adenosylmethionine, which is involved in more than a hundred metabolic reactions (Finkelstein, 1990). Supplementation of RP-Met to transition cows may also have positive epigenetic effects on the immune system of the calf (Alharthi et al., 2019).

PROTEIN AND DRY MATTER INTAKE

NRC (2001) evaluated (without including study effects) the effects of RDP and RUP on milk protein and DMI. When study was accounted for, the effect of RDP on DMI persisted but to a lesser extent (Firkins et al., 2006). Numerous studies used ingredients that were high in RDP to isonitrogenously substitute for either a different protein source or the same source processed to be higher in RUP. Based on a review of the literature, a limitation of RDP decreases MCP flow to the duodenum and DMI (Santos et al., 1998). Many of those studies are in the database used by the current committee. However, many additional studies with greater range and diversity were added for the current work. In essentially all cases, RDP has been estimated using feed library values or by subtraction of observed microbial N and an estimate

of endogenous N from observed total nonammonia flows. Increasing RDP (if limited) increases MCP production and often DMI, and both MCP and DMI increase MP supply even though RDP itself does not contribute directly to MP. In studies designed to test the specific role of RDP directly, RDP had a large influence on MCP flow to the omasum (Reynal and Broderick, 2005). Replacing TP sources of RDP with urea also progressively depressed MCP flow (Brito et al., 2007; Broderick and Reynal, 2009). An RDP limitation depressed DMI linearly (Cyriac et al., 2008).

Dietary CP percentage is positively associated with DMI (Allen, 2000). This association was reinforced, regardless of whether evaluated in crossover or continuous lactation experiments (Zanton, 2016). Although CP typically was measured, RDP typically was not measured. In another meta-analysis using model outputs (Daniel et al., 2016), DMI was related nonlinearly to MP but still not maximized at the centered point of 9.7 percent MP (about 17.2 percent CP). Yet, mechanisms for DMI depression with decreasing CP percent, long related to improvements in fiber digestibility and alleviation of fill, still await further characterization (Sinclair et al., 2014). Some potential mechanisms are discussed in Chapter 2. Increasing RDP from 9.4 to 10.3 percent of DM increased DMI, but DMI decreased when RDP was increased from 11.0 to 12.1 percent of DM (Mutsvangwa et al., 2016). Martineau et al. (2016) suggested that provision of MP from casein via postruminal infusions (not increasing RDP) increased DMI when dietary MP was deficient but induced a satiety effect when dietary MP exceeded recommendations. In the latter case, more ammonia from RUP would need to be cleared by the liver. Mobilization of body protein reserves could contribute to hypophagia in early lactation (see Chapter 2). Carder and Weiss (2017) noted that careful attention to providing metabolizable AAs increased production of milk and milk components in early lactation, apparently sparing body protein mobilization, and even showing potential for a longer-term response after peak milk. Clearly, more research is needed on the role of metabolizable AA supply for cows in the first 30 days of lactation.

Numerous studies have been conducted since NRC (2001) to evaluate lowering dietary CP (typically avoiding limited RDP) while maintaining supply of limiting EAA. Decreasing CP from 17.1 to 15.8 percent decreased DMI, but supplementing RP-Met increased DMI (Broderick et al., 2009). Despite lowering NDF digestibility, feeding MP-deficient diets did not depress DMI, whereas supplementing RP-His to the MP-deficient diet increased DMI and MPY (Giallongo et al., 2015, 2017). When MP was deficient, dairy cattle decreased DMI; however, DMI was numerically but not always significantly recovered by supplementing RP-Met, RP-Lys, and RP-His; when His alone was added, DMI was almost the same as supplementation with all three RP-AAs, which fully recovered MPY (Giallongo et al., 2016). Of the RP-AAs typically studied, the greatest response in DMI appears to be from RP-His (Patton et al., 2015; Giallongo et al., 2017).

When MP was deficient, Met and Lys extraction into tissues was estimated to increase relative to those AAs that were not deficient (Lee et al., 2015). Metabolizable His appears to become increasingly limiting as microbial protein makes up an increasing proportion of the MP (Giallongo et al., 2016), and increased supply of metabolizable His (when it is limited in MP) seems to be directed preferentially toward the mammary gland (Sadri et al., 2016). Thus, increasing MPY could increase mammary extraction of blood EAA in addition to His and pull DMI. Patton et al. (2015) explained the relative constancy of plasma EAA concentrations over the lactation cycle and the challenge to relate concentrations to physiological functions such as control of voluntary feed intake. Clearly, the role of EAA in short- and long-term regulation of milk protein synthesis awaits further understanding (Arriola Apelo et al., 2014b; Cant et al., 2018), and future efforts should help delineate a role for metabolizable AA supply affecting DMI, particularly in longer-term studies (Hristov et al., 2019).

EFFECTS OF PROTEIN ON REPRODUCTION

Based largely on the seminal efforts described by Butler et al. (1998), NRC (2001) discussed studies regarding the negative correlation between excess dietary CP and fertility in dairy cattle. Since then, numerous studies have had specific objectives to address a mechanism for such a response, with likely responses related to urea or ammonia toxicity on oocyte, embryo, and uterine environments (Thatcher et al., 2011; Berry et al., 2016). One meta-analysis supported a role for excess BUN depressing fertility (Lean et al., 2012). In contrast, another meta-analysis (Sinclair et al., 2014) lessened such a role, but the authors noted that many of the studies in the prior meta-analysis used low-producing cows. Berry et al. (2016) noted a clear distinction among production systems in which studies with pasture-fed cattle lacked any negative association even though grazing cattle typically have much higher BUN than those fed total mixed rations. Based on herd records, lowering milk urea N (MUN; a proxy for BUN), within the range of 9.0 to 18.0 mg/dL, was suggested to have only a modest opportunity to improve fertility through dietary adjustments (Guo et al., 2004). Those authors noted that MUN was more related to individual cows within herds than among herds such that dietary formulation per se played a minimal role compared with other factors in the negative association of MUN with fertility. An MP deficiency could promote problems in early lactation and therefore lower fertility (Drackley and Cardoso, 2014), whereas diets with excess MP relative to ME should be avoided (Diskin et al., 2016).

Dairy cows suffer a myriad of issues in the transition period (see Chapter 12) that could influence tissue energy balance and other factors related to reproduction. For example, services per conception leading to pregnancy increased by 0.46 when mastitis was experienced preservice and increased to 0.72 services per conception when both pre- and postservice mastitis was included (Dolecheck et al.,

2019). Energetic efficiency was expected to decrease by up to 15 percent when cows experienced inflammatory responses (Bertoni et al., 2015). However, Bertoni et al. (2015) and Enger (2019) could not attribute quantitative requirements for MP or AAs despite increased synthesis of acute-phase proteins, antibodies, and other proteins during the inflammatory response associated with clinical mastitis. Similarly, the role of mobilization of non-esterified FA in inflammatory responses that depress fertility is well recognized (Le Blanc, 2014; Sordillo et al., 2016), whereas there is a more limited understanding of how inflammatory responses are related to MP supply. Enhancing the postruminal supply of Met and His in the transition cow likely increases the ability to reduce fat infiltration in the liver (Coleman et al., 2020), which likely enhances reproductive success. Those authors also noted that DMI could increase and that inflammatory markers could decrease in the transition period by increasing metabolizable Met supply. Arg and several other AAs (including nonessentials) were inferred to have a role in moderating inflammation, but studies are lacking in ruminants and especially with respect to reproduction.

Appropriate protein feeding in the transition cow is critical to provide metabolizable AA to limit the mobilization of body protein reserves, enhance peak milk production, and maintain higher milk production. The postpartum cow has a supply of labile protein reserves, but these are less than her supply of energy from adipose tissue (Drackley and Cardoso, 2014). As the postpartum cow adapts to high milk production, tissue-mobilized AA must compete for oxidation and gluconeogenesis by the liver and will not necessarily be diverted toward mammary protein synthesis (Larsen et al., 2015). Proper supply of starch should help optimize the efficiency of AA metabolism by the mammary gland (Cantalapiedra-Hijar et al., 2015). Increasing starch supply (without causing ruminal acidosis) should help limit mobilization of AA from muscle or direct more AA toward replenishing peripheral tissues (Nichols et al., 2016) and FAs from adipose tissue (Drackley and Cardoso, 2014) at the same time that increasing insulin secretion helps the reproductive tissues compete for nutrients (Lucy et al., 2014). Thus, MP must be adequate to support the increasing milk production and to stimulate increasing DMI in the postpartum cow (Amanlou et al., 2017). Optimization of metabolizable AA and ME will help to limit fertility issues that have been associated with high BUN.

Concentrations of MUN can be useful at the farm level to evaluate supply of CP. For example, Patton et al. (2014) described opportunities to use MUN in managerial approaches such as to safely lower dietary CP while meeting metabolizable AA targets. However, the usefulness of these values is diminished by variation among cows and herds due to genetic and management factors (Wood et al., 2003; Aguilar et al., 2012). For example, dietary concentrations of Na and K are important determinants of plasma and MUN concentrations

(Spek et al., 2013). These factors likely explain some of the variation among studies (Hristov et al., 2018). Greater attention to herd and other dietary characteristics might help explain variability in MUN and its potential to improve reproductive management. For example, a drop in MUN from before versus after artificial insemination was associated with increased risk for conception failure, which was more important than the MUN concentration per se (Albaaj et al., 2017). Given the current proclivity to decrease CP to minimize environmental pressure and its integration into an improved AA supply-requirement system, the current committee does not support a role of excess protein (i.e., to be beyond a reasonable safety factor) in the impairment of fertility unless the high BUN results from insufficient energy and excessive body tissue mobilization (Patton et al., 2014). Instead, the committee agrees that more attention is needed to assess fertility in light of lower-protein diets. Within a reasonable target for MP supply relative to requirements, fertility should not be impaired.

UREA RECYCLING AND ENERGY COST

Ammonia derived from microbial protein degradation in the gut is absorbed into the portal vein and detoxified by conversion to urea in the liver, whereas excess absorbed AAs that are catabolized in nonhepatic tissues are shuttled to the liver in Ala and Gln and the N removed and converted to urea. In dairy cows, the equivalent of 60 to 85 percent of digested N is converted to urea in the liver (e.g., Lapierre and Lobley, 2001; Ruiz et al., 2002; Recktenwald et al., 2014). To sustain anabolism when protein intake is low, ruminants have evolved to salvage part of the synthesized urea by recycling into the lumen of the gut, either through saliva or by direct transfer from the blood across the epithelial wall (Reynolds and Kristensen, 2008; Batista et al., 2017). Recycled urea is hydrolyzed to ammonia by bacterial ureases (Patra and Aschenbach, 2018), and ammonia can be either reabsorbed into blood as ammonia or used to support microbial protein synthesis. Part of microbial protein is digested and used to support anabolism, and part will be excreted in the feces (undigested rumen microbial protein and most of the hindgut-produced microbial protein). The potential futile cycle of BUN reconversion to ammonia is apparently regulated by expression of urea transporters (Calsamiglia et al., 2010). As explained previously, between 15 percent and 30 percent of rumen microbial N should be derived from BUN in dairy cattle (Li et al., 2019), which agrees with data from other ruminants (Batista et al., 2017).

Although adequate RDP is needed to support MCP production and DMI, excess RDP beyond a reasonable safety factor should be minimized to reduce urea excretion in urine and the environmental impact of dairy production systems (see Chapter 14). Excess dietary protein, particularly RDP, was positively associated with increased heat production

(Reed et al., 2017), although the latter results are inconsistent with earlier work (Tyrrell et al., 1970), which showed a minimal effect on heat production. Although hepatic urea synthesis requires ATP, this cost is minor relative to other energetic costs resulting from deamination of excess AAs and other metabolic processes (Reynolds, 2005). The committee does account for the energy associated with urinary N loss in derivation of metabolizable energy (see Chapter 3) but not any increase in heat production associated with increased urinary N loss. Urinary urea excretion is greater in dairy cattle experiencing heat stress, resulting from metabolic stress responses described by Rius (2019). Therefore, based on limited data, feeding to meet but not exceed RDP and AA requirements is recommended when cows are experiencing heat stress.

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7

Minerals

Not all minerals will be discussed in this chapter. For information on minerals that are primarily a toxicity concern (e.g., aluminum and fluoride) rather than a nutritional concern, see NRC (2005). Mineral requirements specific to preweaned calves are discussed in Chapter 10.

MACROMINERALS

Calcium

Functions

Extracellular calcium (Ca) is essential for formation of skeletal tissues, transmission of nervous tissue impulses, excitation of skeletal and cardiac muscle contraction, blood clotting, and as a component of milk. Intracellular Ca, while 1/10,000th of the concentration of extracellular Ca, is involved in the activity of a wide array of enzymes and serves as an important second messenger conveying information from the surface of the cell to the interior of the cell. About 98 percent of the Ca in the body is located within the skeleton, where Ca, along with phosphate anion, serves to provide structural strength and hardness to bone. The other 2 percent is found primarily in the extracellular fluids. Normally, the concentration of Ca in plasma is 2.2 to 2.5 mM (9 to 10 mg/dL) in the adult cow, with slightly higher values in calves. Between 40 and 45 percent of total Ca in plasma is bound to plasma proteins, primarily albumin, and another 5 percent is bound to organic components of the blood such as citrate or inorganic elements. From 45 percent (at higher blood pH) to 50 percent (at lower pH) of total Ca in plasma exists in the ionized, soluble form. The ionized Ca concentration of plasma must be maintained between 1 and 1.25 mM to ensure normal nerve membrane and muscle end-plate electric potential and conductivity, which has forced vertebrates to evolve an elaborate system to maintain Ca homeostasis. This system attempts to maintain a constant concentration of extracellular Ca by increasing Ca entry into the extracellular fluids whenever there is a loss of Ca from the extracellular compartment. When the loss of Ca exceeds entry, hypocalcemia can occur, resulting in loss of nerve and muscle function, which can lead to recumbency and the clinical condition referred to as milk fever.

Calcium Homeostasis

Ca leaves extracellular fluids during bone formation, in digestive secretions, sweat, and, in specific situations, urine. In lactating cows, secretion of Ca in milk is by far the greatest loss, accounting for 50 to 75 percent of Ca losses. Ca lost via these routes can be replaced from dietary Ca, from resorption of Ca stored in bone, or by resorbing the Ca filtered across the renal glomerulus (i.e., reducing urinary Ca loss). Under most circumstances urinary Ca losses are no more than 1 to 2 percent of absorbed Ca intake (Knowlton and Herbein, 2002; Taylor et al., 2009). When the loss of Ca from extracellular fluids exceeds the amount of Ca entering the extracellular fluids, plasma concentrations decrease. The parathyroid glands monitor the concentration of Ca in carotid arterial blood and secrete parathyroid hormone (PTH) when they sense a decrease in blood Ca. Release of PTH immediately increases renal reabsorption mechanisms for Ca and will stimulate processes to enhance intestinal absorption of Ca and resorption of Ca from bone.

Absorption of dietary Ca can occur by passive (paracellular) transport between epithelial cells across any portion of the digestive tract whenever ionized Ca in the digesta directly over the mucosa exceeds 6 mM (Bronner, 1987). These concentrations are reached when calves are fed all-milk diets and when cows are given oral Ca drenches for prevention or treatment of hypocalcemia (Goff and Horst, 1993). Following drenching, elevated Ca concentrations are short-lived as a result of dilution with digesta and the formation of complexes and chelates that reduce ionized Ca concentrations.

In nonruminants, as much as 50 percent of dietary Ca absorption may be passive (Nellans, 1988), but the amount

of passive absorption of Ca that occurs in dairy cattle is unknown. The diluting effect of the rumen would likely reduce the degree to which passive Ca absorption occurs. When chelates and less-soluble salts such as calcium carbonate ($CaCO_3$) and calcium sulfate ($CaSO_4$) move out of the rumen and interact with hydrochloric acid secreted in the abomasum, ionized Ca increases (Goff, 2018). Ca absorption is tightly regulated and is one of the primary means by which Ca homeostasis is maintained. This suggests that active transport of Ca is the major route for Ca absorption in mature ruminants, and this process is controlled by 1,25-dihydroxyvitamin D, the hormone derived from vitamin D. By carefully regulating the amount of 1,25-dihydroxyvitamin D produced, the amount of dietary Ca absorbed can be adjusted to maintain a constant concentration of extracellular Ca (DeLuca, 1979; Wasserman, 1981; Bronner, 1987). Regulation of Ca absorption in the intestine occurs as 1,25-dihydroxyvitamin D in blood binds vitamin D receptors in the intestinal enterocyte that initiate transcription and translation of several key proteins needed for active transport of Ca (Goff, 2018). These include an apical membrane channel protein that facilitates movement of ionized Ca through the cell membrane; production of calbindin, which binds Ca ions as they pass through the enterocyte membrane; and plasma membrane Ca ATPase that works with a sodium/calcium exchanger that pumps intracellular Ca through the enterocyte basolateral membrane into blood by exchanging 2 moles of Ca for 3 moles of sodium (Na).

When dietary Ca is insufficient to meet the requirements of the animal, Ca will be withdrawn from bone to maintain a normal concentration of extracellular Ca. If dietary Ca is severely deficient for a prolonged period, the animal will develop osteoporosis to the point of developing fractures, but plasma Ca will only be slightly lower than normal. A sudden large increase in loss of Ca from the extracellular pool (e.g., initiation of lactation) can result in acute hypocalcemia before the Ca homeostatic mechanisms can act. This is discussed in the section on milk fever (see Chapter 12).

Requirement for Absorbed Calcium

A factorial system is used to estimate the amounts of Ca required for maintenance, growth, pregnancy, and lactation as in the previous NRC (2001) publication.

Maintenance

The maintenance requirement is the amount of absorbed Ca that is needed to replace endogenous losses in urine and feces. Urinary Ca losses are trivial, and no reliable methods are currently available to predict urinary losses and thus are ignored. Previous estimates for metabolic fecal Ca were based on the fecal appearance of intravenously injected radioisotopes of Ca (Visek et al., 1953; Hansard et al., 1957). The 2001 NRC committee set daily metabolic fecal losses at 0.0154 g Ca/kg body weight (BW) for growing heifers and dry cows (Visek et al.,

1953; Hansard et al., 1957) and 0.031 g Ca/kg BW (Martz et al., 1990) in lactating cows. Metabolic fecal requirements for most minerals should be expressed as a function of dry matter intake (DMI) reflecting increased losses in feces with increased feed consumption. Data from Hansard et al. (1954, 1957), Visek et al. (1953), and Martz et al. (1990, 1999) were pooled and regressed on DMI. The regression equation included a nonsignificant intercept that was dropped resulting in an equation applied to all physiological states:

$$\text{Maintenance requirement} = 0.90\ (\pm 0.034) \times \text{DMI}$$
$$\text{(Equation 7-1)}$$

where maintenance requirement is equivalent to metabolic fecal Ca, g/d; DMI is dry matter intake, kg/d; $R^2 = 0.92$; and standard error (SE) = 1.75 g/d.

In NRC (2001), the absorbed Ca requirements for maintenance for a 300-kg growing heifer and 700-kg dry and lactating cows were 4.6, 10.8, and 21.7 g/d, respectively. In the new system based on DMI and assuming intakes of 7, 13, and 25 kg/d, the new requirements would be 6.3, 11.7, and 22.5 g/d, respectively. Although the equation has changed from NRC (2001), the amounts of absorbed Ca required for maintenance are similar.

Growth

Ca deposition in bone is the primary factor that drives Ca requirement for growth. Growing cattle require more Ca when animals are young and actively accruing bone and less as they approach mature skeletal size. An allometric equation (AFRC, 1991) was used to estimate the Ca requirement of growing calves:

$$\text{Ca, g/d} = ((9.83 \times \text{MatBW}^{0.22}) \times \text{BW}^{-0.22}) \times \text{ADG}$$
$$\text{(Equation 7-2)}$$

where MatBW is mature BW, kg; BW is current body weight, kg; and ADG is average daily gain, kg/d.

Based on that equation, absorbed Ca requirements per unit BW gain decrease with increasing BW. The NASEM (2016) estimates absorbed Ca requirements for growth as 71 g Ca/kg of body protein gain but cited more recent experiments that measured as much as 144 g Ca/kg protein gain. Growth requirements for Ca estimated using the equation above are generally 25 to 30 percent greater than the value used by NASEM (2016) at small BWs relative to mature weight, but as animals get closer to mature weight, the estimated requirements are much greater than those currently used for beef.

Pregnancy

The developing fetus requires a negligible amount of Ca until the last trimester of pregnancy (after day 190 of pregnancy), when the fetal skeleton begins to become calcified.

Fetal skeletal calcification is especially great in the last weeks before parturition, where the absorbed Ca requirement for pregnancy can exceed 10 g/d. The absorbed Ca required for growth of the uterus and conceptus is best described by the exponential equation (House and Bell, 1993) for any given day of gestation beyond day 190 as

$$(Ca\ 0.02456e^{(0.05581 - 0.00007t)t}$$
$$- 0.02456e^{(0.05581 - 0.00007(t-1))(t-1)}) \times (BW / 715)$$

(Equation 7-3)

where t is day of gestation. The average cow in the House and Bell (1993) study weighed 715 kg; therefore, gestation requirement is scaled to that BW.

Lactation

In NRC (2001), the absorbed Ca requirements for lactation were 1.22 g Ca/kg milk for Holstein cows, 1.45 g Ca/kg for Jersey cows, and 1.37 g/kg for other breeds. Castillo et al. (2013) reported a median milk concentration of 1.01 mg Ca/kg in a survey of bulk tank milk concentrations representing more than 30,000 cows in 39 California dairy herds. In more limited data, Carroll et al. (2006) reported a mean milk Ca concentration of 1.10, 1.25, and 1.25 g/kg for Holstein, Jersey, and Brown Swiss cows, respectively. The mean bulk tank milk concentration for 29 Holstein and 3 Jersey herds was 1.04 and 1.13 g Ca/kg, respectively (Robinson et al., 2002). These results implied that the values from previous NRC publications are too high.

Sixty-five percent of milk Ca is contained in the casein micelles in milk (Gaucheron, 2005), and milk Ca concentrations are strongly correlated with milk casein concentrations (Bijl et al., 2012). Survey data from Dutch dairy herds over time have shown that milk Ca has increased from 1.15 to 1.30 g Ca/kg as milk casein has increased from 2.64 to 2.88 percent (Bijl et al., 2012). To correct for differences in milk protein concentration among breeds and to account for the large discrepancy between 2001 dairy NRC milk Ca requirements and more recently measured milk Ca concentrations, a regression equation that related milk Ca concentration to milk true protein was developed using herd means (Robinson et al., 2002; Castillo et al., 2013), treatment means (Kume et al., 1998; Knowlton and Herbein, 2002; Carroll et al., 2006), and extracted individual cow data (Bijl et al., 2012). The resulting equation after adjustment for study effects was

$$Milk\ Ca = 0.295\ (\pm 0.73) + 0.239\ (\pm 0.029)$$
$$\times milk\ true\ protein\ percent \quad (Equation\ 7-4)$$

where Milk Ca is g/kg milk, root mean square error (RMSE) = 0.065, and R^2 = 0.86.

Using published (Animal Improvement Laboratory, 2015) mean protein concentrations of 3.08 and 3.65 percent for Holsteins and Jerseys, respectively, the equation would pre-

dict milk Ca concentrations of 1.03 and 1.17 g Ca/kg, values that are more in line with recently reported values.

Body Tissue Mobilization and Replenishment

Mobilization of body tissue in support of lactation includes the mobilization of bone Ca to support the demand for milk Ca secretion. Each kilogram of body tissue mobilized includes 21 g ash, and Ca accounted for 53 percent of total bone ash in samples taken at 8 days and 11 weeks postpartum (Taylor et al., 2009). This suggests that 11 g of Ca would be provided for each kilogram of body tissue mobilized. Ca balances of −11 to −15 g/d were observed in cows fed 0.52 percent dietary Ca during the first 8 weeks postpartum (Taylor et al., 2009). Ca mobilized at the beginning of lactation needs to be replenished as cows regain mobilized tissue stores over the course of the lactation. Mobilization of skeletal Ca is almost inevitable during early lactation, and cows could lose 800 to 1,300 g of bone Ca in early lactation (Ellenberger et al., 1931). This would require up to 8 g of absorbed Ca/d during the last 20 weeks of lactation to replenish. However, because of the uncertainties involved, no provision for replenishment of skeletal Ca mobilized during early lactation was included in the model.

Calcium Absorption Coefficient

The amount of Ca that must be fed to meet the requirement for absorbed Ca is dependent on the availability of Ca from the diet. The amount of Ca absorbed will generally equal the requirement for Ca if the diet contains enough available Ca. The proportion of dietary Ca absorbed will decrease as dietary Ca increases above requirements. As vitamin D–mediated Ca absorption from the intestine is tightly regulated, the determination of the efficiency of absorption of Ca from a diet requires that animals be fed at or near their Ca requirement. This will ensure that intestinal Ca absorption mechanisms are fully activated. Few studies fulfill this criterion, suggesting that published absorption data may often underestimate the availability of Ca. Furthermore, measuring Ca availability of specific ingredients is extremely difficult, and data must be extrapolated from total diets.

Ca absorption is usually measured using digestion experiments in which apparent Ca absorption equals Ca intake minus fecal Ca, both expressed in g/d. Since fecal Ca includes both undigested feed and metabolic fecal Ca, true Ca absorption is estimated by adding metabolic fecal Ca to apparently absorbed Ca. The absorption coefficient (AC) for a diet or feed is calculated as true Ca absorbed (g/d) divided by Ca intake (g/d).

Previous committees that authored *Nutrient Requirements of Dairy Cattle* publications (NRC, 1971, 1978, 1989) used a single AC for all diets. The 1971 and 1978 publications assumed an AC of 0.45, whereas the 1989 publication used 0.38. The AC was reduced in the 1989 *Nutrient Requirements of Dairy Cattle* partly in response to reports that cows in

early lactation were less able to utilize dietary Ca (Ramberg, 1974; van't Klooster, 1976), making use of a lower coefficient prudent. The 0.38 AC was based largely on a summary of 11 experiments with lactating dairy cows in which the average apparent absorption of dietary Ca was 0.38 (Hibbs and Conrad, 1983). In the majority of these 11 experiments, cows were fed diets well in excess of their Ca requirements, but in 3 of the experiments, the cows were in negative Ca balance, and the AC was still less than 0.40. In those experiments, alfalfa and brome hay supplied most of the Ca. The 2016 *Nutrient Requirements of Beef Cattle* uses a mean true Ca availability from the diet of 0.50, and the INRA system (INRA, 2018) uses values between 0.30 and 0.55. The 2001 Dairy NRC adopted a system where Ca availability was based on AC of individual feeds rather than using a single dietary average because availability of Ca from forages and individual mineral supplements varies widely (Hansard et al., 1957; Ward et al., 1972; Martz et al., 1990).

To accommodate a system based on AC of individual feeds, AC based on work of Hansard et al. (1957) and summaries of the relative availabilities of mineral supplements compared to either calcium chloride ($CaCl_2$) or calcium carbonate ($CaCO_3$) were adopted. A major factor limiting Ca absorption is the solubility of the Ca from the mineral source, and $CaCl_2$ represents a source of highly soluble Ca. In NRC (2001), $CaCl_2$ was assigned an AC of 0.95. That value was based on studies in which $^{45}CaCl$ was used as a tracer to measure Ca absorption by young calves (Hansard et al., 1954). Hansard et al. (1957) demonstrated that $CaCl_2$ is between 1.2 and 1.32 times more absorbable than $CaCO_3$. Therefore, the efficiency of absorption of Ca from $CaCO_3$ was set at 75 percent. Finally, a list of common supplemental sources of Ca and an estimate of the efficiency of absorption of Ca from each source were developed using data summarized by Soares (1995a) based on their efficiency of absorption relative to $CaCO_3$. In summary, ACs for all mineral supplements were based the assumption that $CaCl_2$ had an AC of 0.95. Unfortunately, there were very little data in the literature at the time with diets fed at near estimated Ca requirements to determine whether the adopted ACs agreed with actual measured values. However, since the 2001 publication, several appropriate experiments have been conducted.

To evaluate NRC (2001) ACs, 45 treatment means from experiments with high-producing cows in early lactation were assembled (Wohlt et al., 1986; Martz et al., 1999; Knowlton and Herbein, 2002; Moreira et al., 2009; Taylor et al., 2009). Apparent Ca absorption from those studies was converted to true Ca absorption by subtracting metabolic fecal Ca from fecal Ca output (metabolic fecal Ca, g/d = 0.9 × DMI, kg/d). Diet ingredient information from those experiments was entered into NRC (2001) software to generate a predicted AC for Ca for each diet, and those were compared with the actual measured values.

The mean 2001 NRC predicted and actual true ACs (based on measured apparent absorption and estimated metabolic fecal Ca) were 0.60 and 0.45, respectively. Interestingly, the mean (± SD) measured value (0.45 ± 0.047) for true Ca absorption was identical to the average value adopted by the 1971 and 1979 committees (NRC, 1971, 1978). The range in Ca concentration in the diets summarized was from 0.17 to 1.03 percent, and it is known that excess Ca intake will reduce Ca absorption. However, the within-study change in true Ca availability was from 0.8 to 5.9 g/100 g Ca intake, which is equivalent to a reduction in the ACs of 0.008 to 0.059 units/100 g Ca intake. This suggests that the effect of Ca intake on absorption was small. The predicted intercept at zero Ca intake was 50.2 (± 1.5) equivalent to a true AC of 0.50 and varied little among studies. These observations suggest that the previously adopted ACs for feedstuffs and mineral supplements were too high.

Part of the overprediction of Ca absorption likely stemmed from the use of 0.95 true AC for $CaCl_2$ based on values observed in young (<30 days old) calves prior to weaning (Hansard et al., 1954). In that same study, subsequent measurements of absorption from $CaCl_2$ were much lower in animals ranging in age from 6 to 190 months. The measured AC for Ca from $CaCl_2$ was 0.60 and 0.53 in young and mature steers, respectively (Hansard et al., 1957). The relative availability of $CaCO_3$ was based on the 0.95 AC of $CaCl_2$ (which was too high), and the ACs for other mineral supplements were based on their availability relative to $CaCO_3$, resulting in an overestimation of the Ca availability for all mineral supplements.

To correct the observed overprediction of Ca availability, the ACs for each of the feed classes and mineral supplements were reviewed and adjusted where deemed appropriate. A comparison of adjusted AC to the other coefficients and literature values (Hansard et al., 1957; NRC, 2001; Kiarie and Nyachoti, 2010) is shown in Table 7-1. The availability of $CaCl_2$ was reduced from 0.95 to 0.60, which is more in line with measured values in functioning ruminants (Hansard et al., 1957). For most supplements, the ACs were reduced by about 25 percent. In the 2001 NRC, the AC for corn silage was set at 0.60, but the mean measured value across three treatments (Martz et al., 1990, 1999) was 0.425. Therefore, a more conservative value of 0.40 was assigned. Legume silages and hays because of their high Ca concentration can be significant contributors of Ca, but for reasons discussed above, the AC was held at 0.30. For all other forages, the AC was raised to 0.40, a value that is more consistent with values observed for grasses and hays reported by Hansard et al. (1957). The coefficient for cereal grains and protein supplements was maintained at 0.60. Although there is little experimental basis for assigning this value, these feedstuffs are generally low in Ca and are only minor contributors to overall Ca intake. Most nonforage feedstuffs will contain only small amounts of Ca. A notable exception is for Ca soaps of palm oil fatty acids (FAs), which can be 7 to 9 percent Ca. Although the FAs in this product are approximately 76 percent digestible and digestion can only occur following dissociation of the Ca from the palmitate in the small intestine, it is

TABLE 7-1   Revised AC for Ca from Mineral Supplements and Feed Ingredient Classes

| Mineral Element Source | AC of Ca | | | |
| | 2001 Dairy NRC AC of Primary Element | Adjusted ACs | Hansard et al. (1957) AC | Kiarie and Nyachoti (2010) Literature AC |
Calcium Sources				
Bone meal, steamed	0.95	0.60	0.61	—
Calcium carbonate, $CaCO_3$	0.75	0.50	0.46	0.59
Calcium chloride anhydrous, $CaCl_2$	0.95	0.60	0.57	0.63
Calcium chloride dihydrate, $CaCl_2\ 2H_2O$	0.95	0.60	—	0.63
Calcium hydroxide, $Ca(OH)_2$	0.55	0.60	—	—
Calcium oxide, CaO	0.50	0.33	—	—
Calcium phosphate (monobasic), Ca $(H_2PO_4)_2$	0.95	0.60	0.56	0.55
Calcium sulfate dihydrate, $CaSO_4 \cdot 2H_2O$	0.70	0.60	—	—
Curacao, phosphate	0.70	0.45	—	—
Dicalcium phosphate (dibasic) $CaHPO_4$	0.94	0.60	0.47	0.73
Dolomitic limestone (magnesium)	0.60	0.35[a]	—	0.50
Limestone, ground	0.70	0.45	0.41	0.50
Magnesium oxide, MgO	0.70	0.45	—	—
Oystershell, flour (ground)	0.75	0.50	—	—
Phosphate, defluorinated	0.70	0.45	—	—
Phosphate rock	0.30	0.22	—	—
Phosphate rock, low fluorine	0.30	0.22	—	0.48
Soft rock phosphate colloidal clay	0.30	0.22	—	—
Mean for all mineral supplements (except rock phosphates)	0.86	0.55	0.52	0.57
Legume forages	0.30	0.30	0.36	0.58
Corn silage	0.60	0.40	—	0.52
Grass hays	0.30	0.40	0.45	0.04
All other forages	0.30	0.40	0.45	—

[a]Absorption value for dolomite from Gerken and Fontenot (1967).
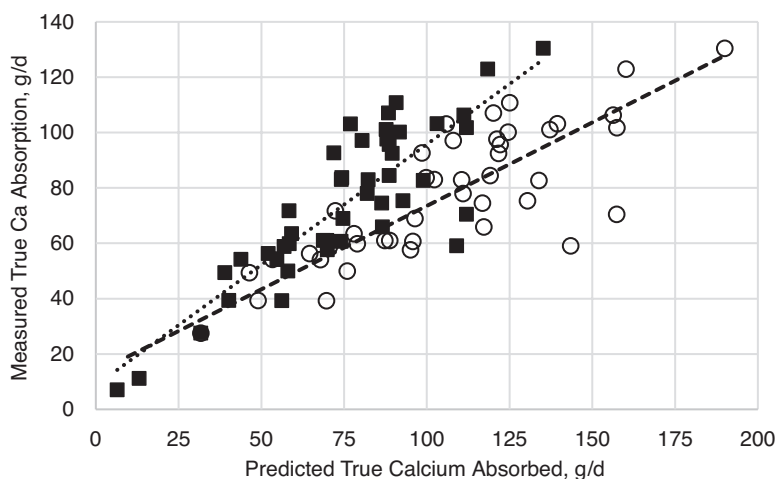
FIGURE 7-1   Comparison of true Ca absorption (g/d) (measured apparent absorption adjusted for estimated metabolic fecal Ca) versus predicted 2001 NRC values (open circles) and the adjusted (solid squares) absorption coefficients for individual feeds as shown in Table 7-1. For measured versus 2001 NRC, the regression equation was as follows: Absorbed Ca, g/d $= 8.6\ (\pm 4.80) + 0.668\ (\pm 0.04514) \times$ Predicted; $P < 0.001$, $R^2 = 0.84$, RMSE $= 11.2$ g/d. For measured versus adjusted, the regression equation was as follows: Absorbed Ca, g/d $= 2.9\ (\pm 5.24) + 0.973$ $(\pm 0.0674) \times$ Predicted; $P < 0.001$, $R^2 = 0.84$, RMSE $= 11.4$ g/d.

not likely that Ca absorption would exceed that of $CaCl_2$, so an availability of 0.60 was adopted. The accuracy of the new ACs was examined by entering the values in the 2001 NRC software for each of the feed ingredients in the diets from experiments with high-producing cows in early lacta-

tion (Wohlt et al., 1986; Martz et al., 1999; Knowlton and Herbein, 2002; Moreira et al., 2009; Taylor et al., 2009) and then comparing true Ca absorption (measured apparent plus estimated metabolic fecal Ca) with predicted absorbed Ca (see Figure 7-1). Both sets of coefficients were correlated

with measured Ca absorption. As discussed, use of the 2001 NRC ACs overpredicted Ca absorption at high intakes of absorbed Ca (slope = 0.67). After adjustment of the ACs (see Table 7-1), measured and predicted Ca absorption were in good agreement where the slope (0.97) and intercept of the predicted versus actual regression equation did not differ from 1 and 0, respectively. The Ca-to-phosphorus (P) ratio was once thought to affect absorption of Ca and P, but data suggest that the ratio is not critical, unless the ratio is >7:1 or <1:1 (ARC, 1980; Miller, 1983) and the model does not adjust AC for that ratio.

Although adjustments in the ACs improved the accuracy of prediction of Ca absorption, the committee recognizes that there is a dearth of measured availability data on individual feeds and mineral supplements. This is particularly important for comparing mineral supplements and for feeds that can provide substantial dietary Ca such as legume forages and canola meal. Major sources of variation for the AC of supplements have not been quantified. For example, particle size of limestone can affect rumen pH (Keyser et al., 1985), but it is unknown whether particle size (within typical ranges) affects the AC. The increased availability of stable isotopes of Ca such as ^{42}Ca and ^{44}Ca and higher sensitivity of mass spectrometer measurements should allow for improved estimates of Ca availability and metabolic fecal Ca losses.

Effects of Physiologic State

The amount of available Ca that will be absorbed varies with the physiologic state of the animal. Hansard et al. (1954) and Horst et al. (1978) reported that the efficiency of absorption of Ca decreases markedly as animals become older. As animals age, there is a decline in vitamin D receptors in the intestinal tract (Horst et al., 1990), which is thought to reduce the ability to respond to 1,25-dihydroxyvitamin D. The difference in efficiency of Ca absorption in beef steers from 1 to 6 years of age is nearly negligible (Hansard et al., 1954). Age was not included as a factor to adjust dietary Ca requirement in cattle >200 kg BW.

In early lactation, nearly all cows are in negative Ca balance (Ellenberger et al., 1931; Ender et al., 1971; Ramberg, 1974). As feed and Ca intake increase, most cows transition into positive Ca balance about 6 to 8 weeks into lactation (Ellenberger et al., 1931; Hibbs and Conrad, 1983). Cows in the first 10 days of lactation are at greatest risk of being in negative Ca balance (Ramberg, 1974), and many are subclinically hypocalcemic during this period (Reinhardt et al., 2011). Ramberg (1974) reported that the rate of entry of Ca into the extracellular fluid pool from the intestine increased about 1.55-fold from the day before parturition until 10 days in milk. Thereafter, the rate of entry of Ca into the extracellular pool from the intestine was constant. A study by van't Klooster (1976) demonstrated that Ca absorption increased from 22 percent in late gestation to 36 percent by day 8 of lactation, after which it remained relatively constant. This represented a 1.6-fold increase in efficiency of Ca absorption over this 8-day period. Regression analysis of data of Ward et al. (1972) predicted that cows need to be fed 5 g Ca/kg milk in early lactation to avoid negative Ca balance. However, there was no evidence to demonstrate that negative Ca balance in early lactation was detrimental to the cow provided the concentration of Ca in plasma remained normal (i.e., lactational osteoporosis ensures adequate entry of Ca from bone into the extracellular Ca pool).

Calcium Deficiency

A deficiency of dietary Ca in young animals leads to a failure to mineralize new bone and contributes to retarded growth. Rickets is more commonly caused by a deficiency of vitamin D or P, but a deficiency of Ca can contribute to rickets as well. In older animals, a deficiency of dietary Ca forces the animal to withdraw Ca from bone, which causes osteoporosis and osteomalacia, making bones prone to spontaneous fractures. The concentration of Ca in milk is not altered even during a severe dietary deficiency of Ca (Becker et al., 1933).

Excess Dietary Calcium

Feeding excessive dietary Ca is generally not associated with any specific toxicity. The maximum tolerable level (MTL) for Ca in ruminants was set at 1.5 percent of dietary dry matter (DM; NRC, 2005). Feeding excessive Ca could interfere with trace mineral absorption (especially zinc [Zn] and selenium [Se]) and dilutes energy and protein the animal might better utilize for increased production.

Phosphorus

Physiologic Roles

P has more known biological functions than any other mineral. About 80 percent of the body's P is in bones and teeth principally as apatite salts and as calcium phosphate. It is in every cell of the body, and almost all energy transactions involve formation or breaking of high-energy phosphate bonds (such as those in adenosine triphosphate [ATP]). Phosphorylation is a primary regulator of numerous enzymes. P also is involved in acid-base balance of blood and other bodily fluids, as well as in cell differentiation, and is a component of cell walls and cell contents as phospholipids and nucleic acids.

Normal P concentration in blood plasma of dairy animals is 1.3 to 2.6 mmol/L (4 to 8 mg/dL), and whole blood concentrations are six to eight times greater (Goff, 2004). Plasma concentrations decrease with increasing age and are lower in early lactation than later lactation (Forar et al., 1982). For a 600-kg cow, approximately 1 to 2 g of inorganic phosphate is circulating in blood plasma, 5 to 8 g of inorganic P is in the extracellular pool, and total intracellular P is about 155 g

(Goff, 1998). The intracellular concentration of P is about 10 times greater than the concentration in plasma (Goff, 1998).

Rumen microorganisms also require P (Burroughs et al., 1951; Breves and Schröder, 1991), and it is supplied to the rumen by the diet and recycling via saliva. Using various techniques, estimates of P recycling in lactating dairy cows fed adequate or excess P range from about 30 to 75 g/d (Kebreab et al., 2005; Puggaard et al., 2011). Inadequate supply of P to the rumen can reduce fiber digestibility. A diet with 0.24 percent P had lower neutral detergent fiber (NDF) digestibility than a similar diet with 0.34 percent P when fed to dairy cows (Puggaard et al., 2011). Digestibility of NDF was reduced even though the concentration of P in rumen fluid was 3 to 4 mmol/L, which is greater than the concentrations (0.6 to 2.5 mmol/L) that maximized cellulose digestibility in vitro (Hall et al., 1961; Chicco et al., 1965). However, no evidence is available showing improved ruminal digestion once the cow's P requirement is met.

Phosphorus Homeostasis

Blood plasma P concentrations are controlled via alterations in intestinal absorption, P recycling via saliva, renal excretion, and bone resorption. Absorption of P from the intestines is much less regulated than absorption of Ca. Net absorption of P occurs mainly in the small intestine (Grace et al., 1974; Reinhardt et al., 1988), with only small amounts absorbed from the rumen, omasum, and abomasum. Absorption is thought to occur mainly in the duodenum and jejunum (Care et al., 1980; Scott et al., 1984); however, little is known about absorption anterior to the small intestine (Breves and Schröder, 1991). Presumably, as in nonruminants, absorption occurs via two distinct mechanisms. A saturable vitamin D–dependent active transport system is operative when animals are fed low P diets. Synthesis of 1,25-dihydroxyvitamin D can be stimulated when blood P is low, resulting in more efficient absorption (Horst, 1986). Feeding 25-hydroxyvitamin D increases circulating 1,25-dihydroxyvitamin D and plasma P concentrations (Wilkens et al., 2012; Weiss et al., 2015b), which could indicate increased intestinal absorption of P. Passive absorption predominates when adequate or excessive amounts of potentially absorbable P are consumed, and absorption is proportional to the concentration gradient between the lumen of the small intestine and blood plasma (Wasserman and Taylor, 1976). However, data suggest that this process is saturable (Mogodiniyai Kasmaei and Holtenius, 2013), and current ruminant P models use Michaelis–Menten kinetics to describe intestinal absorption (Kebreab et al., 2004; Hill et al., 2008).

Renal clearance is usually a minor contributor to P homeostasis, but both urinary concentration and excretion of P increase as the supply of absorbable P to dairy cows increases (Knowlton and Herbein, 2002; Guyton et al., 2003; Knowlton et al., 2005; Puggaard et al., 2011; Mogodiniyai Kasmaei and Holtenius, 2013). The increase in urinary P

excretion as P intake increases is usually less than 10 percent of the increase in P intake. P recycling via saliva is the major homeostatic mechanism for P (Breves and Schröder, 1991). P absorbed from the intestine in excess of requirement elevates blood P, which is then transferred to saliva and reenters the rumen. Salivary and plasma concentrations of P have a strong positive correlation (Valk et al., 2002), but the mass of P recycled via saliva is not necessarily correlated with plasma P concentrations when cows are fed marginal amounts of P (Puggaard et al., 2011). Recycled P can be used by ruminal microorganisms; a portion of it will be reabsorbed by the intestines, and a portion will pass out in feces. Fecal excretion of recycled P is one reason why apparent absorption of P does not reflect true absorption of dietary P.

Requirements for Absorbed Phosphorus

As described previously (NRC, 2001), the factorial approach was used to estimate the requirement for absorbed P by summing requirements for maintenance, growth, pregnancy, and lactation.

Maintenance

The maintenance requirement of P is the endogenous fecal loss (inevitable fecal loss) plus endogenous urinary loss when P supply just meets the true requirement. Previously (NRC, 2001), endogenous urinary P was estimated as 2 mg P/kg BW. However, studies with dairy cows, steers, and goats fed diets that were at or below requirements consistently reported lower losses of P in urine than estimated by that equation (Bortolussi et al., 1996; Rodehutscord et al., 2000; Knowlton and Herbein, 2002; Kebreab et al., 2005; Puggaard et al., 2011). In those studies, urinary loss of P ranged from 0.2 to 0.9 mg/kg BW (mean = 0.5; SD = 0.28). For the three studies using lactating dairy cows, the range was 0.24 to 0.58 mg P/kg BW (mean = 0.4; SD = 0.17). Endogenous urinary P loss was set at 0.6 mg absorbed P/kg BW (i.e., the highest reported endogenous urinary P loss in dairy cows).

About half of the inevitable fecal loss of P is associated with microbial debris and purines and pyrimidines of nucleic acids. The other portion of endogenous fecal P includes sloughed cells, digestive secretions, and unabsorbed recycled P. As discussed previously (NRC, 2001), endogenous fecal loss should be expressed as a function of DMI. Although the amount of endogenous P derived from microbes may be more related to intake of fermentable organic matter than DMI (Rodehutscord et al., 2000), accurately estimating fermentable matter is difficult. Any gain in accuracy in estimating endogenous fecal P by calculating it from intake of fermentable matter may be lost by the error associated with estimating fermentability. Therefore, endogenous fecal P was estimated from DMI as done previously (NRC, 2001).

Based on data from growing bulls and steers (Bortolussi et al., 1996; Klosch et al., 1997), NRC (2001) set the absorbed

P requirement for endogenous fecal P for growing cattle at 0.8 g/kg DMI. Using data from experiments (Speikers et al., 1993; Valk et al., 2002; Puggaard et al., 2011) in which lactating cows were fed at or below estimated P requirements, fecal excretion ranged from 0.95 to 1.4 g P/kg DMI (mean = 1.2, SD = 0.14 from 14 treatment means). If the absorbability of dietary P is assumed to equal 0.80, then endogenous fecal P equals 1.0 g/kg DMI, which is identical to the endogenous fecal P requirement from NRC (2001). Myers and Beede (2009) varied DMI of lactating dairy cows over a wide range and measured inevitable fecal loss of P. For cows fed ad libitum (ca. 25 kg/d DMI), endogenous fecal loss equaled 1.04 g P/kg DMI. In that study, inevitable fecal loss of P increased to 1.36 and 1.19 g P/kg DMI when intake was restricted to 50 and 75 percent of ad libitum DMI, respectively. The severe DMI restriction imposed likely affected feeding behavior, rate of eating, rumination time, and so on, which could affect salivary flow and intestinal secretion, so the data from cows fed the restricted treatments were not used in establishing endogenous fecal requirement. Limited data are available regarding endogenous fecal P excretion by dry cows. Based on isotope dilution, endogenous fecal P was 0.4 g P/kg DMI for a corn silage, corn cob diet and 0.5 g P/kg DMI for a diet with 90 percent corn silage (Martz et al., 1999). With more practical diets, endogenous fecal P for dry cows fed low P diets (assumed true absorption of dietary P was 0.8) averaged 1 g of absorbed P/kg DMI (two treatment means: 0.92 and 1.07 g/kg DMI) (Valk et al., 2002). The almost 2-fold difference in estimated endogenous fecal P between those methods is difficult to explain but was not caused by differences in DM digestibility (similar between studies). Because of the atypical diets used in the Martz et al. (1999) study, the data from Valk et al. (2002) were used to set the endogenous fecal P requirement for dry cows at 1.0 g absorbed P/kg DMI (i.e., the same as for lactating cows).

Maintenance requirement for absorbed P (endogenous fecal and urinary losses):

$$\text{Growing heifers (g/d): } 0.8 \text{ g P/kg DMI} \\ + 0.0006 \text{ g P/kg BW} \quad \text{(Equation 7-5a)}$$

$$\text{Adult cows (g/d): } 1.0 \text{ g P/kg DMI} \\ + 0.0006 \text{ g P/kg BW} \quad \text{(Equation 7-5b)}$$

Growth

The requirement for growth is the amount of absorbed P accreted in soft tissues plus that deposited in skeletal tissue. Skeletal growth comprises a larger portion of live weight gain in younger heifers than in older heifers; therefore, the grams of P required per kilogram of growth are higher in younger animals. The 2016 beef cattle requirement (NASEM, 2016) for retained P was set at 3.9 g/100 g of retained protein. Because younger animals deposit greater amounts of protein/kg of ADG, this approach will result in higher P requirements

for younger animals compared with older animals. The main problem with that approach is that protein concentration in live weight growth must be known or estimated accurately. Therefore, the allometric equation used previously (NRC, 2001) was retained:

$$P, \text{ kg/d } (1.2 + ((4.635 \times \text{MatBW}^{0.22})(\text{BW}^{-0.22}))) \times \text{ADG} \\ \text{(Equation 7-6)}$$

where MatBW and BW are in kg; BW is current body weight, kg; and ADG is average daily gain, kg/d.

Pregnancy

No new data are available on conceptus P accretion; therefore, the NRC (2001) pregnancy requirement was retained. Quantitatively, the requirement for P for pregnancy is low until the last trimester. House and Bell (1993) measured accretion of P in conceptuses (fetus, fetal fluids, and membranes, placentomes, and uterine tissues) of 18 multiparous Holstein cows slaughtered at varying times from 190 to 270 days of gestation. Changes in fetal mass and P content across the sampling period were similar to earlier data (Ellenberger et al., 1950). The requirement for absorbed P to meet demands of the conceptus for any day beyond 190 days of gestation is

$$\text{Absorbed P, g/d} = (0.02743 \text{ e}^{(0.05527 - 0.000075t)t} \\ - 0.02743\text{e}^{(0.05527 - 0.000075(t-1))(t-1)}) \times (\text{BW} / 715) \\ \text{(Equation 7-7)}$$

where t is day of gestation (House and Bell, 1993). The average cow weighed 715 kg in that study; therefore, the requirement was scaled to 715 kg.

Estimates of rates of P accretion in conceptuses of Holstein cows increase from 1.7 g/d at 190 to 5.4 g/d at 280 days of gestation. The P requirement of the conceptus at <190 days was set to zero in the model.

Lactation

The daily requirement for absorbed P for lactation is equal to the amount of P secreted in milk daily. Mean (e.g., treatment groups or farms) P content of milk ranged from 0.83 to 1.00 g/kg (Speikers et al., 1993; Wu et al., 2000; Castillo et al., 2013). For individual cows, milk concentration ranged from about 0.7 to 1.2 g P/kg (Klop et al., 2014). NRC (2001) used a value of 0.90 g P/kg of milk, and newer data (Klop et al., 2013) support that value. Concentrations of protein and P in milk are correlated, and milk P can be estimated from milk protein (Klop et al., 2013). The lactation requirement for absorbed P (g/d) is set at

$$\text{Milk protein is unknown: Milk yield (kg/d)} \times 0.90 \\ \text{(Equation 7-8a)}$$

Milk protein is known: Milk yield $\times$ [0.49 + 0.13 $\times$ Milk true protein (%)]   (Equation 7-8b)

Using Equation 7-8b (Klop et al., 2013) with an average milk true protein of 3.1 percent yields an estimated milk concentration of 0.88 g P/kg.

Dietary Requirement and Efficiency of Absorption

The dietary requirement is the total requirement for absorbed P divided by the AC for P from the diet. The use of feed (or feed class)–specific AC was introduced in NRC (2001), and that approach has been expanded. The AC for P in NRC (2001) was set at 0.64 for all forages except corn silage and 0.70 for all other feeds. The AC for P supplements ranged from 0.30 to 0.90, and the AC for total diets (weighted average from the dietary ingredients) was usually around 0.70.

To accurately determine the AC for a specific feedstuff or mineral source, P must be fed in an amount close to the animal's true requirement, and P recycling must be accurately quantified. Most studies do not satisfy these experimental specifications. Furthermore, even simple diets will contain multiple sources of P, and accurately partitioning the overall dietary AC into AC for ingredients is not possible. In the previous edition (NRC, 2001), the AC for P for all feedstuffs other than mineral supplements was based on data for alfalfa hay and corn silage. An alternative to assuming all feedstuffs have essentially the same AC is to analytically partition dietary P into fractions and estimate the AC for each fraction via modeling (Hill et al., 2008; Feng et al., 2015, 2016). This is the approach used for basal ingredients (described below).

The AC for P supplements from NRC (2001) was retained because newer data are not available. These values were tabulated from Soares (1995b), Peeler (1972), and other sources in the literature and are used in the model. Values determined using ruminants, especially cattle, were given preference whenever possible in tabulation. Dicalcium phosphate (calcium phosphate dibasic) with an AC of 0.75 in cattle (Tillman and Brethour, 1958; Challa and Braithwaite, 1988), phosphoric acid with an AC of 0.90 in cattle (Tillman and Brethour, 1958), and monosodium phosphate with an AC of 0.90 in sheep (Tillman and Brethour, 1958) were taken as reference standards. The ACs of P in other mineral sources were set based on these reference standards (Soares, 1995b).

Form of Dietary Phosphorus

For the current model, feed P is analytically partitioned into inorganic P (blue molybdovanadate method; AOAC, 2000) and organic P (total P − inorganic P). A model on P metabolism and absorption (Hill et al., 2008; Feng et al., 2015, 2016) also included a phytate P fraction, but its absorption coefficient was similar to that of the nonphytate organic P fraction (0.66 versus 0.7). Therefore, those two fractions were combined into organic P, which simplifies analytical

requirements. Using the above P model, the AC is 0.84 for inorganic P and 0.68 for organic P fraction (i.e., average for phytate and nonphytate organic P).

The weighted average AC is then calculated based on the size of the two P fractions, which is the AC values in the feed library. For feeds that did not have P fraction data, AC values from similar feeds were used, or the AC was set at the default of 0.72. Additional analytical data are needed regarding P fractions of different feedstuffs. Feeds can be assayed for total P and inorganic P and those values entered in the feed library, but at the time of publication, most commercial labs did not conduct those assays. Factors other than form of P can affect AC; however, these effects have not been adequately quantified and cannot be modeled.

Phosphorus Intake

Although not as tightly regulated as Ca, true absorption of P decreases as P intake increases above requirements (Challa and Braithwaite, 1988; Challa et al., 1989; Martz et al., 1999); however, adequate data are not available to accurately quantify that effect. Because salivary P typically supplies at least 2-fold greater amounts of P to the lumen of the small intestine than does dietary P, the efficiency of absorption of salivary P is important. Salivary P is in the form of sodium and potassium phosphate salts. The AC of salivary endogenous P recycled to the small intestine was 0.68 to 0.81 in bull calves (Challa et al., 1989). Excessive dietary P relative to the requirement reduced the efficiency of absorption of inorganic or salivary P (Challa et al., 1989). The AC shown in Table 19-3 for mineral supplements and the AC values used for the various P fractions outlined above should be considered maximum absorption. If P is fed in excess of requirements, those ACs will overestimate actual absorption; however, because this occurs once the P requirement is met, it will not affect the amount of dietary P needed to meet requirements for absorbed P.

Use of Phytase

Phytate phosphorus (inositol polyphosphate) is the common storage form of P in many plants and usually comprises the largest proportion of organic P in concentrates (Nelson et al., 1968; Morse et al., 1992). Forages (or vegetative matter) usually have low concentrations of phytate. Normal ruminal metabolism breaks down most of the phytate; however, exogenous phytase can increase phytate breakdown in the rumen (Brask-Pedersen et al., 2013). Feeding supplemental phytase to dairy cows has not consistently reduced fecal excretion of P, and most studies reported no effect (Guyton et al., 2003; Kincaid et al., 2005; Knowlton et al., 2005; Knowlton et al., 2007).

Dietary Calcium

When cows are fed P at or above requirements, Ca intake ranging from deficient to excess usually has not affected

efficiency of P absorption (Hibbs and Conrad, 1983; Moreira et al., 2009; Taylor et al., 2009; Herrera et al., 2010). Solubility of supplemental Ca (CaCl$_2$ versus limestone) did not affect P absorption by dairy cows (Herrera et al., 2010). However, Ca and P apparent digestibility are positively correlated (Hibbs and Conrad, 1983).

Animal Responses to Varying Dietary Phosphorus

Production responses by growing and lactating cattle to differing dietary P concentrations were reviewed in the previous edition; therefore, only more recent studies will be reviewed in detail in this version. In growing heifers, diets with 0.3 to 0.34 percent P generally resulted in maximum gain, adequate blood P concentrations, and adequate bone strength compared with animals fed diets with lower concentrations of P (NRC, 2001). Newer data support that conclusion. A study with Holstein and Holstein × Jersey crossbred heifers that started at 4 months of age and ended at 22 months of age found no differences in growth (weight and stature), reproductive measures, or bone strength between heifers fed 0.3 or 0.4 percent P (Esser et al., 2009; Bjelland et al., 2011).

The review conducted previously (NRC, 2001) concluded that for lactating cows, diets with 0.32 to 0.42 percent P for the entire lactation were sufficient depending on milk yield potential. Furthermore, they concluded that no benefits on lactational performance occurred when cows were fed diets with >0.42 percent P. Because of the ability to mobilize P from bone, longer-term performance studies evaluating effects of differing concentrations of dietary P on lactating cows are more meaningful than short-term studies. Newer studies lasting from 9 weeks to two lactations largely support the conclusions reached by the previous committee. Grazing dairy cows were fed diets with approximately 0.22 or 0.31 percent P starting at about 30 days in milk through about 90 days in milk (Reid et al., 2015), and no effects on milk yield, milk composition, or feed intake were observed. Dietary P concentration of 0.33 or 0.42 percent did not affect milk yield (35.1 versus 35.4 kg/d), DMI, or milk composition of mid-lactation Holstein cows fed diets for 14 weeks (Wu et al., 2003). However, Holstein cows fed diets with 0.32 percent P had reduced yields of fat-corrected milk (40.3 versus 44.3 kg/d) and DMI (25.0 versus 26.5 kg/d) compared with cows fed diets with 0.44 percent P for 10 weeks. The diets with 0.32 percent P did not meet the P requirement based on the current model. In a 23-week experiment (Lopez et al., 2004a,b), DMI, milk yield (35.1 versus 34.9 kg/d), milk composition, health disorders (except occurrence of eye inflammation, which was statistically greater in cows fed high P), and reproductive measures did not differ between Holstein cows fed diets with 0.37 or 0.57 percent P starting immediately after parturition. Similar results were obtained when Swedish Red and White cattle were fed diets with 0.32 or 0.42 percent P during the first 4 months of lactation (Ekelund et al., 2006). Based on bone markers, cattle in both groups exhibited bone P resorption, but resorption was similar between treatment groups.

Two multilactation studies evaluated effects of varying dietary P concentrations on long-term health and production of dairy cows. In one study, dairy cows (breed not reported, approximately 600 kg BW) were fed diets with 0.24, 0.28, or 0.33 percent starting in mid-lactation and continuing through a dry period and then for the entire next lactation and the subsequent dry period (Valk and Sebek, 1999). No treatment effects were observed in the first lactation period on milk yield (26.8, 25.9, and 27.5 kg/d, respectively), milk composition, or DMI. During the first dry period, cows fed the lowest P diet had reduced DMI. During the second lactation, cows fed the lowest P diet produced significantly less milk, consumed less DM, and were losing BW, and because of animal welfare concerns, that treatment was terminated after cows were on the treatment for approximately 12 months. No differences in milk yield (33.0 versus 34.1 kg/d), DMI, milk composition, or BW were observed between cows fed 0.28 or 0.33 percent P during the second lactation of the experiment. In another study, milk production (36.4 versus 35.4) and milk composition did not differ between Holstein cows fed diets with 0.35 or 0.42 percent P (Odongo et al., 2007) over two lactations. However, first-lactation, but not multiparous, cows fed the low P diet had lower DMI than first-lactation cows fed 0.42 percent P. BW and body condition were also lower for first-lactation cows fed the low P diet, indicating 0.35 percent dietary P was not adequate for first-lactation cows. Data from that experiment could not be evaluated with the current model because adequate parity data were not included in the study. But overall, data from longer-term production studies support the P requirements calculated using the current model.

Phosphorus and Reproduction

The previous edition (NRC, 2001) reviewed published research reports from 1923 through 1999 to assess the effects of dietary P on reproductive performance of cattle, and studies published after 1999 have been added to this review. In some studies, but not all, severe deficiency of dietary P caused infertility or reduced reproductive performance of cattle (Alderman, 1963; Morrow, 1969; McClure, 1994). Typically, P concentration was <0.20 percent of dietary DM, the deficient diet was fed for an extended length of time (1 to 4 years), and where measured, feed intake was depressed, causing coincidental deficiencies of energy, protein, and other nutrients. Low body condition generally is considered the main cause of reduced reproductive efficiency in P-deficient cows (Holmes, 1981). Little (1975) demonstrated that deficiencies of P and protein were additive on failure to exhibit first postpartum estrus in grazing multiparous beef cows.

In growing heifers, experimentally induced reproductive failure caused by a dietary P deficiency has been very difficult to produce. The studies reviewed by NRC (2001)

reported no adverse effects on reproduction in heifers when they were fed diets with as little as 0.15 percent P for several months (some studies lasted more than 1 year). With lactating dairy cows, evidence from available research to support feeding P in excess of requirements to improve reproduction is virtually nonexistent. Results of 10 studies can be summarized very succinctly (Steevens et al., 1971; Carstairs et al., 1980; Call et al., 1987; Brodison et al., 1989; Brintrup et al., 1993; Valk and Sebek, 1999; Wu and Satter, 2000; Wu et al., 2000; Lopez et al., 2004a,c; Odongo et al., 2007). All measures of reproductive performance compared within each study were not affected by the concentration of dietary P with one exception. In the study by Steevens et al. (1971), services per conception were greater in the second year for cows fed 0.40 versus 0.55 percent P, but not in the first year of study. Among these seven studies, dietary P ranged from 0.24 to 0.62 percent of dietary DM, length of feeding different dietary P concentrations ranged from the first 12 weeks of lactation to as long as three consecutive lactations, and average milk yields ranged from 15 to 37 kg/d. As long as dietary P was greater than 0.31 percent, reproductive performance was normal and not improved with increased concentrations of P. Cows in some of the studies would not be considered high-producing cows by modern standards. However, the more recent studies used cows producing more than 35 kg, and no effects of dietary P on reproduction were observed in those studies. The preponderance of data does not support feeding dietary P at concentrations in excess of those needed to meet dietary requirements to improve reproductive performance.

Phosphorus Deficiency

Detailed description of occurrence, etiology, clinical pathology, diagnosis, and treatment of P deficiency in ruminants has been described (Goff, 1998). Signs of deficiency may occur rather quickly if dietary P is insufficient. Deficiency is most common in cattle grazing forages on soils low in P or in animals consuming excessively mature forages or crop residues with low P content. Dairy cows do not seem to have the ability to self-select appropriate intakes of P or other minerals (Muller et al., 1977). Hypophosphatemia can also occur when a cow develops a displaced abomasum (Grünberg et al., 2005). Nonspecific chronic signs of deficiency include unthriftiness, inappetence, poor growth, and reduced milk yields, but signs are often complicated by coincidental deficiencies of other nutrients such as protein or energy. Animals may be chronically hypophosphatemic (<4 mg/dL in plasma), but the concentration of P in milk remains within the normal range. Hemoglobinuria (Jubb et al., 1990) and liver dysfunction (Grünberg et al., 2005) are associated with hypophosphatemia. In severe deficiency cases, bone mass is lost, and bones become weak. Severe clinical manifestations of P deficiency include acute hypophosphatemia, rickets in young growing animals, and osteomalacia in adults. Cows may also exhibit pica.

Acute hypophosphatemia (less than 2 mg P/dL of plasma) may occur when cows are fed marginally low dietary P and challenged by extra demand for P in late pregnancy with accelerated fetal growth, especially with twin fetuses and with colostrum and milk formation during early lactation. The disease usually is complicated with concurrent hypocalcemia, hypomagnesemia, and possibly hypoglycemia.

Concentrations of P in plasma often fall below the normal range in the periparturient period (Grünberg, 2008). In other mammals, physiologic correction can occur rather rapidly as P absorption is responsive to renal production of 1,25-dihydroxyvitamin D, which is stimulated by low P in the blood (Reinhardt et al., 1988; Goff, 1998). Feeding peripartum dairy cows 25-hydroxyvitamin D increased plasma 1,25-dihydroxyvitamin D and elevated plasma P (Wilkens et al., 2012; Weiss et al., 2015b). Secretion of cortisol around parturition may depress concentrations of P in plasma. Intravenous Ca to correct hypocalcemia usually results in a rise in P in plasma because parathyroid hormone secretion is lowered, reducing urinary and salivary loss of P. It also stimulates resumption of gut motility, recycling of salivary P, and absorption. Oral or intravenous administration of a soluble form of P such as sodium monophosphate can help correct hypophosphatemia. In some cows with severe cases of clinical milk fever, protracted hypophosphatemia (P in plasma <1 mg/dL) occurs with recumbency; even with successful treatment for hypocalcemia, P in blood remains low. This disorder is not well understood. However, increasing the amount or concentration of P in the diet in excess of requirement in late pregnancy or early lactation will probably not correct hypophosphatemia in the periparturient period, as this disorder seems to occur secondary to hypocalcemia.

When young calves are fed P-deficient diets, rickets occurs from a failure of mineralization in osteoid and cartilaginous (growth plate) matrices during bone remodeling. In contrast, in mature animals (no active growth plates), osteomalacia occurs over time with P deficiency with failure of mineralization of the remodeled osteoid matrix. In the adult, P in bone released during remodeling is used to maintain concentrations of P in blood rather than being reincorporated into bone. In young animals, bone cartilage remains unmineralized, resulting in bone that can be flexed without breaking.

Maximum Tolerable Level

NRC (2005) set the MTL of P for cattle at 0.7 percent of diet DM. That concentration was chosen because studies feeding higher concentrations were lacking, not because data were available showing negative effects when cattle were fed diets with >0.7 percent P. Long-term feeding of excess P can cause problems with Ca metabolism, inducing excessive bone resorption and urinary calculi, secondary to the elevated concentrations of P in blood (NRC, 2005). Most often, P toxicity is complicated with low dietary Ca, but ruminants can tolerate a wide ratio of Ca-to-P as long as P and Ca

are adequate. Supplemental phosphates given in large oral doses are not considered highly toxic but can result in mild diarrhea and abdominal distress. Dairy cattle are quite adept at excreting excess absorbed P to maintain concentrations of P in blood within a normal range via salivary secretion and fecal excretion (Challa et al., 1989). Urinary excretion of P also may increase, although its quantitative importance is small relative to fecal excretion. Feeding 0.69 percent P to Holstein–Friesian cows for 14 weeks prepartum through 22 weeks of lactation caused no problems or signs of toxicity (De Boer et al., 1981). In contrast, a meta-analysis determined that even moderate overfeeding of P during the prepartum period was a risk factor for hypocalcemia (Lean et al., 2006). High P intake (>80 g/d) by cows approaching parturition increased blood P and incidences of milk fever and hypocalcemia (Reinhardt and Conrad, 1980). High (0.64 percent versus 0.22 percent) dietary P reduced apparent absorption of magnesium (Mg) in pregnant dairy heifers (Schonewille et al., 1994).

Magnesium

Mg is a major intracellular cation that is a cofactor for enzymatic reactions in every major metabolic pathway. Extracellular Mg is vital to normal nerve conduction, muscle function, and bone mineral formation and is involved in Ca and P homeostasis. Low concentrations of serum Mg attenuate PTH release in response to low serum Ca (Takatsuki et al., 1980), and in humans and laboratory animals, low Mg status results in lower serum concentrations of 1,25-dihydroxyvitamin D and can result in vitamin D insensitivity and perhaps PTH insensitivity (Rude and Gruber, 2004; Sahota et al., 2006). The concentration of Mg in plasma of cows is normally between 0.75 and 1.0 mmol/L (1.8 and 2.4 mg/dL). In an adult cow, 60 to 70 percent of the body's Mg is in bone (200 to 250 g), a small amount is in the blood and other extracellular fluid (<4 g), and the remainder is inside cells (~90 g) (Storry and Rook, 1962). Bone is not a significant source of Mg that can be utilized in times of deficit. Maintenance of normal concentration of Mg in plasma is nearly totally dependent on absorption of dietary Mg.

Magnesium Requirement

A factorial approach was taken to describe the Mg requirements of dairy cattle.

Maintenance

Fecal loss of endogenous Mg was set at 0.3 g Mg/kg DMI as explained below. When cows display signs of clinical hypomagnesemia, urinary Mg loss is essentially zero, but for cows near the threshold of hypomagnesemia, urinary loss in adult dairy cows was approximately 0.0007 g Mg/kg BW (Schonewille et al., 2000b), which was set as the obligate urinary loss.

Growth

In heifers, the Mg content of the body decreases from about 0.65 g Mg/kg at birth to about 0.2 g/kg at 500 kg BW (Blaxter and McGill, 1956); therefore, the value of 0.45 g Mg/kg ADG used in the 2001 NRC is a reasonable average growth requirement.

Pregnancy

In pregnant animals, fetal-placental accretion of Mg is about 0.18 g/d in Holsteins from day 190 until the end of pregnancy (House and Bell, 1993). However, based on the Mg concentration in the body of a newborn calf (Blaxter and McGill, 1956), estimated accretion rate for Mg was about 0.3 g/d in late gestation. Considering the problems associated with hypomagnesemia at parturition, 0.3 g/d is used to describe the fetal requirement for Mg, and requirements are scaled to 715 kg maternal BW.

Lactation

Milk has an average Mg concentration of about 0.11 g (Hermansen et al., 2005; van Hulzen et al., 2009; Castillo et al., 2013). Colostrum contains about 0.38 g Mg/kg (see Chapter 12). Because cows have limited stores of labile Mg, diets for late-gestation cows must be formulated to provide adequate Mg for colostrum synthesis.

Summary of Equations (g absorbed Mg/d)

$$Maintenance = 0.3 \times DMI + 0.0007 \times BW$$
(Equation 7-9)

$$Growth = 0.45 \times ADG \quad \text{(Equation 7-10)}$$

$$Gestation \ (>190 \ d \ pregnant) = 0.3 \times (BW \ / \ 715)$$
(Equation 7-11)

$$Lactation = 0.11 \times Milk \quad \text{(Equation 7-12)}$$

where DMI, ADG, and milk are in kg/d, and BW is in kg.

Absorption and Dietary Requirements

Dietary requirements, not absorbed requirements, are generally similar to NRC (2001); however, the previous version included a substantial safety factor. If a similar safety factor was included, dietary requirements would be approximately 25 percent greater than the previous version.

Mg is absorbed primarily from the small intestine of young calves. As the rumen and the reticulum develop, they become the main site for Mg absorption (Pfeffer et al., 1970; Martens and Rayssiguier, 1980), but some absorption may

occur in the large intestine. In adult ruminants, the small intestine is a site of net secretion of Mg, but absorption may still occur in that site (Greene et al., 1983). Absorption of Mg from the rumen mostly occurs via two active mechanisms (Leonhard-Marek et al., 2010; Fach, 2015; Martens et al., 2018). One mechanism (potential difference-dependent uptake) is driven by an electrical gradient at the apical (luminal) membrane and is an active process inhibited by elevated potassium (K) concentrations in rumen fluid (Leonhard-Marek and Martens, 1996; Leonhard-Marek et al., 2010; Fach, 2015). This is a high-affinity, low-capacity transporter system. The second system (low affinity, high capacity) is driven by the Mg concentration gradient that can exist between the rumen contents and the epithelial cell and is independent of the electrical potential difference and not sensitive to K concentrations. This transport system is active and electrically neutral; therefore, it involves either cotransport of an anion (e.g., Cl^- or HCO_3^-) or an exchange with intracellular protons (Leonhard-Marek et al., 2010; Fach, 2015). The exact mechanism is not known at this time.

Factors Affecting Absorption

Absorption of Mg does not appear to be under any type of hormonal regulation; excess absorbed Mg is filtered by the kidney and excreted. A major driver of Mg absorption is the gradient between intracellular Mg and rumen contents. An increase in Mg intake usually linearly increases the concentration of Mg in rumen fluid, which usually increases apparent and calculated true absorption of Mg (Jittakhot et al., 2004a,b,c). However, Mg absorption might be saturable. Increasing dietary Mg concentrations above 1.1 percent continued to increase the concentration of soluble Mg in rumen fluid but did not increase apparent or true absorption of Mg by dry dairy cows (Jittakhot et al., 2004b).

Martens et al. (2018) reviewed Mg absorption by ruminants and antagonists to absorptions in great detail. Dietary K is a significant antagonist to Mg absorption because ruminal K disrupts the electrical gradient needed to drive Mg absorption (Fisher et al., 1994; Ram et al., 1998; Schonewille et al., 1999, 2008; Jittakhot et al., 2004c; Weiss, 2004). Inadequate intake of Na increases the concentration of K in rumen fluid (Bailey, 1961; Martens et al., 1987) and reduces absorption of Mg (Martens et al., 1987). However, once the Na requirement is met, dietary Na does not appear to affect Mg absorption. High dietary P concentration (ca. 0.6 percent) reduced apparent Mg absorption in heifers by about 18 percent (Schnewille et al., 1994), but within typical dietary concentrations, effects of dietary P are probably small.

Abrupt elevation in concentrations of ruminal ammonia reduces Mg absorption; however, chronic elevation (i.e., several days) did not affect Mg absorption (Gäbel and Martens, 1986). High concentrations of ruminal ammonia reduce the electrical potential, but the change probably is not great enough to affect Mg absorption. The adaption response suggests the involvement of inducible transport proteins, and alteration of the Na/proton pump has been implicated (Fach, 2015). Dietary changes that cause an abrupt increase in ruminal ammonia (e.g., initial turnout onto high-protein pasture) should be avoided; however, once animals are adapted, high ruminal ammonia does not appear to affect Mg absorption.

Rumen pH is negatively correlated with Mg solubility and under in vitro and other experimental situations, a small drop in pH within the normal physiological range (6.5 to 5.5) has increased Mg solubility by more than 50 percent (Dalley et al., 1997). When rumen pH was reduced by more realistic diet manipulation (i.e., increased starch concentrations), ruminal Mg concentrations increased (Schonewille et al., 2000a), but the effect was less consistent than with in vitro systems. Furthermore, the effect of ruminal pH on absorption of Mg was less dramatic than changes in solubility (Horn and Smith, 1978). In addition to Mg solubility, pH may have direct effects on Mg absorption systems. In cell culture experiments, Mg permeability through a protein channel increased markedly as pH decreased below 7 (Li et al., 2007). Increasing the dietary concentration of readily fermentable carbohydrates can increase apparent absorption of Mg. Adding 30 percent starch to a diet increased apparent Mg absorption by 50 percent (0.37 versus 0.24) or 28 percent (0.25 versus 0.20) when goats were fed low (0.8 percent) or high (3.4 percent) K diets, respectively (Schonewille et al., 1997). However, apparent Mg absorption did not differ when dairy cows were fed diets with 10 or 20 percent starch (Schonewille et al., 2000a). More data with cattle are needed before the effects of dietary starch can be modeled.

Supplemental dietary fat can reduce apparent digestibility of Mg, but the reduction was not related to the concentration of supplemental fat in the diet (Jenkins and Palmquist, 1984; Rahnema et al., 1994). Apparent Mg absorption decreased about 20 percent when cattle were fed diets that contained 2.5 to 5 percent added fat compared with control diets with no added fat. Supplementing up to 5 percent added fat from whole cottonseed did not affect apparent Mg absorption (Smith et al., 1981). Although data are limited, assuming a 20 percent reduction in absorption of Mg when supplemental fat is fed is recommended but was not included in the software. Feeding ionophores increased apparent absorption of Mg by beef cattle and dairy cattle by 10 to 28 percent when magnesium oxide (MgO) was fed (Greene et al., 1986a; Spears et al., 1989; Tebbe et al., 2018). However, monensin reduced absorption of Mg by 23 percent when magnesium sulfate was fed (Tebbe et al., 2018). Effects of monensin on Mg absorption are not included in the model.

The availability of Mg from MgO is affected by particle size (smaller particles enhance absorption), calcination temperature, and origin (Jesse et al., 1981; van Ravenswaay et al., 1989; Xin et al., 1989; Hemingway et al., 1998). Particle size also likely affects Mg availability from magnesium carbonate ($MgCO_3$), magnesium hydroxide ($Mg(OH)_2$), and dolomitic limestone.

TABLE 7-2   Description of Data Used to Generate Mg Equations[a]

	Mean	SD	Minimum	Maximum
Dry matter intake, kg/d	13.6	6.49	5.8	26.1
Diet K, g/kg	24.9	12.7	6.9	75.6
Diet Mg, g/kg	3.60	2.75	1.08	17.3
Mg intake, g/d	42.6	23.4	11.8	124.3
Supplemental Mg, percent of total Mg	27.0	28.1	0	90
True absorption of Mg	0.26	0.10	0.07	0.47

[a]Ninety-seven treatment means.

Quantifying Absorption

In NRC (2001), inadequate data were available for a rigorous evaluation of Mg absorption, but a substantial number of studies have since been published. However, quantitative estimates of the true absorption of Mg are still difficult to obtain because of the uncertainty regarding the daily loss of endogenous fecal Mg. Endogenous fecal Mg has been expressed relative to BW, and typical estimates were 2 to 5 mg Mg/kg BW (Greene et al., 1986b; NRC, 2001; Schonewille et al., 2008). However, saliva and digestive secretions are important contributors to endogenous fecal Mg, and these are related more to DMI than BW, especially when comparing across physiologic states (e.g., dry versus lactating cow). Therefore, data from two meta-analyses (Weiss, 2004; Schonewille et al., 2008) were used to estimate endogenous fecal Mg as a function of DMI. Dietary Mg (g/kg of diet DM) was regressed on concentration of apparently digested Mg (g/kg) with trial as a random effect, but because of the negative effect of K, only studies with dietary K ≤2 percent were used. The absolute value of the intercept, 0.3 g Mg/kg DMI, is an estimate of endogenous fecal Mg. In sheep, loss of endogenous fecal Mg was positively correlated with serum concentrations of Mg (Allsop and Rook, 1979). If this is true for dairy cattle, cows consuming less than adequate Mg could have a lower loss of endogenous fecal Mg than cows fed adequate Mg, but no adjustment was made to endogenous fecal Mg loss based on Mg status of the cow.

Adequate data were available to quantify the relationship between dietary K concentration and Mg absorption by dairy cows. Data from studies using heifers, dry cows, and lactating cows (Weiss, 2004; Holtenius et al., 2008; Schonewille et al., 2008) were combined (see Table 7-2). If the amount of supplemental Mg as a percentage of total diet Mg could not be calculated, the study was deleted. The final data set contained 97 treatment means from 23 studies. True absorption of Mg was calculated as described above, and only dietary K concentration and percentage of total Mg provided by supplemental sources (MgO was the source of supplemental Mg in all studies except for three) were statistically related to it. The effect of dietary K was not linear; transforming to the natural logarithm provided the best fit. The resulting equation (trial was included as a random effect) was

$$\text{True Mg absorption} = (44.1 - 5.42 \times \ln(K) - 0.08 \\ \times \text{Supplemental}) / 100 \quad \text{(Equation 7-13)}$$

where K is expressed as g/kg total diet and Supplemental = percentage of dietary Mg provided by MgO. Standard errors associated with the coefficients are 4.8, 1.54, and 0.034 for intercept, K, and supplemental coefficients, respectively.

A potential problem with this equation is the collinearity between dietary Mg concentration and supplementation ($r = 0.70$); however dietary Mg concentration was not statistically related with true absorption of Mg. Setting supplemental Mg at 0 and basal dietary K as 12 g/kg of diet DM (approximate K requirement), true absorption of Mg from basal diet = 0.31, which was assigned as the default for all feeds. Setting supplemental Mg at 100 percent and dietary K at 12 g/kg yields an estimate of 0.23 as the default availability for Mg from MgO, which is 26 percent lower than true absorption of Mg from basal feeds. This agrees with individual studies (van Ravenswaay et al., 1989; Davenport et al., 1990; Holtenius et al., 2008) in which apparent absorption of Mg was measured for diets with and without supplemental MgO and with <20 g of K/kg DM. In those studies, true absorption of Mg from MgO (calculated using the difference method) was 22 to 45 percent lower than the true absorption of Mg from the basal diet. The prediction error associated with Equation 7-13 is high (95 percent prediction interval associated with estimated ACs is + 0.16); users may wish to adjust ACs based on risk tolerance. In the previous NRC, the default AC for basal ingredients was reduced by 1-SD unit from the mean.

Absorption coefficients for common Mg supplements are in Table 19-3 (see Chapter 19). The default value for MgO reflects the average of the MgO used in the experiments; however, substantial variation exists among MgO sources, which can influence Mg availability as discussed above. High-quality MgO (e.g., small particle size and proper calcination procedures) may have greater availability than the default value. The proportion of particles <0.25 mm in MgO is positively correlated, and the proportion of particles >1.0 mm is negatively correlated with apparent absorption of Mg. Solubility of Mg from MgO in various solutions (water, citric acid, weak hydrochloric acid, buffered rumen fluid) is positively correlated with Mg absorption, but current data are not adequate to use solubility to quantify or adjust the ACs.

Few data are available for other Mg supplements. Relative to MgO, calculated true absorption of Mg was 1.7 times greater (van Ravenswaay et al., 1989) for magnesium sulfate ($MgSO_4$), about the same for $Mg(OH)_2$ (Davenport et al., 1990; Hemingway et al., 1998) and reagent-grade $MgCO_3$ (Ammerman et al., 1972), about 0.5 times for dolomite limestone (Gerken and Fontenot, 1967), and 0.2 times for magnesite (Ammerman et al., 1972). However, Tebbe et al. (2018) reported that in diets without monensin, apparent absorption of Mg when $MgSO_4$ was fed was only about 10 percent greater than that from MgO. Based on available data and because efficiency of Mg absorption differs between

sheep and cattle, data from dairy cows were given more weight than data from sheep, and the true absorption of Mg from $MgSO_4$ was assumed to be 20 percent greater than that from MgO. With monensin, apparent absorption of Mg when $MgSO_4$ was fed was about 30 percent lower than when MgO was fed (this effect is not included in the model). Data are not available for $MgCl_2$, but because of similar solubility to $MgSO_4$, they were assigned the same AC.

Magnesium Deficiency

A deficiency of Mg is of greater practical concern than deficiency of most other minerals because of the limited labile stores of Mg within the body and because of the commonly occurring antagonists of Mg absorption discussed above. A clinical deficiency of Mg results in muscle twitching, hyperexcitability, convulsions, and often death (Martens et al., 2018) and is commonly referred to as grass or lactation tetany because it often occurs in spring when cattle are first let out to graze, and it is more common in lactating than nonlactating cattle. The direct cause of clinical signs is low concentration of Mg in cerebrospinal fluid. Low concentrations of Mg in plasma (less than approximately 0.7 mmol/L) are not associated with any specific clinical signs but are a risk factor for clinical hypocalcemia (discussed in more detail in Chapter 12).

Maximum Tolerable Level

Cattle can excrete large amounts of Mg in urine, so Mg toxicity is not a practical problem in dairy cattle. Although an MTL of 0.6 percent has been established (NRC, 2005), negative effects in cattle have been observed only when dietary concentrations are >1 percent. The negative effects of high Mg are generally reduced feed intake, reduced diet digestibility, and osmotic diarrhea.

The Strong Ions: Sodium, Potassium, and Chloride

Na, K, and chloride (Cl^-) are completely dissociated in body fluids (Stewart, 1978) and are the major contributors to blood and cellular strong ion difference. Their relative concentrations in various body tissues are tightly regulated since they serve as osmoregulators that modulate water absorption and movement between extracellular and intracellular fluids and across the rumen and intestinal wall, and they have large impacts on systemic acid-base balance (Hu and Murphy, 2004). The dietary strong ions are absorbed with true absorptions of 0.9 or greater. Therefore, fecal strong ion excretion is primarily of metabolic origin. Regulation of strong ion balance occurs mostly via the kidney through urinary excretion. When cattle are fed typical diets, strong cation (K^+ and Na^+) excretion far exceeds strong anion (Cl^-) excretion. This results in increased urinary bicarbonate ion excretion to maintain electrochemical neutrality. Because of this, cattle and other ruminants generally excrete an alkaline urine (pH 7.5 to 8). When Cl^- is fed in excess of needs and insufficient cations (Na^+ and K^+) are available to balance excretion of Cl^-, there is a reduction in urinary bicarbonate excretion and urine pH decreases. Thus, shifts in the relative amounts of excess Na^+, K^+, and Cl^- that are excreted in the urine can have profound effects on acid-base status. Dietary cation–anion difference (DCAD), measured in mEq/kg diet DM, is a frequently used measure of the relative balance among the strong cations Na^+ and K^+ and strong anions (Cl^- and sometimes S^{-2}) (Ender et al., 1971; Mongin et al., 1981). DCAD is strongly associated with urinary pH (Constable et al., 2009) and acid-base status of the cow (Hu and Murphy, 2004) and is used in transition cow feeding to reduce incidence of hypocalcemia at calving (see Chapter 12).

Because strong ion intakes in excess of the requirements are excreted in the urine, urine volume and, correspondingly, water intake are directly related to strong ion intake. Bannink et al. (1999) showed a direct linear relationship between urine volume and strong ion intake exists in lactating cows. The increased urine volume dilutes the nitrogen (N) concentration in urine. Correspondingly, increasing dietary sodium chloride (NaCl) (Spek et al., 2012) and potassium sesquicarbonate (Iwaniuk et al., 2014) linearly decreases milk urea N concentrations.

Fecal Sodium, Potassium, and Chloride

Ruminants evolved consuming forages that were high in K (>20 g K/kg DM), low in Na (≤1 g Na/kg DM), and moderate in Cl (3 to 6 g Cl/kg DM). Therefore, their requirements reflect the differences in relative K, Na, and Cl concentrations of feeds. Dairy cow feces contain approximately 85 percent water. Fecal water output was strongly related to the sum of Na, K, and Cl fecal excretion when expressed on an equivalent weight basis in 122 balance experiments with dairy cows with a mean fecal strong ion excretion rate of 3.47 (± 1.24) equivalents per day, where Fecal H_2O, L/d = 15.5 (± 1.78) + 5.88 (± 0.385) × Fecal Strong Ions (Eq/d); RMSE = 3.89; R^2 = 0.861; $P < 0.001$. Because of the relationship between strong ion and fecal water excretion, the committee suggests that metabolic fecal requirements for Na, K, and Cl are likely due to the need to maintain osmotic balance and consistent fecal moisture content.

Sodium

Cattle evolved on feeds that are low in Na; hence, they developed efficient absorptive processes and a tenacious ability to conserve Na via the kidney, but they have only a small reservoir of Na in a form that is readily available for metabolism.

Physiologic Roles

Na is the primary extracellular cation (Aitken, 1976). In addition, 30 to 50 percent of total body Na is in a

nonexchangeable fraction in the crystalline structure of bone (Eldman et al., 1954). The exchangeable fraction of Na modulates extracellular fluid volume and acid-base equilibrium (Stewart, 1983; McKeown, 1986). Heart function and nerve impulse conduction and transmission are dependent on the proper balance of Na and K. Na also plays an indispensable role in sodium–potassium adenosine triphosphate enzyme (Na-K ATPase) responsible for creating electrical gradients for nutrient transport. The Na–K pump is essential for all eukaryotic cells, enabling transport of glucose, amino acids (AAs), and phosphate into cells and hydrogen (H), Ca, bicarbonate, K, and Cl ions out of cells (Lechene, 1988). Sodium bicarbonate ($NaHCO_3$) is a major component of saliva that helps buffer acids produced during rumen fermentation (Erdman, 1988).

Typical Na concentrations in blood plasma are 150 mEq/L and 160 to 180 mEq/L in saliva. Na is the predominant cation in rumen fluid with a typical content of 80 to 90 mEq/L, but the range can be from 50 to 140 mEq/L (Bennink et al., 1978; Catterton and Erdman, 2016). Ruminal concentrations of Na and K are strongly negatively correlated (Catterton and Erdman, 2016). Increased dietary K results in increased rumen K concentrations, which stimulate Na absorption across the rumen wall and reduce rumen Na concentration to maintain electrical and osmotic neutrality (Martens and Blume, 1987).

Sodium Utilization and Homeostasis

Absorption occurs throughout the digestive tract, and dietary Na generally is assumed to be almost completely available. Absorption occurs by an active transport process in the reticulorumen, abomasum, omasum, and duodenum. Passive absorption also occurs through the intestinal wall, so there is a tendency toward equal concentrations in intestinal and fecal fluids. However, substantial active absorption against a sizable concentration gradient occurs in the lower small intestine and large intestine (Renkema et al., 1962).

Na and K can interchange such that in Na-deficient animals, K excretion is increased, providing a mechanism that helps ensure that ruminants can subsist on feeds low in Na over long periods of time. Na concentrations in blood and tissues are maintained principally via reabsorption and excretion by the kidneys. Excretion of Na, K, and Cl⁻ is closely synchronized. Na is the central effector of ion excretion, and changes in renal reabsorption are chief determinants of Na excretion. Endocrine control via tissue receptors and the renin–angiotensin system, aldosterone, and atrial natriuretic factor monitor and modulate Na concentrations in various tissues, which consequently control fluid volume, blood pressure, K concentrations, and renal processing of other ions. When cattle are depleted of Na, salivary glands decrease secretion of Na in saliva. The decrease in Na content is replaced reciprocally by nearly the same concentration of K (van Leeuwen, 1970; Morris and Gartner, 1971).

Requirement for Absorbed Sodium

Maintenance

The factorial method was used to derive the absorbed Na requirement. The maintenance requirement for absorbed Na is equal to the inevitable losses in feces and urine of animals fed very near their true requirement. In the review of the literature on Na, the 2001 NRC committee recognized that previous suggested maintenance requirement (0.015 g Na/kg BW) used for growing heifers and mature cows would be insufficient for lactating cows and would result in clinical signs of deficiency or reduced milk yields. Therefore, the maintenance requirement was empirically set for mature cows at 0.038 g Na/kg BW.

Urinary excretion of Na is dependent on the relative excretion rates of the other strong ions (K^+ and Cl^-) to maintain electrochemical neutrality in the urine and the acid-base balance of the cow. Because of these interrelationships, it is not possible to develop a consistent estimate of endogenous urinary excretion of Na or strong ions. Therefore, the committee's estimate of the maintenance requirement is based solely on the inevitable losses of Na in feces. Metabolic fecal excretion of Na was estimated from the results of 137 individual Na digestibility measurements from eight experiments in which cows were fed diets ranging from 0.27 to 1.17 percent Na. The metabolic fecal requirement was determined by regression of absorbed Na on Na intake, both expressed as grams per kilogram (g Na/kg) of diet DM. The resulting regression equation was as follows: Absorbed $Na = -1.45$ (± 0.25) $+ 0.98$ (± 0.036) Na Intake; RMSE $= 0.52$; $R^2 = 0.91$; $P < 0.001$. Metabolic fecal Na equals 1.45 g/kg DMI. The slope was not different from 1; therefore, absorption efficiency is assumed to be 1.0 (see below).

This maintenance requirement was adopted for both growing and lactating animals. For a 650-kg cow consuming 28 kg of feed DM, the metabolic fecal requirement for Na would be 41 g of dietary Na/d. This is a higher but also a more theoretically based value compared with the previous 2001 NRC estimate of 30 g/d for a lactating cow of the same size (0.038 g Na/kg BW × 700 kg/0.90 AC). A 300-kg growing animal consuming 7 kg DM/d would have a maintenance requirement of 10 g/d (0.145 percent of diet DM) or double the maintenance requirement of 4.5 g from the 2001 NRC report. Maintenance requirements for dry cows are also about doubled compared with the previous version. A review of Na requirements for beef cattle suggested that Na requirements for lactating and growing beef cattle were 0.10 and 0.07 percent of diet DM, respectively (Morris, 1980). Since osmoregulation in the feces is being used as the basis for the new maintenance requirement and K can replace Na in that role, diets with lower Na concentrations (0.07 percent) can likely be fed without affecting animal performance, assuming that the diet contains more than adequate amounts of K.

Effect of Environmental Temperature

Sweating, for aid in heat balance, includes secretion of Na (Jenkinson and Mabon, 1973) and other electrolytes. Based on the Agricultural Research Council (ARC, 1980) recommendations, the previous committee suggested that an additional increment of 0.10 and 0.50 g Na/100 kg BW be fed to animals maintained at ambient temperatures of 25°C to 30°C and >30°C, respectively. For a 700-kg cow, this would translate into an additional 0.7 and 3.5 g/d of absorbed Na for cows housed at 25°C to 30°C and >30°C, respectively. The losses of electrolytes in sweat are dependent on an animal's sweating rate and the concentration of minerals in the sweat, which have been shown to change with the rate of secretion (Sonner et al., 2015). In humans, Na^+ and Cl^- content increases from 30 to 90 mEq/L as sweating rate increases, whereas K^+ content decreases from 20 to 5 mEq/L (Sonner et al., 2015). There are little reliable data on the composition of sweat in cattle. Jenkinson and Mabon (1973) suggested that Na and Cl excretion rate decreased in relation to K in Ayrshire cattle, but reevaluation of their data suggested that there were no changes in sweat composition in animals that were actually heat stressed (>25°C, temperature humidity index [THI] ≥72). The mean Na^+ concentration was 0.25 g/L (11 mEq/L).

Jenkinson and Mabon's (1973) Na excretion data were fit to an exponential equation related to THI, where Na excretion, g per/M^2/d $= 0.198e^{0.044 \times THI}$, $R^2 = 0.9687$. Using surface area calculated by the Brody (1945) equation ($M^2 = 0.147 \times BW^{0.56}$), a 700-kg cow would be expected to have a surface area of 5.8 m^2. Combined with the predicted Na excretion rate per unit body surface area, the expected Na excretion in sweat was small (0.64 to 1.10 g) per day in cows housed between 25° and 35°C and a THI from 72 to 85. The measured sweating rates in Jenkinson and Mabon's (1973) study ranged from 5 to 66 mL/m^2/h (0.12 to 1.9 L/d). This is similar to the more recently reported range of sweating rates observed in lactating Holstein cows under heat stress with either a shade cloth (19 to 33 g/m^2/h) (Dikmen et al., 2014) or evaporative cooling (5 to 25 g/m^2/h) (Dikmen et al., 2015).

Those sweating rates are on the very low end of the reported range (14 to 600 g/m^2/h) in a meta-analysis of sweating rates in cattle (Thompson et al., 2011). This suggests that actual Na loss in sweat could be as much as 5- to 10-fold greater in heat-stressed cows, assuming the Na content in sweat does not change. Based on reported sweating rates in dairy cows during heat stress that was abated by evaporative cooling (Dikmen et al., 2015), Na losses in sweat would be minimal. The committee emphasizes the need for more reliable data using simultaneously measured sweating rates and sweat composition before a Na requirement during heat stress can be established. No provision is provided in the model to do so.

Growth

The requirement of absorbed Na for growth was set at 1.4 g/kg of ADG for animals weighing between 150 and 600 kg live BW (Gueguen et al., 1989).

Pregnancy

Slaughter data from 18 multiparous pregnant Holstein cows were used to quantify the requirement for absorbed Na of the conceptus during the last trimester (House and Bell, 1993). Requirements for all mineral elements are negligible to about 190 days of gestation. The Na requirement of the conceptus is 1.4 g/d × (BW / 715) from 190 to 270 days of gestation (the BW term scales values to the average BW in that study) but should not be used to compute the Na requirement for days of gestation <190 (House and Bell, 1993).

Lactation

The previous report set the absorbed Na requirement for milk at 0.65 g/kg, which was based on the average Na concentration in milk from several studies (0.63 g/kg) as reported by the ARC (1965). However, the weighted average milk Na concentration summarized across several more recent studies (Fisher et al., 1994; Sanchez et al., 1994a,c, 1997; Silanikove et al., 1997; Kume et al., 1998; Robinson et al., 2002; van Hulzen et al., 2009; Castillo et al., 2013; Khelil-Arfa et al., 2014; Visentin et al., 2016) was 0.41 (± 0.037) g Na/kg, nearly 40 percent lower than the previous 2001 NRC value. Milk Na is related to incidence of mastitis and increases with elevated milk somatic cell count (SCC) (Harmon, 1994). With greatly improved management techniques for prevention of mastitis, milk Na concentrations would be expected to have decreased during the past 50 years. The absorbed Na requirement for lactation was set at 0.4 g/kg milk.

Summary of Equations (g absorbed Na/d)

$$Maintenance = 1.45 \times DMI \quad \text{(Equation 7-14)}$$

$$Growth = 1.4 \times ADG \quad \text{(Equation 7-15)}$$

$$Gestation\ (>190\ d\ pregnant) = 1.4 \times (BW\ /\ 715) \quad \text{(Equation 7-16)}$$

$$Lactation = 0.4 \times Milk \quad \text{(Equation 7-17)}$$

where DMI, ADG, and milk are in kg/d, and BW is in kg.

Dietary Requirement and Efficiency of Absorption

The regression coefficient of 0.98 for absorbed Na versus dietary Na was not statistically different from 1, implying

that true absorption is 100 percent. The previous committee (NRC, 2001) set the Na absorption rate at 90 percent, which seemed too low given the ruminant animals' ability to survive on extremely low Na diets. In addition, Na from typical feeds is solubilized and released in the liquid matrix of digesta and is readily available for absorption. Feedstuffs commonly used in diets for dairy cattle do not contain enough Na to meet requirements, and supplemental sources typically account for the majority of an animal's total Na intake.

NaCl is the most often used supplement, and its Na is considered 100 percent available. The efficiency of absorption of Na from other salts (e.g., $NaHCO_3$, sodium carbonate [Na_2CO_3], sodium sesquicarbonate [$Na_3H(CO_3)_2$]) is also considered essentially 100 percent. When some animal by-product feedstuffs containing bone are fed, Na would be less available as it is tightly bound in the crystalline structure. However, these sources represent rare circumstances and are minor Na sources compared to typical supplemental Na salts. Therefore, the committee set the AC for Na at 1.00 for all feeds.

For a 650-kg cow consuming 28 kg/d of feed DM, the metabolic fecal requirement for Na (28 kg × 1.45 g Na/kg) would be 41 g of dietary Na/d. The milk production requirement for a cow producing 45 kg/d milk would be 18 g/d (45 kg milk × 0.4 g Na/kg in milk) for a total Na requirement of 59 g/d or 0.21 percent Na in the diet DM. This compares with the previous requirement of 36 g (50 × 0.65 / 0.90) for milk production and 27 g (700 × 0.038 / 0.90) for maintenance for a total of 63 g Na/d (0.25 percent of diet DM). While the maintenance requirement for Na has increased, the reduced requirement for Na in milk more than compensated, such that total Na requirements are slightly lower than in the 2001 NRC.

Lactational Responses to Varying Dietary Sodium Concentrations

Kemp and Geurink (1966) reported that 0.14 percent Na in grazed forage was sufficient to support more than 30 kg of milk production per day. However, feeding lactating dairy cows a diet with no supplemental NaCl (0.16 percent Na, dry basis) resulted in marked depressions in DMI and milk yield after just 1 to 2 weeks of feeding (Mallonee et al., 1982a). Empirical modeling of data from 15 experiments with lactating cows conducted in either cool or warm seasons suggested that DMI and milk yield were improved by dietary concentrations of Na well above those needed to meet requirements (Sanchez et al., 1994b,c). DMI and milk yield responses over a range of dietary Na concentrations (0.11 to 1.20 percent, dry basis) were curvilinear, with maximum performance at 0.70 to 0.80 percent Na. Concentrations of Na, K, Cl^-, Ca, and P in diet DM ranged from below those needed to meet requirements to concentrations considerably higher. Thus, there is a potential confounding between Na and DCAD, and it is not known whether the optimal Na concentration would vary if

the dietary concentrations of other macrominerals would have been closer to requirements. There were interactions of Na with K, Cl^-, and P on DMI, indicating that responses to Na differed over the range of dietary concentrations to those minerals. In addition, interactions of dietary Na with K, Cl^-, and P on DMI differed in experiments conducted in the cool or warm season. In hot weather, milk yield and DMI increased when Na increased from basal (0.18 percent Na, dry basis) to 0.55 percent dietary Na with either NaCl or $NaHCO_3$; Cl^- was equalized among diets (Schneider et al., 1986).

Little evidence exists for a Na-by-K interaction when dietary Cl^- was held constant, and Na and K were fed at or above their estimated requirement. Na and K were equally effective when dietary anion cation difference was increased by addition of either cation. In only one study (Iwaniuk and Erdman, 2015) was Na more effective than K in maintaining milk fat, but cation had no effect on milk yield or intake. In other experiments (West et al., 1992; Sanchez et al., 1997; Hu and Kung, 2009), the K/Na ratio did not affect intake or milk production. Wildman et al. (2007) showed a quadratic effect of the K/Na ratio on milk production but no effect on intake or milk composition.

Sodium Deficiency

Babcock (1905) fed a diet very low in Na to dairy cows and described intense craving for salt, licking and chewing various objects, and general pica. Deficiency signs were manifested within 2 to 3 weeks. Na deficiency signs may not develop for weeks to months, depending on rate of milk production. However, feed intake and milk yield began to decline 1 to 2 weeks after cows were fed a diet without supplemental NaCl (0.16 percent Na), and pica and drinking of urine of other cows were observed (Mallonee et al., 1982a). Although dietary Cl^- concentration was not measured in that study, potassium chloride (KCl) was supplemented (1.0 percent total dietary K), so Cl^- deficiency was probably not the cause of the condition. The condition was reversed quickly by inclusion of NaCl in the diet. Other deficiency signs include loss of appetite; rapid loss of BW; an unthrifty, haggard appearance; lusterless eyes; and rough hair coat (Underwood, 1981). More extreme signs of deficiency include incoordination, shivering, weakness, dehydration, and cardiac arrhythmia leading to death.

Free-Choice Feeding of Sodium Chloride and Sodium (Sodium Chloride) Toxicity

Cattle consume salt liberally when it is available. Smith et al. (1953) found that lactating cows consumed more salt when provided free-choice in granular versus block form, but consumption of block was sufficient to meet needs for lactation. Demott et al. (1968) fed lactating cows 4 percent NaCl in a grain mix at 1 kg of grain for each 2 kg of 4 percent fat-corrected milk yield for 2 weeks without ill effects on milk

yield, BW, or general health. Although total DMI was not measured, the Na concentration of the total diet DM would have been about 0.8 to 1.0 percent. High intake of NaCl can increase the incidence and severity of udder edema (Randall et al., 1974). Feeding diets with 0.88 percent Na from NaCl or $NaHCO_3$ to mid-lactation Holstein cows did not cause toxicity or reduce feed intake and milk yield compared with 0.55 percent Na (Schneider et al., 1986).

A major factor influencing the degree of exhibition of NaCl toxicosis is the availability and quality of drinking water. Extensive discussion of the effects of high Na and Cl^- concentrations in drinking water is provided in Chapter 9. NRC (2005) set the MTL of NaCl at 3 percent of diet DM for lactating cattle and 4.5 percent for growing cattle.

Chloride

The requirements for Cl^- for various classes of dairy cattle are the least studied of any strong ion. Nonetheless, its physiologic roles and interrelationships with Na and K are extremely important. Typically, Cl^- is provided in the diet as NaCl, which is solubilized, releasing the negatively charged Cl^- ion for absorption. Cl^- is functionally important because of its propensity to accept electrons during metabolism.

Physiologic Roles

Cl^- is the major anion in the body involved in regulation of osmotic pressure, making up more than 60 percent of the total anion equivalents in the extracellular fluid. As a strong anion, it always is dissociated in solution. It is essential for transport of carbon dioxide and oxygen, the chief anion in gastric secretions, and accompanied by H^+ in nearly equivalent amounts. It is needed for activation of pancreatic amylase, and chlorinated compounds are produced by some phagocytic cells to kill pathogens. Typical concentrations of Cl^- are from 90 and 110 mEq/L in blood plasma and 10 to 30 mEq/L in ruminal fluid. The concentration of Cl^- in cattle was estimated to be about 1.2 to 1.4 g/kg over the range of 100 to 500 kg empty body weight (EBW; ARC, 1980).

Utilization and Homeostasis

About 80 percent of the Cl^- entering the digestive tract arises from digestive secretions in saliva, gastric fluid, bile, and pancreatic juice. Cl^- is absorbed throughout the digestive tract. It, like Na, is absorbed mainly from the upper small intestine by passive diffusion following Na along an electric gradient. Cl^- is transported across the ruminal wall to blood against a wide concentration gradient (Sperber and Hyden, 1952). Martens and Blume (1987) showed that Cl^- was cotransported actively with Na across the rumen wall, although the exact mechanism is unclear. Substantial absorption of Cl^- from gastric secretions (hydrochloric acid) occurs in the distal ileum and large intestine by exchange with secreted

bicarbonate. Appreciable quantities of Cl^- are excreted in the feces, in part to maintain osmatic balance along with the other strong ions (Na^+ and K) to maintain fecal moisture content. In the short term, relatively large day-to-day differences in dietary intake of Cl^- have little effect on the total Cl^- entering the digestive tract. Much smaller amounts of Cl^- are lost in sweat mainly as NaCl or KCl. Cl^- fed in excess of needs for maintenance and milk production is primarily excreted in the urine.

Tight regulation of the concentration of Cl^- in extracellular fluid and its homeostasis is coupled intimately to that of Na. The role of Cl^- in maintaining ionic and fluid balance was thought to be passive to that of Na and K. However, Fettman et al. (1984b) showed that during Cl^- deficiency, the ion functioned independently to mediate Cl^- conservation. Cl^- was conserved by reducing excretion by the kidney, as well as in feces and milk. Excess Cl^- intake is excreted mainly in urine of steers and sheep (Nelson et al., 1955), but in lactating cows, a significant amount of Cl^- is excreted via feces (Coppock, 1986). Normally, anion concentration in extracellular fluid is regulated secondarily to cation concentrations, and when the amount exceeds reabsorption capability of the kidney, excess Cl^- is excreted in urine (Hilwig, 1976). Cl^- excretion is tied to excretion of strong cations, acid-base balance, and maintenance of electrochemical neutrality of the urine (Stewart, 1981). Under normal circumstances, excess cations are secreted in conjunction with Cl^-, and bicarbonate ion excretion increases to maintain electrochemical balance, resulting in alkaline urine. If bicarbonate or electrolyte cations need to be conserved in relation to Cl^-, Cl^- excretion is accompanied by ammonium ions and urine pH decreases to maintain systemic acid-base balance.

Requirement for Absorbed Chloride

Maintenance

The factorial method was used to derive the absorbed Cl^- requirement. In the previous report (NRC, 2001), the maintenance requirement for Cl^- was set at 2.25 g/100 kg BW. This requirement was based on the suggestion that inevitable endogenous losses of Cl^- in feces and urine on a mass basis are about 50 percent higher than that of Na (Gueguen et al., 1989), but no experimental evidence for that was given. Fettman et al. (1984b) showed that urinary excretion of Cl^- was minimal (<2 g/d) during Cl^- deficiency. Urinary excretion of Cl^- is dependent on the relative urinary excretion rates of K and Na (Stewart, 1981) such that it is not possible to develop a consistent estimate of endogenous urinary excretion of Na.

With the relationship between fecal water excretion and total strong ion excretion (see Na discussion), the committee's estimate of the maintenance requirement for Cl^- is based on inevitable losses in feces. Metabolic fecal excretion of Cl^- was estimated from the results of 144 individual Cl^- digestibility measurements from nine experiments in

which cows were fed diets ranging from 0.25 to 0.61 percent Cl^-. The metabolic fecal requirement was determined by regression of apparently absorbed Cl^- on Cl^- intake, both expressed as g Cl^-/kg of feed DM. The resulting equation was as follows: Absorbed $Cl^- = -1.11$ ($\pm$ 0.25) $+ 0.92$ ($\pm$ 0.075) Cl^- Intake; RMSE $= 0.52$; $R^2 = 0.87$; $P < 0.001$. This equation indicates a metabolic fecal requirement of 1.11 g Cl^-/kg diet DM (0.11 percent Cl^- in the diet DM) and an average AC of 0.92.

Using an AC of 92 percent, a 700-kg cow consuming 25 kg of feed DM would have a metabolic fecal Cl^- requirement of 30.1 g/d. This value is more theoretically grounded but is also 76 percent greater than the NRC (2001) estimate for maintenance of 17.5 g/d.

Effect of Environmental Temperature

No provision was provided for Cl^- losses in sweat during heat stress in the 2001 NRC. Reexamination of the data of Jenkinson and Mabon (1973) with Ayrshire calves suggested that the mean Cl^- concentration in sweat was 0.28 g/L. Using the surface area calculations and the sweating rates described for Na, an estimated Cl^- excretion rate is $0.198e^{0.045 \times THI}$ ($R^2 = 0.93$), suggesting that Cl^- losses are similar to Na. The projected Cl^- losses would be 0.7 to 1.2 g/d for a 700-kg cow exposed to a THI ranging from 72 to 85. These losses are small and subject to substantial uncertainty; therefore, effects of temperature were not included in the model. Assuming a 5-fold increase in actual sweating rate reported by Thompson et al. (2011) compared to those by Jenkinson and Mabon (1973), Cl^- losses would be much greater. More reliable data on sweating rates and the Cl^- concentration in sweat are needed to establish a requirement for Cl^- losses during heat stress. The loss would be negligible when effective heat abatement technologies are used.

Growth

For cattle with BW between 150 and 600 kg, the requirement for absorbed Cl^- for growth was set at 1.0 g Cl^-/kg of ADG (Gueguen et al., 1989).

Pregnancy

No research is available to directly establish the requirement for absorbed Cl^- for pregnancy. However, based on consideration of the daily Na accretion rate of the conceptus and the fetus separately (House and Bell, 1993), and assuming that the relative proportions of Cl^- and Na in the fetus and in a newborn calf (41.5 percent Cl^- and 58.5 percent Na; ARC, 1980) are similar, Adequate Intake (AI) for pregnancy from 190 days of gestation to parturition was set at 1.0 g/d $\times$ (BW / 715). The average BW in House and Bell (1993) was 715 kg, and requirements are scaled to that.

Lactation

Cl^- exists in milk almost entirely as the free ion (Holt, 1985). Cl^- is highest in colostrum, declines rapidly to average concentrations after lactation commences, and increases toward the end of lactation (Flynn and Power, 1985). The previous report set the absorbed Cl^- requirement for milk at 1.15 g Cl/kg based on the average Cl^- concentration in milk from several studies reported by ARC (1965). Milk Cl^- is strongly related to incidence of mastitis and is elevated in cows with high milk SCC (Harmon, 1994). With improved management techniques for prevention of mastitis, milk Cl^- concentrations would be expected to have decreased during the past 50 years. The weighted average milk Cl^- concentration across several more recent studies (Fisher et al., 1994; Sanchez et al., 1994a, 1997; Silanikove et al., 1997; Kume et al., 1998; Robinson et al., 2002; van Hulzen et al., 2009; Castillo et al., 2013; Khelil-Arfa et al., 2014; Visentin et al., 2016) was 0.97 ($\pm$ 0.06) g Cl/kg. Therefore, the absorbed Cl^- requirement for milk production was set at 1.0 g Cl/kg.

Summary of Equations (g absorbed Cl^-/d)

$$\text{Maintenance} = 1.11 \times \text{DMI} \quad \text{(Equation 7-18)}$$

$$\text{Growth} = 1.0 \times \text{ADG} \quad \text{(Equation 7-19)}$$

$$\text{Gestation (>190 d pregnant)} = 1.0 \times (\text{BW / 715}) \quad \text{(Equation 7-20)}$$

$$\text{Lactation} = 1.0 \times \text{Milk} \quad \text{(Equation 7-21)}$$

where DMI, ADG, and milk are in kg/d, and BW is in kg.

Dietary Requirement and Efficiency of Absorption

Little research has been done in ruminants to measure the true AC for Cl^- principally due to the widespread availability of good, inexpensive inorganic sources (e.g., NaCl). Cl^- from inorganic sources and common feedstuffs is freely released into the liquid phase of the digesta and readily absorbed (Underwood, 1981). Apparent absorption of Cl^- in lactating cows fed fresh forage ranged from 71 to 95 percent and averaged 88 percent (Kemp, 1966). This is comparable to other estimates of absorption efficiency of 85 to 91 percent in cattle and sheep fed mixed diets (ARC, 1980). Paquay et al. (1969b) found that apparent absorption of Cl^- was not influenced by intake of Cl^- but was correlated negatively with intakes of DM, energy, and pentosan, as well as positively correlated with intakes of K and N. Factors such as lactation, pregnancy, and growth affecting the requirement for Cl^- do not appear to alter the efficiency of Cl^- absorption. Overall, the absorption efficiency for Cl^- in ingredients commonly fed to dairy cattle is usually ≥ 90 percent (Henry, 1995b).

The committee estimated AC from 144 Cl⁻ balance studies was 92 percent. Therefore, an AC for Cl⁻ of 0.92 was assigned for all dietary ingredients.

For a 650-kg cow consuming 28 kg/d DMI and producing 45 kg/d milk, the new requirement for Cl⁻ includes 34 g/d for maintenance (28 kg DMI×1.11 / 0.92) plus 49 g Cl⁻ required for milk production (45 kg milk×1 / 0.92) for a total of 83 g dietary Cl⁻. This compares with the previous dietary Cl⁻ requirement of 81 g/d (NRC, 2001). While the maintenance requirement has increased, milk production requirements have decreased such that the total Cl⁻ requirement for lactating cows has changed little compared to the previous NRC.

Lactation and Growth Responses to Varying Dietary Chloride

Coppock (1986) reviewed the estimated requirements of dietary Cl⁻ for lactating dairy cows in studies in which milk production ranged from 24 to 32 kg/d. Holstein cows fed a diet with 0.18 percent Cl⁻ conserved Cl⁻ by dramatically reducing excretion of Cl⁻ in urine and feces and tended to reduce Cl⁻ output in milk; however, intakes of feed and water, as well as milk yield and composition, did not differ from cows fed 0.40 percent Cl⁻ (Coppock et al., 1979). Half of the cows in each treatment group had free access to a trace-mineral salt block, and cows fed the diet low in Cl⁻ consumed more of the salt block. Fettman et al. (1984a) fed diets containing 0.10, 0.27, and 0.45 percent Cl⁻ for the first 8 to 11 weeks of lactation. Cows fed 0.10 percent Cl⁻ rapidly exhibited clinical signs of Cl⁻ deficiency and poor performance compared with those fed medium and high concentrations of dietary Cl⁻. Health, feed intake, and yield and composition of milk by cows fed the medium and high concentrations of dietary Cl⁻ were similar. Empirical models with a large data set showed that increasing dietary Cl⁻ over a range of 0.15 to 1.62 percent decreased DMI and milk yield of mid-lactation cows (Sanchez et al., 1994b). The negative effects of increasing dietary Cl⁻ were more dramatic in hot summer weather than in winter. This is consistent with the results of Escobosa et al. (1984) showing profound exacerbating effects of high dietary Cl⁻ on acid-base balance (metabolic acidosis) and lactation performance during heat stress. Adding Cl⁻ to the diet when the other strong ions (Na⁺ and K⁺) are held constant reduces DCAD, and the decrease in DCAD was likely the cause of the negative effect of high Cl⁻ (discussed in the DCAD section below).

Feeding diets with 0.038 percent Cl⁻ for 7 weeks to male Holstein calves did not produce clinical deficiency or depress feed intake, growth rate, or digestibility of feed compared with calves fed 0.50 percent Cl⁻ (Burkhalter et al., 1979). Calves fed the low Cl⁻ diet adapted by reducing urinary excretion of Cl⁻, and their water intake and urine output were greater than that of calves fed more Cl⁻. Calves fed a low Cl⁻ (0.038 percent) diet developed mild alkalosis, but it did not affect growth, and calves adapted to the low intake of Cl⁻ (Burkhalter et al., 1980).

If NaCl is used to meet the Na requirement, generally the Cl⁻ requirement is met or exceeded. However, if NaHCO₃ or some other Na-containing salt is used to supply Na, it may be necessary to meet the Cl⁻ requirement with another supplement (e.g., KCl). Research is needed to establish more accurate requirements and appropriate dietary concentrations of Cl⁻ (and Na) for all classes of dairy cattle. If current estimates are too high, it could contribute to soil salinity when manure is applied (Coppock, 1986). Cl⁻ in drinking water also may make a major contribution to intake of Cl⁻. In a survey of 39 California dairy herds by Castillo et al. (2013), inclusion of the Cl⁻ in water increased estimated total Cl⁻ intake by 6.5 percent.

Chloride Deficiency

Cl⁻ deficiency was created in young calves (100 kg BW) by feeding a diet with 0.063 percent Cl⁻ and removing about 600 g of abomasal contents daily (Neathery et al., 1981). Clinical signs were anorexia, weight loss, lethargy, mild polydipsia, and mild polyuria. In latter stages, severe eye defects and reduced respiration rates occurred, and blood and mucus appeared in feces. Deficiency of Cl⁻ resulted in severe alkalosis and hypochloremia, which manifested in secondary hypokalemia, hyponatremia, and uremia. Control calves also had abomasal contents removed daily but were fed a diet with 0.48 percent Cl⁻, and they grew normally and showed no signs of deficiency. During the first 8 to 11 weeks of lactation, dairy cows fed low (0.1 percent, dry basis) Cl⁻ exhibited dramatic and progressive declines in intakes of feed and water, BW, milk yield, and electrolyte concentrations in blood serum, saliva, urine, milk, and feces (Fettman et al., 1984b).

A significant decline of Cl⁻ in blood serum was found within 3 days after switching cows from a diet containing 0.42 percent to a diet with 0.10 percent Cl⁻ (Fettman et al., 1984b). Clinical signs of deficiency were depraved appetite, lethargy, hypophagia, emaciation, hypogalactia, constipation, and cardiovascular depression. Metabolic alterations were severe primary hypochloremia, secondary hypokalemia, and metabolic alkalosis (Fettman et al., 1984a,b,c). Cl⁻ deficiency, resulting from an inadequate dietary supply or loss of gastric juices, can lead to alkalosis due to an excess of bicarbonate, because inadequate Cl⁻ is partially compensated for by bicarbonate.

Chloride Toxicity

High systemic concentrations of Cl⁻, in the absence of a neutralizing cation (e.g., Na⁺), can cause disturbance of normal acid-base equilibrium (Stewart, 1981; Escobosa et al., 1984), but the maximum tolerable concentration of Cl⁻ in the diet has not been determined. The maximum tolerable concentration of dietary NaCl was set at 3.0 percent (dry basis) for lactating dairy cows and 4.5 percent for growing cattle (NRC, 2005).

Potassium

Physiologic Roles

K is the third most abundant mineral in the body. It must be supplied daily because there is little storage in the body and the animal's requirement for K is high. K is involved in osmotic pressure and acid-base regulation, water balance, nerve impulse transmission, muscle contraction, and oxygen and carbon dioxide transport; as an activator or cofactor in many enzymatic reactions; in cellular uptake of AAs and synthesis of protein; in carbohydrate metabolism; and in maintenance of normal cardiac and renal tissue (Stewart, 1981; Hemken, 1983). It is the major intracellular electrolyte with concentrations in the range of 150 to 155 mEq/L. In contrast to Na^+ and Cl^-, extracellular concentrations of K^+ are low (about 5 mEq/L). Saliva typically contains <10 mEq/L, whereas concentrations in ruminal fluid range from 40 to 100 mEq/L (Bennink et al., 1978; Catterton and Erdman, 2016). Blood plasma contains 5 to 10 mEq/L. The vast majority of K in blood is located within red blood cells (Aitken, 1976; Hemken, 1983). About 80 percent of the K in the body is associated with lean tissue and bone. Gastrointestinal contents account for an additional 15 percent of body K and are affected by the K content of the diet (Belyea et al., 1978).

Potassium Utilization and Homeostasis

K is absorbed primarily in the duodenum by simple diffusion, and some absorption occurs in the jejunum, ileum, and large intestine. The main excretory route of excess absorbed K is via urine. This route is primarily under regulation by aldosterone, which increases Na reabsorption in the kidney with the concomitant excretion of K. Blood acid-base status also affects urinary excretion of K (McGuirk and Butler, 1980). With the onset of an alkalotic condition, intracellular H^+ are exchanged with K^+ in plasma as part of the regulatory mechanisms to maintain blood pH. A large gradient exists between intracellular renal tubule concentrations of K and that of luminal fluid (urine). This gradient affects the passage of K from the tubular cells into urine.

There is a distinct relationship between excess cations such as Na^+ and K^+ and urinary pH (Hu and Murphy, 2004). Excess K and Na are excreted in the urine and result in increased urinary bicarbonate secretion. Because K^+ is the primary cation in dairy cattle diets, intake responses to K may be directly related to changes in urinary acid-base balance. Fecal K is primarily from endogenous losses as true digestibility of K approaches 100 percent.

Requirement for Absorbed Potassium

The factorial method was used to derive the absorbed K requirement. In the previous report (NRC, 2001), the maintenance requirement for K was set at 0.038 g K/kg BW plus 6.1 g

K/kg DMI. These requirements were based on suggested endogenous urinary losses of 0.038 g K/kg BW and endogenous fecal losses of 2.6 g K/kg of dietary DM (Gueguen et al., 1989) coupled with an empirical adjustment of the endogenous fecal losses of an additional 3.5 g K/kg diet DM for a total 6.1 g K/kg. The empirical adjustment to fecal losses was added because based on production responses, the initial requirement was not adequate (Dennis et al., 1976; Dennis and Hemken, 1978; Erdman et al., 1980; Sanchez et al., 1994b,c). Generally, as dietary K increased from 0.5 to 1.2 percent of dietary DM, feed intake was consistently increased. The previous committee suggested that a higher maintenance requirement for absorbed K for lactating cows compared with nonlactating animals was justified based on K's role in dynamic processes associated with ruminal function at higher levels of feed intake and maintenance of systemic acid-base balance.

However, the adjustment was applied to the metabolic fecal K requirement. Applying the adjustment in this way implied much greater fecal losses than the actual measured losses. A metabolic fecal requirement of 6.1 g K/kg diet DM in a diet containing 1.2 percent K would have implied an apparent AC of 0.49, which is far below any measured values in the literature.

As it is difficult to formulate a diet with less than 1 percent K using traditional forages, the studies used to determine the intake and milk production responses to K often fed atypical diets that were high in cereal grains, by-product feeds such as brewers dried grains, distillers grains, and cottonseed hulls as a forage substitute to achieve a low K basal diet (Dennis et al., 1976; Dennis and Hemken, 1978; Erdman et al., 1980; Sanchez et al., 1994b,c). In most cases, the calculated DCAD of the basal diets was low (0 to 50 mEq per kilogram diet DM) using the Ender et al. (1971) equation, which includes sulfide (S^{2-}). In addition, dietary K was increased by addition of KCl, which would not change the DCAD concentration. Therefore, the committee is uncertain whether these results could be applied to more typical diets where basal DCAD is 200 mEq/kg or greater. Since only dietary K and Na can be used to increase DCAD, the question is whether Na^+ could replace K^+ as a urinary cation to maintain an alkaline urine once the needs for metabolic fecal and milk K secretion have been met.

Metabolic fecal excretion of K was estimated from the results of 149 individual K digestibility measurements from nine experiments in which cows were fed diets ranging from 0.96 percent to 1.86 percent K. The metabolic fecal requirement was determined by regression of apparently absorbed K on K intake, both expressed as grams per kilogram of feed DM. The regression equation was as follows: Absorbed K $= -2.48 \, (\pm 0.74) + 1.02 \, (\pm 0.056)$ K Intake; RMSE $= 0.52$; $R^2 = 0.93$; $P < 0.001$. This equation suggests a metabolic fecal requirement of 2.48 g K/kg DMI and a true absorption of 1.02, which was not different from 1. Therefore, the metabolic fecal requirement was set at 2.5 g K/kg DMI, similar to previous estimates (Paquay et al., 1969a; Gueguen et al., 1989), and an AC of 1.0 was assigned to dietary K.

Urinary excretion of K is dependent on the relative excretion rates of Na and Cl⁻ (Stewart, 1981). Dairy cattle typically secrete an alkaline urine with a pH of ~8.0 (Hu and Murphy, 2004) because of the need to excrete excess cations (K^+ and Na^+) in relation to anions (Cl⁻), which in turn increases urinary bicarbonate secretion to maintain the electrochemical balance in the urine. As previously indicated, a maintenance requirement based on the measured metabolic fecal K alone would result in a diet that is too low in K compared to the observed experimental responses to dietary K with respect to feed intake and milk production (Dennis et al., 1976; Dennis and Hemken, 1978; Erdman et al., 1980; Sanchez et al., 1994b,c). Therefore, the committee arbitrarily set an AI to meet the endogenous urinary K needs at 0.2 g/kg BW. This will usually result in a minimum dietary K concentration of 1.00 percent of diet DM for lactating dairy cows. The AI to meet endogenous urinary K need for growing heifers and dry cows was set at 0.07 g K/kg BW to maintain a minimum total dietary K of 0.60 percent.

Effect of Environmental Temperature

NRC (2001) set absorbed K requirements for thermoregulation (sweating) at 0.04 and 0.36 g/100 kg BW for cattle maintained at environmental temperatures of 25° to 30°C and >30°C, respectively. This is equivalent to 0.28 and 2.5 g/d, respectively, in a 700-kg dairy cow. Reexamination of the data of Jenkinson and Mabon (1973) with Ayrshire calves suggested that the mean K concentration in sweat was 0.45 g/L. Using the surface area calculations and the sweating rates previously described for Na, an estimated K excretion rate (g/M²/d) would be $0.08e^{0.091 \times THI}$ ($R^2 = 0.93$), suggesting that K losses at the upper end of the THI range would be 0.8 to 2.5 g/d for a 700-kg cow exposed to a THI ranging from 72 to 85. At the upper end of the THI range, these losses are approximately 2-fold greater than those for Na and Cl⁻. Assuming a 5-fold increase in actual sweating rate reported by Thompson et al. (2011) compared to those reported by Jenkinson and Mabon (1973), K losses could be much greater. The measured average sweating losses from 0900 to 2200 h in heat-stressed Holstein cows (Mallonee et al., 1985) was 0.20 g/h. While estimated K^+ losses are approximately 2-fold greater than those for Na and Cl⁻, these losses are negligible in relation to typical K intakes in lactating dairy cows of 250 to 350 g/d (<1 percent of K intake). These losses would be minimal where evaporative cooling was used as a means of heat abatement. The committee concluded that more reliable data on sweating rates and the K concentration in sweat are needed to establish a requirement for K losses during heat stress. Therefore, no provision is provided in the model for sweating losses for K.

Growth

The previous committee (NRC, 2001) set the requirement of absorbed K for growth at 1.6 g/kg ADG based on the es-

timate of Gueguen et al. (1989) for cattle with BW between 150 and 500 kg. That value seemed low when compared to measured values. The K content in mature Holstein heifers (Belyea et al., 1978) fed diets that varied in K was 2.0 (± 0.08) and 1.95 (± 0.06) g K/kg total BW and EBW, respectively. The K content in growing cattle decreased from 2.20 to 1.96 g/kg EBW as slaughter weight increased from 252 and 454 kg (Lohman and Norton, 1968), but there was no effect of slaughter weight on K concentration in the total body (2.49 g/kg). Since K retention at any given range includes retention in the gastrointestinal tract, the K requirement for growth was set at 2.5 g K/kg gain.

Pregnancy

Slaughter data from 18 multiparous pregnant Holstein cows were used to quantify the requirement for absorbed K for conceptus accretion during the last trimester of pregnancy (House and Bell, 1993). Requirement for K is negligible up until about 190 days of gestation. After 190 days of gestation, the requirement of the conceptus for absorbed K is 1.03 g/d × BW / 715 (the BW term scales values to the average cow in that study).

Lactation

The K concentration in milk is constant even under conditions of widely varying K intakes (Sasser et al., 1966). The previous report set the absorbed K requirement for milk at 1.5 g/kg. The weighted average milk K concentration summarized across several more recent studies (Fisher et al., 1994; Sanchez et al., 1994a, 1997; Silanikove et al., 1997; Kume et al., 1998; Robinson et al., 2002; van Hulzen et al., 2009; Castillo et al., 2013; Khelil-Arfa et al., 2014; Visentin et al., 2016) was 1.49 (± 0.11) g K/kg. Therefore, the absorbed K requirement for milk production was maintained at 1.5 g K/kg milk.

Summary of Equations (g absorbed K/d)

$$\text{Maintenance (lactating cows)} = 2.5 \times \text{DMI} + 0.2 \times \text{BW}$$
(Equation 7-22a)

$$\text{Maintenance (nonlactating animals)} = 2.5 \times \text{DMI} + 0.07 \times \text{BW}$$
(Equation 7-22b)

$$\text{Growth} = 2.5 \times \text{ADG} \quad \text{(Equation 7-23)}$$

$$\text{Gestation (>190 d pregnant)} = 1.03 \times \text{BW} / 715$$
(Equation 7-24)

$$\text{Lactation} = 1.5 \times \text{Milk} \quad \text{(Equation 7-25)}$$

where DMI, ADG, and milk are in kg/d, and BW is in kg.

Dietary Requirement and Efficiency of Absorption

Hemken (1983) indicated that K is almost completely absorbed with a true digestibility of 95 percent or greater for most feedstuffs. Paquay et al. (1969a) found that the apparent absorption of K by dairy cows fed alfalfa silage, clover silage, and cabbage silage ranged from 87 to 94 percent. Apparent absorption was slightly lower in four tropical forages fed to sheep, but efficiency of absorption was not affected by maturity of the forage (Perdomo et al., 1977). Average apparent absorption of K in eight forages fed to cattle and sheep was 85 percent (Miller, 1995).

Because K is excreted mainly in urine, urinary excretion and apparent absorption are reliable criteria for estimation of efficiency of absorption. Supplemental K from inorganic sources such as potassium carbonate, KCl, and potassium sulfate is highly soluble and readily available for absorption (Peeler, 1972; Miller, 1995). In the model, an AC value of 1.00 for K was used for all feedstuffs and mineral sources.

For growing heifers weighing 300 kg, gaining 1 kg BW/d and consuming 7 kg DM/d, the previous requirements (NRC, 2001) was 35 g (0.49 percent K in diet DM). The new requirement is 41 g (0.59 percent K in diet DM). The dietary K requirement from the previous report (NRC, 2001) for a 650-kg lactating dairy cow producing 45 kg milk and consuming 28 kg diet DM/d was 265 g (0.94 percent K in diet DM). The new dietary requirement is 268 g (0.96 percent K in diet DM). Essentially, the total requirements for K have not changed substantially, but the route of excretion has shifted from metabolic fecal to endogenous urinary excretion.

Production Responses to Varying Concentrations of Dietary Potassium

Growth

Growth of dairy calves was maximized with 0.58 percent dietary K, and no benefits were noted with higher concentrations (Bigelow et al., 1984). Weil et al. (1988) found no differences in BW gain (average 0.73 kg/d) or DMI when feeding diets with 0.55 to 1.32 percent K (dry basis) to Holstein and Jersey calves starting at 4 weeks of age, but ADG and feed intake were greater for calves fed 0.58 percent K than for those fed 0.34 percent. Tucker et al. (1991) fed diets with 0.4 or 0.6 percent dietary K (supplemented from KCl) and 0 or 2.0 percent NaHCO$_3$ to growing calves (76 kg BW) and found no effects on feed intake. However, ADG increased with higher dietary K and tended to be reduced by addition of NaHCO$_3$. Feedlot cattle require 0.55 to 0.60 percent K (NASEM, 2016), but for cattle under range conditions with slower growth rates, 0.3 to 0.4 percent K appears adequate.

Lactation

The secretion of K in milk necessitates higher dietary concentrations for lactating cows compared with growing cattle. Early research indicated that 0.75 and 0.70 percent dietary K (dry basis) was sufficient for early and mid- to late-lactation cows averaging 24 and 29 kg/d milk production, respectively (Dennis et al., 1976; Dennis and Hemken, 1978). Feed intake generally increased as dietary K concentration was increased up to about 1.0 percent of diet DM, but milk responses were small (Dennis et al., 1976; Dennis and Hemken, 1978; Erdman et al., 1980; Mallonee et al., 1985). Sanchez et al. (1994b,c), using data from 15 experiments with mid-lactation dairy cows (1,444 cow-period observations) conducted in either cool or warm seasons, showed that intake and milk yield were improved with concentrations of dietary K well above those needed to meet requirements. Intake and milk yield responses over a range of dietary K concentrations (0.66 to 1.96 percent, dry basis) were curvilinear, with maximum performance at 1.50 percent K in the cool season. In the warm season, DMI and milk yield increased over the range of dietary K concentrations in the data set. Because of the numerous interactions observed, optimal concentration of K likely varies depending on other minerals. For example, higher dietary Cl$^-$ would result in an increased cation response from either Na or K due to effect of DCAD (Hu and Murphy, 2004; Iwaniuk and Erdman, 2015). Interactions of dietary K with Na and Cl$^-$ on DMI differed in cool versus warm season experiments. In a winter study in Florida, Mallonee (1984) found no benefit of increasing dietary K from 1.07 to 1.58 percent (dry basis) on intake or lactation performance of mid-lactation Holstein cows; however, there were interactions with dietary Na (0.16 to 0.70 percent). Feeding diets with excess K relative to requirement increased intake and milk yield in heat-stressed cows (Beede et al., 1983; Schneider et al., 1984; Mallonee et al., 1985; Schneider et al., 1986; West et al., 1987; Sanchez, 1994a). A dietary K concentration of 1.5 percent (dry basis) during heat stress maximized lactation performance (Beede and Shearer, 1991).

Potassium Deficiency

Signs of severe K deficiency were manifested in lactating dairy cattle fed diets with 0.06 to 0.15 percent K (Pradhan and Hemken, 1968; Mallonee et al., 1982b). Marked decline in feed and water intake, reduced BW and milk yield, pica, loss of hair glossiness, decreased pliability of the hide, lower concentrations of K$^+$ in plasma and milk, and higher blood hematocrit readings occurred within a few days to a few weeks after cows were offered the K-deficient diets. Rate of occurrence and severity of deficiency signs appear to be related to rate of milk production, with higher-yielding cows affected more quickly and severely than lower-yielding cows. With severe K deficiency, cows will be profoundly weak or recumbent with overall muscular weakness and poor intestinal tone (Sielman et al., 1997). In this case, hypokalemia syndrome was associated with treatment of ketosis.

When diets contained 0.5 to 0.7 percent K, the only apparent sign of inadequacy in lactating cows was reduced feed intake with corresponding lower milk yield compared with cows fed adequate K. Because many forages contain high concentrations of K, severe K deficiency would be extremely rare. However, marginal deficiency can occur if corn silage is the sole forage and no supplemental K is fed.

Potassium Toxicity

The dietary concentration of K that leads to toxicity is not well defined (Ward, 1966b). K toxicosis is unlikely to occur under natural conditions but could occur as a result of excess supplementation. Acute toxicity (Ward, 1966a) and death (apparently cardiac arrest) occurred when 501 g of K as KCl was given by stomach tube to a cow (475 kg BW). This amount was approximately the daily amount consumed by similar cows fed 15 kg of alfalfa that was consumed without ill effects. Dennis and Harbaugh (1948) administered 182 and 240 g of K as KCl without detectable clinical signs of toxicity, but 393 g by stomach tube to cattle weighing about 300 kg resulted in one death, two that required treatment, and two exhibiting no signs of toxicity. When 4.6 percent dietary K (via supplemental potassium carbonate) was fed to cows during early lactation, feed intake and milk yield were reduced, and water intake and urinary excretion were increased (Fisher et al., 1994). NRC (2005) set the maximum tolerable concentration at 2.0 percent of diet DM based on indexes of animal health. However, cattle are known to tolerate high concentrations (>3 percent of DM) of K such as are seen in early spring pastures for extended periods of time. Dietary K depresses Mg absorption and is a risk factor for grass tetany. Feeding K in excess of that needed to meet requirements can present metabolic and physiologic challenges to cattle and will increase excretion of K into the environment.

Dietary Cation–Anion Difference

DCAD was discussed in the 2001 NRC, but no requirements were suggested. The DCAD is calculated as the difference between the sum of the major cations (Na^+ and K^+) and the sum of the major anions (Cl^- and sometimes S^{2-}) and is expressed in milliequivalents (mEq) per kg or per 100 g of diet DM. The simplest calculation of the DCAD equation (Mongin, 1981) includes dietary Na, K, and Cl^- and was developed for use in poultry diets. Ender et al. (1971), in early work related to the use of DCAD for prevention of milk fever, proposed a DCAD equation that included sulfur (S) assumed to be S^{2-}, as the second anion. When discussing specific dietary values, DCAD calculated using Ender et al. (1971) will be referred to as DCAD-S, whereas the term DCAD refers to the Mongin (1981) equation. For a diet containing 1.11 percent K, 0.25 percent Na, 0.33 percent Cl^-, and 0.20 percent S, the DCAD and DCAD-S concentrations would be 300 and 185 mEq/kg DM, respectively.

Inclusion of other dietary cations (Ca^{2+} and Mg^{2+}) and anions (P^{3-}) have been suggested to be included in the DCAD equations. However, the contribution of those ions to urine net acid excretion is dependent on their relative absorption rates and the degree of urinary excretion. Constable et al. (2009) found that K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and sulfate (SO_4^{2-}) ion accounted for most of the strong ion effects on urine pH in cattle. However, urinary Ca and Mg losses in lactating cows are minimal such that the relative amounts of K, Na, Cl^-, and SO_4^{2-} excretion have the greatest impact on urine pH.

Cattle routinely consume diets that are high in DCAD, resulting in urinary excretion of excess strong cations (primarily K^+) relative to strong anions (Cl^-). In high DCAD diets, urinary electrochemical neutrality is maintained by increased bicarbonate secretion. Addition of a dietary cation source such as $NaHCO_3$ (Erdman et al., 1982) to lactating dairy cows resulted in decreased urinary net acid excretion, increased urinary bicarbonate secretion, and increased urine pH. Feed intake declined as urine pH dropped from 8 to 7 (Hu and Murphy, 2004) with decreasing DCAD. When negative DCAD diets are fed, urinary excretion of excess Cl^- relative to K^+ and Na^+ occurs, resulting in decreased urinary bicarbonate secretion (Tucker et al., 1988) and reduced urinary pH. Reduction in urinary pH below 7 is associated with increased Ca excretion in the urine (Constable et al., 2009). Feeding low DCAD diets to prepartum dairy cows reduces prevalence of milk fever (see Chapter 12).

Much of the research on the effects of DCAD in lactating dairy cows has used cation sources such as sodium and potassium bicarbonate, carbonate, and sesquicarbonate salts to increase the DCAD concentration in the diet. The anion components of these salts can also act as buffering agents in the rumen and contribute to the increase in rumen pH and acetate/propionate ratio in part because of their buffering effect. A 100 mEq/kg DM in DCAD resulted in a linear (0.03) increase in rumen pH (Iwaniuk and Erdman, 2015). Thus, the overall effect of DCAD effects on animal performance is likely due to a combination of their effects on rumen fermentation and acid-base status of the cow.

Lactation Responses to Dietary Cation–Anion Difference

Maximum DMI, milk yield, and 4 percent fat-corrected milk yield occurred at a DCAD of 380 mEq/kg diet DM (Sanchez et al., 1994b). Similar results were observed when data were subdivided by season with maximal DCAD for maximal DMI and 4 percent fat-corrected milk yield. However, maximum response appeared to occur at a slightly higher DCAD in winter-fed versus summer-fed cows.

Since the last report (NRC, 2001), two meta-analyses of published literature on DCAD effects on lactating dairy cattle (Hu and Murphy, 2004; Iwaniuk and Erdman 2015) have been conducted. Hu and Murphy (2004) reported that

DCAD had significant effects on production and acid-base responses in lactating cows. The data set included 17 experiments and 69 treatment means using DCAD calculated with the Mongin (1981) equation. Comparison of response curves for DMI and urine pH revealed a 1.5-kg/d decline in feed DMI and a 1 pH-unit drop in urine pH as DCAD decreased from 340 to 120 mEq/kg diet DM. The close relationship between reduced DMI and urine pH suggests that urinary acid-base status is related to DMI. The maximal intake and yields of milk, fat-corrected milk, and fat occurred at DCAD of 396, 336, 489, and 550 mEq/kg diet DM, respectively, and the DCAD at which 80 percent of the maximum response occurred was 200, 185, 190, and 305 mEq/kg DM, respectively.

Iwaniuk and Erdman (2015) conducted a much larger meta-analysis of data collected from 43 published studies that included 196 treatment means. This analysis included data previously summarized by Hu and Murphy (2004) and incorporated earlier and later published work where supplements such as sodium and potassium bicarbonate, carbonate, and sesquicarbonate salts were fed. Data for DMI, milk production, and 3.5 percent fat-corrected milk were fitted to an asymptotic model where $Y = a + b \ (1 - e^{(-k \times \text{DCAD-S})})$, where a = intercept, b = maximal response to DCAD-S, and k is the rate constant for the effect of DCAD-S (mEq/kg diet DM) on the response.

For DMI, the response equation was DMI, kg/d = 18.44 $(\pm 0.389) + 1.11\,(\pm 0.468)\,(1 - e^{(-0.0038\,(\pm 0.002) \times \text{DCAD-S})})$, $R^2 = 0.41$, RMSE = 0.53. Eighty percent and 66 percent of the maximal intake response to DCAD-S occurred at 425 and 290 mEq/kg diet DM, respectively. Milk production responses to DCAD-S were relatively small with a maximal response of 1.1 kg/d.

Milk fat percentage and milk fat yield increased linearly with increased DCAD with 0.1 percentage unit and a 39-g/d increase in fat percentage and fat yield per 100-mEq/kg diet DM increase in DCAD-S. Rumen pH (83 treatment means) increased linearly with increasing DCAD-S, with the changes in pH being consistent with the effects on biohydrogenation of FA intermediates that are known to inhibit milk fat synthesis (see Chapter 4).

The response function for 3.5 percent fat-corrected milk was 3.5 percent FCM, kg/d = 25.49 $(\pm\ 0.751) + 4.82$ $(\pm 1.57) \times (1 - e^{(-0.0024\,(\pm 0.001) \times \text{DCAD-S})})$, $R^2 = 0.48$, RMSE = 0.73. For 3.5 percent FCM, 80 percent and 66 percent of the maximal response to DCAD occurred at a DCAD-S of 675 and 450 mEq/kg diet DM, respectively. However, 675 mEq/kg was outside of the range of inference in the data set. The changes in 3.5 percent fat-corrected milk production reflected the curvilinear response in milk yield and the linear response in fat yield to increasing DCAD-S.

With fewer observations (52 and 42, respectively), a 100-mEq/kg diet DM increase DCAD-S resulted in a 0.73 and 1.54 percentage unit increase in DM and NDF digestibility, respectively. The change in NDF digestibility accounted for approximately two-thirds of the increase in DM digestibility.

Dietary Cation–Anion Difference Requirements for Lactating Cows

There is no fixed requirement for DCAD in lactating cows. Rather, the feeding level chosen should be determined based on the incremental production responses (milk and fat yield) in relation to the incremental added costs (DMI and mineral salt supplementation) according to the response equations outlined by Hu and Murphy (2004) and Iwaniuk and Erdman (2015).

An absolute practical minimum or AI for DCAD would be based on the minimum requirements for the minerals, K, Na, Cl⁻, and S. For example, a 700-kg dairy cow producing 50 kg milk will require a diet containing 1.11 percent, 0.25 percent, 0.33 percent, and 0.20 percent K, Na, Cl⁻, and S, respectively. The calculated DCAD-S using those requirements would be 174 mEq/kg diet DM or 301 mEq/kg using the Mongin (1981) equation. If measured Cl⁻ and S concentration of the diet exceeds the minimum requirement, then additional K or Na may be needed for an acceptable DCAD and DCAD-S.

Growth Responses to Dietary Cation–Anion Difference

Few studies have examined the influence of DCAD on growth of calves. Calves (1 to 12 weeks of age) grew faster when fed a 200-mEq DCAD-S diet than calves fed a −100-mEq diet (Xin et al., 1991). In another study, Holstein and Jersey calves averaging 56 to 70 days of age were fed diets containing −180, 45, 225, and 383 mEq/kg DCAD-S (Jackson et al., 1992). Feed intake and ADG responded quadratically, being greatest at 225 mEq and lowest with −180 mEq. In a follow-up study, intake, growth rate, and Ca metabolism were compared for Holstein calves (56 to 70 days of age) fed diets with −180 or 130 mEq DCAD-S/kg of dietary DM in a factorial arrangement of treatments with 0.42 and 0.52 percent dietary Ca (Jackson and Hemken, 1994). Feed intake did not differ due to DCAD, but growth rate was increased with the 130 mEq/kg DCAD-S; dietary Ca had no effect. Urinary Ca excretion was greater for calves fed diets with −180 mEq compared with diets with 130 mEq. Breaking strength of the ninth rib was greater for calves fed the 130-mEq treatment compared with the −180-mEq treatment; breaking strength of the seventh rib was greater when calves were fed either higher DCAD or higher Ca. Based on these studies, the AI of DCAD-S for growing calves is 150 to 200 mEq/kg of diet DM, which is the approximate value obtained when calves are fed their minimum requirements for K, Na, and Cl⁻.

No studies were identified in which DCAD was varied in growing dairy heifers. Growing (Ross et al., 1994b) and finishing (Ross et al., 1994a) beef steers were fed diets containing 0, 150, 300, and 450 mEq/kg DCAD (Mongin, 1981 equation), and a DCAD of 150 mEq/kg maximized feed intake and growth rate. In the absence of experiments with growing dairy heifers, an adequate DCAD (Mongin, 1981 equation) would be 150 mEq/kg diet DM.

Sulfur

Function

About 0.15 percent of the body is S, predominantly in the form of S AAs (S-AAs) and the amino sulfonic acid, taurine, but S is also a component of thiamin and biotin, structural compounds (e.g., chondroitin sulfate), and other biologically important molecules. Met, thiamin, and biotin cannot be synthesized by cattle; they must either be supplied in the diet or synthesized by ruminal microbes. The sulfate ion (SO_4^{2-}) is found in cellular and extracellular spaces, and concentrations are likely under homeostatic control via renal clearance and perhaps other mechanisms (Markovich, 2001). S is not a major factor in acid-base balance, but the primary function of SO_4^{2-} is likely acid-base balance.

Requirement

The dietary requirement for S by the cow is primarily to provide adequate substrate to ensure maximal microbial protein synthesis, which in turn will increase the supply of the S-containing compounds required by cows. Based on in vitro and in vivo studies, maximum fiber digestibility usually occurs when diets contain 0.15 to 0.25 percent total S (Guardiola et al., 1983; Qi et al., 1994). Bouchard and Conrad (1973a,b) determined that 0.20 percent dietary S (supplemental S provided by Na, Ca, K, or $MgSO_4$) was adequate to sustain maximal S retention in mid-lactation dairy cows producing 30 to 37 kg milk/d. Based on the lack of any newer data, the S requirement for all classes of dairy cattle (excluding preruminant calves) remained at 0.2 percent of diet DM or

$$\text{Total S, g/d} = \text{DMI} \times 2.0 \quad \text{(Equation 7-26)}$$

where DMI is kg/d, and S is total dietary, not absorbed.

Historically, a dietary N to S ratio of 10:1 to 12:1 has been considered optimal (Bouchard and Conrad, 1973a). However, no evidence is available indicating that the ratio is important when the dietary S requirement is met. Low-protein diets may benefit from S supplementation, but that is because dietary S probably was also low.

Sources

The S content of feedstuffs is positively correlated to the protein concentration; however, the use of S-based fertilizers can increase S concentrations of forages without a concomitant increase in protein concentrations (Spears et al., 1985; Arthington et al., 2002). S is not routinely assayed by many feed-testing labs, and table averages will likely underestimate concentrations for forages that have been fertilized with S. Most of the S in plants is in S-containing AAs, and those AAs reach the intestine to be absorbed, are used by rumen microbes to synthesize bacterial AAs, or are degraded.

Within the rumen, S is released from degradation of S-AAs, but the rate of release is much greater for cysteine than for Met (Bird, 1972b). Furthermore, in vitro ruminal degradation of Met is slower than breakdown of other AAs (Mbanzamihigo et al., 1997). Theoretically, a diet with very low protein degradability could be limiting in rumen-available S, even though total dietary S is adequate; however, under practical situations, this is unlikely to occur.

Cows can consume inorganic S (usually as sulfate, SO_4^{2-}) via forages that have been fertilized with S-containing fertilizers, distillers grains (Nietner et al., 2015), water (see Chapter 9), and from S supplements (e.g., $MgSO_4$, $CaSO_4$, and K_2SO_4). Based on in vitro rumen measures, in vivo S balance, and ruminant growth studies (mostly with sheep), the different sources of inorganic Sr have similar biological value (Henry and Ammerman, 1995).

Within cells and extracellular spaces of the cow, SO_4^{2-} is involved with acid-base balance and perhaps other functions. Oxidation of S-AAs within cells is a major source of SO_4^{2-}, but SO_4^{2-} transporters also exist in the intestine (Markovich, 2001), and SO_4^{2-} can be absorbed by ruminants (Bird and Moir, 1971). However, much of the ingested SO_4^{2-} is probably reduced to hydrogen sulfide within the rumen.

Excess Sulfur

Excess ingested S (includes S from the diet and drinking water) causes indirect and direct negative effects on cow health and productivity. Excess intake of S can lead to deficiencies or reduced status of many trace minerals. Providing approximately 0.2 percent added S from SO_4^{2-} (total diet S at approximately 0.4 percent) reduces the absorption of copper (Cu) and Se (van Ryssen et al., 1998; Ivancic and Weiss, 2001; Richter et al., 2012), and newer data suggest it may also negatively affect manganese (Mn) and Zn retention in cattle (Pogge et al., 2014). Negative effects of dietary S probably occur at concentrations less than 0.4 percent of the diet. Increasing dietary S from 0.13 percent up to 0.35 percent by increasing dietary inclusion of distillers grains linearly decreased liver Cu concentrations in feedlot lambs (Felix et al., 2012).

Excess SO_4^{2-} added to rations can reduce feed intake and performance without eliciting any signs of clinical toxicity (Kandylis, 1984) perhaps mediated via a reduction in DCAD (discussed above). Diets with 0.2 percent added $SO_4^{2-}-S$ reduced DMI by lactating dairy cows (Ivancic and Weiss, 2001; Tebbe et al., 2018). High concentrations of SO_4^{2-} in water can reduce water intake (see Chapter 9).

Clinical S toxicity causes neurologic changes, including blindness, coma, muscle twitches, and recumbency (Kandylis, 1984). Many of those clinical signs are consistent with polioencephalomalacia (Gould, 1998). Postmortem examination reveals severe enteritis, peritoneal effusion, and petechial hemorrhages in many organs, especially kidneys (Bird, 1972a). Often the breath will smell of hydrogen

sulfide (H_2S)—which is likely the toxic principal in S toxicosis. Much of the ingested SO_4^{2-} and S from ruminally degraded S-AAs is reduced to H_2S. When dietary S concentrations are close to requirement (i.e., 0.2 percent), the H_2S is used by ruminal bacteria to synthesize S-AAs and other organic S-containing compounds (i.e., assimilatory pathway), resulting in low ruminal concentrations of H_2S. However, when higher concentrations of dietary S are fed (usually from SO_4^{2-} sources), dissimilatory reduction of SO_4^{2-} by SO_4^{2-} reducing bacteria occurs, and ruminal H_2S concentrations become elevated (see review by Drewnoski et al., 2014). The generally accepted etiology of H_2S toxicity is that at low rumen pH (pKa of H_2S is about 7), much of the H_2S produced remains as H_2S, which is volatile and can be eructated. After eructation, it can be inhaled, enter the circulation, and reach the brain, causing brain damage and polioencephalomalacia (Bird, 1972a). In beef cattle fed high-grain finishing diets without any forage, the risk of polioencephalomalacia increases greatly when total dietary S is greater than 0.42 percent (water assumed to provide trivial amounts of S), but when the diet contained 8 percent forage NDF, dietary S had to be closer to 0.6 percent to increase the risk (Nichols et al., 2012). Higher dietary fiber should increase ruminal pH, causing more of the H_2S to be dissociated (HS^-) and not volatile. Because diets fed to dairy cows typically have substantially more forage than feedlot diets, polioencephalomalacia is not likely to be observed in dairy cattle even at very high concentrations of dietary S. NRC (2005) set the MTL of dietary S (water assumed to be a trivial source of S) at 0.3 percent for diets with 85 percent concentrate and at 0.5 percent for diets with at least 40 percent forage (more representative of dairy cow diets).

Sulfate anions have been added to rations of dry cows before calving to decrease the DCAD to help prevent milk

fever (see Chapter 12), often to levels above 0.5 percent S. That concentration of S in high-forage diets should not cause clinical toxicity; however, Se and Cu absorption will likely be reduced, but because these diets are typically only fed for a few weeks, the overall effect on Se and Cu status is small. Longer-term feeding of high S diets (e.g., 0.5 percent) is not recommended.

MICROMINERALS

Chromium

Chromium (Cr) is an essential nutrient, although requirements have not been quantified for cattle. An AI for Cr of 20 to 44 μg/d has been established for humans (NRC, 2006). Several forms of supplemental Cr have been fed to cattle, including chromium chloride, chromium picolinate, chromium nicotinate, chromium-enriched yeast, chromium Met, and chromium propionate. At the present time (2021), the only approved Cr supplement that can be fed to cattle in the United States is chromium propionate, and the maximum legal rate is 0.5 mg supplemental Cr/kg of diet DM.

Reliable data on the Cr concentrations in feeds are difficult to obtain because concentrations are very low (μg/kg DM range) and contamination occurs readily during sample processing (e.g., using a steel grinder). For example, concentrations of Cr in ground corn grain and ground soybean seed samples were twice the concentration measured in their unground counterparts (see Table 7-3). The lack of reliable data on basal concentrations of Cr in diets greatly limits the ability to establish AI or requirements for Cr.

Biochemistry and Absorption

In mammalian tissues, the primary active form of Cr is as a component of a small peptide called chromodulin. Chromodulin contains only four AA residues but can bind 4 Cr (Cr^{3+}) ions and has been isolated from bovine liver (Davis and Vincent, 1997) and colostrum (Yamamoto et al., 1988). This compound is thought to bind to insulin-activated insulin receptors and stimulate tyrosine kinase, resulting in enhanced responses to insulin (Vincent, 2000). It may have other functions related to insulin activity. Cr also is likely involved in gene regulation.

Little is known regarding the absorption mechanism for Cr, but in nonruminants, absorption is usually <1 percent of Cr intake (Lukaski, 1999). Absorption of Cr from organic sources in nonruminants is greater (~3 percent of intake) than that for inorganic sources (Cefalu and Hu, 2004). Potential antagonists to Cr absorption include high concentrations of dietary Fe and perhaps Zn and phytate (Pavlata, 2007). Organic sources of Cr may also be converted more quickly within the body to biologically active forms, and organic forms usually have greater biological effects than inorganic sources (Vinson and Hsiao, 1985; Balk et al., 2007). Quantitative data on absorption of any form of Cr by ruminants are not available.

TABLE 7-3   Concentrations of Cr in Common Feeds (mg Cr/kg DM)[a]

Feed[b]	Mean	SD	Range
Alfalfa hay or silage*	0.522	0.220	0.199–0.889
Beet pulp*	1.222	0.386	0.776–1.451
Corn grain, ground*	0.049	0.031	0.014–0.114
Corn grain, whole	0.026	0.015	0.008–0.054
Corn silage*	0.220	0.087	0.105–0.441
Cottonseed*	0.094	0.086	0.033–0.155
Dried distillers grains	0.160	0.056	0.084–0.238
Grass hay*	0.155	0.093	0.098–0.320
Oats, whole	0.025	0.008	0.021–0.034
Soybean, whole	0.069	0.035	0.034–0.122
Soybean hulls, loose	0.262	0.073	0.191–0.336
Soybean hulls, pelleted*	0.550	0.175	0.309–0.705
Soybean meal	0.208	0.050	0.154–0.286
Wheat	0.041	0.014	0.029–0.062
Wheat middlings	0.084	0.031	0.044–0.132

[a]Source of data: Spears et al. (2017).
[b]Feeds with an asterisk were ground through a Wiley mill prior to Cr analysis and contamination is likely (Spears et al., 2017).

Cattle Responses to Supplemental Chromium

An extensive review of animal responses to Cr was conducted in 1997 (NRC, 1997), and another review limited to cattle was published in 1999 (Kegley and Spears, 1999). This section will concentrate on more recent publications. Studies have evaluated the effects of supplemental Cr on production measures, glucose and lipid metabolism, and immune function. Source of Cr and supplementation rate varied among studies, but most studies used chromium picolinate, chromium Met, or chromium propionate, and supplementation rates were usually 4 to 10 mg/d (approximately 0.5 mg Cr/kg diet DM when fed to lactating dairy cows). The effect of supplemental Cr on insulin sensitivity depends on the physiological state of the animal. Within 1 week or so prior to parturition, supplemental Cr often reduces insulin sensitivity in multiparous cows, as measured by an increased plasma insulin to plasma glucose ratio (Hayirli et al., 2001; Pechova et al., 2002). With primiparous animals, supplemental Cr starting 6 weeks prepartum increased insulin sensitivity when measured 2 weeks prepartum but reduced it when measured 2 weeks postpartum (Subiyatno et al., 1996). Increased plasma insulin prepartum can stimulate lipogenesis and suppress lipolysis, which can explain why supplemental Cr prepartum often reduces plasma nonesterified fatty acids (NEFAs) (Hayirli et al., 2001; Bryan et al., 2004). Elevated plasma NEFA prepartum is a risk factor for several health disorders in dairy cows (Roberts et al., 2012; McArt et al., 2013; Qu et al., 2014). Cr supplementation of growing beef and dairy animals enhances insulin sensitivity (Sumner et al., 2007; Spears et al., 2012). This should result in increased glucose uptake and increased protein synthesis by skeletal muscle. In early lactation dairy cows, supplemental Cr increased insulin responsiveness in one study (Hayirli et al., 2001), but in a limited study, supplemental Cr reduced insulin sensitivity (Subiyatno et al., 1996). Supplementation rates were similar between studies, but source of Cr differed. Supplemental Cr (approximate intake of 6 to 9 mg/d) usually increases milk yields in early lactation, higher-producing (>30 kg/d) cows (NRC, 1997; Hayirli et al., 2001a; AlSaiady et al., 2004; Smith et al., 2005; Sadri et al., 2009; Vargas-Rodriguez et al., 2014), but not in lower (ca. 30 kg/d)–producing cows (Bryan et al., 2004).

Supplemental Cr has improved certain measures of immune function in beef and dairy cattle (reviewed by Weiss and Spears, 2005). The most consistent effect has been increased blastogenesis of cytotoxic T-lymphocytes, which may be modulated via reduced cortisol concentrations. Neutrophil function has not been affected by Cr (Weiss and Spears, 2005); however, concentrations of proinflammatory cytokines in activated neutrophils were greater when cows were supplemented with Cr (Yuan et al., 2014). This may enhance overall immune response, but in a clinical trial, Cr did not affect incidence of mastitis (Chang et al., 1996).

Although production and immune function responses to supplemental Cr are often positive and Cr has been shown to have metabolic effects, establishing a requirement for Cr is not possible because total intakes of Cr (basal plus supplemental) have not been measured. Glucose and insulin often respond to Cr supplementation, but results are not linear with dose. The lowest rate of supplementation (0.01 mg Cr/kg BW for growing cattle or 0.006 mg Cr/kg BW for lactating cow) usually was adequate for maximal response (Hayirli et al., 2001; Spears et al., 2012). Whether the changes observed in insulin and glucose represent improvements in metabolism are not known. Milk yield has responded linearly and quadratically to increasing supplemental Cr (Hayirli et al., 2001; Smith et al., 2008). The maximal response occurred at a supplementation rate of approximately 0.01 mg Cr/kg BW (~6 mg/d) but in the study that reported a linear response that was the highest rate tested. Although an AI for Cr cannot be established based on only two titration studies with lactating cows, supplementation of approximately 0.01 mg Cr/kg BW often increases milk yield in early lactation.

Maximum Tolerable Level

The maximum tolerable dietary concentration for Cr^{3+} from soluble forms was set at 100 mg Cr/kg of diet DM (NRC, 2005), but data are very limited regarding adverse responses to high dietary concentrations of Cr^{3+}. Hayirli et al. (2001) reported that milk yield responded quadratically to increasing Cr and feeding cows 0.025 mg Cr/kg BW (15 mg/d) reduced milk yields to values similar to the control. Concentrations of Cr in milk, muscle, and body fat were not greater in cows fed 2 mg Cr/kg of diet DM compared with cows fed no supplemental Cr; however, Cr concentrations in liver and kidney were two to three times greater (Lloyd et al., 2010). In vitro, Cr picolinate can increase production of the hydroxyl radical, which can negatively affect immune function, damage DNA, and oxidize membrane FAs (Vincent, 2000). The concentration of Cr at which this occurs in vivo is unknown, and it is not clear whether this effect is unique to Cr picolinate.

Cobalt

Function

The primary function of cobalt (Co) is to serve as a precursor for vitamin B_{12} (cobalamin) synthesis in the rumen. Rumen microbes can usually produce adequate vitamin B_{12} if adequate Co is available in the diet (see Chapter 8 for details). In addition to synthesizing vitamin B_{12}, bacteria can synthesize vitamin B_{12} analogues, which are not biologically active. The presence of these vitamin B_{12} analogues in liver and blood reduces the utility of vitamin B_{12} determination to assess the status of dietary Co (Halpin et al., 1984). However, hepatic vitamin B_{12} concentrations below 0.1 µg/g wet weight are indicative of Co deficiency (Smith, 1987). A portion of dietary Co can be absorbed in the cation form (Smith, 1987); however, it has no known function and, once absorbed, does not appear capable of reentering the rumen

so microbes could use it. Most is excreted in the urine, and a smaller amount exits with the bile (Underwood, 1981).

Cobalt carbonate, chloride, nitrate, sulfate, and glucoheptonate all appear to be suitable sources of Co for ruminants. Cobalt oxide, which is much less soluble, resulted in much lower vitamin B_{12} concentrations during in vitro rumen fermentation (Kawashima et al., 1997), and was somewhat less available (Henry, 1995a). Cobalt oxide pellets and controlled-release glass pellets containing Co that remain in the rumen–reticulum have been used successfully to supply Co over extended periods of time to ruminants on pasture, although regurgitation can cause loss of some types of pellets (Poole and Connolly, 1967).

Deficiency

Ruminants appear to be more sensitive to vitamin B_{12} deficiency than nonruminants (see Chapter 8 for details). This is likely because vitamin B_{12} is a key component in the pathways for gluconeogenesis and de novo methyl group synthesis. Ruminants are dependent on gluconeogenesis for meeting needs of tissues for glucose with ruminally derived propionate serving as a primary glucose precursor. A breakdown in propionate metabolism where methylmalonyl-CoA is converted to succinyl-CoA is a primary defect arising from a deficiency of vitamin B_{12}. Inadequate dietary Co elevates plasma homocysteine concentrations in growing beef cattle, suggesting a deficiency in methyl group availability for resynthesis of Met from homocysteine (Stangl et al., 2000). Stores of vitamin B_{12} in the liver of adult ruminants are usually sufficient to last several months when they are placed on a Co-deficient diet. Hepatic concentrations of vitamin B_{12}, urinary and plasma concentrations of methylmalonic acid, and serum concentrations of homocysteine have been used to evaluate Co (and vitamin B_{12}) status of cattle (Stangl et al., 2000).

Young animals are more sensitive to dietary insufficiency of Co because they have lower reserves of vitamin B_{12} in the liver. Early signs of a deficiency of Co include failure to grow, unthriftiness, and loss of weight (Smith, 1997). More severe signs include fatty degeneration of the liver, anemia with pale mucous membranes (Underwood, 1981), and reduced resistance to infection as a result of impaired neutrophil function (MacPherson et al., 1987; Paterson and MacPherson, 1990). Although cows may have adequate stores of vitamin B_{12} to last several months, ruminal microbes do not. Within a few days of feeding a diet deficient in Co, ruminal concentrations of succinate rise. This may be the result of a blockade of microbial conversion of succinate to propionate, or a shift in ruminal bacterial populations toward succinate production rather than propionate production (Kennedy et al., 1996).

Requirement

The dietary requirement for Co was previously estimated to be 0.11 mg/kg of dietary DM. This was based on the amount of Co that must be supplied to keep plasma concentrations of vitamin B_{12} above 0.3 µg/L (Marston, 1970). However, depending on the biochemical response criteria, adequate dietary Co (basal plus supplemental) ranged from 0.13 to 0.25 mg Co/kg diet DM for growing beef cattle (Stangl et al., 2000). Maximal growth in beef cattle occurred when the diet contained between 0.15 and 0.18 mg Co/kg DM (Schwarz et al., 2000; Tiffany et al., 2003). Liver vitamin B_{12} and folate concentrations were maximized at 0.24 and 0.19 mg Co/kg, respectively, whereas plasma vitamin B_{12} was maximized at a dietary Co concentration of 0.26 mg/kg (Stangl et al., 2000). The dietary Co concentrations required to minimize plasma homocysteine and methyl malonic acid concentrations were 0.16 and 0.12 mg/kg of diet DM, respectively. Milk yield responses to increasing dietary Co have not been finely titrated. Few positive production responses have been reported when Co was supplemented to dairy cows, but in those studies, the lowest concentration evaluated was approximately 0.20 mg Co/kg of diet DM (Kincaid et al., 2003; Kincaid and Socha, 2007; Akins et al., 2013). Feeds are not commonly assayed for Co (sensitivity is an issue), but in the studies above, basal concentrations averaged about 0.1 mg Co/kg DM. Because of the lack of adequate data on basal feed and the variable concentrations required for maximal growth and biochemical indicators responses, the AI of total Co (basal + supplemental) is set at 0.2 mg Co/kg of diet DM or

$$\text{Cobalt AI (mg/d total Co)} = 0.2 \times \text{DMI} \quad \text{(Equation 7-27)}$$

where DMI is kg/d.

Beef steers fed barley in high-grain diets (85 percent of diet DM) had lower concentrations of vitamin B_{12} in rumen fluid and in plasma than steers fed a corn-based diet at similar concentrations of supplemental Co (Tiffany and Spears, 2005). Whether type of grain affects Co conversion to vitamin B_{12} when fed in typical dairy cow diets (usually < 40 percent starch grains) is unknown.

Special Properties of Cobalt

Dietary Co may also have some effects independent of its necessity for production of vitamin B_{12}. Co fed at 0.25 to 0.35 mg/kg of dietary DM, well above those required for sufficient vitamin B_{12} synthesis, seems to enhance ruminal digestion of feedstuffs, especially lower-quality forages (Saxena and Ranjhan, 1978; Lopez-Guisa and Satter, 1992). This effect may be due to selection of certain microbial populations with a higher Co requirement or may be a result of the divalent Co cation forming crosslinks between negatively charged bacteria and negatively charged forage particles, which allows bacteria to attach to forage particles more efficiently (Lopez-Guisa and Satter, 1992). Cu, Ca^{2+}, and Mg^{2+} are divalent cations that may have some of the same ability (Storry, 1961; Somers, 1983). Addition

of Co has increased total anaerobic bacteria in the rumen by 50 percent and lactic acid production in the rumen by 86 percent (Young, 1979).These results suggest that ruminal microbes may require greater concentrations of dietary Co than the cow. However, the general lack of effects on DMI, milk composition, and milk yield when diets contained 0.15 to 0.20 mg Co/kg DM suggests that the AI of 0.20 mg Co/kg is also adequate for ruminal bacteria.

Toxicity

Co toxicity causes reduced feed intake, loss of BW, and eventually anemia—signs similar to those seen in Co deficiency (Ely et al., 1948; Keener et al., 1949; NRC, 2005). The maximal tolerable dietary Co concentration has been set at 25 mg/kg of dietary DM (NRC, 2005).

Copper

Function

Cu is a component of several proteins, including cytochrome c oxidase (required for aerobic respiration), lysyl oxidase (required for formation of collagen and elastin), and tyrosinase (necessary for production of melanin pigment). Cu is required for hemoglobin synthesis and is involved with iron (Fe) metabolism (e.g., as a component of ceruloplasmin). Cu, along with Zn, is a component of cytosolic superoxide dismutase, which protects cells from the toxic effects of reactive oxygen species (ROS). This is particularly important in phagocytic cells and may be a primary mode of action for reduced infectious disease when adequate Cu is fed.

Absorption of Dietary Copper

Intestinal absorption of Cu by humans and rodents appears to be under homeostatic control and is upregulated when low Cu diets are fed and downregulated with high intakes of Cu (Lönnerdal, 2008). Whether homeostatic regulation of absorption occurs in ruminants has not been determined. A weak negative relationship was observed between initial concentration of Cu in liver and rate of accumulation of liver Cu, but over a wide range of initial concentrations (60 to 230 mg Cu/kg of liver dry weight), rate of liver accumulation was essentially constant in nonlactating dairy cows (Balemi et al., 2010). That range encompasses what is considered adequate Cu status. Age of the animal, chemical form of dietary Cu, and the presence of antagonists affect intestinal absorption of Cu. In calves without functioning rumens, the Cu AC can be as high as 0.70. Bremner and Dalgarno (1973a,b) found that 50 to 60 percent of dietary Cu (supplied as Cu sulfate) was retained in liver for calves between 3 and 14 weeks of age. As the rumen starts functioning, absorption of Cu decreases substantially, and the AC for Cu is usually ≤0.05 in adult cattle.

Effect of Other Minerals on Copper Absorption

S, molybdenum (Mo) in combination with S, and Fe antagonize Cu absorption in ruminants. Zn concentrations need to be 10 to 20 times requirement before antagonism is observed in ruminants (Miller et al., 1989), so Zn antagonism is not of practical importance. Antagonism of Cu by Fe could occur via competition for intestinal binding sites (e.g., divalent metal transporter or DMT1), and Fe may exacerbate the reaction between Cu and S within the rumen (Gould and Kendall, 2011). Fe antagonism of Cu absorption usually requires supplemental Fe at concentrations exceeding 250 mg Fe/kg of diet DM (Chase et al., 2000; Mullis et al., 2003). Dietary Fe concentrations can be that high because of the Fe in forages. High Fe concentrations in forages are likely caused by soil contamination and may not greatly affect Cu absorption because the Fe is mostly in the form of ferric oxide, which is essentially inert. However, high-Fe soil has been implicated in reducing Cu status of grazing sheep (Suttle et al., 1984). Because of acid conditions in silages, over time, the Fe in silages may become more reactive (Hansen and Spears, 2009) and might cause increased antagonism. Data are not available showing lower Cu status in cattle fed silages with high concentrations of Fe.

Increased consumption of S (dietary and via drinking water) in the absence of high Mo concentrations reduces Cu status of cattle (Arthington et al., 2002; Pogge et al., 2014). The antagonism may occur because of formation of Cu sulfides within the rumen. The dietary concentration of S required to reduce Cu absorption or Cu status has not been titrated, but liver concentrations of Cu in beef cattle decreased when diets contained 0.5 to 0.6 percent total S (Arthington et al., 2002; Pogge et al., 2014). Water that contained approximately 500 mg S (as sulfate)/L also reduced liver Cu concentrations in growing beef heifers (Wright et al., 2000). Mo interacts with S and exacerbates the antagonism. Within the rumen, S and Mo can form thiomolybdates and bind soluble Cu, but thiomolybdates can be absorbed into the circulation and bind Cu compounds within the animal (Gould and Kendall, 2011). Evidence that dietary Mo in the absence of high dietary S interferes with Cu absorption is lacking (Gardner et al., 2003). Many of the negative responses observed when dietary Mo is elevated may actually be signs of molybdenosis. An equation to estimate absorption of Cu based on dietary S and Mo has been developed using data from sheep experiments (McLauchlan and Suttle, 1976):

$$\text{Cu Absorption} = 10^{(-1.153 - 0.0019 \times \text{Mo} - 0.076 \times \text{S} - 0.0131 \times \text{S} \times \text{Mo})}$$

(Equation 7-28)

where Cu absorption = g absorbed/g of total Cu; S = dietary S (g/kg of diet DM) and Mo = dietary Mo in mg/kg of diet DM.

The accuracy of this equation has not been evaluated with cattle and was not included in the software. However, based on that equation, Cu absorption from diets with low Mo

($\leq$1 mg Mo/kg) and dietary S at concentrations of 0.3, 0.4, and 0.5 percent would be reduced approximately 15, 30, and 43 percent compared with a diet with 0.22 percent S. With 4 mg Mo/kg, estimated absorption would be reduced another approximately 25 percent at each of those S concentrations.

Other Factors Affecting Absorption of Copper

Soil consumption at rates that may occur during grazing reduced absorption of Cu by 50 percent in sheep (Suttle, 1975) and cattle (Dewes, 1996). The effect of grazing on Cu absorption is likely a function of stocking rate and height of the sward (lower sward and greater stocking rate will increase soil consumption) and type of soil (Grace et al., 1996). Soils with clays may have a greater negative effect on Cu absorption. The model does not include an adjustment of Cu availability for grazing cattle because of the numerous uncertainties; however, users may wish to increase intake of Cu by cattle grazing short swards on clay soils.

Breed differences exist among cattle in susceptibility to Cu toxicity. Jersey cattle fed the same diet as Holstein cattle accumulated more Cu in their livers (Du et al., 1996; Morales et al., 2000). Whether this reflects differences in feed intake, efficiency of Cu absorption, hepatic storage, or biliary excretion of Cu is not known. However, differences in abundance of a Cu transporter in intestinal cells are likely a major cause of differences in Cu metabolism among beef cattle breeds (Fry et al., 2013). Because of a lack of data on Cu absorption by different breeds, the model does not include breed effects when calculating Cu requirements or Cu supply. Requirements and supply were calculated largely using data from Holsteins; therefore, absorbed supply of Cu may be underestimated, and requirements may be overestimated for Jersey cattle.

Effect of Source of Copper

Cu is found in most common feedstuffs in the range of 4 to 15 mg Cu/kg DM, and true absorption of Cu in those feeds was set at 0.05 (Buckley, 1991), assuming total diet had <0.22 percent S and <1 mg Mo/kg. In the seventh revised edition (NRC, 2001), the AC for feedstuffs was set at 0.04. Inorganic Cu is usually supplemented in the sulfate, chloride, carbonate, or oxide forms. Several commercial products in which the Cu is chelated or associated with organic compounds (e.g., AAs or carbohydrates) are also available. Very little data on actual true absorption of Cu from these sources are available, but several studies have been conducted to evaluate relative bioavailability by comparing changes in hepatic Cu concentrations when cattle are fed different Cu sources. Other biomarkers have been used to calculate relative bioavailability, but their accuracy and sensitivity are uncertain. The AC for Cu from Cu sulfate ($CuSO_4$) was set at 0.05 in the previous edition (NRC, 2001), and that value was retained. The AC for other Cu supplements was based on

relative bioavailability studies using liver Cu concentrations when available.

The bioavailability of Cu from dietary copper oxide (CuO) is almost zero (Langlands et al., 1989; Kegley and Spears, 1994); however, ruminal boluses containing CuO can be effective sources of Cu (Parkins et al., 1994). The long-term exposure (weeks or months) of CuO in a bolus to the ruminal environment increases its availability, whereas finely ground CuO does not stay in the rumen long enough to be solubilized. The bioavailability in Cu from copper chloride ($CuCl_2$) is similar to that of $CuSO_4$ (Ivan et al., 1990). In many studies, indicators of Cu status were not different in cattle fed $CuSO_4$ or proprietary Cu supplements, including Cu proteinates, Cu-AA complexes and tribasic $CuCl_2$ (Wittenberg et al., 1990; Ward and Spears, 1993; Du et al., 1996; Arthington et al., 2003; Spears et al., 2004; Correa et al., 2014). However, in other studies, proprietary products were significantly more available than $CuSO_4$ (Kincaid et al., 1986; Rabiansky et al., 1999; Hansen et al., 2008). Reasons for the inconsistent results are not clear, but relative availability of proprietary forms of supplemental Cu probably depends on the presence of antagonists (e.g., S and Mo), Cu status, and specific product being fed. For example, tribasic $CuCl_2$ was almost twice as effective at increasing liver Cu concentration as $CuSO_4$ in the presence of S and Mo, but when those Cu sources were fed to cattle in low Cu status without antagonists, they had equal bioavailability (Spears et al., 2004). Inadequate data are available to quantify factors affecting relative bioavailability of proprietary Cu supplements; therefore, the generic commercial Cu supplement in the feed library was assigned the same AC as $CuSO_4$. Users can modify the value based on available data for the specific product and situation.

Copper Requirements

Endogenous losses of Cu were assumed to be predominantly via bile and are expressed relative to BW. Using Cu isotopes (Buckley, 1991), biliary and urinary loss of Cu was approximately 0.0145 mg Cu/kg BW. This is more than twice the value used in the seventh revised edition (NRC, 2001). Cu content of growing tissues, when the liver is included as part of the carcass, is 2 to 2.5 mg Cu/kg based primarily on studies of sheep and beef cattle (Grace, 1983; Miranda et al., 2006; García-Vaquero et al., 2011). Because of concerns with the consumption of excessive Cu, the growth requirement was set at 2.0 mg Cu/kg live weight change. Cu content of milk is about 0.04 mg/kg when diets contain typical concentrations of Cu (Castillo et al., 2013; Faulkner et al., 2017), but it can be as high as 0.2 mg/kg when animals are fed a high Cu diet (Schwarz and Kirchgessner, 1978). The requirement for absorbed Cu for lactation was set at 0.04 mg Cu/kg milk produced. This is about a 70 percent decrease compared to the 0.15 mg Cu/kg milk produced used by the seventh revised edition (NRC, 2001). The conceptus at 190 days of gestation of Holstein cows (average BW = 715 kg) contained approxi-

mately 20 mg Cu (House and Bell, 1993). Because data are lacking, the committee assumed no Cu accumulated during the first trimester, and accumulation was linear between 90 and 190 days of gestation, so that gestation requirement for absorbed Cu was (0.2 mg Cu accumulated/d from 90 to 190 days of gestation or 0.3 µg Cu/kg maternal BW/d). From 190 days of gestation until calving, Cu accumulation in conceptus was 1.6 mg/d or 2.3 µg Cu/kg of maternal BW/d (House and Bell, 1993).

For an average lactating Holstein cow (35 kg of milk, 650 kg BW, 150 days pregnant), the total requirement for absorbed Cu is 11.0 mg/d compared with 11.4 mg obtained using the seventh revised edition (NRC, 2001). Assuming an intake of 23 kg and a dietary AC of 0.045, dietary Cu concentration of about 11 mg/kg would meet her requirement. Requirements for a 700-kg nonlactating cow at 260 days of gestation is 11.7 mg/d (approximately 20 mg dietary Cu/kg assuming an intake of 13.5 kg) compared with 7 mg/d calculating using the seventh revised edition (NRC, 2001).

Summary of Equations (mg absorbed Cu/d)

$$\text{Maintenance} = 0.0145 \times \text{BW} \quad \text{(Equation 7-29)}$$

$$\text{Growth} = 2.0 \times \text{ADG} \quad \text{(Equation 7-30)}$$

$$\text{Gestation (90 to 190 d pregnant)} = 0.0003 \times \text{BW}$$
$$\text{(Equation 7-31)}$$

$$\text{Gestation (>190 d pregnant)} = 0.0023 \times \text{BW}$$
$$\text{(Equation 7-32)}$$

$$\text{Lactation} = 0.04 \times \text{Milk} \quad \text{(Equation 7-33)}$$

where ADG and milk are in kg/d, and BW is in kg.

Signs of Copper Deficiency

Clinical signs of Cu deficiency are generally nonspecific (i.e., reduced growth rate, ill-thrift, increased prevalence of disease, reduced reproductive efficiency), but Cu deficiency can result in a loss of hair pigment or loss of hair. Diarrhea can also occur with Cu deficiency, but that may be related to excess Mo. Anemia (hypochromic macrocytic), fragile bones and osteoporosis, and cardiac failure also are observed in Cu deficiency (Underwood, 1981). Inadequate supply of Cu reduces the killing ability of phagocytic cells of cattle, but responses to supplemental Cu on cellular and humoral immunity have been inconsistent (Weiss and Spears, 2005). Impaired immune function is likely the reason cows fed inadequate Cu have more severe mastitis than those fed adequate Cu (Scaletti et al., 2003). The control diets in essentially all studies that showed improved immune function and reduced infectious disease would not have met current Cu requirements.

Assessing Copper Adequacy

Dietary concentrations of Cu are of limited value in assessing adequacy of Cu supply because of variable dietary and water concentrations of antagonists (discussed above). Plasma concentrations of Cu <0.5 mg/L are generally considered indicative of clinical Cu deficiency. However, other than confirming overt Cu deficiency, plasma concentrations are not useful in assessing status, including situations with excessive stores of Cu in the liver (López-Alonso et al., 2006). Activities of Cu-containing proteins (e.g., ceruloplasmin or superoxide dismutase) are generally considered unreliable biomarkers of Cu status in cattle (Mulryan and Mason, 1992; López-Alonso et al., 2006; Hepburn et al., 2009). Concentrations of the Cu chaperon protein may have potential as a marker of Cu status, but additional research is needed (Hepburn et al., 2009). Concentrations of Cu in liver are the standard for assessing Cu status in cattle, although recommended reference values vary. Concentrations of Cu in liver <10 mg/kg on a DM basis are generally considered indicative of impending clinical deficiency, and values less than about 35 mg Cu/kg DM are generally considered suboptimal (Smart et al., 1992; Underwood and Suttle, 1999). However, in the presence of high dietary Mo and S, which promote formation and absorption of thiomolybdates into the blood, Cu in liver may not accurately reflect Cu status (Suttle, 1991). The concentration of Cu in liver indicative of excess has not been definitively identified, but field reports indicate Cu toxicity occurs with liver concentration of 300 to 350 mg Cu/kg dry weight (Auza et al., 1999; Grace and Knowles, 2015).

Copper Toxicity

Cu toxicosis can occur in cattle that consume excessive amounts of supplemental Cu or feeds that have been contaminated with Cu compounds used for other agricultural or industrial purposes (Underwood and Suttle, 1999). When cattle consume excessive Cu, they accumulate large amounts of Cu in the liver before toxicosis becomes evident. Stress or other factors may result in the sudden liberation of large amounts of Cu from the liver to the blood, causing a hemolytic crisis. Such crises are characterized by considerable hemolysis, jaundice, methemoglobinemia, hemoglobinuria, generalized icterus, widespread necrosis, and often death (Steffen et al., 1997; Underwood and Suttle, 1999; Johnston et al., 2014). Because of antagonists (e.g., S and Mo) and because Cu continues to accumulate in the liver when excess Cu is fed (Balemi et al., 2010), defining a dietary concentration that will result in toxicity is not possible. NRC (2005) set the MTL for dietary Cu for cattle at 40 mg Cu/kg DM. However, growth rate and feed conversion were reduced in beef cattle with liver Cu concentrations of 290 mg/kg dry weight and fed diets with 20 mg supplemental Cu/kg DM for a total dietary concentration of 30 mg Cu/kg (Engle and Spears, 2000), and

fiber digestibility by dairy cows was reduced when $CuSO_4$ was supplemented to increase total dietary Cu to 20 mg/kg (Faulkner and Weiss, 2017).

Iodine

Function

The sole role of iodine (I) as a required nutrient is for the synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) that regulate energy metabolism. The amount of I incorporated into thyroid hormones was about 0.25 mg/d in calves weighing 45 kg and increased to 1.4 mg I/d in nonpregnant heifers weighing 400 kg (Mixner et al., 1966). Late-gestation cows incorporate about 1.5 mg I/d into thyroid hormone (Miller et al., 1988). Thyroid hormone production increases during lactation, especially in high-producing cows, and I incorporation into thyroid hormones may reach 4 to 4.5 mg I/d (Sorensen, 1962). Thyroid hormone production also is increased during cold weather to stimulate an increase in basal metabolic rate (Goodman and Middlesworth, 1980). About 80 to 90 percent of dietary I is absorbed, and most of the I not taken up by the thyroid gland is excreted in urine and milk (Miller et al., 1988). The I content of milk is a reasonable indicator of I status because it increases as dietary I intake increases (Berg et al., 1988). The availability of assays for thyroxine and thyroxine-stimulating hormone (TSH) might provide an accurate assessment of thyroid function and the causes of thyroid dysfunction. Alternatively, blood TSH concentrations might be used as a biomarker for thyroid function to reevaluate the minimum I requirements in dairy cattle.

When the I content of the diet is adequate or excessive, less than 20 percent of the dietary I will be incorporated into the thyroid gland (Sorensen, 1962). Under conditions where intake of dietary I is marginal, the thyroid gland will incorporate about 30 percent of the dietary I into thyroid hormones (Miller et al., 1975). When severely I deficient, the hyperplastic thyroid can bind up to 65 percent of the I consumed by the cow (Lengemann and Swanson, 1957).

Adequate Intakes

I requirements in previous reports were based on a limited number of thyroxine production rates measured in cattle during the 1960s and 1970s. Due to the time that has elapsed since those studies were conducted and the limited number of measurements, the committee concluded that there were insufficient data to determine an estimated average requirement for I. Therefore, AIs, rather than requirements, were determined.

Total daily thyroxine secretion rate (TSR) increased at a decreasing rate in growing heifers ages 77 to 686 days (Mixner et al., 1962) and varied from 0.008 to 0.0030 mg/kg BW. In lactating cows, the mean thyroxine secretion rate was about 0.30 mg/100 kg BW (Mixner et al., 1962; Miller et al.,

1975). The previous report listed separate requirements for maintenance, pregnancy, and lactation. It was suggested that the thyroxine secretion rate was 2.5-fold greater in lactating cows than in dry cows (Sorenson et al., 1962), yet limited evidence (Swanson et al., 1972) showed only a 25 percent increase from late pregnancy to lactation. The primary determining factor for TSR was BW. Therefore, the AI for maintenance for all groups of animals is based on TSR related to BW. In a summary of the studies of Swanson et al. (1972) and Mixner et al. (1962), TSR, $mg/d = 0.0653 \times BW^{0.528}$ ($R^2 = 0.96$). Thyroxine (T4) contains 66 percent I and Miller et al. (1975) suggested that under conditions where I was not limiting, 20 percent of dietary I was used by the thyroid gland to synthesize thyroxine. Therefore, the maintenance AI for I can be predicted by the following equation: I, $mg/d = 0.216 \times BW^{0.528}$.

In addition to thyroxine synthesis, I is also secreted in milk (discussed below), and for lactating cows, this loss must be replaced by dietary intake. At low I intake, milk contains about 0.05 mg I/L, and transfer of dietary I to milk is about 0.5 (Swanson et al., 1972). Therefore, the AI for lactation was set at 0.1 mg/L of milk. The equation for total AI for all classes of cattle except calves is

$$\text{Dietary I, } mg/d = 0.216 \times BW^{0.528} + 0.1 \times \text{Milk}$$
(Equation 7-34)

where BW is kg and milk is kg/d.

The AI for I for nonruminating calves was based on the AI established for human infants and was set at 0.8 mg I/kg DMI. Once calves are ruminating, Equation 7-34 should be used.

This amount of I will likely not be adequate when diets contain goitrogenic feeds such as canola meal (Pappas et al., 1979). Diets with canola meal decreased transfer of I into milk by 50 percent (discussed below). Assuming thyroxine synthesis is affected similarly, the AI for animals fed diets with goitrogenic feeds would be twice that calculated above. However, because of limited data, this adjustment is not included in the model, but users should consider adjusting supplementation when goitrogens are fed. Based on typical DMI, a dry cow (700 kg BW) and an average lactating cow (650 kg BW and 35 kg/d of milk) would need to be fed diets with 0.51 and 0.48 mg I/kg DM when diets did not contain goitrogens and 1.02 and 0.96 mg I/kg DM with goitrogenic diets. Because of human health concerns related to excess I intake and the fact that milk I concentrations increase with increased I concentration in the cow's diet, supplemental I should not exceed amounts deemed as AI for the cow (see toxicity section).

Factors Affecting Iodine Needs

Goitrogens are compounds that interfere with the synthesis or secretion of thyroid hormones and cause hypothyroidism. Goitrogens fall into two main categories: (1) cyanogenic

goitrogens impair iodide (I^{-1}) uptake by the thyroid gland. Cyanogenic glucosides can be found in many feeds, including raw soybeans, beet pulp, corn, sweet potato, white clover, and millet, and once ingested are metabolized to thiocyanate and isothiocyanate. These compounds alter I^{-1} transport across the thyroid cell membrane, reducing I retention; (2) Progoitrins and goitrins found in cruciferous plants (rape, kale, cabbage, turnips, and mustard) and aliphatic disulfides found in onions that inhibit thyroperoxidase prevent formation of mono- and diiodotyrosine (Ermans and Bourdoux, 1989). With goitrins, especially those of the thiouracil type, hormone synthesis may not be restored by dietary I supplementation, and the offending feedstuff needs to be reduced or removed from the diet.

Canola meal derived from low erucic acid varieties of rapeseed contains glucosinolates that can be converted into thiocyanate during seed processing. Recent studies (Franke et al., 2009a,b; Weiss et al., 2015a) demonstrated that when canola meal substitutes for soybean meal in diets, milk I concentrations are reduced. While milk I continued to increase with increasing dietary I concentration (up to 5 mg/kg diet DM) when diets contained canola meal, the rate of increase in milk I was reduced by 50 percent or more depending on the amount of canola meal fed (Franke et al., 2009a; Weiss et al., 2015a). However, blood serum I concentrations were similar to controls (Weiss et al., 2015a), and urinary I excretion was increased, suggesting I absorption is not impaired (Franke et al., 2009b). The negative effects of dietary goitrogens can be overcome by increasing the concentration of dietary I or removal of the feeds containing goitrogens. Diets that contain goitrogenic ingredients may need more I than the recommended AI.

Supplemental I in the form of ethylenediamine dihydriodide (EDDI) has been used to decrease foot rot in beef cattle (Maas et al., 1984) and more recently has been suggested as a possible treatment for digital dermatitis in dairy cattle (Gomez et al., 2014). Dietary EDDI may also have value in treating ringworm in young cattle (Cam et al., 2007). However, concerns about excessive milk I concentrations with supplemental I would prevent the use of EDDI for these purposes in lactating dairy cows.

Sources of Iodine

Most sources of I are readily absorbable, and the iodides of Na, K, and Ca are commonly used. Potassium iodide is easily oxidized and volatilizes before the animal can ingest it. Pentacalcium orthoperiodate and EDDI are more stable and less soluble and are commonly used in mineral blocks and salt licks exposed to the weather. Concentrations of I in forage are variable and dependent on the I content of the soil. Soils near the oceans tend to provide adequate I in plants. However, in the Great Lakes regions and Northwest United States, I concentrations in forages are generally low enough to result in a deficiency of I unless I is supplemented.

Milk Iodine

Typical range in milk I concentration is 100 to 300 µg/L (Flachowsky et al., 2014), and I in dairy products is readily absorbed by humans. Milk and dairy products are major sources of I intake in the United States and Europe, where dairy products make up a significant portion of the average diet. Dairy products may account for 30 to 74 percent of I intake in the United States (Murray et al., 2008). The recommended dietary allowance for I intake ranges from 70 µg/d in infants to 290 µg/d in pregnant and nursing women (Swanson et al., 2012). However, the upper tolerable limit for I for humans is only 2.2- to 3.5-fold greater than recommended intake. A suggested limit for milk is 500 µg I/kg. While there have been concerns about excess I intake from milk, more recent evidence based on urinary I excretion suggests that a significant portion of individuals with high I requirements (pregnant and nursing women) may not be consuming adequate I. In part, this is due to the reduced consumption of iodized table salt in the United States. Milk and dairy products will continue to make an important contribution to I intake in the general population.

Flachowsky et al. (2014) reviewed factors that affected milk I concentration. By far, dietary concentration of I is the primary factor that affects milk I concentration. Berg et al. (1988) demonstrated a linear increase in milk I excretion with increasing supplementation of I in the form of EDDI with 26 to 39 percent of the supplemental I excreted in the milk. Other factors such as I source, I antagonists (discussed above), farm management practices including teat dipping with I-containing substances, and the use of I sanitizers in milk processing can affect milk I concentrations (Flachowsky et al., 2014). I present in iodophor-containing teat dips and udder disinfectants were shown to be absorbed through the teat skin and markedly increased milk I concentrations. However, use of iodophors was discontinued in dairy teat dips and disinfectants in the late 1980s such that milk I concentrations have gradually declined (Flachowsky et al., 2014).

Deficiency Symptoms

I deficiency reduces production of thyroid hormones, slowing the rate of oxidation of all cells. Often the first indication of I deficiency is enlargement of the thyroid (goiter) of newborn calves (Miller et al., 1968). Calves also may be born hairless, weak, or dead. Fetal death can occur at any stage of gestation, but often the cows will appear normal (Hemken, 1970). In adult cattle, I deficiency can cause enlarged thyroid glands, reduced fertility (males and females), and increased morbidity. Under conditions of marginal or deficient dietary I, the maternal thyroid gland becomes extremely efficient in removing I from the plasma and recovering I during the degradation of spent thyroid hormone and thyroglobulin. Unfortunately, this leaves little I for the fetal thyroid gland, and the fetus becomes hypothyroid. The goiter condition is

the hyperplastic response of the thyroid gland to increased stimulation of thyroid growth by thyroid-stimulating hormone produced in the pituitary gland. Under mild I deficiency, the hyperplastic thyroid gland can compensate for the reduced absorption of I (Hetzel and Welby, 1997).

Toxicity

The maximum tolerable limit was set at 50 mg I/kg of diet DM (NRC, 2005). Clinical signs of I toxicity in ruminants include excessive nasal and ocular discharge, hyperthermia, salivation, decreased milk production, coughing, and dry, scaly coats (Paulikova et al., 2002). As discussed earlier, high concentrations of dietary I in the diet increase I concentrations in milk, and because humans are much more sensitive to I toxicity than cows, the danger of excess dietary I fed to cattle is a public health issue (Hetzel and Welby, 1997). Current U.S. Food and Drug Administration (FDA) regulations set the maximum limit of I supplementation in cattle from EDDI at 50 mg/d, which in a lactating cow consuming 25 kg of DM/d would correspond to a maximum concentration of 2.0 mg I/kg DM, roughly three times the amount considered to be needed.

Iron

Functions and Measures of Adequacy

Fe has a multitude of functions within the body, including oxygen transport (component of heme found in hemoglobin and myoglobin), electron transport (e.g., ferredoxins and cytochrome P-450 enzymes), immunity (e.g., myeloperoxidase and catalase), energy metabolism (e.g., aconitase), and gene regulations (Beard, 2001; Templeton and Liu, 2003). Fe deficiency results in hypochromic microcytic anemia due to failure to produce hemoglobin. Light-colored veal is due to low muscle myoglobin as a result of restricted dietary Fe. In young dairy cattle (<24 months old), serum ferritin had a stronger correlation with concentrations of Fe in liver and spleen than did hemoglobin (Miyata and Furugouri, 1987). However, over a lactation cycle, serum ferritin did not appear to reflect changes in Fe stores in dairy cows (Furugouri et al., 1982). Other measures of Fe status (e.g., Fe binding capacity of serum, plasma Fe) can change in response to factors that are independent of Fe status such as inflammation (Baydar and Dabak, 2014) and parturition (Miltenburg et al., 1991).

Because Fe deficiency is very rare in adult cattle, data evaluating the accuracy and sensitivity of Fe status indicators are not available. Common measures of Fe status did not change over the dry and early lactation (up to 60 days in milk) periods and were not affected when dry and lactating cows were fed 30 mg/kg of supplemental Fe from and Fe-AA complex (Weiss et al., 2010). Fe supplementation did not affect any production measure other than a small, but statistically significant, decrease in milk SCC. In Fe-deficient calves, morbidity and mortality associated with depressed immune responses occurred prior to any observed change in packed cell volume (Mollerberg and Moreno-Lopez, 1975). In lactating goats, hemoglobin concentrations were negatively related to SCC (Atroshi et al., 1986). Beard (2001) suggested that changes in functional measurements such as immune measures and performance during exercise in humans occurred prior to changes in hemoglobin and other measures of Fe status.

Requirement

Because of the uncertainty regarding the true absorption of Fe from feeds and the amount of Fe needed for maintenance, the factorial approach was used to generate an AI. During tissue and protein turnover, the majority of Fe is effectively recovered and recycled so that maintenance requirements for Fe are negligible. Milk contains about 1 mg Fe/kg (Aleixo and Nóbrega, 2003; Schnell et al., 2015). The absorbed Fe requirement of the conceptus of the pregnant cow between day 190 of gestation and the day of calving has been estimated to be 0.025 mg Fe/kg maternal BW or 18 mg/d for a 715-kg late-gestation Holstein cow (House and Bell, 1993). Increased vascularization of the uterus and other reproductive organs and fetal hematopoiesis would increase the gestation requirement for Fe earlier than 190 days of gestation, but data are unavailable to quantify this. Estimates of Fe content in the body range from 18 to 34 mg Fe/kg BW of calves (Bremner and Dalgarno, 1973c). The absorbed Fe requirement for growth of cattle has been set at 34 mg Fe/kg ADG.

Summary of Equations (mg absorbed Fe/d)

$$\text{Maintenance} = 0 \qquad \text{(Equation 7-35)}$$

$$\text{Growth} = 34 \times \text{ADG} \qquad \text{(Equation 7-36)}$$

$$\text{Gestation (>190 d pregnant)} = 0.025 \times \text{BW}$$
$$\text{(Equation 7-37)}$$

$$\text{Lactation} = 1.0 \times \text{Milk} \qquad \text{(Equation 7-38)}$$

where ADG and milk are in kg/d, and BW is in kg.

Absorption

Fe in the ferric form (Fe^{3+}) is poorly absorbed from the intestinal tract; however, reductases exist on the surface of enterocytes that convert ferric Fe to ferrous (Fe^{2+}), allowing for absorption. Fe-deficient calves absorbed Fe provided by ferric chloride but not from ferric oxide (Ammerman et al., 1967). Some Fe^{3+} can be reduced to Fe^{2+} on reaction with the acid of the abomasum (Wollenberg and Rummel, 1987).

The concentration of soluble Fe in corn silage also increased markedly following in vitro simulated abomasal exposure (Hansen and Spears, 2009).

Form of dietary Fe affects absorption, but Fe absorption by animals is a tightly regulated process, and Fe status interacts with form of Fe to ultimately determine the amount of Fe absorbed. Absorption of radioactive Fe from ferric chloride was three to five times greater by Fe-depleted calves than by Fe-sufficient calves (Ammerman et al., 1967). Calves fed diets with 750 mg Fe (from ferrous sulfate)/kg DM downregulated expression of a duodenal Fe importer (DMT1) found on the luminal side of the enterocyte and ferroportin, an exporter of Fe found on the basal-lateral membrane of enterocytes (Hansen et al., 2010a). The DMT1 may also be involved in absorption of Cu and Mn, which could be a reason high Fe can antagonize absorption of those two metals. Based on changes in expression of many proteins involved with Fe metabolism and a decrease in plasma Fe concentrations (Hansen et al., 2010b), a diet that is deficient in Cu but extremely excess in Mn may induce Fe deficiency in ruminants. In nonruminants, dietary Zn (Lind et al., 2003), phytate (Gillooly et al., 1983), phosphate, and Ca (Monsen and Cook, 1976) can reduce Fe absorption, and dietary β-carotene, vitamin A, and vitamin C can increase Fe absorption (García-Casal et al., 1998). Elevated dietary P reduces hepatic Fe concentrations in steers (Standish et al., 1971), but whether the other factors listed above affect Fe absorption in ruminants is unknown.

Assigning ACs for Fe to basal ingredients and supplements is plagued by a lack of data and because absorption is dependent on Fe supply and Fe status of the animal. Maximum absorption efficiency occurs when animals are deficient in Fe, which is very rare in adult ruminants. Maximum absorption can occur in calves because diets are inherently low in Fe, and deficiency is possible if animals are not supplemented. Absorption of Fe by deficient calves fed low Fe liquid-based diets ranges from 0.55 to 0.72 (Matrone et al., 1957; Bremner and Dalgarno, 1973a,b; Miltenburg et al., 1993). An analysis of balance studies done in growing calves suggests that the AC of soluble Fe from liquid diets declined from 0.40 to 0.15 as dietary Fe increased from 40 to 100 mg/kg (ARC, 1980).

Once the animal is ruminating, the efficiency of Fe absorption is considerably lower, in part because diets are generally excess in Fe and because much of the dietary Fe is provided by forages. Mechanically harvested forages can be high in Fe because soil contamination occurs during harvest. Much of that Fe is likely ferric oxide, which, based on data from calves and sheep, is essentially unavailable (Ammerman et al., 1967; van Ravenswaay et al., 2001). Based on in vitro tests and solubility, Fe from soil contamination has low bioavailability, but after soil-contaminated forage is stored as silage (pH ~4), solubility of Fe was about 20 times greater than in preensiled samples (Hansen and Spears, 2009). Whether that results in increased absorption of Fe is unknown.

Studies using radioactive Fe determined that Fe absorption efficiency was less than 0.02 in adult cattle fed a diet that supplied much more Fe than was needed (van Bruwaene et al., 1984). When pregnant ewes were fed diets that contained 20 mg Fe/kg diet (much lower than most practical diets fed dairy cows), absorption of dietary Fe was 0.21 (Hoskins and Hansard, 1964). Because data are not available to contradict the value used previously (NRC, 2001) for Fe absorption from basal ingredients (0.10), that value was retained. However, because of the typically high concentrations of Fe in most dairy diets and because much of the Fe is likely from soil contamination, an AC less than 0.10 is probably more accurate. Based on limited data from calves and sheep, ferrous sulfate has the greatest relative bioavailability, followed by ferrous carbonate (Ammerman et al., 1967; van Ravenswaay et al., 2001). In NRC (2001), Fe supplements were given ACs of 0.4 to 0.6, which may be appropriate for preruminant cattle but are likely much too high for adult ruminants fed practical diets. No data are available on actual absorption of Fe by ruminants; ferrous sulfate was assumed to have an absorption of 0.20 based on data from Hoskins and Hansard (1964), and based on relative availability, ferrous carbonate was assigned an absorption value of 0.10. Many mineral supplements are contaminated with low concentrations of Fe, and because of a total lack of data, the Fe was assumed to be mostly Fe oxide and given an AC of 0.01.

A 650-kg cow producing 25 kg of milk/d at 205 days of gestation and consuming 20 kg/d DM needs to absorb only 41 mg Fe/d or be fed a diet (AC = 0.1) with just 20 mg Fe/kg DM. Most feedstuffs will contain adequate Fe to meet the Fe requirements of adult cattle. Milk-fed calves are the only group of cattle that ordinarily require Fe supplementation. Feeding veal calves <15 weeks of age as little as 39 mg Fe/kg DM will allow calves to grow at a normal rate, but the muscles remain pale and the animals remain slightly anemic (Bernier et al., 1984). A study by (Lindt and Blum, 1994) suggests that a 50-mg Fe/kg diet is adequate to maintain physiological function in growing veal calves.

Toxicity

NRC (2005) set the MTL for cattle at 500 mg Fe/kg of diet DM; however, that value is dependent on source. The MTL assumes the Fe is from a readily available source; therefore, if the Fe is predominantly from forage, diets in excess of that value likely will cause little problem. However, when readily available sources of Fe are fed, the MTL established by NRC (2005) may be too high. Milk yield and DMI decreased linearly when cows were fed diets with 0, 250, or 400 mg supplemental Fe from Fe sulfate (McCaughey et al., 2005). Diets supplemented with 500 mg Fe (from Fe sulfate) decreases measures of Cu status (Chase et al., 2000). Excess Fe can lead to oxidative stress by increasing the generation of ROS and reactive N species (Meneghini, 1997). High Fe

(from Fe sulfate) reduced in vitro ruminal digestibility of DM (Harrison et al., 1992). Fe in water can affect water intake and increase measures of oxidative stress in dairy cows (see Chapter 9).

Manganese

Function and Measures of Adequacy

Mn is a cofactor in a host of enzymes and other proteins that are needed for normal metabolism of AAs, carbohydrates, and lipids and is required by every organ system in the body. Mn-dependent transferases are vital for cartilage production and bone development, and a common sign of Mn deficiency is skeletal abnormalities. Clinical signs of these abnormalities include enlarged joints, shortened and weak bones, superior brachygnathism, ataxia, and deafness and equilibrium problems caused by improper development of bones in the middle ear. The skeletal system of the gestating fetus is especially sensitive to Mn deficiencies, and the newborn calf can show clinical signs of deficiency while its dam appears normal (Hansen et al., 2006a). The majority of calves born from beef cows (Rojas et al., 1965) and heifers (Hansen et al., 2006a) fed diets with approximately 16 mg Mn/kg diet DM for the entire gestation had bone deformity signs indicative of a Mn deficiency, whereas the dams appeared clinically normal.

Reproductive efficiency can be reduced when cows are fed diets with inadequate Mn; however, the concentration of dietary Mn at which this occurs is poorly defined (Hidiroglou, 1979). In an old study (Bentley and Phillips, 1951), heifers fed diets with approximately 10 mg Mn/kg had poor fertility, and the calves that were born had bone disorders. When the diet contained 30 to 40 mg Mn/kg DM, fertility improved and no bone disorders were noted. More recently, beef heifers fed a basal diet with 16 mg Mn/kg had similar reproductive measures as did heifers fed diets with 26 mg Mn/kg; however, heifers fed diets with 56 to 66 mg Mn/kg had a substantial but statistically insignificant increase in pregnancy rate compared with heifers fed 16 or 26 mg Mn/kg (Hansen et al., 2006b).

At the current time, no sensitive indicators of Mn status have been identified for cattle. Manganese superoxide dismutase (Mn-SOD) is in the mitochondria and works in concert with other antioxidants to minimize accumulation of ROS. In some species, Mn-SOD activity responds to changes in Mn intake (de Rosa et al., 1980). Data are lacking relating Mn intake to Mn-SOD activity in cattle, but steers injected with a mix of trace minerals including Mn had higher Mn-SOD activity in red blood cells than steers injected with saline (Genther and Hansen, 2014). In humans, Mn-SOD activity of lymphocytes responded to changes in Mn intake (Davis and Greger, 1992), and in rats, Mn intake was related to several measures of immune function (Son et al., 2007). Arginase is a Mn-dependent enzyme in the urea cycle, and rats fed Mn-deficient diets had reduced arginase activity,

which resulted in higher concentrations of plasma ammonia and lower concentrations of plasma urea (Brock et al., 1994). Plasma urea concentrations in cattle have not been shown to respond to changes in dietary Mn. Arginase activity also affects concentrations of nitric oxide in certain tissues, which could explain some of the effects Mn has on immune function (Chang et al., 1998). Concentrations of Mn in plasma or whole blood did not differ between cattle fed diets with wide ranges in Mn concentrations (Weiss and Socha, 2005; Hansen et al., 2006a,b), indicating that it is not a good indicator of status in clinically normal cattle. Newborn calves that were displaying clinical signs of Mn deficiency had lower whole-blood Mn than calves born from heifers fed adequate Mn during gestation (Hansen et al., 2006a). In beef cattle, the concentration of Mn in liver responded linearly to increasing dietary concentrations of Mn (16 to 66 mg/kg), but liver Mn was only about 10 percent greater (9.4 versus 8.2 mg Mn/kg of liver DM), whereas intake of Mn varied more than 4-fold (Hansen et al., 2006b). The lack of sensitive status indicators and established reference ranges for liver concentrations make quantifying the Mn requirement difficult.

Manganese Absorption and Homeostasis

Intestinal absorption of Mn is thought to occur mainly via DMT1, a protein that is also involved with Cu, Zn, and especially Fe absorption (Garrick et al., 2003). Expression of DMT1 in the duodenum of humans experiencing an Fe deficiency is increased (Zoller et al., 2001), and it is downregulated in calves fed high Fe (Hansen et al., 2010a). Whether Mn status affects expression is unknown. Although data with cattle are lacking, high Co, Fe, and Cu can inhibit uptake of Mn in other species (Garrick et al., 2003). In humans, high Ca reduced absorption of Mn, but P and Mg did not (Davidsson et al., 1991). In chickens, high Ca had no effect on Mn absorption, whereas phosphate significantly reduced Mn absorption (Wedekind et al., 1991). Whether any of these antagonisms of Mn absorption occur in cattle are unknown. Elevated dietary S reduced apparent absorption of Mn by steers (Pogge et al., 2014).

Measuring the true absorption of Mn is especially difficult because the majority of dietary Mn that is absorbed is removed from the portal circulation by the liver and is excreted back into the intestine via bile. Using radioactive Mn ($MnCl_2$), Mn absorption by humans ranged from 1.0 to 2.5 percent (Davidsson et al., 1991; Finley et al., 1994). In rats, Mn ($MnCl_2$) absorption averaged 1.8 percent (Chua and Morgan, 1997). In a very limited study (two cows), absorption of radioactive Mn (from $MnCl_2$) averaged about 0.5 percent (Vagg, 1976), and in another limited study (two cows) based on portal vein data, Sansom et al. (1978) calculated an absorption rate of 0.5 percent for $MnCl_2$. Across studies, the AC in cattle was about one-fourth the value measured in nonruminants. That may be a true species difference or it could be caused by differences in intake of Mn. Absorp-

tion and turnover of Mn are positively correlated (Britton and Cotzias, 1966). When Mn intake increases, more Mn is absorbed, but biliary excretion also increases so that very little net change in body Mn content occurs.

Data are not available regarding absorption of Mn from basal feeds or from inorganic sources other than $MnCl_2$. In the previous edition (NRC, 2001), the AC for Mn from the Cl^- and SO_4^{-2} salts was set at 0.01 and 0.0075 for all basal feedstuffs. The same relative differences between supplemental Mn sources and basal feedstuffs were retained in this edition; however, AC of Mn from $MnCl_2$ was set at 0.005 based on the limited cow data that are available. Manganese sulfate was assigned the same AC value (0.005), and basal ingredients were assigned an AC of 0.004 (i.e., 0.005×0.75). The ACs of other sources of supplemental Mn were set at Mn carbonate (0.0015) and Mn monoxide (0.003) based on relative bioavailability studies (Henry, 1995c).

Manganese Requirements

An AI is used for Mn because inadequate data are available to establish a requirement. The amount of Mn needed by a dairy cow for maintenance has not been experimentally measured. Approximate intakes of 250 to 300 mg total Mn by gestating cattle were not adequate to prevent deficiency signs in their offspring (Hansen et al., 2006a; de Carvalho et al., 2010). Assuming an AC for dietary Mn of 0.004 and that approximately 50 mg Mn was needed for gestation (see below), the maintenance requirement for absorbed Mn must be greater than 0.0016 to 0.002 mg Mn/kg BW. Weiss and Socha (2005) determined an intake of approximately 580 mg Mn/d was necessary to maintain lactating dairy cows in zero Mn balance. In that study, average secretion of Mn in milk was 0.6 mg/d, which equals 150 mg dietary Mn (assumed AC = 0.004). Subtracting that value from 580 mg yields an estimated maintenance requirement of 430 mg for a 650-kg cow or 0.0026 mg absorbed Mn/kg BW. Because of a lack of other data, that value was set as the maintenance AI for absorbed Mn. This is 30 percent higher than the requirement estimated in the previous version (i.e., 0.002 mg Mn/kg BW).

House and Bell (1993) determined that 0.3 mg Mn/d was deposited into the fetus from 190 days of gestation until parturition for Holstein cows weighing 715 kg. Therefore, the daily gestation requirement (starting at 190 days of gestation) for absorbed Mn was set at 0.00042 mg/kg BW of the dam. The lactation requirement is equal to the amount of Mn secreted in milk daily. The concentration in milk ranges from about 0.016 to 0.050 mg Mn/kg (Gunshin et al., 1985; Erdogan et al., 2004; Pechova et al., 2008; Castillo et al., 2013). The weighted average was 0.027, which is essentially equal to 0.03 mg Mn/kg, the value used in the previous edition (NRC, 2001). Therefore, the lactation requirement for absorbed Mn was set at 0.03 mg Mn/kg of milk. The concentration of Mn in carcasses of calves averages about 2.5 mg/kg of total carcass on a DM basis (Suttle, 1979). Assuming the

carcasses used in these experiments were 27 percent DM, the Mn requirement for growth can be estimated to be 0.7 mg Mn/kg BW gain. No new data were found on tissue accretion of Mn or whole-body Mn concentrations in cattle; therefore, the growth requirement was not changed.

The AI for absorbed Mn for a late-gestation Holstein cow weighing 700 kg would be 1.8 mg/d for maintenance plus 0.3 mg/d for gestation for a total of 2.1 mg absorbed Mn. This is about 490 mg total dietary Mn (assumed AC = 0.0042). Assuming a DMI of 12.5 kg/d, a concentration of 40 mg total Mn/kg of diet DM would meet the requirement. This is substantially greater than in the previous version. For a 650-kg cow producing 45 kg of milk/d, the maintenance requirement would be 1.7 mg and 1.35 mg for lactation for a total absorbed Mn requirement of 3.1 mg/d. Assuming a DMI of 26 kg/d, dietary concentration of total Mn to meet the requirement would be approximately 27 mg Mn/kg (assumed AC of 0.0042).

Summary of Equations (mg absorbed Mn/d)

$$Maintenance = 0.0026 \times BW$$
(Equation 7-39)

$$Growth = 2.0 \times ADG \quad \text{(Equation 7-40)}$$

$$Gestation\ (>190\ d\ pregnant) = 0.00042 \times BW$$
(Equation 7-41)

$$Lactation = 0.03 \times Milk \quad \text{(Equation 7-42)}$$

where ADG and milk are in kg/d, and BW is in kg.

Maximum Tolerable Level

Because absorption of dietary Mn is extremely low, Mn toxicity in ruminants is unlikely to occur, and the few documented incidences with adverse effects are limited to reduced feed intake and growth (Jenkins and Hidiroglou, 1991). The maximum tolerable amount of Mn as given by NRC (2005) is 2,000 mg Mn/kg of diet DM. However, diets with 500 mg Mn/kg exacerbated the negative effects of feeding a Cu-deficient diet (Hansen et al., 2009). Furthermore, cattle fed a Cu-deficient diet with 500 mg Mn/kg DM displayed some indications of an Fe deficiency (Hansen et al., 2010b).

Selenium

Functions and Animal Response

The only known nutritional function of Se is as a component of specific selenoproteins. Essentially, any protein can contain Se because of nonspecific incorporation of selenomethionine (replacing Met) into the polypeptide chain. However, selenoproteins require selenocysteine in a specific

location within the peptide chain to be functionally active. Selenocysteine is identical to cysteine, except a Se molecule replaces the sulfur molecule. To be inserted into the proper location in a peptide, selenocysteine must be synthesized from serine that is joined to a specific transfer RNA (tRNA; UGA codon). Selenocysteine that is absorbed by the intestine cannot be directly inserted into the active site within a polypeptide chain. In humans, at least 25 genes for selenoproteins have been identified (Lu and Holmgren, 2009), but the functions of many of the resulting selenoproteins are unknown. Glutathione peroxidases (GPx) are a family of selenoproteins that reduce hydrogen peroxide to water or phospholipid hydroperoxides to phospholipids, and increasing Se intake of cattle often increases the activity of these enzymes. Iodothyronine deiodinases are a family of selenoenzymes that activates thyroxin by deiodinating T_4 into T_3, and Se supplementation has increased serum T_3 concentrations in cattle (Awadeh et al., 1998; Contreras et al., 2002). The enzyme can also inactivate T_3 by further deiodination. Thioredoxin reductases (TRx) are selenoenzymes that reduce thioredoxin, which is involved in regulation of the redox potential of cells. Two other selenoproteins (selenoprotein P and selenoprotein W) have been found in bovine tissue, but their functions are unclear.

White muscle disease or nutritional muscular dystrophy is the classical sign of a clinical Se deficiency. Signs of this disease include leg weakness and stiffness, flexion of the hock joints, and muscle tremors (NRC, 1983). Cardiac and skeletal muscles have chalky striations and necrosis, and animals often die from cardiac failure. In cattle, improved Se status has increased growth rates (Wichtel et al., 1994; Reis et al., 2008) and reduced prevalence of retained fetal membranes (reviewed by Hemingway [1999] and Jovanovic et al. [2013]). Most studies evaluating effects of Se on clinical and subclinical mastitis have reported positive results (Smith et al., 1985; Erskine et al., 1987, 1989, 1990; Weiss et al., 1990; Wichtel et al., 1994; Jukola et al., 1996; Malbe et al., 2003; Kommisrud et al., 2005). Other health problems that have responded to Se supplementation include metritis, cystic ovaries (Harrison et al., 1984; Enjalbert et al., 2006), and udder edema (Miller et al., 1993). The likely mode of action of Se for these health disorders is via regulation of cellular concentrations of ROS via GPx and TRx. Se supplementation of cattle has improved the function of immune cells, including neutrophil (Hogan et al., 1990; Cebra et al., 2003), macrophage (Ndiweni and Finch, 1995), and lymphocyte (Stabel et al., 1990; Cao et al., 1992; Pollock et al., 1994). Concentrations of specific ROS within cells affect inflammatory responses, arachidonic acid metabolism, and production of prostaglandins and numerous cytokines (Salman et al., 2009; Sordillo, 2013).

Sources

Concentrations of Se in plants are positively correlated with those in the soil (Whelan et al., 1989; Hall et al., 2011). In general, feeds grown in the central plains of the United States and Canada contain more than 0.1 mg Se/kg DM, and feeds grown east of the Mississippi River and west of the Rocky Mountains typically contain <0.1 mg Se/kg DM (NRC, 1983). Except for Se accumulator plants (e.g., *Astragalus bisulcatus*), the predominant form of Se in plants is selenomethionine plus minor amounts of selenocysteine and selenite (Whanger, 2002). Se concentration of feeds is positively correlated with protein concentrations, and plant parts that are higher in protein also are higher in Se. Leaves of forage plants contain 1.5 to 2 times more Se than do stems (Gupta and Winter, 1989).

Based on current regulations of the U.S. FDA (1997, 2003), the only forms of Se that can be added legally to diets in the United States are sodium selenite, sodium selenate, and Se-enriched yeast at levels not to exceed 0.3 mg supplemental Se/kg DM. Other sources of supplemental Se that have been evaluated in cattle include barium selenate (Ceballos et al., 2010) and Se dioxide (Grace et al., 1995).

Efficiency of Absorption

Apparent absorption of Se from diets without supplemental Se is between 0.30 and 0.60 for sheep, goats, and nonlactating dairy cows (Harrison and Conrad, 1984a,b; Aspila, 1988; Koenig et al., 1997; Gresakova et al., 2013). Apparent absorption of Se in diets that contain selenite and selenate in diets ranged from 0.36 to 0.51 (Harrison and Conrad, 1984b; Ivancic and Weiss, 2001; Gresakova et al., 2013), and for diets with Se yeast, apparent absorption ranged from about 0.57 to 0.62 (Walker et al., 2010; Gresakova et al., 2013). In a direct comparison, apparent absorption of Se from Se yeast was about 24 percent greater (0.62 versus 0.50) than that of selenite when fed to sheep (Gresakova et al., 2013). Because of endogenous fecal losses, true absorption of Se is greater than apparent absorption. True absorption estimated using the regression method averaged about 0.5 in dairy cows fed inorganic Se (Harrison and Conrad, 1984a; Ivancic and Weiss, 2001). No data are available on estimated true absorption of Se from yeast. Because Se from Se yeast is retained in cellular proteins to a greater extent than that from inorganic Se, endogenous fecal losses of Se when Se yeast is fed may be greater, but the true absorption would also be greater.

Factors Affecting Absorption

Intestinal absorption of selenate is greater than selenite in sheep and rat intestinal in vitro models (Ardüser et al., 1986) and in the human Caco-2 cell model (Gammelgaard et al., 2012); however, because most but not all of the selenate is reduced to selenite within the rumen, absorption of selenate is probably only slightly greater than selenite in cattle (Podoll et al., 1992). The predominant form of Se in Se yeast is selenomethionine, which is absorbed via the same mechanism as Met. Intestinal absorption of selenomethionine is greater than absorption of inorganic Se sources (Gammelgaard

et al., 2012). Assuming differences in apparent absorption accurately reflect differences in true absorption, absorption of Se from high-quality (i.e., high proportion of Se as selenomethionine) Se yeast is at least 1.2 times that of inorganic Se. Se uptake by ruminal microorganisms is much greater when Se yeast is fed compared with selenite (Mainville et al., 2009), but form of Se did not affect measures of ruminal fermentation (Panev et al., 2013).

Dietary sulfate added to increase concentrations of dietary S by 0.2 and 0.4 percentage units (Ivancic and Weiss, 2001) and low (<0.6 percent of DM) and high (>1.0 percent of DM) concentrations of dietary Ca (Harrison and Conrad, 1984a) reduce absorption of inorganic Se by cattle. Supplementation of S from anionic salts (approximately 0.6 percent total diet S) for the last 3 weeks of gestation did not influence Se status of nonlactating cows (Gant et al., 1998). Long-term feeding of diets that contained approximately 0.3 percentage units of added sulfate–sulfur to beef cattle did not affect concentrations of Se in blood or the activity of GPx (Khan et al., 1987). In an in vitro sheep intestinal model, thiosulfate and molybdate reduced absorption of inorganic Se (Ardüser et al., 1986). In nonruminants, Se absorption was not affected by dietary Cu, Fe, Mo, and Mn over a wide range of concentrations (Abdel Rahim et al., 1986). In dairy cows, increased dietary Cu did not affect measures of Se status (Koenig et al., 1991). Rats that were Mg deficient had significantly lower Se absorption than rats adequate in Mg (Jiménez et al., 1997), and elevated dietary Zn reduced Se absorption in rats (House and Welch, 1989). Whether Mg or Zn affects Se absorption in cattle is unknown. Antagonists to absorption of inorganic Se may affect absorption of Se yeast differently.

Indicators of Selenium Status

Se status can be assessed by concentrations of Se in tissue and blood, activity of glutathione peroxidase, and various immune cell assays. Few differences in blood and tissue concentrations of Se occur between different inorganic sources of Se when fed to dairy cattle (Podoll et al., 1992; Gibson et al., 1993; Ortman and Pehrson, 1999; Ortman et al., 1999). Se from Se yeast or from basal ingredients with higher concentrations of Se usually increases concentrations of Se in blood and activity of GPx more than diets with inorganic Se (Conrad and Moxon, 1979; Ortman and Pehrson, 1997; Knowles et al., 1999; Ortman et al., 1999; Weiss and Hogan, 2005; Juniper et al., 2008; Phipps et al., 2008; Koenig and Beauchemin, 2009). Blood concentrations and GPx activity average 20 to 25 percent greater when Se yeast is fed (Weiss, 2003; Juniper et al., 2006, 2008; Phipps et al., 2008; Koenig and Beauchemin, 2009) similar to the difference in apparent absorption. On average, milk Se is about 1.9 times greater when Se yeast is fed compared with inorganic Se, but a meta-analysis determined that depending on supplementation rate, the difference could be more than three times (Ceballos et al., 2009). Feeding rumen-protected

Met reduces the concentration of Se in milk when Se yeast is fed (Weiss and St-Pierre, 2009).

Requirements and Factors Affecting Requirements

The seventh revised edition (NRC, 2001) defined the Se requirement as 0.3 mg/kg of dietary DM for all classes of dairy cattle. No new data are available to dispute this requirement. However, most of the data supporting this requirement were generated from experiments in which selenite or selenate/kg of dietary DM (DM basis) was fed, and total dietary Se ranged from 0.35 to 0.40 mg/kg.

Establishing requirements for Se using the factorial approach is difficult because the deposition of Se in body tissues, conceptus, and milk is dependent on Se intake and Se source, and essentially no data are available on endogenous fecal and urinary losses. Assuming a cow is fed a diet with approximately 0.3 mg Se from inorganic sources/kg of dietary DM, the conceptus will accumulate 0.055 mg Se/d during the last trimester of gestation (House and Bell, 1994). Comparable data when Se yeast is fed are not available, but accumulation in swine fetuses when Se yeast was fed was approximately 1.3 times greater than when selenite was fed (Ma et al., 2014).

Se concentrations vary across tissues in cattle, with kidney usually having the highest concentrations and muscle having lower concentrations (Lawler et al., 2004; Juniper et al., 2008), but muscle contained about 0.3 mg Se/kg DM when growing cattle were fed a diet with 0.3 mg Se/kg from selenite (Juniper et al., 2008) and 0.4 to 0.8 mg Se/kg dry weight when Se yeast was fed (Juniper et al., 2008; Richards et al., 2011). Concentrations of Se in various tissues of growing beef animals were 1.25 times greater when Se yeast was fed compared with selenite (Juniper et al., 2008). Se concentration of milk averages about 0.02 mg/kg and 0.04 mg/kg when selenite and Se yeast is fed at approximately 0.3 mg Se/kg of diet, respectively (Ceballos et al., 2009). Using the regression method, endogenous fecal and urinary losses varied by more than a factor of two depending on the source of data (Harrison and Conrad, 1984b; Ivancic and Weiss, 2001). Endogenous cells sloughed by cows that are deficient in Se would likely have lower concentrations of Se than cells sloughed by cows adequate in Se. No data are available on endogenous losses when Se yeast is fed but would likely be greater because of greater Se concentrations in cells.

Current FDA regulations limit Se supplementation to 0.3 mg/kg of diet (assumed air dried or approximately 90 percent DM basis) in the United States (FDA, 1997), and in most situations, that amount of supplemental Se will maintain dairy cattle in good Se status. Based on the effect of Se on mastitis, concentrations of Se in whole blood should be greater than about 0.18 µg/mL or approximately 0.075 µg/mL for plasma when inorganic Se is fed (Jukola et al., 1996). Intake of approximately 6 mg/d of inorganic Se should maintain those blood concentrations (Maus et al., 1980). Less Se

may be needed when Se yeast is fed; however, more Se is also secreted in milk and retained in the body (nonspecific proteins) when Se yeast is fed. Based on available data, the AI of Se for all classes of cattle was set at

$$\text{Selenium AI, mg/d} = 0.3 \times \text{DMI}$$
(Equation 7-43)

where DMI is in kg/d, and basal diet is generally assumed to provide no Se.

Most of the studies with Se for dairy cattle were conducted in areas with low soil Se so that basal Se was usually <0.1 mg Se/kg DM. This means that intake of supplemental Se was approximately equal to intake of total Se. In areas where soil has higher Se concentrations (e.g., North and South Dakota), users are advised to analyze locally grown forages for Se and include basal Se in the calculation of Se supply. Since Se concentrations in feedstuffs are low, specialized equipment is needed for the assay, which many labs do not have. The Se concentrations in the feed library included in the model are means and can differ greatly from feeds grown on specific farms.

Toxicity

Alkali disease and blind staggers result from Se toxicity. Clinical signs include sloughing of hooves, lameness, loss of hair, and emaciation. Most cases of Se toxicity have been related to consumption of Se-accumulating plants (e.g., *Astragalus* sp.), and much of the Se in those plants is found in methyl-selenium compounds. Similar clinical signs were also caused by feeding high doses of selenomethionine (10 mg Se/kg diet DM) or selenite (25 mg Se/kg diet DM) to yearling cattle for 120 days (O'Toole and Raisbeck, 1995). Acute toxicity can occur when cows are fed 10 to 20 mg Se/kg BW from selenite. An injection of about 0.5 mg Se/kg BW to young cattle (ca. 200 kg) resulted in a 67 percent mortality rate (NRC, 1983). The current MTL for dietary Se is 5 mg/kg of diet DM (NRC, 2005) or about 16 times greater than the recommended dietary concentration.

Zinc

Function

Zn is a component of more than 200 enzymes, including oxidoreductases (e.g., Cu–Zn superoxide dismutase), transferases (e.g., RNA polymerase), hydrolases (e.g., alkaline phosphatase and carboxypeptidase), lyases (carbonic anhydrase), and ligases (e.g., tRNA synthetase) (Kidd et al., 1996). Zn is involved with macronutrient metabolism, numerous aspects of the immune system, gene regulation, hormonal regulation, neurotransmission, and apoptosis. The effects of Zn on immune function have received substantial attention (Fraker and King, 2004). Historically, these effects

were thought to be manifested via Zn-containing enzymes such superoxide dismutase (which usually does not show a change in activity when dietary Zn intake is altered). However, accumulating data indicate that changes in Zn concentrations within immune cells are a major regulatory mechanism that may affect the entire immune system (Haase and Rink, 2009). The ubiquitous functions of Zn are likely the primary reason identifying markers of Zn status has been so difficult.

Absorption

Molecular and cellular mechanisms of absorption of dietary Zn have not been studied to any degree in the bovine, but substantial information is available from rodents and poultry models. Whether information from those species reflects mechanisms in cattle is unknown. In rats and poultry, Zn absorption occurs throughout the small intestine and perhaps in the large intestine by two different mechanisms: a saturable, transport-mediated absorption system and nonsaturable diffusion. In poultry, transport-mediated absorption occurred primarily in the duodenum and jejunum, and nonsaturable diffusion occurred primarily in the ileum (Yu et al., 2008). In rats, saturable absorption of Zn was found in all segments of the small intestine. The saturable transporters probably belong to the Zip family, and expression is downregulated when dietary Zn supply is high and upregulated when supply is low (Liuzzi and Cousins, 2004; Lichten and Cousins, 2009). Based on poultry and rodent data, at low dietary concentrations of Zn, high-affinity transporters become important (jejunum and ileum) but at high concentrations of Zn, diffusion in ileum and colon likely would predominate because of transporter saturation and downregulation. Although some regulation of Zn absorption probably occurs, based on the putative absorption processes, when excess-absorbable Zn is fed, cows will absorb Zn in excess of requirement. Export of Zn out of enterocytes appears to be regulated and is used to maintain Zn homeostasis. Expression of metallothionein genes or synthesis of the protein in intestinal cells is upregulated when excess Zn is provided (Tran et al., 1998; Shen et al., 2008), which likely is one mechanism of increased fecal excretion of endogenous Zn.

Factors Affecting Zinc Absorption

Measuring the true absorption of Zn or relative availability of Zn is exceedingly difficult because fecal excretion of Zn is used to maintain Zn homeostasis, and good markers of Zn status are lacking. Lactating cows that were adapted to a Zn-deficient diet (6 mg Zn/kg diet DM) absorbed nearly 50 percent of dietary Zn (Kirchgessner and Schwarz, 1976). However, maximizing Zn absorption by feeding deficient diets is clearly not desirable. Based on isotope studies, Zn absorption by ruminating cattle fed practical diets that were likely adequate in Zn ranged from 12 to 33 percent (Miller and Cragle, 1965; Hansard et al., 1968; Miller et al., 1968).

However, these studies are decades old and were done with cattle with low DMI compared with modern lactating cows. Whether DMI affects Zn absorption is unknown, but a positive correlation between DM digestibility and Zn absorption has been observed (Miller and Cragle, 1965). Because new data are lacking, the AC for zinc chloride ($ZnCl_2$; the Zn source used in the above studies) used in the previous version (NRC, 2001) was retained (i.e., 0.20). Absorption of Zn by rats fed radioactive Zn from $ZnCl_2$ or from soy flour produced by plants fertilized with radioactive $ZnCl_2$ were the same (Stuart et al., 1986). Similar results were found when preruminant calves were fed $ZnCl_2$ or corn plants grown with radioactive Zn (Neathery et al., 1972). These studies, at least for nonruminants, indicate that Zn contained in basal ingredients has similar absorbability as $ZnCl_2$ (ca. 0.20). In agreement, true absorption of Zn was 0.182 by adult goats fed a diet in which about 50 percent of the Zn was from basal ingredients and 50 percent from $ZnCl_2$ (Hattori et al., 2010). Therefore, Zn in basal ingredients was assigned an AC of 0.20. Absorption coefficients for Zn from other supplements were estimated from studies measuring relative bioavailability. $ZnCl_2$ and zinc sulfate ($ZnSO_4$) had similar bioavailability in calves with a functioning rumen (Kincaid, 1979). Zinc oxide had a bioavailability of approximately 80 percent that of $ZnSO_4$ (Sandoval et al., 1997) so that its AC was set at 0.16. Zinc carbonate had the same bioavailability as $ZnSO_4$ in sheep (Sandoval et al., 1997). Several proprietary supplemental Zn sources are available, but published data on measures of relative bioavailability are limited (Cao et al., 2000). The limited data indicate slightly higher (10 to 20 percent) greater bioavailability for some proprietary compounds compared with $ZnSO_4$. A problem with most relative bioavailability studies is that very high concentrations of Zn are fed, which can affect results.

Several dietary components can interfere with Zn absorption or increase body losses of Zn, but most of the studies have been done with nonruminants (Lönnerdal, 2000). Phytate clearly reduces Zn absorption in nonruminants. Zn absorption by calves fed milk averaged about 50 percent (Miller and Cragle, 1965) but was reduced by more than half when soybean protein was included in the diet, likely because of the phytic acid in the soybean product (Miller et al., 1968). Phytase has little effect on P absorption (see P section), suggesting that in functioning ruminants, phytic acid probably does not greatly affect Zn absorption. High-fiber diets can reduce Zn absorption in nonruminants, but that affect is often confounded with effects of phytate. Diets that differed greatly in concentration of undigested NDF did not affect apparent Zn absorption in dairy cows (Faulkner et al., 2017). Ca can reduce Zn absorption in nonruminants, but that may be an indirect effect caused by the effects of Ca on phytate (Lönnerdal, 2000). In cattle, supplementation of Ca was associated with a reduction of Zn in serum of yearling steers and calves (Mills et al., 1967; Perry et al., 1968), but no deleterious effects of increased dietary Ca on

metabolism of Zn or growth in sheep were observed (Pond and Wallace, 1986).

Cadmium markedly reduces Zn absorption in rats (Evans et al., 1974). High dietary Fe (1,000 mg Fe/kg of diet provided by ferrous sulfate) reduced liver Zn concentration in steers by about 18 percent (Standish et al., 1971). Zn and Cu are antagonistic to one another. Very high Cu can interfere with Zn metabolism; however, very low Zn-to-Cu ratios (0.15:1) are likely necessary to produce antagonism (Oestreicher and Cousins, 1985). Diets with 40 mg Cu/kg and 50 mg Zn/kg (0.8:1 Cu-to-Zn ratio) did not reduce plasma Zn in growing steers (Gooneratne et al., 1994). Elevated dietary S (approximately 0.5 percent) has increased urinary loss of Zn and reduced Zn absorption in beef cattle (Gooneratne et al., 2011; Pogge et al., 2014). Conversely, feeding monensin may increase absorption of Zn (Spears, 1990), and with nonruminants, certain proteins such as whey or beef increase the absorption of Zn, but other proteins such as casein and isolated soy protein (phytase treated) reduce Zn absorption (Lönnerdal, 2000). Based on currently available information, most practical diets should not contain adequate concentrations of antagonistic substances to reduce Zn absorption with the possible exception of excess S.

Dietary Zinc Requirement

A factorial approach was taken to determine the dietary requirement for Zn. In the previous edition (NRC, 2001) endogenous fecal and the obligate urinary loss of Zn was set at 0.033 mg Zn/kg BW and 0.012 mg Zn/kg BW, respectively, for a total maintenance requirement of 0.045 mg Zn/kg BW. The data used to generate those equations came from a study with growing heifers (ca. 300 kg BW) fed radioactive Zn (Hansard et al., 1968). Newer data do not support the value used for the obligate urinary loss and bring into question the value used for endogenous fecal loss. Growing cattle (ca. 300 kg) fed low but not deficient Zn diets excreted 0.003 mg Zn/kg BW in the urine (Gooneratne et al., 2011; Pogge et al., 2014), and lactating cows fed low Zn diets (30 mg Zn/kg DM) excreted 0.0016 mg Zn/kg BW in urine (Faulkner et al., 2017). Because urinary excretion of Zn is so low, the obligate urinary loss was set at zero. A stable isotope study with goats fed at maintenance estimated endogenous fecal loss of Zn at 0.17 mg/kg BW (Hattori et al., 2010). An earlier study with 300-kg beef heifers estimated endogenous fecal loss at 0.027 mg Zn/kg BW (Hansard et al., 1968). On a DMI basis (intake was <2 percent of BW in both studies), endogenous fecal Zn ranged from 2.0 (heifers) to 8.6 (goats) mg Zn/kg DMI. The extremely limited data and the great differences between studies make it difficult to estimate this requirement. In addition, the diets were not typical of what is fed to dairy cows. One reason for the disparate results is that endogenous fecal loss of Zn reflects Zn status. As more Zn is fed, Zn bound to metallothionein is excreted via feces to maintain Zn homeostasis. Based on Zn intake, the goats

TABLE 7-4   Comparison Between Current and Previous (NRC, 2001) Zn Requirements for Dry and
Lactating Cows

	650 kg Cow, 30 kg Milk/d, DMI = 22.4 kg	650 kg Cow, 50 kg Milk/d, DMI = 29.4 kg	700 kg Dry Cow, 270 Days of Gestation, DMI = 13 kg
Total absorbed requirement			
Current, mg/d	232	347	65
NRC 2001, mg/d	149	229	44
Total dietary requirement[a]			
Current, mg/d	1,160	1,735	325
NRC, 2001, mg/d	993	1,526	293

[a]Absorption coefficient was 0.20 for current requirements and 0.15 for NRC (2001).

(Hattori et al., 2010) were likely in greater Zn status than the heifers (Hansard et al., 1968). Data on endogenous fecal excretion of Zn (and most trace minerals) by dairy cows are clearly needed to improve estimates of maintenance requirements. Therefore, the committee decided to use the mean value (rounded to the nearest whole number) from the two experiments and set the endogenous fecal Zn requirement as

Endogenous fecal loss = Maintenance requirement
= 5 mg Zn/kg DMI.

No new data are available on Zn accretion by the conceptus; therefore, the gestation requirement was not changed and set at 0.017 mg Zn/kg of maternal BW, which equals 12 mg Zn/d between day 190 of gestation and the end of gestation for a 715-kg Holstein cow (House and Bell, 1993). Newer data support retaining the lactation requirement for Zn at 4 mg/kg of milk, but concentrations can range from about 3 to 6 mg Zn/kg (Schwartz and Kirchgessner, 1975; Kinal et al., 2007; Castillo et al., 2013; Faulkner et al., 2017). The amount of Zn retained during growth of body tissues averages 24 mg Zn/kg ADG (range, 16 to 31 mg) (Miller, 1970; Kirchgessner and Neesse, 1976). Zn accretion in growing sheep averaged about 28 mg Zn/kg of empty BW but was 24 mg Zn/kg in young sheep (15 kg BW) and increased to 30 mg Zn/kg in sheep weighing about 50 kg (Bellof and Pallauf, 2007). Whether the growth requirement (per kg of BW) increases as growing cattle get larger is not known; therefore, a single growth requirement (24 mg Zn/kg daily gain) was used.

Summary of Equations (mg absorbed Zn/d)

$$\text{Maintenance} = 5.0 \times \text{DMI} \quad \text{(Equation 7-44)}$$

$$\text{Growth} = 24 \times \text{ADG} \quad \text{(Equation 7-45)}$$

$$\text{Gestation (>190 d pregnant)} = 0.017 \times \text{BW} \quad \text{(Equation 7-46)}$$

$$\text{Lactation} = 4 \times \text{Milk} \quad \text{(Equation 7-47)}$$

where DMI, ADG, and milk are in kg/d, and BW is in kg.

The maintenance requirement for absorbed Zn was greatly increased compared with the previous version, but the AC for basal Zn was also increased. A comparison between NRC (2001) and current requirements is in Table 7-4.

Deficiency

Cattle that are deficient in Zn quickly exhibit reduced DMI and growth rates. With a more prolonged deficiency, the animals exhibit reduced growth of testes, weak hoof horn, and parakeratosis of the skin on the legs, head (especially nostrils), and neck. On necropsy, thymic atrophy and lymphoid depletion of the spleen and lymph nodes are evident (Miller and Miller, 1962; Mills et al., 1967; Mayland et al., 1980). A genetic defect that greatly reduces absorption of Zn has been identified in Dutch–Friesian cattle, and they become severely deficient in Zn unless fed extremely large amounts of dietary Zn (Flagstad, 1976). Marginal deficiency of Zn may increase the risk of mastitis and other infectious disease. Dairy cows fed diets with approximately 41 mg Zn/kg diet DM had higher milk SCC than cows fed diets with 63 mg/kg Zn (Cope et al., 2009). The diet with 41 mg Zn/kg would not meet the current Zn requirements.

Concentrations of Zn in serum are normally between 0.7 and 1.3 µg/mL, and concentrations below 0.5 µg/mL are often considered deficient. However, stress or disease can cause a rapid redistribution of Zn out of extracellular fluids, causing concentrations of Zn in serum to fall into the "deficient" range even when dietary Zn is adequate (Goff and Stabel, 1990). Liver Zn concentrations are not reflective of Zn intake but will decline with prolonged periods of dietary deficiency (Herdt and Hoff, 2011). Increased liver Zn concentrations have been observed in sheep fed supplemental Zn compared to no added dietary Zn (Cao et al., 2000), and the effects are greater when fed zinc lysine compared to zinc sulfate, zinc oxide, and zinc Met (Rojas et al., 1995). However, no differences were observed in liver Zn concentrations of adult cattle fed 360 mg/d of supplemental Zn compared to no supplemental Zn (Rojas et al., 1996), and source of supplemental Zn had no effect on liver Zn concentrations (Rojas et al., 1996; Siciliano-Jones et al., 2008). Liver Zn concentrations in cattle are also affected

by age and perhaps other factors (Puschner et al., 2004). Carbonic anhydrase and alkaline phosphatase activities in blood have been used to assess Zn status, but these are difficult to interpret because concurrent disease can affect these enzymes as much as a deficiency of Zn (Mills, 1987). No widely agreed-on status indicator is available for Zn.

Toxicity

Cattle can generally tolerate high concentrations of dietary Zn. Clinical toxicity was observed in cattle fed a 900-mg Zn/kg diet (Ott et al., 1966a,b). Feed intake, milk production, and Cu status were reduced when cows were fed diets with 2,000 mg Zn/kg (from $ZnSO_4$) but not when fed diets with 1,000 mg Zn/kg (Miller et al., 1989). NRC (2005) established an MTL for cattle at 500 mg Zn/kg diet DM. However, dairy cows can likely tolerate greater concentrations.

Arsenic, Molybdenum, Nickel, and Vanadium

These elements can be found in minute amounts in the tissues of animals. In rodents, some of these elements have been demonstrated to be essential. Data on essentiality in dairy cattle are nonexistent, and practical diets would not be expected to result in deficiency of any of these elements. Most of these elements are toxic at levels occasionally occurring under field conditions.

The current understanding of metabolism does not include any specific role for arsenic (As). Goats fed a diet with 0.35 mg As/kg DM had more kids, and more of the kids survived through weaning compared with goats fed a diet with 0.035 mg As/kg (Anke, 1986). Organic arsenicals, as well as inorganic forms of As, are well absorbed and can cause toxicosis when feedstuffs are accidentally contaminated with As. Inorganic arsenicals are more toxic than organic arsenicals. The maximal tolerable level was set at 30 mg As/kg diet DM (NRC, 2005).

Mo is an essential mineral and a cofactor of xanthine oxidase, aldehyde oxidase, and sulfite oxidase (Rajagopalan, 1988). However, clinical signs of a deficiency of Mo have not been produced in any animal, making supplementation unnecessary. Because of its antagonistic effects on Cu absorption (see discussion in Cu section above), clinical signs of Mo toxicity are similar to those of a Cu deficiency. Increasing dietary Cu concentrations will usually decrease or eliminate clinical signs of Mo toxicity.

Nickel (Ni) is an essential nutrient for ruminants, although deficiencies are extremely difficult to produce (Spears, 1984). One function of Ni is as a cofactor for some forms of urease. Ruminal urease activity was stimulated when lambs were fed a diet supplemented with 5 mg Ni/kg (from nickel chloride) compared to basal with 0.06 mg Ni/kg (Spears et al., 1977). Feeding diets with 0 to 3 mg/kg of supplemental Ni to young heifers (125 kg of BW) linearly increased plasma urease activity, DMI, and ADG (Singh et al., 2019). Studies on the effects of Ni at nutritionally relevant supplementation rates on lactating dairy cows are lacking. Ni is relatively nontoxic with maximal tolerable dietary concentrations of 100 mg Ni/kg for cattle (NRC, 2005). However, no effects were noted on performance variables when lactating cows were fed diets with 0, 50, or 250 mg Ni/kg DM (O'Dell et al., 1970).

Vanadium (V) may have insulin-like activity (Heyliger et al., 1985). When V (as vanadyl sulfate) was supplemented to dairy cow diets at 0 to 0.12 mg V/kg $BW^{0.75}$ from 4 weeks pre-partum to 4 weeks postpartum, milk yield increased quadratically with a maximum at 0.04 mg V/kg $BW^{0.75}$ (Heidari et al., 2016). Milk composition, BW, and DMI were not affected. Blood glucose concentrations followed a pattern similar to milk yield, and the insulin-to-glucose ratio postpartum was reduced by all V treatments compared to control. The basal concentration of V was 0.89 mg/kg DM. In goats, supplemental V at 2 mg/d increased glucose clearance rate and increased average daily gain and feed efficiency (Zarqami et al., 2018). Although these studies show promise, inadequate data are available to determine an AI for V. Although it is poorly defined, the MTL is 50 mg V/kg of diet (NRC, 2005). However, ruminal function (DM digestibility) in lambs was disrupted with just 7 mg vanadium/kg of diet (Williams, 1973).

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8

Vitamins

INTRODUCTION

Historically, vitamin requirements (or recommendations) and dietary concentrations were expressed on an international unit (IU) basis. This was done because early assays relied on biological response and did not directly measure mass of a compound and because multiple forms and sources of many vitamins are available, and they yield different potencies when evaluated using bioassays. The conversion factors for calculating IU for various forms of vitamins are in Table 8-1. These standard conversion factors have been used in previous editions.

FAT-SOLUBLE VITAMINS

Vitamin A and β-Carotene

Sources

Vitamin A can be provided by preformed supplemental vitamin A or by the enzymatic conversion of consumed carotenoids (predominantly β-carotene). The common forms of supplemental vitamin A used in the United States are all-*trans* retinyl acetate and all-*trans* retinyl palmitate, and vitamin A activity is defined in retinol equivalents. Retinol is not found in plants, but many feeds contain β-carotene (Lindqvist et al., 2012, 2014; Pickworth et al., 2012). Carotenoids other than β-carotene can be converted to vitamin A by animals, but conversion efficiency appears to be poor, and most common feeds do not contain substantial amounts of those carotenoids. Forages can contain substantial amounts of β-carotene but most grains and grain by-products are practically void of β-carotene. Corn silage contains extremely variable concentrations of β-carotene depending on duration of storage, amount of grain in the silage, and other factors (Pickworth et al., 2012). β-Carotene concentrations decrease as forages mature (Park et al., 1983). β-Carotene is easily oxidized, and once plants are cut, concentrations decrease quickly so that stored forages (silage and hay) have lower concentrations of β-carotene than fresh forage (Bruhn and Oliver, 1978; Park et al., 1983). Under ideal wilting and ensiling conditions, loss of β-carotene for legume and grass mixtures averaged 15 percent but was as high as 25 percent (Lindqvist et al., 2012). The length of time forages are stored is negatively correlated with β-carotene concentrations (Bruhn and Oliver, 1978; Pickworth et al., 2012).

Bioavailability and Factors Affecting Supply

Bioavailability of vitamin A is defined as the proportion of vitamin A consumed that is absorbed into the body and is available to cells, but for ruminants, absolute bioavailability data do not exist. Bioavailability of vitamin A depends on the degree of ruminal destruction and on absorption efficiency by the small intestine. Based on in vitro data and data with non-ruminants, bioavailability for vitamin A is probably substantially less than 100 percent. Ruminal destruction of vitamin A can be extensive; approximately 60 percent of supplemental vitamin A was destroyed in the rumen when steers were fed hay and corn grain diets (Warner et al., 1970). Similar values have been obtained using in vitro rumen systems (Rode et al., 1990; Weiss et al., 1995). In vitro ruminal destruction of vitamin A was approximately 20 percent with high-forage diets but increased to about 70 percent with 50 to 70 percent concentrate diets. In vitro and in vivo studies suggest that between about 0 and 50 percent of dietary β-carotene is destroyed in the rumen (Potanski et al., 1974; Fernandez et al., 1976; Noziere et al., 2006), but the β-carotene contained within forages is more resistant to ruminal degradation than is supplemental β-carotene. Essentially no reliable data are available on the intestinal absorption of dietary retinyl esters in cattle, but data collected from humans and rats suggest 20 to 60 percent of it is absorbed (Blomhoff et al., 1991; Harrison, 2005). Prior to absorption, the esters are cleaved and then retinol is absorbed by what appears to be diffusion. Absorption of retinol is enhanced by increased intake of fat.

TABLE 8-1   Factors for Converting Common Sources of Vitamins into IU

Vitamin	Source	Mass/1 IU	
		Standard	Used in This Edition
A	β-carotene	2.5 microgram	2.5 microgram[a]
	All-*trans* retinol	0.3 microgram	0.3 microgram
	All-*trans* retinyl acetate	0.344 microgram	0.344 microgram
	All-*trans* retinyl palmitate	0.550 microgram	0.550 microgram
D	Cholecalciferol (vitamin D_3)	0.025 microgram	0.025 microgram
	Ergocalciferol (vitamin D_2)	0.025 microgram	0.025 microgram[a]
E	*RRR*-tocopherol	0.67 milligram	0.45 milligram[b]
	RRR-tocopheryl acetate	0.74 milligram	0.50 milligram[b]
	RRR-tocopheryl succinate	0.83 milligram	0.55 milligram[b]
	All-*rac* tocopherol	0.91 milligram	0.91 milligram
	All-*rac* tocopheryl acetate	1.00 milligram	1.00 milligram
	All-*rac* tocopheryl succinate	1.12 milligram	1.12 milligram

[a]Potency is likely overestimated using this conversion factor but data are inadequate to quantify (see text).

[b]Based on cattle data, the *RRR* forms of vitamin E are more potent than the standard conversion factors (see text).

Apparent absorption of β-carotene from a variety of forages averaged 77 percent in dairy steers (Wing, 1969), but Cohen-Fernandez et al. (1976) reported that fecal recovery (indigestibility) of radiolabeled β-carotene was about 90 percent in sheep. In human and rodent models, intestinal absorption of β-carotene is a saturable process (von Lintig, 2010), suggesting that absorption efficiency may be less when cows are consuming large amounts of β-carotene (e.g., grazing) compared with cows consuming hay-based diets.

In addition to ruminal metabolism, the bioavailability of β-carotene as a source of vitamin A depends on the efficiency of converting it to retinol. β-Carotene is predominantly converted to retinol by an enzyme located in intestinal mucosal cells. Retinoic acid, at least in humans and rodents, regulates this conversion process; animals with high concentrations of retinoic acid convert less β-carotene into retinol. If this regulation occurs in cattle, cows in good vitamin A status will convert less β-carotene into retinol, which means tissue concentrations of β-carotene may increase when β-carotene is fed, whereas cows in low vitamin A status may convert much of the β-carotene they absorb into retinol and have lower β-carotene concentrations in tissues.

The vitamin A activity of β-carotene for cattle is in Table 8-1. Previously, the conversion efficiency used for humans was much greater than that used for cattle, but the assumed efficiency for humans has been reduced and is now 1 mg β-carotene (in food) = 277 IU of vitamin A or 83 μg retinol (IOM, 2000a), which is less than the value used for cattle (1 mg β-carotene = 400 IU of vitamin A). Absorption of β-carotene in fruits and vegetables by humans was much less than previously thought (IOM, 2000b). The defined activity of β-carotene for cattle is based largely on experiments using lambs fed corn silage (Martin et al., 1968). Studies are needed to reevaluate the conversion efficiency of cattle in light of the changes made to the human conversion efficiency factor.

Vitamin A is less stable than many other vitamins. When supplemental vitamin A is mixed in premixes without added trace minerals, loss of activity during storage under ideal conditions is similar to other vitamins at about 3.5 percent per month, but if the premix contains supplemental inorganic trace minerals (copper, iron, manganese, selenium [Se], and zinc), loss in activity was about 9 percent per month (Shurson et al., 2011). Pelleting and extrusion cause very substantial losses in vitamin A activity, and improper environmental conditions during storage (e.g., heat and exposure to sunlight) will increase loss of activity during storage (Coelho, 2002).

Relative bioavailability of vitamin A supplements can be evaluated by monitoring retinol concentrations in liver, and significant differences were found between commercial sources of supplemental vitamin A when fed to feedlot cattle (Alosilla et al., 2007). These differences could be caused by loss of activity during storage or differences in ruminal destruction or intestinal absorption. The relative dose–response assay has been used to assess dietary effects on vitamin A bioavailability and vitamin A status in calves (Hammell et al., 2000) and adult cattle (Westendorf et al., 1990), but it may lack adequate sensitivity. Because of the invasive nature or limited sensitivity of current assays, data on factors affecting bioavailability of vitamin A and β-carotene are limited. High supplementation rates of vitamin E (6,000 IU/d) tended to reduce vitamin A status in feedlot cattle (Westendorf et al., 1990), and 2,500 IU/d of supplemental vitamin E reduced plasma and tissue concentrations of β-carotene in grazing cattle (Yang et al., 2002). Feeding supplemental fat increased β-carotene concentration in plasma of dairy cows (Weiss et al., 1994).

Functions and Animal Responses

One specific function of vitamin A (retinaldehyde) is the production of rhodopsin (a vision pigment) that is necessary for low-light vision. However, because vitamin A (retinoic acid) is a major regulator of gene transcription, it is involved in a multitude of cellular and tissue functions, including spermatogenesis, female reproduction, fetal development, and maintenance of skeletal and epithelial tissue. It also is a major regulator of immune cell function and has profound effects on the immune system (Stephensen, 2001; Mora et al., 2008). Vitamin A status in cattle is positively related to various measures of immune function (Yano et al., 2009), and vitamin A supplementation enhances the function of different immune cells (Tjoelker et al., 1988a,b, 1990; Meyer et al., 2005). Cows that eventually developed retained fetal membranes had lower serum concentrations of retinol prepartum than did healthy cows (LeBlanc et al., 2004), and cows with retained fetal membranes had lower serum retinol concentrations postpartum (Akar and Gazioglu, 2006). In one study (LeBlanc et al., 2004), but not in another (Rezamand et al., 2007), cows that developed an intramammary gland infection in early lactation had lower serum retinol than healthy cows. Lower concentrations of plasma retinol were associated with more severe lameness in cows (Sadeghi-nasab et al., 2013). Stillborn calves, but not aborted fetuses, were deficient in vitamin A (Waldner and Blakley, 2014). Overall, the preponderance of data indicates that cows in suboptimal vitamin A status are at higher risk for numerous health disorders than cows in adequate vitamin A status.

Requirements

Inadequate data are available to establish a requirement for vitamin A, and because the β-carotene content of diets is highly variable and almost never known in commercial situations, an Adequate Intake (AI) was established for supplemental vitamin A rather than total vitamin A (see Chapter 1 for discussion regarding AI). Fresh forage (e.g., pasture) can have high concentrations of β-carotene; therefore, the amount of supplemental vitamin A needed when fresh forage is fed will be less than for cattle consuming conserved forages. The AI presented below assumes conserved forages are fed and are probably in excess of requirements for grazing cattle.

The vitamin A requirement established in 2001 (NRC, 2001) for growing heifers, dry cows, and lactating cows was 110 IU of supplemental vitamin A/kg body weight (BW) and was based on cerebrospinal fluid pressure, the presence of papillary edema of the eye, milk yield, immune function, mammary gland health, and reproduction (NRC, 2001). The requirement also incorporated expected ruminal destruction of a portion of the supplemental vitamin A when higher concentrate diets are fed.

The AI for supplemental vitamin A for growing heifers was kept at 110 IU/kg BW because of a lack of new data; however, the AI of vitamin A for replacement heifers remains especially poorly defined. Holstein steers fed a high-grain diet that provided approximately 110 IU of supplemental vitamin A/kg BW had greater average daily gain than steers fed no supplemental vitamin A, but the vitamin A treatment was confounded with a vitamin E treatment (Salinas-Chavira et al., 2014). Feeding finishing diets (i.e., high concentrate) void of supplemental vitamin A has increased intramuscular fat deposition in beef steers and tended to decrease rib fat thickness compared with steers fed approximately 70 IU/kg BW (Gorocica-Buenfil et al., 2007). Whether the effects of low vitamin A intake on fat deposition occur in heifers fed lower-energy diets is not known. If inadequate vitamin A does affect fat deposition in growing dairy heifers, this could be detrimental to future milk yields if the fat is deposited in the developing mammary gland. In the beef study (Salinas-Chavira et al., 2014), the feeding period was approximately 170 days, and no adverse effects were reported in cattle not fed supplemental vitamin A. Because of reproduction demands, the lack of adverse effects when no supplemental vitamin A was fed to steers may not extend to replacement heifers.

In NRC (2001), the vitamin A requirement for adult cattle (lactating and dry) was set at 110 IU/kg BW. Milk represents a significant loss of retinol from the cow, with concentrations ranging from about 3 to 11 mg/kg of milk fat (Jensen and Nielsen, 1996; Jensen et al., 1999; Shingfield et al., 2005; Noziere et al., 2006). This is equivalent to approximately 0.1 to 0.4 mg/kg of milk with 3.7 percent fat, or about 1,000 IU of vitamin A/kg of milk. Using the average concentration, a cow producing 35 kg of milk with 3.7 percent fat would secrete about 10 mg of retinol into milk daily, which is equivalent to 30,000 IU, which is substantial relative to the AI (ca. 69,000 IU for a 625-kg cow). In addition, lactating cows are typically fed higher-concentrate diets than growing heifers and dry cows. These facts could argue for increased AI for vitamin A for lactating cows relative to the AI for heifers and dry cows and that the AI should be related to milk fat yield. The preponderance of available data on production, health, and reproduction indicates that approximately 110 IU of vitamin A/kg BW for lactating cows is adequate, but most studies used cows producing <35 kg of milk per day. Based on current data and expected loss of retinol in milk, the daily AI for vitamin A was set as follows:

If milk yield ≤35 kg/d (including dry cows),
$$AI = 110 \text{ IU/kg BW} \quad \text{(Equation 8-1a)}$$

If milk yield >35 kg/d, $AI = 110$ IU/kg BW + 1000 IU
$$\times (\text{milk yield} - 35) \quad \text{(Equation 8-1b)}$$

Although lactating cows secrete substantial amounts of retinol into milk, several arguments exist for setting the AI for dry cows equal to that of lower-producing cows. First, the developing fetus requires vitamin A. Second, the concentration of retinol in colostrum is positively correlated with vitamin A intake during the dry period (Puvogel et al., 2008). Colostrum contains substantial amounts of retinol (see Chapter 12), and colostrum synthesis causes a sig-

nificant reduction in plasma concentrations of retinol (Goff et al., 2002). Calves are born with low vitamin A status, and increased concentrations of retinol in colostrum improve vitamin A status of the newborn calf (Puvogel et al., 2008). Third, cows fed 170,000 IU of vitamin A/d during the dry period and early lactation period produced more milk than cows fed no supplemental vitamin A (Oldham et al., 1991). Fourth, late-gestation dry cows with lower vitamin A status have increased risk of retained fetal membranes and intramammary gland infections in early lactation (LeBlanc et al., 2004). Because of a lack of new data, the AI for vitamin A for dry cows was retained at 110 IU/kg BW.

β-Carotene

β-Carotene has functions other than serving as a precursor for retinol. Responses to supplemental β-carotene by dairy cows have been inconsistent and could reflect differences in basal β-carotene intake or vitamin A status. The preponderance of studies has found no effect of supplemental β-carotene on milk production (de Ondarza et al., 2009). In an older review, Hurley and Doane (1989) reported that supplemental β-carotene (usually at 300 to 400 mg/d) improved some measure of reproductive efficiency in 12 of 22 studies, but when studies conducted only in North America were summarized, β-carotene had no effect on reproduction in 4 of 5 studies. Newer data show similar inconsistencies. Kawashima et al. (2009) reported cows that were anovulatory had lower concentrations of β-carotene in plasma, whereas Kaewlamun et al. (2011) reported that 1 g/d of supplemental β-carotene had no effect on ovarian activity and uterine involution. Some data have shown a relationship between low plasma concentrations of β-carotene prepartum and increased incidence of retained fetal membranes (Inaba et al., 1986; Akar and Gazioglu, 2006), whereas LeBlanc et al. (2004) found no relationship. β-Carotene, independent of its pro–vitamin A function, is an antioxidant and can enhance the killing ability of neutrophils (Chew, 1993). In some (Chew, 1987) but not all (Michal et al., 1994) studies, supplementing between 300 and 600 mg β-carotene/d reduced the incidence of intramammary gland infections and mastitis. These studies were conducted with cows at dry-off or peripartum cows. Newer data on effects of β-carotene on mammary gland health are limited. Supplementing 425 mg β-carotene/d to lactating cows did not affect somatic cell count (SCC) (de Ondarza et al., 2009). Jukola et al. (1996) reported increased mastitis in cows with low plasma β-carotene, whereas LeBlanc et al. (2004) reported no relationship. Considering the extremely inconsistent data with respect to β-carotene supplementation, an AI could not be established for β-carotene.

Maximum Tolerable Level for Vitamin A

Feeding approximately 500,000 IU of vitamin A/d (approximately $6.5 \times$ current AI) during the dry period reduced milk yield in the subsequent lactation possibly because of increased mammary cell apoptosis (Puvogel et al., 2005). Based on older data, cattle consuming approximately 1,300 IU of vitamin A/kg BW (approximately $12 \times$ current AI) developed signs of osteoporosis (NRC, 1987). One-week-old calves fed 3 million IU of vitamin A/d for 10 days developed hyena disease (premature growth plate closure) (Takaki et al., 1996). In humans and other nonruminants, excess intakes of vitamin A can cause problems with bone metabolism, including osteoporosis (Penniston and Tanumihardjo, 2006); negatively affect immune function and increase incidence of certain infectious diseases (Field et al., 2002); and cause fetal abnormalities (Azaïs-Braesco and Pascal, 2000). With improved sensitivity of measurements, negative effects of excessive vitamin A for humans are being observed at much lower intakes of vitamin A than previously. For example, markers of osteoporosis in humans may develop when vitamin A intake is about twice the recommended daily allowance, whereas previously, 10 times requirement was considered necessary to see negative effects (Penniston and Tanumihardjo, 2006). Because of ruminal metabolism and multiple other differences, human toxicity data cannot be extrapolated to cows, but nutritionists should be aware that negative effects of excess vitamin A may occur at lower intakes of vitamin A than previously thought.

Vitamin D

Sources and Factors Affecting Supply

Vitamin D can be produced within the skin of most mammals, including cattle, as a result of the photochemical conversion of 7-dehydrocholesterol to vitamin D_3. In plants, ultraviolet irradiation causes photochemical conversion of ergosterol to vitamin D_2. Although some feeds contain vitamin D (Horst et al., 1984), there are almost no data on vitamin D concentrations of feeds published within the past 20 years (Kalac, 2012). Therefore, basal ingredients are assumed to be an unreliable source of vitamin D, and the AI is expressed on a supplemental vitamin D basis. Vitamin D_2, the form associated with plants, and vitamin D_3, the form associated with vertebrates, are both used for supplementation of diets. The biological activity of the two forms was generally considered equal in cattle; however, Littledike and Horst (1982) demonstrated reduced efficacy of the vitamin D_2 form in cattle. Presumably, this is because reduced binding of vitamin D_2 metabolites to vitamin D–binding proteins in blood leads to more rapid clearance of vitamin D_2 metabolites from plasma. Vitamin D_3 was about twice as effective at elevating the concentration of 25-hydroxyvitamin D (i.e., calcidiol) in plasma of dairy cows as vitamin D_2 (Hymøller and Jensen, 2010). In humans, the value of vitamin D_2 as a vitamin D supplement is questionable (Houghton and Vieth, 2006). Vitamin D_3 is the predominate source of supplemental vitamin D used for livestock, and the AI for supplemental vitamin D assumes vitamin D_3 will be used. If vitamin D_2 is used, supplementation rates

probably should be increased. Calcidiol is commercially available and can be used as a source of vitamin D. Most of the research has been with transition cows (see Chapter 12), and at this time, the relative activity (i.e., IU per unit mass) of calcidiol is not known.

Dietary vitamin D can be metabolized in the rumen by bacteria to inactive metabolites (Horst and Reinhardt, 1983), but the degree of this metabolism is unclear. Hymøller and Jensen (2010) reported that concentrations of vitamins D_2 and D_3 in rumen fluid (in vitro) were constant over time (up to 30 hours). However, concentrations were expressed per unit of dry matter (DM), which suggests vitamin D degradation occurred at a rate similar to DM digestion. Both forms of vitamin D followed similar time profiles.

In vivo synthesis of vitamin D_3 depends on the duration and intensity of exposure to solar radiation, and solar intensity depends on latitude, season, and cloud cover. Cattle housed outside have higher concentrations of 25-hydroxyvitamin D in plasma during the summer compared with winter (Hymøller et al., 2009; Edrington et al., 2012; Casas et al., 2015). To maintain adequate plasma concentrations of 25-hydroxyvitamin D, dairy cows (56° latitude) in June required about 90 minutes of sun exposure (centered on approximately 1300 h) per day (Hymøller and Jensen, 2012). Based on human vitamin D synthesis rates, the required duration of sun exposure (assumed latitude of 40°) to obtain adequate vitamin D could be several times greater during spring and fall and unobtainable during the winter (Webb and Engelsen, 2006). Supplemental vitamin D is probably not needed during summer months for cattle that graze for several hours during daylight hours. However, as the date deviates from the summer solstice, sun exposure becomes an unreliable source of vitamin D for grazing cattle.

Physiology and Function

Vitamin D is a prohormone, a necessary precursor for the production of the calcium (Ca) regulating hormone 1,25-dihydroxyvitamin D. Absorbed vitamin D enters the circulation but is rapidly converted to 25-hydroxyvitamin D within the liver by vitamin D 25-hydroxylase, which is then released into the blood. Concentrations of vitamin D in blood are not a good indicator of status because of rapid removal, and blood levels usually are 1 to 2 ng vitamin D/mL plasma (Littledike and Horst, 1982). The production of 25-hydroxyvitamin D within the liver is dependent on vitamin D supply (dietary and in vivo synthesis). Thus, plasma 25-hydroxyvitamin D concentration is a good indicator of vitamin D status of an animal (Horst et al., 1994). However, in humans with high serum concentrations of 25-hydroxyvitamin D (i.e., good status), increased intake of vitamin D increased serum concentrations of vitamin D at twice the rate as the increase in 25-hydroxyvitamin D (Heaney et al., 2008).

The 25-hydroxyvitamin D circulates to the kidney, where it can be converted to the hormone 1,25-dihydroxyvitamin D. This hormone acts to increase the active transport of Ca and phosphorus (P) across the intestinal epithelial cells and potentiates the action of parathyroid hormone (PTH) to increase bone Ca resorption. Both functions are vital for Ca and P homeostasis. The influence of vitamin D on Ca and P metabolism has been studied for decades, but vitamin D receptors are found throughout the body and regulate a multitude of genes involved in a host of functions in addition to Ca and P metabolism (Christakos et al., 2013). Vitamin D or, more precisely, 1,25-dihydroxyvitamin D has substantial involvement in maintaining and regulating immune function (Reinhardt and Hustmyer, 1987; Nelson et al., 2012). In bovine cell systems and in vivo, vitamin D regulates both innate (Nelson et al., 2010; Téllez-Pérez et al., 2012; Alva-Murillo et al., 2014) and adaptive immunity (Nelson et al., 2011). Intramammary infusion of 25-hydroxyvitamin D reduced the severity of experimentally induced bacterial mastitis (Lippolis et al., 2011). However, intramuscular injections of vitamin D_3 to cows with clinical mastitis did not improve measures of mammary gland health (Shahmohammadi et al., 2014).

Renal production of 1,25-dihydroxyvitamin D is tightly regulated under most situations. Activity of 25-hydroxyvitamin D-1-α-hydroxylase of the kidney is stimulated by PTH, which is released in response to declining concentrations of Ca in blood (DeLuca, 1979). In the absence of PTH, when an animal is in positive Ca balance, 25-hydroxyvitamin D can be hydroxylated in the kidney to 24,25-dihydroxyvitamin D as a primary step in the inactivation and catabolism of vitamin D. The vitamin D catabolic enzymes also function to deactivate 1,25-dihydroxyvitamin D. These catabolic enzymes exist in tissues throughout the body. In these tissues, the catabolic pathway is generally stimulated by 1,25-dihydroxyvitamin D as a negative feedback to reduce high concentrations of 1,25-dihydroxyvitamin D in plasma (Reinhardt and Horst, 1989; Goff et al., 1992). Dietary supplementation of 25-hydroxyvitamin D, at least to peripartum cows, can overwhelm the feedback mechanism and significantly elevate plasma concentrations of 1,25-dihydroxyvitamin D (Wilkens et al., 2012; Weiss et al., 2015). Increased concentrations of 1,25-dihydroxyvitamin D under that situation resulted in some transient increases in plasma Ca concentrations but did not reduce clinical or subclinical hypocalcemia postpartum.

A low concentration of P in blood also can enhance renal production of 1,25-dihydroxyvitamin D, even when the concentration of Ca in plasma is normal or above normal (Gray and Napoli, 1983). Also, higher than normal concentrations of P in blood can inhibit renal production of 1,25-dihydroxyvitamin D, which can be a factor contributing to hypocalcemia in the periparturient cow (Barton et al., 1987).

Vitamin D deficiency reduces the ability to maintain Ca and P homeostasis, resulting in a decline for P and less often a decrease for Ca in plasma. This eventually causes rickets in young animals and osteomalacia in adults; both are bone diseases in which the primary lesion is failure to mineralize the organic matrix of bone. In young animals, rickets causes

enlarged and painful joints; the costochondral joints of the ribs are often readily palpated. In adult cattle, lameness and pelvic fracture are a common sequelae of vitamin D deficiency. Vitamin D deficiency in humans, as determined by low plasma concentrations of 25-hydroxyvitamin D, is a risk factor for numerous health disorders, including cancers, cardiovascular disease, diabetes, and immune dysfunction (Holick, 2007).

Requirements

The amount of dietary vitamin D required to provide adequate substrate for production of 1,25-dihydroxyvitamin D is difficult to define; therefore, the committee established an AI, rather than a requirement for vitamin D. Animals exposed to adequate sunlight may not require any dietary vitamin D, but this is highly dependent on the latitude, exposure time, and season. Sun-cured hay provided adequate vitamin D to prevent symptoms of vitamin D deficiency in young growing calves, but the hay made up most of the diet (Thomas and Moore, 1951). Other feeds are likely to provide inadequate vitamin D.

The movement away from pasture feeding systems and toward confinement and feeding of stored feeds and by-products has increased the need for dietary supplementation of vitamin D for dairy cows. The contribution of sunlight and sun-cured forage to the supply of vitamin D for the cow is not considered in this publication, and the AI for vitamin D is expressed as IU of supplemental vitamin D (assumed to be vitamin D_3). However, as discussed above, cattle that are grazing during the summer probably do not need supplemental vitamin D.

Horst et al. (1994) determined that plasma 25-hydroxyvitamin D concentrations below 5 ng/mL are indicative of vitamin D deficiency, and concentrations of 200 to 300 ng/mL would indicate vitamin D toxicosis. For humans, optimal plasma concentration of 25-hydroxyvitamin D is in the range of 30 to 50 ng/mL based on a variety of health outcomes (Bischoff-Ferrari, 2008). An optimal range of 25-hydroxyvitamin D plasma concentrations has not been established for cattle but likely is similar to the optimal range for humans. However, bovine macrophage function in vitro improved linearly as 25-hydroxyvitamin D concentrations increased up to 100 ng/mL (Nelson et al., 2010). Cattle with low exposure to sunlight and not supplemented with vitamin D generally have plasma concentrations of 25-hydroxyvitamin D less than 20 ng/mL (McDermott et al., 1985; Vinet et al., 1985; Hymøller et al., 2009). Supplementation of 10,000 to 50,000 IU/d of vitamin D (ca. 15 to 75 IU/kg BW) usually (McDermott et al., 1985; Vinet et al., 1985; Nelson et al., 2016) but not always (Hymøller et al., 2009) maintained plasma concentrations of 25-hydroxyvitamin D greater than 30 ng/mL. Cows in early lactation (<30 days in milk) had lower concentrations of 25-hydroxyvitamin D in plasma than cows in later lactation (Nelson et al., 2016), but whether this was a physiological response or reflected changes in intake is not known.

Under most circumstances, 10,000 IU/d (16 IU vitamin D/kg BW) should provide adequate vitamin D with respect to Ca metabolism for dairy cows during late gestation. Astrup and Nedkvitne (1987) reported that lactating cows producing about 20 kg of milk/d required about 10 IU vitamin D/kg BW to maintain normal concentrations of Ca and P in blood. These studies were conducted in Norway in winter and spring, when effective sunlight exposure should have been minimal. Effects on immunity and other health measures were not evaluated in those studies. However, Ward et al. (1971) reported that cows fed an alfalfa hay–concentrate diet receiving 300,000 IU vitamin D_3 once each week (43,000 IU/d) returned to estrus 16 days earlier than cows given no supplement. Ward et al. (1972) also demonstrated that cows receiving 300,000 IU vitamin D_3/wk had improved absorption of dietary Ca. Hibbs and Conrad (1983) summarized the results of several experiments and concluded that cows supplemented with 40,000 IU vitamin D_2/d (50 to 70 IU vitamin D/kg BW) produced more milk and generally ate more than cows fed the same diets with no vitamin D supplementation or supplemented with 80,000 or more IU vitamin D/d. Reduced milk production, which could be interpreted as the beginning of vitamin D toxicosis, was observed when cows were fed 80,000 IU vitamin D/d (120 to 140 IU/kg BW). In those studies, vitamin D_2 was used and 40,000 IU of vitamin D_2 may be substantially less active than 40,000 IU of vitamin D_3.

The previous vitamin D requirement (NRC, 2001) was set at 30 IU/kg BW for all classes of dairy cattle (approximately 20,000 IU/d for a typical Holstein cow). Based on a limited number of studies, for most cows, this rate of supplementation should maintain plasma concentrations of 25-hydroxyvitamin D at about 30 ng/mL, which appears adequate (Nelson et al., 2016). However, some lactating cows had plasma concentrations less than 30 ng/mL when the group was fed a diet formulated to provide 20,000 IU of supplemental vitamin D_3, but herds fed diets formulated to provide approximately 30,000 IU of supplemental vitamin D_3 per day consistently maintained plasma concentrations of 25-hydroxyvitamin D >30 ng/mL (Nelson et al., 2016). In addition, newer studies have identified positive effects of vitamin D on immune function.

Therefore, the AI for supplemental vitamin D was set as follows:

For replacement heifers and dry cows: Vitamin D AI,
$$\text{IU/d} = 30 \times \text{BW, kg} \qquad \text{(Equation 8-2a)}$$

For lactating cows: Vitamin D AI, IU/d = 40
$$\times \text{BW, kg} \qquad \text{(Equation 8-2b)}$$

Although Ca metabolism can differ between some breeds (see Chapters 7 and 12), based on serum concentrations of 25-hydroxyvitamin D (Nelson et al., 2016) and number of vitamin D receptors in intestinal tissues (Liesgegang et al.,

2008), breed differences in vitamin D nutrition have not been shown. Additional experimentation is needed to determine optimal plasma concentrations of 25-hydroxyvitamin D with respect to immune function and diseases not directly related to Ca and P status. New data are needed to better titrate vitamin requirements.

Maximum Tolerable Level

Very little new information is available regarding the maximum tolerable level for vitamin D in dairy cattle. The maximum tolerable amount of vitamin D is inversely related to dietary concentrations of Ca and P. Short-term studies by McDermott et al. (1985) suggest that 50,000 IU vitamin D_3/d (80 IU/kg BW) is well tolerated while 250,000 IU vitamin D_3/d (400 IU/kg BW) is not. Hibbs and Conrad (1983) reported a slight decline in milk production when cows were fed 80,000 IU D_2/d (160 IU/kg BW). In nonruminants, the maximum tolerable level for vitamin D_2 is much greater than that for vitamin D_3. NRC (1987) suggested the maximal tolerable level of vitamin D is 2,200 IU/kg diet when fed for long periods (more than 60 days) and 25,000 IU/kg diet when fed for shorter periods of time. Vitamin D intoxication is associated with reduced DM intake (DMI), polyuria initially followed by anuria, dry feces, and reduced milk production. On necropsy, calcification of kidneys, aorta, abomasum, and bronchioles is evident (Littledike and Horst, 1982). Finishing beef steers fed 500,000 to 5,000,000 IU of vitamin D_3 the last 8 days of life had significantly greater concentrations of Ca in muscle, but no other negative effects were reported (Montgomery et al., 2004). The maximal tolerable dose of parenterally administered vitamin D is at least 100-fold lower than the maximal tolerable oral dose, and repeated injections can be especially toxic (Littledike and Horst, 1982).

Vitamin E

Sources

Vitamin E is a generic name for a series of lipid-soluble compounds called tocopherols and tocotrienols. The most biologically active form of vitamin E is α-tocopherol; it is also the most common form of vitamin E found in most feedstuffs. α-Tocopherol has three chiral centers and can exist in eight stereoisomeric forms. Plants only make the *RRR* isomer of α-tocopherol, but chemical synthesis produces all eight isomers in equimolar concentrations.

The concentration of *RRR*-α-tocopherol in plants is highly variable, but generally, it is associated with metabolically active tissues (i.e., leaves) and fat storage depots (oilseeds or seed germ). Forages and intact oilseeds (e.g., soybeans, canola, cottonseed) are the only feedstuffs with appreciable concentrations of α-tocopherol. Grains and oilseed meals

generally contain <6 mg α-tocopherol/kg of DM (McMurray et al., 1980), but dried distillers grains (ca. 12 percent oil) can contain up to 20 mg/kg (Winkler et al., 2007). Generally, the concentration of α-tocopherol in concentrate feeds is positively correlated with fat concentration. Whole soybeans contain between 5 and 30 mg α-tocopherol/kg DM (Seguin et al., 2010; Carrera and Seguin, 2016). Other oilseeds probably have similar variable concentrations of α-tocopherol. α-Tocopherol is labile, and roasting or heat processing and long exposure to oxygen destroy it (Francois et al., 2006).

Fresh forage can be an excellent source of α-tocopherol, but concentrations are extremely variable, ranging from about 20 to 150 mg α-tocopherol/kg DM (Tramontano et al., 1993; Lindqvist et al., 2012, 2014; Elgersma et al., 2013). Plant species (grasses tend to have higher concentrations than legumes), maturity (concentrations decrease as maturity increases), climate, and numerous other factors contribute to the variation. Wilting and ensiling decrease α-tocopherol concentrations by 25 to 65 percent (Müller et al., 2007; Lindqvist et al., 2012). Short wilting periods and practices that encourage rapid fermentation generally reduce losses of α-tocopherol when forages are stored as silage. Less data are available on α-tocopherol concentrations in corn silage, but values range from about 9 to 20 mg/kg DM (O'Sullivan et al., 2002; Weiss et al., 2009; Kalac, 2012). Hay usually has lower concentrations of α-tocopherol than hay crop silages with typical values <25 mg/kg of DM (Kalac, 2012), but some hays may contain twice that concentration (Weiss et al., 2009).

The form of supplemental vitamin E usually fed to dairy cows is all-*rac*-α-tocopheryl acetate. The esterified form of the vitamin is more stable than the alcohol form; expected losses in biological activity from premixes containing all-*rac*-α-tocopheryl acetate are 1 or 2 percent per month under most storage conditions, but extruded products containing all-*rac*-α-tocopheryl acetate may have storage losses of 6 percent per month (Coelho, 2002; Shurson et al., 2011). *RRR*-α-tocopheryl acetate (or the free alcohol form) is also available commercially as a vitamin E supplement.

Bioavailability

In vitro and in vivo experiments have shown that commercial forms of supplemental vitamin E are stable in the rumen over a wide range of diets (Leedle et al., 1993; Weiss et al., 1995; Chikunya et al., 2004; Hymøller and Jensen, 2010). Data are not available on the efficiency of intestinal vitamin E absorption in ruminants, but in humans, less than 70 percent of ingested vitamin E is likely absorbed (Kayden and Traber, 1993). Efficiency of absorption increases as dietary fat concentration increases, and because cattle are usually fed diets with much less fat than typical human diets, vitamin E absorption by cattle may be less.

The United States Pharmacopeia (USP) has defined the factors to convert mass of the common types of supplemental

vitamin E into units related to bioavailability (see Table 8-1). Those conversion factors are based largely on research with laboratory rodents conducted decades ago, and newer data with humans and cattle have brought those conversion factors into question. The relative difference in conversion factors between the alcohol and ester forms within the main vitamin E form is likely correct (Hidiroglou et al., 1988, 1989) and simply represents dilution by the acetate moiety. The difference in bioactivity or bioavailability between the *RRR* and all-*rac* forms, however, is likely greater than the USP conversions indicate. Cattle (Weiss et al., 2009) as well as other animals (Lauridsen et al., 2002; Cortina et al., 2004; Jensen et al., 2006) have higher concentrations of α-tocopherol in blood and tissues when fed the *RRR* form of vitamin E compared with the all-*rac* form, even though on an IU basis, intake of vitamin was the same. The vitamin E requirement for humans in the United States assumes that only 2R isomers (i.e., *SRS*, *SRR*, *RRS*, and *RRR*) of vitamin E are biologically active (IOM, 2000b). Data with dairy cows and calves support that assumption (Eicher et al., 1997; Meglia et al., 2006; Weiss et al., 2009). This means that *RRR* forms of vitamin E are twice as biologically active than the all-*rac* forms (see Table 8-1), and that difference was used for this publication. However, feed labeling regulations require that the standard conversion factors be used for different forms of *RRR*-tocopherol. When using these forms of supplemental vitamin E, users will need to convert the labeled IU to units used in Table 8-1.

Functions and Animal Responses

The best understood function of vitamin E is as a lipid-soluble cellular antioxidant that is especially reactive with fatty acid (FA) peroxyl radicals. These compounds are produced by peroxidation of polyunsaturated FAs. Via this function and perhaps others, vitamin E is involved in the maintenance of cellular membranes, arachidonic acid metabolism, immunity, and reproductive function. Most of the research on dairy cow response to vitamin E supplementation has concentrated on reproduction and health measures, such as mastitis, retained fetal membranes, and metritis.

White muscle disease is a classic sign of a clinical deficiency of vitamin E, and it was prevented in preweaned calves when 50 IU of vitamin E/d were supplemented to a vitamin E–free diet based on skim milk (Blaxter et al., 1952). Dietary or parenteral supplementation of vitamin E to dairy cows during the peripartum period has consistently improved the function of neutrophils and sometimes macrophages (Hogan et al., 1990, 1992; Politis et al., 1995, 1996, 2001, 2004; Suwanpanya et al., 2007). In those studies, the amount of supplemental vitamin E fed per day during the prepartum period varied between 1,000 IU/d and 3,000 IU/d. In all studies, cows were fed stored forages.

Feeding approximately 1,000 IU/d of supplemental vitamin E (usually all-*rac*-α-tocopheryl acetate) to dry cows

when adequate Se was supplemented reduced the prevalence of retained fetal membranes in most (Harrison et al., 1984; Miller et al., 1993) but not all (Wichtel et al., 1996) studies. When vitamin E was injected (usually in combination with Se), about half the time, there was no effect for prevalence of retained fetal membranes, and about half the time, there was a positive response (Miller et al., 1993). More recent studies have tended to be positive (Erskine et al., 1997; Bourne et al., 2008), especially when cows had low plasma concentrations of α-tocopherol prior to injection (LeBlanc et al., 2002). In the older studies, the typical treatment was a single injection of approximately 700 IU vitamin E and 50 mg Se, but in the more recent studies, 2,000 to 3,000 IU vitamin E were injected. A meta-analysis determined that vitamin E supplementation during the prepartum period significantly reduced the risk of cows having retained fetal membranes (Bourne et al., 2007).

The majority of studies evaluating effects of supplemental vitamin E on mastitis have been positive (Smith et al., 1985; Weiss et al., 1997; Valle et al., 2000; Politis et al., 2004; Chatterjee et al., 2005; Rajiv and Harjit, 2005). Supplementation was usually between 1,000 and 3,000 IU/d during the dry period or peripartum period. Rates of new infection, SCCs, and the severity and duration of mastitis have been reduced with vitamin E supplementation. However, a study conducted in Canada (Batra et al., 1992) found that about 1,000 IU/d of supplemental vitamin E did not reduce the incidence of clinical mastitis. Based on the concentrations of Se in the plasma (<35 ng/mL), cows in that study were deficient in Se. In contrast to the positive studies, a large, replicated field study found that supplementing dry cows for approximately 60 days with 3,000 IU of vitamin E per day (control treatment provided 135 IU of supplemental vitamin E/d) significantly increased the risk of developing mastitis during early lactation (Bouwstra et al., 2010b). Most of the positive studies supplemented vitamin E at lower rates (1,000 IU/d) or at similar rates for shorter periods of time (14 to 45 days). Case definitions also differed between studies. Clinical data are lacking evaluating effects of supplemental vitamin E during later lactation on mastitis and other health measures.

Low plasma concentrations of α-tocopherol, especially during the peripartum period, have been related to increased risk of health problems, including mastitis, high SCCs, displaced abomasum, and retained fetal membranes (Weiss et al., 1997; LeBlanc et al., 2004; Nyman et al., 2008; Politis et al., 2012; Qu et al., 2013). However, Jukola et al. (1996) reported no relationships between plasma α-tocopherol concentrations and mammary gland and reproductive health measures, and Bouwstra et al. (2010a) reported that high α-tocopherol concentrations in plasma were a risk factor for increased mastitis.

Concentrations of α-tocopherol in plasma drop precipitously shortly before calving and remain low for a few days postpartum (Goff and Stabel, 1990; Weiss et al., 1990). This

coincides with a period of reduced immune function in dairy cows (reviewed by Sordillo, 2005). Vitamin E supplementation has improved various measures of immune function, especially in the peripartum cow (Hogan et al., 1990, 1992; Politis et al., 1996, 2001, 2004; Chandra et al., 2014). Supplementing 2,000 to 4,000 IU of vitamin E per day during the last 2 weeks of gestation reduced mammary gland infection rates, clinical mastitis, or SCCs compared with cows given 1,000 IU of supplemental vitamin E during that period (Weiss et al., 1997; Baldi et al., 2000). However, a field study on commercial farms (Persson Waller et al., 2007) found no benefit of supplementing 1,600 mg *RRR*-α-tocopherol per day (approximately 3,500 IU of vitamin E using the conversion factor discussed above) during the last 4 weeks of gestation on mammary gland health postpartum, but stillbirths were reduced.

Extremely high supplementation rates of vitamin E (generally 3,000 to 10,000 IU/d) have been used to reduce the development of spontaneously oxidized flavor in milk (Nicholson et al., 1991). More recently, high rates of vitamin E supplementation (3,000 to 11,000 IU/d) have been used to reduce milk fat depression associated with diets containing polyunsaturated oils, but results have been mixed. Vitamin E did not prevent or reduce milk fat depression induced by feeding diets with high inclusion rates of oil (>6 percent added oil) from rapeseed (Givens et al., 2003; Deaville et al., 2004). At more modest inclusions (<3 percent added oil), high rates of vitamin E supplementation have reduced but not eliminated milk fat depression (Focant et al., 1998; Bell et al., 2006; Pottier et al., 2006; O'Donnell-Megaro et al., 2012). In a short-term experiment, vitamin E did not reduce milk fat depression when oil supplementation started before vitamin E supplementation (Zened et al., 2012).

Requirements

Inadequate data are available to determine a requirement for vitamin E, but based mainly on cow health, an AI for vitamin E can be established. Many common feeds fed to dairy cows can contain appreciable concentrations of α-tocopherol, but the highly variable concentrations result in substantial uncertainty regarding basal concentrations. In addition, in commercial situations, few feeds are actually assayed for α-tocopherol. Therefore, the AI for vitamin E is expressed as supplemental vitamin E, not total dietary vitamin E. Because of the lack of new data, the AI for dry and lactating cows was the same as in NRC (2001). Dairy cows in the immediate (ca. 2 weeks) prepartum period benefit from increased supplementation of vitamin E (3.2 to 6.4 IU/kg BW); however, differences in supplementation rates make establishing an AI for peripartum cows difficult. The lowest supplementation rate that observed benefits (Baldi et al., 2000) was 3.0 IU/kg BW or about 2,000 IU/d during the last 2 to 3 weeks of gestation, which was used for the AI.

Dry cows: Vitamin E AI, $IU/d = 1.6 \times BW$, kg

(Equation 8-3a)

Prepartum animals within 3 weeks of calving: Vitamin E AI, $IU/d = 3.0 \times BW$, kg   (Equation 8-3b)

Lactating cows and growing heifers, Vitamin E AI, $IU/d = 0.8 \times BW$, kg   (Equation 8-3c)

This is approximately equal to 1,000, 2,000, and 500 IU of supplemental vitamin E per day for dry, prefresh, and lactating cows, respectively. Fresh forage is an excellent source of vitamin E, and the need for supplemental vitamin E by grazing cattle is substantially less than those presented for cattle fed conserved forages. To account for increased supply of α-tocopherol when cows consume fresh forage, fresh forage was assumed to supply 35 mg/kg (50 IU/kg) more α-tocopherol than hay and silage. The requirement for supplemental vitamin E was reduced by 50 IU/d for every kilogram of fresh pasture DM consumed by a cow.

The difference between the AI for vitamin E for dry and lactating cows is mainly caused by expected differences in intake of vitamin E from basal feedstuffs and potentially reduced absorption of vitamin E by cows fed conventional dry cow diets (i.e., low-fat concentration). Based on typical DMI and average vitamin E concentrations in feedstuffs, the recommended amount of total vitamin E (supplemental plus vitamin provided by feedstuffs) is approximately 2.6 IU/kg BW during the late gestation and for lactating dairy cows. Of that amount, the basal diet will provide on average about 1.8 IU/kg BW for lactating cows (ranges from about 0.8 for cows fed diets based on severely weathered hay to about 2.8 IU/kg BW for cows fed diets based on pasture) and about 1 IU/kg BW (ranges from 0.5 to about 2.3 IU/kg BW) for dry cows. Colostrum synthesis during the immediate prepartum period increases the need for vitamin E. Cows may secrete 5 to 7.5 mg α-tocopherol/kg of colostrum (see Table 12-1 in Chapter 12). This is equivalent to 100 to 150 mg (or IU) of all-*rac* tocopheryl acetate per 10 kg of colostrum. However, plasma concentrations of α-tocopherol in mastectomized cows decrease markedly around calving (Goff et al., 2002), indicating colostrum synthesis is not the only reason peripartum cows require additional vitamin E.

Maximum Tolerable Level

Toxicity studies have not been conducted with ruminants, but data from rats suggest an upper limit of approximately 75 IU/kg BW per day (NRC, 1987). Lesser amounts of supplemental vitamin E (2,500 to 6,000 IU/d) fed to cattle had reduced vitamin A and β-carotene concentrations in tissues (Westendorf et al., 1990; Yang et al., 2002). Dry dairy cows fed 3,000 IU of supplemental vitamin E per day during the

dry period (ca. 60 days) had a higher risk of having mastitis than cows fed 135 IU/d (Bouwstra et al., 2010b).

WATER-SOLUBLE VITAMINS

B Vitamins

B vitamins, with the possible exceptions of niacin, biotin, and vitamin B_{12}, are often not considered in diet formulation and are rarely supplemented because signs of B vitamin deficiencies are rarely observed in adult ruminants, and feeds and synthesis by ruminal microbes provide a substantial supply. However, changes in diet composition may have changed vitamin supply. In addition, marginal deficiency signs may be subtle and only cause biochemical perturbations and cellular dysfunction without observable clinical signs. The substantial increase in milk yields by today's dairy cows and the need to maximize metabolic efficiency likely have increased the demand for B vitamins. In addition, increasing vitamin concentrations in colostrum and milk may have benefits to the health of the calf and to humans consuming dairy products.

Ruminal Metabolism of B Vitamins

In ruminants, B vitamin supply cannot be calculated exclusively from B vitamin intake; significant synthesis and destruction of these vitamins by the ruminal microflora occur. Hunt et al. (1954) stated, "Members of the vitamin B-complex are synthesized in the rumen of the bovine, but our knowledge of the factors which affect these syntheses are rather limited." Table 8-2 illustrates the great variability of intake, duodenal flow, and apparent synthesis of B vitamins in rumen of dairy cows. Negative values for apparent ruminal synthesis indicate that the amount of vitamin destroyed in the rumen is greater than the amount of vitamin ingested. Absorption of B vitamins across the rumen wall has been demonstrated when the rumen is emptied of its content and filled with an aqueous solution of vitamins (Rérat et al., 1958), but in fed animals, no ruminal absorption of B vitamins is detectable (Rérat et al., 1959). As B vitamin absorption takes place mostly in the small intestine, duodenal flow of B vitamins represents the amount of vitamins potentially available for absorption by the cow. Overall, because of analytical challenges and other issues, current estimates of B vitamin synthesis, degradation, and absorption need to be improved to increase the ability to accurately determine when supplementation is warranted and will elicit a positive response.

Thiamin (B_1)

The main forms of thiamin are free thiamin and its mono-, di-, and triphosphorylated forms. Thiamin diphosphate is essential for carbohydrate metabolism (pyruvate dehydroge-

TABLE 8-2 Intake, Duodenal Flow, and Apparent Ruminal Synthesis[a] of B Vitamins in Dairy Cows (mg/kg of DMI)[b]

	Intake	Duodenal Flow	Apparent Synthesis in Rumen
Thiamin	1.3 to 3.8	0.8 to 7.8	−1.5 to 4.2
Riboflavin	4 to 106	3 to 87	−50 to 29
Niacin	22 to 170	47 to 146	−123 to 120
Vitamin B_6	2.6 to 17.6	0.7 to 7.7	−14.1 to 1.3
Biotin	0.2 to 7.0	0.2 to 6.6	−0.9 to 0.2
Folates	0.2 to 1.1	0.9 to 2.4	0.5 to 1.7
Vitamin B_{12}	—[c]	0.1 to 4.8	0.1 to 4.8[d]

[a]A negative value indicates that the amount of vitamin degraded in the rumen is greater than the amount of vitamin ingested.
[b]Steinberg and Kaufman, 1977; Breves et al., 1981; Santschi et al., 2005a; Schwab et al., 2006; Lebzien et al., 2006; Niehoff et al., 2013; Beaudet et al., 2016; Castagnino et al., 2016a,b, 2017. No data available on pantothenic acid.
[c]Under or close to the level of detection.
[d]Dietary concentrations of cobalt: 0.17 to 2.5 mg/kg DM.

nase and two transketolases in the pentose–phosphate pathway), energy metabolism (α-ketoglutarate dehydrogenase in the Krebs cycle), and catabolism of branched-chain amino acids (AAs; branched-chain α-ketoacid dehydrogenases). Thiamin triphosphate is required by a peroxisomal enzyme complex for FA oxidation. Thiamin is involved in regulation of the immune system, acts as an anti-inflammatory factor, and has antioxidant properties (Manzetti et al., 2014).

Given the importance of glucose as an energy supply for the brain and because thiamin is intricately involved in several of the energy-producing reactions, thiamin deficiency causes central nervous system disorders. Polioencephalomalacia (PEM) is the most common thiamin deficiency disorder. Clinical signs include a profuse but transient diarrhea, listlessness, circling movements, opisthotonus, and muscle tremors. If treated promptly by parenteral injections of thiamin (2 mg/kg BW), the condition can be reversed (NASEM, 2016). Thiamin deficiency has been observed when thiaminases, associated with either feeds or produced from altered ruminal fermentation, destroy thiamin or produce antimetabolites of the vitamin that block thiamin-dependent reactions (Combs, 2012). Thiaminases have been detected in bracken ferns and some raw fish products. Feeding high-sulfate diets can also cause a thiamin deficiency (Gould et al., 1991), increases the need for thiamin diphosphate by the brain, and increases the risk of developing PEM (Amat et al., 2013). Thiamin is generally considered atoxic. In three short-term (3- to 4-week periods) experiments, supplemental dietary thiamin, at doses of 150 and 300 mg/d, increased milk yield in one experiment, increased milk protein yield in two experiments, and did not affect, increased, or decreased milk fat yields (Shaver and Bal, 2000). Supplementation of thiamin

in low fiber diets was more positive than when diets contained adequate fiber.

Sources of thiamin include grains, grain by-products, soybean meal, and brewer's yeast. Thiamin concentrations in rumen contents (Tafaj et al., 2004, 2006), duodenal flow (Breves et al., 1981), and apparent ruminal synthesis of the vitamin (Schwab et al., 2006; Castagnino et al., 2016a,b) are negatively correlated with ruminal pH. Between 52 and 68 percent of dietary supplemental thiamin escaped destruction in rumen (Zinn et al., 1987; Santschi et al., 2005a).

Riboflavin (B$_2$)

Riboflavin is the essential component of two coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide, involved with more than 100 enzymes in oxidation-reduction reactions. The coenzymes are essential for catabolism of certain AAs and purines, β-oxidation of FAs, and dehydrogenation of succinate into fumarate in the Krebs cycle. Riboflavin is also involved in the reduction of oxidized glutathione (glutathione reductase) and in the activation of pyridoxine (vitamin B$_6$) and folates into their coenzyme forms (Combs, 2012).

Deficiency symptoms have been described in very young milk-fed calves (Wiese et al., 1947), but no deficiency or toxicity symptoms have been reported in adult ruminants. A single intramuscular injection of riboflavin (10 mg/kg for calves and 5 mg/kg for mature cows) increased neutrophil counts and enhanced neutrophil function (Osame et al., 1995). The effects of supplemental riboflavin on lactation performance have not been studied. Forages are good sources of riboflavin, although it is rapidly destroyed by sun-drying. Almost all of the riboflavin in dietary supplements (99 percent) is destroyed in the rumen (Zinn et al., 1987; Santschi et al., 2005a).

Niacin (B$_3$)

The generic term "niacin" covers two molecules: nicotinic acid and nicotinamide. Niacin is the essential component of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are involved in more than 200 reactions in the metabolism of carbohydrates, FAs, and AAs and in all redox reactions. Each form has specific metabolic roles; NAD is involved in glycolysis, lipolysis, and the Krebs cycle. As such, NAD$^+$ is reduced into NADH and works in synchrony with FAD, which is the ion acceptor. On the other hand, NADP is involved in the pentose–phosphate pathway and FA synthesis and acts as coenzyme of the glutathione reductase and dihydrofolate reductase. At high doses, nicotinic acid possesses antilipolytic and vasodilatory activities.

Niacin does not completely fit the definition of vitamin because in most mammals, the molecule is synthesized from tryptophan (Trp). In rats, ketone bodies (Shastri et al., 1968) and fatty liver (Fukuwatari and Shibata, 2013) suppress con-

version of Trp into niacin. The importance of endogenous synthesis of niacin differs among species (Combs, 2012). In preruminant calves, endogenous synthesis of niacin is sufficient to avoid clinical deficiency signs if the diet provides sufficient Trp (Hoppner and Johnson, 1955), but the importance of the Trp–niacin pathway for dairy cows is unknown.

Supplementation of nicotinic acid frequently increased the number of ruminal protozoa and microbial protein synthesis in vitro and in vivo (Schüssler et al., 1978; Riddell et al., 1980, 1981; Dennis et al., 1982; Shields et al., 1983; Brent and Bartley, 1984; Horner et al., 1988a,b; Erickson et al., 1990; Ottou and Doreau, 1996; Aschemann et al., 2012; Niehoff et al., 2013). According to a meta-analysis (Schwab et al., 2005) using data from 27 studies, 6 g/d of supplemental nicotinic acid did not affect lactation performance of dairy cows, but 12 g/d resulted in modest increases in yields of fat, protein, and fat-corrected milk. Feed efficiency (milk yield/DMI) tended to increase with supplemental niacin.

Supplemental niacin can have pharmacological effects on lipolysis and vasodilation, the first one to counteract the effects of lipid mobilization in early lactation and the second one to reduce the consequences of heat stress on lactating dairy cows. However, results have not been consistent. Decreases in plasma concentrations of FAs and β-hydroxybutyrate and increases in plasma glucose are the most frequently reported responses following use of nicotinic acid supplements (dose ranging from 6 to 12 g/d), although the response is highly variable among studies (Schwab et al., 2005; Niehoff et al., 2009; Pescara et al., 2010). Supplementary nicotinic acid (doses range from 12 to 36 g/d) increases vasodilation, enhancing heat loss during periods of heat stress in some studies (Di Costanzo et al., 1997; Niehoff et al., 2009; Pescara et al., 2010; Zimbelman et al., 2010, 2013; Wrinkle et al., 2012; Pineda et al., 2016) but not in others (doses varying from 4 to 24 g/d; Lohölter et al., 2013; Rungruang et al., 2014). In nonruminant animals, toxicity of niacin is low, at least 10- to 20-fold the estimated requirements (Combs, 2012).

Brewer's yeast and distillers grains are good sources and forages are considered fair sources of niacin (McDowell, 2000). Concentrations of niacin in cereals are often high, but a large proportion is covalently linked to small peptides and carbohydrates, which markedly impairs its availability, at least in nonruminant animals (Combs, 2012). Availability of those complexes to ruminants is not known. Destruction in rumen of supplementary niacin, given as nicotinic acid or nicotinamide, is greater than 90 percent (Zinn et al., 1987; Santschi et al., 2005a). However, production responses to supplementation of rumen-protected (RP) forms of niacin have been small (Yuan et al., 2012; Pineda et al., 2016).

Pantothenic Acid (B$_5$)

Pantothenic acid is an essential component of coenzyme A (CoA) and the acyl carrier protein (ACP). ACP is at the center of the multienzyme complex, FA synthase, and as such, its

component, 4′-phosphopantetheine, acts as an arm to allow the binding and transfer of acyl units for the elongation of the FA chain. Coenzyme A (CoA) is essential for numerous enzymatic reactions within cells, including the Krebs cycle, lipid metabolism, and AA catabolism, and acts as a global regulator of energy metabolism. CoA cannot pass through cell membranes, but all tissues can synthesize it using pantothenic acid. Conservation of CoA within cells is due to a tight control on CoA synthesis but also to efficient recycling of phosphopantetheine formed during catabolism of CoA and ACP (Bender, 1999).

Deficiency symptoms have been described in calves fed a pantothenic-free synthetic milk (Sheppard and Johnson, 1957), but no deficiency or toxicity symptoms have been reported in adult ruminants. Dietary supplementation of 1 g/d pantothenic acid decreased the efficiency of ruminal microbial protein synthesis of cows fed a low-forage diet, whereas it increased the amount of organic matter ruminally fermented with a high-forage diet (Ragaller et al., 2011). In the same study, pantothenic acid decreased plasma glucose with the low-forage diet and decreased milk protein content and increased lactose content with the high-forage diet. In a field study, supplements of pantothenic acid protected (50, 100, or 200 mg/d) or not protected (200 mg/d) from degradation in rumen increased milk production, milk fat and protein contents, and plasma concentration of glucose in cows during the first 5 months of lactation (Bonomi, 2000). However, supplementation of unprotected pantothenic acid (21 mg/kg DM) fed alone or in combination with biotin (0.87 mg/kg DM) for 18 days had no effect on DMI and yields of milk and milk components (Ferreira et al., 2015).

Pantothenic acid, usually in its bound forms (CoA, CoA esters, ACP), is widely present in feed ingredients from plant and animal origins. In sheep, the amount of free pantothenic acid reaching the duodenum is positively correlated with its intake, whereas the amount of CoA reaching the duodenum is positively correlated with the amount of microbial DM synthesized in the rumen (Finlayson and Seeley, 1983). In steers, only 22 percent of supplemental pantothenic acid escaped degradation in the rumen (Zinn et al., 1987). Similarly, only 15 percent of a pantothenic acid supplement was not degraded in an artificial rumen (Völker et al., 2011).

Vitamin B$_6$

There are six vitamers with vitamin B$_6$ activity: pyridoxine, pyridoxamine, and pyridoxal and their respective phosphorylated forms. Pyridoxal-5-phosphate (P-5-P) is a coenzyme for more than 120 enzymes and is involved in most reactions in AA metabolism. Due to its critical roles in AA metabolism, vitamin B$_6$ requirements of nonruminants are increased by high-protein diets (Okada et al., 1998). The vitamin is also essential for glycogen utilization; synthesis of histamine, hemoglobin, and sphingolipid; and modulation of expression of some genes. In nonruminants, symptoms of deficiency are nonspecific neurologic and dermatologic changes. There is no report of deficiency symptoms in adult ruminants. The effects of vitamin B$_6$ supplementation on lactation performance of dairy cows have not been studied. In nonruminants, toxicity of vitamin B$_6$ is low, although it is neurotoxic at excessively high doses, over 1,000 times the reference nutrient intake (Bender, 1999; Combs, 2012).

Forages and grains are good sources of vitamin B$_6$, but diet composition does not have a major effect on vitamin B$_6$ ruminal concentrations (Kon and Porter, 1953; Briggs et al., 1964; Lardinois et al., 1994; Santschi et al., 2005b) probably because apparent ruminal synthesis of vitamin B$_6$ is negatively correlated with B$_6$ intake (Beaudet et al., 2016; Castagnino et al., 2016a,b, 2017). However, 60 to 100 percent of supplemental B$_6$ escaped destruction in rumen (Zinn et al., 1987; Santschi et al., 2005a).

Biotin

Biotin plays key roles in lipid, AA, and energy metabolism due to its function as coenzyme for five carboxylases that catalyze the incorporation of the most oxidized form of one-carbon units (i.e., bicarbonate). Two of these carboxylases (pyruvate carboxylase and propionyl-CoA carboxylase) are likely of major importance for ruminants due to their role in gluconeogenesis. Methylcrotonyl-CoA carboxylase is involved with the catabolism of leucine (Leu), and two forms of acetyl-CoA carboxylase (mitochondrial and cytosolic) are involved with FA synthesis and oxidation. Biotin is involved in regulation of gene expression of many enzymes that play critical roles in glucose metabolism.

In many species, the major sign of a biotin deficiency is skin lesions. In vitro, omission of biotin from the culture media markedly reduces ruminal cellulose digestion and volatile FA production, especially propionate (Milligan et al., 1967). However, biotin supplementation has not improved in vitro and in vivo fiber digestibility (Majee et al., 2003; Rosendo et al., 2003). Two meta-analyses (Chen et al., 2011; Lean and Rabiee, 2011) evaluated the effects of supplemental biotin on milk production (some data were used by both analyses) with similar conclusions. Biotin supplements, at a dose of 20 mg/d, increased DMI, milk production, and fat and protein yields but did not affect milk fat and protein concentrations. Numerous studies report an improvement in hoof health when 10 to 20 mg/d supplemental biotin is fed (Lean and Rabiee, 2011). High doses of biotin are considered atoxic.

Yeast is a good source of biotin, and oilseed meals contain more biotin than cereals, with corn being a better source than wheat and barley. In feeds, biotin is present as free biotin and as biocytin, biotin bound to protein lysyl residues by an amide link. This bond can only be broken by the enzyme, biotinidase, present in intestinal mucosa and pancreatic juice. Biotinidase is rarely used for sample preparation because no pure preparation of the enzyme is available commercially; therefore, differences in extraction methods leading to incomplete liberation of free

biotin exacerbate the variability among studies. Duodenal flow of biotin is related to the amount of fermented organic matter and microbial protein synthesis (Lebzien et al., 2006). Bioavailability of dietary supplements of biotin has been estimated around 45 percent (Frigg et al., 1993; Santschi et al., 2005a).

Folates

Folic acid is used either as the generic name of the vitamin or, specifically, for the synthetic form of the vitamin, pteroylmonoglutamatic acid. The term "folates" applies to the numerous biologically active forms: dihydrofolate and several forms of tetrahydrofolate. The length of the glutamate chain can vary from one to seven glutamate molecules. In mammals, folic acid accepts and releases one-carbon units in biochemical reactions. Cellular tetrahydrofolate accepts one-carbon units from donors such as serine or formate and transfers them for thymidylate and purine synthesis. Therefore, folic acid is crucial for DNA synthesis, replication, and repair. A folic acid deficiency causes an imbalance in DNA precursors, uracil misincorporation, and chromosome breakage. Tetrahydrofolate can also transfer methyl groups to homocysteine for regeneration of methionine (Met) under the action of a vitamin B_{12}–dependent enzyme, Met synthase. In the methylation cycle, the role of folate coenzymes is to provide one-carbon units to ensure a constant supply of S-adenosylmethionine, which is the primary methylating agent. Reactions mediated by S-adenosylmethionine include DNA methylation, which controls gene transcription and genetic stability, as well as synthesis of phosphatidylcholine, choline, creatine, and several neurotransmitters.

Weekly intramuscular injections of 40 mg folic acid given to dairy heifers from 10 days until 16 weeks of age increased average daily gain by 8 percent during the 5 weeks following weaning (Dumoulin et al., 1991), suggesting that folic acid may be deficient in young calves around weaning when the ruminal microbial populations are not fully established. Daily dietary supplements of folic acid (2 to 6 mg/kg BW of unprotected folic acid or 1 to 3 g of a RP product) usually (Girard and Matte, 1998; Graulet et al., 2007; Girard et al., 2009a; Li et al., 2016) but not always (Girard et al., 2005) increase milk production and milk protein yield during the first part of the lactation. Except for one study (Li et al., 2016), none of these studies observed an increase in DMI, suggesting that supplemental folic acid increases metabolic efficiency. Li et al. (2016) also reported improved reproductive efficiency when cows were supplemented with RP folic acid. Dietary supplements of folic acid have little effects on ruminal fermentation (Chiquette et al., 1993; Girard et al., 2009a; Ragaller et al., 2010). High doses of folic acid have no negative effects in nonruminant animals, except in the presence of vitamin B_{12} deficiency (Selhub et al., 2007; Combs, 2012).

Oilseeds and brewer's yeast are major dietary sources. Disappearance of supplementary folic acid before the duo-

denal cannula is 97 percent but 25 percent of a dose of folic acid infused in the abomasum disappears before the duodenal cannula, probably absorbed in the proximal part of the duodenum (Santschi et al., 2005a). Based on the latter, destruction of a dietary supplement of folic acid can be estimated around 72 percent of the amount ingested.

Vitamin B_{12}

"Vitamin B_{12}" is a generic term used to describe all corrinoids containing an atom of cobalt (Co) and exhibiting the biological activity of cyanocobalamin. Cyanocobalamin is the synthetic form of vitamin B_{12} present in most supplements. The cyanide group is added to stabilize the molecule, but the molecule is not biologically active until the cyanide group is enzymatically removed. In mammals, the major cobalamin vitamers are methylcobalamin, adenosylcobalamin, and hydroxocobalamin.

Several vitamin B_{12}–dependent metabolic reactions have been identified in microorganisms, but in mammals, only two such reactions exist. One of the two vitamin B_{12}–dependent enzymes, Met synthase, is the critical interface between folic acid and vitamin B_{12} metabolism. Met synthase transfers a methyl group from 5-methyl-tetrahydrofolate (producing tetrahydrofolate) to homocysteine producing Met. In a vitamin B_{12} deficiency, all available one-carbon units are diverted toward the synthesis of 5-methyl-tetrahydrofolate, which cannot be demethylated by Met synthase in absence of vitamin B_{12}, leading to a secondary folate deficiency. Besides its role in the methylation cycle and folate metabolism, vitamin B_{12} plays a key role for the entry of propionate in the Krebs cycle and gluconeogenesis, through the mitochondrial vitamin B_{12}–dependent enzyme, methylmalonyl-CoA mutase.

Vitamin B_{12} deficiency has been demonstrated in preruminant calves fed diets devoid of animal protein (Lassiter et al., 1953). In ruminants, vitamin B_{12} deficiency is the major consequence of an insufficient supply in Co. However, even with sufficient dietary Co, plasma concentrations of vitamin B_{12} are low during the first weeks of lactation (Elliot et al., 1965; Mykkänen and Korpela, 1981; Girard and Matte, 1999; Kincaid and Socha, 2007). Dietary or parenteral supplemental vitamin B_{12} when cows are fed adequate Co has minor or no effects on production responses (Elliot et al., 1979; Croom et al., 1981; Kincaid and Socha, 2007; Grace and Knowles, 2012; Akins et al., 2013). However, a combined supplement of folic acid and vitamin B_{12} given from 3 weeks before calving until 8 or 16 weeks of lactation (usually given parenterally once weekly) increased milk production and energetic efficiency in early lactation (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a,b; Ghaemialehashemi, 2013; Duplessis et al., 2014a; Gagnon et al., 2015). Perhaps via improved energy status, the combined vitamin supplement has improved various measures of reproductive efficiency (Ghaemialehashemi, 2013;

Duplessis et al., 2014b; Gagnon et al., 2015). No toxicity of vitamin B_{12} has been reported. Parenteral supplementation of a commercial mixture of butophosphan (an organic P compound) and vitamin B_{12} before calving or in early lactation decreased plasma concentrations of nonesterified FAs and β-hydroxybutyrate (Fürll et al., 2010; Rollin et al., 2010; Kreipe et al., 2011; Pereira et al., 2013; Nuber et al., 2016). Production responses to that mixture are variable.

Vitamin B_{12} is not synthesized by plants; it is produced only by bacteria and archaebacteria when Co supply is adequate (Martens et al., 2002). Only 11 percent of the Co ingested is used for ruminal synthesis of corrinoids, of which only 4 percent is incorporated into vitamin B_{12} (Girard et al., 2009b). Ruminal bacteria use dietary Co to produce vitamin B_{12} analogues, which are devoid of biological activity. The production of biologically active vitamin B_{12} usually increases with Co intake, generally at the expense of analogue synthesis (Hedrich et al., 1973; Bigger et al., 1976; Tiffany et al., 2003, 2006; Stemme et al., 2008). Apparent ruminal synthesis of vitamin B_{12} is correlated positively with Co intake (Beaudet et al., 2016; Castagnino et al., 2016b, 2017). Apparent ruminal synthesis of vitamin B_{12} in the rumen is generally positively correlated with fiber intakes (Sutton and Elliot, 1972; Schwab et al., 2006; Beaudet et al., 2016; Castagnino et al., 2016a,b, 2017) and negatively correlated with the amount of starch digested in rumen (Sutton and Elliot, 1972; Schwab et al., 2006; Beaudet et al., 2016).

Choline

Choline is not a vitamin in a traditional sense because it can be synthesized by cows (i.e., not dietary essential) and it is required in gram rather than milligram or microgram amounts. Johnson et al. (1951) produced a choline deficiency in week-old dairy calves using synthetic milk replacer diets containing 15 percent casein. Choline requirements estimated from that experiment were 260 mg/L of milk replacer (1,733 mg/kg DM). Current estimates of requirements for the calf are 1,000 mg/kg DM. The predominant sign of choline deficiency in most animals is fatty liver; in calves, other deficiency signs include muscular weakness and renal hemorrhage.

Both naturally occurring choline in feeds, predominantly found in phospholipids (lecithin), and dietary choline from supplements such as choline chloride are extensively degraded in the rumen (Neill et al., 1979; Sharma and Erdman, 1988a,b). Microbial degradation of choline in the rumen results in the production of acetaldehyde and trimethylamine. Methyl group carbon from trimethylamine is subsequently degraded to methane (Neill et al., 1978). Supplementation of choline in an unprotected form is useless because of extensive ruminal degradation.

Because of extensive degradation of dietary choline, methyl groups for synthesis of methyl-containing metabolites in the dairy cow are presumably produced via methylation pathways involving Met and the enzyme, S-adenosylmethionine methyl transferase. Sources of methyl groups for ruminants would include intestinally absorbed Met, betaine resulting from degradation of choline, and de novo synthesized methyl groups produced through 5-methyl tetrahydrofolate. Approximately one-third of the Met methyl groups were transferred to choline in studies with lactating dairy goats (Emmanuel and Kennelly, 1984). Intravenous infusion of choline and carnitine reduced the irreversible loss of Met by 18 to 25 percent in sheep, suggesting that Met could be spared with the addition of methyl-group–containing metabolites (Lobley et al., 1996).

Choline concentration of milk ranges from about 70 to 100 mg/L (Deuchler et al., 1998; Pinotti et al., 2003; Elek et al., 2008) and increases 25 to 40 percent when RP choline is fed (ca. 15 g/d actual choline). This suggests that secretion of choline into milk could be qualitative indicator of postruminal choline supply. Daily excretion rates vary from about 2 to 6 g/d depending on milk yield and whether RP-choline was fed.

The dairy cow evolved under circumstances where intestinally absorbed choline is almost nonexistent; hence, choline supply is dependent on the ability of the cow to synthesize it from serine or from feeding RP forms. Since publication of the first article on the use of RP-choline (Erdman and Sharma, 1991), adequate studies have now been published to allow for meta-analyses. Sales et al. (2010) used data from 11 different publications and Arshad et al. (2020) used data from 20 publications, including most of the publications used by Sales et al. (2010). Results were in general agreement: supplementing approximately 13 g of actual choline (in RP form) increased milk yield about 1.5 kg/d, increased DMI about 0.5 kg/d, and had little effect on milk composition but increased milk fat and protein yields. Most studies conducted with RP-choline only involve transition cows (supplementation usually started a few weeks prepartum and usually ended 3 to 4 weeks postpartum). Data on production responses to RP-choline later in lactation are limited and inconsistent. The predicted milk yield response (in early lactation) to RP-choline was greater when calculated (not measured) supply of metabolizable Met was low and response decreased as metabolizable Met supply increased (Arshad et al., 2020). This meta-analysis and individual studies indicate that RP-choline may reduce the amount of Met being used as a methyl donor, allowing more to be used for protein synthesis. In an experiment where methyl transfer from Met was inhibited but choline was provided, fat-correct milk yield increased, suggesting the importance of Met in methyl group metabolism in the dairy cow (Sharma and Erdman, 1988b). However, in studies in which both RP-choline and RP-Met have been fed, few interactions have been observed (Sun et al., 2016; Zhou et al., 2016).

Because the role choline has in hepatic lipid metabolism (Piepenbrink and Overton, 2003), RP-choline has

been investigated as a means of reducing incidence and severity of ketosis. Although a meta-analysis (Arshad et al., 2020) found that peripartum cows fed RP-choline had significantly higher blood glucose concentrations and lower blood concentrations of FAs and β-hydroxybutyrate, the mean differences were clinically insignificant, and several individual studies report no differences. However, several studies have reported improved health of peripartum cows when RP-choline was supplemented. Improvements included fewer cases of retained placenta, less mastitis, and displaced abomasum (Ardalan et al., 2010; Lima et al., 2012). Although health data are limited, a meta-analysis (Arshad et al., 2020) reported that RP-choline significantly reduced the incidence of retained placenta and mastitis but did not affect prevalence of other health disorders, including ketosis and fatty liver. Limited data also suggest that positive health effects are more likely when cows rather than prepartum heifers are supplemented with RP-choline (Lima et al., 2012).

Supplementing peripartum cows with an effective source of RP-choline (i.e., not degraded in rumen but available for intestinal absorption) is expected in increased production measures and can reduce the prevalence of some health disorders, but the committee did not establish a dietary requirement for choline because it is synthesized by cows and because of potential variability in commercial products.

Vitamin C

Vitamin C or ascorbic acid is synthesized from L-gulonic acid within the liver of ruminants. Calves cannot synthesize ascorbic acid until approximately 3 weeks of age (Cummins and Brunner, 1991). Hence, vitamin C is not considered an essential nutrient for healthy cattle that are older than about 3 weeks. Most orally ingested ascorbic acid is destroyed in the rumen, but some commercial formulations of vitamin C may provide varying degrees of protection from ruminal metabolism. Oral supplementation of modified forms of vitamin C designed to reduce ruminal degradation has increased or tended to increase plasma ascorbic acid in sheep (Hidiroglou et al., 1997), steers (Pogge and Hansen, 2013), and dairy cows (Weiss, 2001).

Ascorbic acid functions as a water-soluble cellular antioxidant and is involved numerous biochemical pathways (Smirnoff, 2018). Vitamin C is needed for collagen synthesis, iron absorption, and phagocytic cell function, among other functions. Stressful (e.g., poor housing conditions, heat stress) and inflammatory events (e.g., mastitis) reduce plasma concentrations of ascorbic acid in calves and cows (Hidiroglou et al., 1977; Cummins and Brunner, 1989; Weiss et al., 2004; Padilla et al., 2006). Clinical ketosis did not affect plasma ascorbic acid concentrations (Padilla et al., 2005). Although dietary supplementation of some forms of vitamin C can increase concentrations of ascorbic acid in blood, and lower plasma concentrations of ascorbic acid are associated with mastitis, dietary supplementation

of vitamin C to cattle has had little to no effect on diseases and immune cell function (Santos et al., 2001; Chaiyotwittayakun et al., 2002; Naresh et al., 2002; Weiss and Hogan, 2007). No growth response has been reported when calves were supplemented with vitamin C. Immunoglobulin titers in calves were generally not affected by vitamin C supplementation (Cummins and Brunner, 1989; Hidiroglou et al., 1995). Current data do not support routine supplementation of vitamin C to calves or adult cattle.

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9

Water

INTRODUCTION

Of all of the nutrients consumed by dairy cattle, water is consumed in the greatest amounts. Water is essential for life and only follows oxygen in importance. In addition, no nutrient is found in greater concentrations in either the body of a mature cow (~65 percent), her fetus (~80 percent), or her milk (85 percent), and the body recycles no nutritional element more so than water. Even small changes in body water can result in important changes in animal health and performance. Water flux in a lactating dairy cows averages about 30 percent (Beede, 2012), which is greater than any other domesticated ruminant (Woodford et al., 1984a).

Water possesses unique physical and chemical properties that allow it to act as a solvent and to support life. Two important characteristics of water are that (1) the molecule is electrically *polar* and that (2) an unshared pair of electrons on the oxygen atom can bond with a hydrogen (H) atom on another molecule, creating a hydrogen bond. A consequence of these two characteristics is that water molecules are attracted to each other. Water also possesses several properties that contribute to the effectiveness in regulating body temperature (Denny, 1993). First, compared to virtually all other liquids at room temperature, water has a high specific heat (at 0°C, it is 4,218 J kg^{-1} K^{-1}). Thus, to alter its temperature, large amounts of heat need to be added or removed. In addition, the thermal conductivity of water is 0.565 W m^{-1} K^{-1} at 0°C and aids the dissipation of heat from the body (Denny, 1993). Water has a high latent heat of vaporization, thus allowing for the evaporation of water from the skin and respiratory tract. This characteristic of water creates a notable route of heat loss for cattle (Monteith, 1972; Squires, 1988).

The true number of functions served by water is not fully known, but major functions include regulating body temperature, supporting intermediary metabolism by acting as a solvent to dissolve substances, transporting nutrients and metabolites throughout the body, and eliminating waste materials in urine, feces, and respiration. Last, water serves as a lubricant in joints and in many organs. In cerebrospinal fluid, water acts as a cushion for the brain and brain and spinal tissue (Roubicek, 1969). Given the number of functions related to water, restricting water intake results in rapid but often reversible reductions in feed intake and milk yield (Burgos et al., 2001).

POOLS OF BODY WATER

Total Body Water

Total body water (TBW) is composed of intracellular fluid water and extracellular fluid water (ECW). ECW can be broken down into blood plasma water, interstitial water, and transcellular water (Hix et al., 1959; Murphy, 1992). The intracellular pool is the largest pool at approximately 40 percent of body weight (BW; Murphy, 1992). The ECW pool includes water contained in saliva, plasma, and interstitial fluid. The plasma volume of water in lactating cows is about 6.4 percent of BW (Woodford et al., 1984a). Because milk contains roughly 85 percent water, the ECW pool, which includes milk, is proportionally large. The proportion of ECW within the gastrointestinal tract is located mostly in the rumen and is about 65, 62, and 61 percent of total ECW in cows on −7, 63, and 269 days postpartum, respectively (Andrew et al., 1995).

Water enters the reticulo-rumen pool through saliva, through swallowed water, and by consuming feed containing water (Appuhamy et al., 2014). A portion of drinking water may pass directly into the abomasum through the esophageal groove (Woodford et al., 1984b) but likely is only 11 to 22 percent of the total drinking water intake (Woodford et al., 1984b; Café and Poppi, 1994).

Empty Body Water

Empty body water (EBWtr) is the proportion of water contained in the animal minus that contained in the ingesta.

In general, EBWtr decreases with increasing body fat content (Maeno et al., 2013); thus, in calves, EBWtr is approximately 70 percent (Chapman et al., 2017), and as they age, EBWtr decreases before reaching a relatively constant value on physiological maturity (Lohman, 1971). TBW is lower for fat dry cows and higher in lactating cows (Aschbacher et al., 1965; Murphy, 1992). Andrew et al. (1995) reported that the EBWtr content at −7, 63, and 269 days postpartum is 59, 66, and 60 percent of BW, which are similar to more recent data for lactating cows (64.7 ± 3.02 percent of BW; Agnew et al., 2005).

WATER BALANCE

Cattle lack the ability to store bulk volumes of water for extended periods of time (Dukes, 1955). Provided that water is available throughout the day, the volume of water in the body remains relatively consistent (Reece, 2004). When needed, the animal gains water by ingestion via drinking and consuming feed containing water and through metabolic oxidation. Water is lost via feces, urine, and sweating and respiration, while a small volume is also lost through saliva (Holter and Urban, 1992). When lactating, milk is a major route of water loss; however, the proportion of water in milk is highly regulated and does not fluctuate with changes in whole-animal fluid balance (Olsson, 2005).

Water Intake

Thirst and Drinking Behavior

Physiologically, homeostatic factors regulate pH, osmotic pressure, and acid-base balance and are modulated by the movement of ions such as sodium (Na), potassium (K), chloride (Cl⁻), and bicarbonate through intracellular and extracellular fluids containing water and solutes (Reece, 2004; Hogan et al., 2007). Consequently, the gain and loss of body water plays a major role in maintaining homeostasis.

Thirst is defined as a "longing or compelling desire to drink" and is stimulated by either extracellular or cellular dehydration (Hogan et al., 2007). Thirst may be triggered by a reduction in salivary secretion and dryness of the throat and mouth. The hypothalamic region of the brain controls thirst and drinking behavior and is mediated by angiotensin 11 (Hogan et al., 2007).

Osborne et al. (2002) observed that cows consumed 40 percent of their daily water intake within 2 hours of each feeding and milking time. Cattle generally use the musculature found in their cheeks to create suction that draws water upward. This water is then directed by the tongue and transported intraorally (Reis et al., 2010). Drinking is composed of one cycle in which one aliquot of water is first sucked up and then swallowed (Hiemae and Crompton, 1985). The frequency of water consumption varies depending on an array of factors that most notably include water availability,

and lactating cows should have access to unlimited amounts of water throughout the day (Radostits and Blood, 1985), especially under hot conditions (West, 2003). The frequency of discrete water consumption episodes varies from five for cows in late lactation (Jago et al., 2005) to almost eight in low stocking environments (Cardot et al., 2008). The frequency of water consumption is reduced when animals are on pasture compared to those in confinement (Jago et al., 2005). In some cases (e.g., winter), beef cattle have been known to survive as many as 3 to 5 days without water (Siebert and Macfarlane, 1975; Squires, 1988). In lactating dairy cattle, the total time spent drinking ranges between 13 and 17 min/d (Thomas et al., 2007). The volume of water consumed is positively correlated with the animal's standing of social dominance within the herd (Andersson and Lindgren, 1987). Pinheiro Machado Filo et al. (2004) observed that cows consume more water from large deeper troughs.

Free Water Intake

Free water intake (FWI) is defined as water that is consumed directly from a water store or watering device.

Lactating Cows

Several factors that affect daily FWI by dairy cows have been identified. Table 9-1 is a list of published equations used to predict FWI (kg/d) in lactating and dry dairy cows. The previous report (NRC, 2001) recommended the equation of Murphy et al. (1983); however, newer equations that attempt to identify more factors and account for more variation have been developed. Controlled studies to quantify the effects of ambient temperature, temperature humidity index (THI), solar radiation, relative humidity, wind speed, and precipitation on FWI are needed, but some of these variables are used in predictive equations (see Table 9-1). In general, because mean and minimum and maximum daily temperatures are closely correlated, one measure is probably suitable for predictive purposes (Murphy et al., 1983). Recently, Appuhamy et al. (2016) evaluated published equations used to predict FWI and developed and evaluated new predictive equations (see Table 9-1). Recommendations of Appuhamy et al. (2016) and those adopted for this report are that when reliable estimates of dry matter intake (DMI) are available, Equation 9-1 (see Table 9-1) be used to predict FWI while Equation 9-2 (see Table 9-1) be used when reliable estimates of DMI are not available. Both Equations 9-1 and 9-2 are unique to others because they include dietary K. K has been shown to positively affect both water consumption (Meyer et al., 2004; Fraley et al., 2015) and ruminal liquid passage rates (Fraley et al., 2015). Given the lack of data available to develop these equations, seven alternative equations are also listed. Appuhamy et al. (2006) noted that the equation of Murphy et al. (1983) and Meyer et al. (2004) both required DMI and, when evaluated, performed well. In

TABLE 9-1   Equations Used to Predict FWI (kg/d) in Dairy Cattle[a]

Equation	Reference	Models for Predicting FWI (kg/d)[b]
Recommended equations		
Lactating cows		
Equation 9-1	Appuhamy et al. (2016)	$= -91.1 + (2.93 \times DMI) + (0.61 \times DM\%) + (0.062 \times NaK) + (2.49 \times CP\%) + (0.76 \times TMP)$
Equation 9-2	Appuhamy et al. (2016)	$= -60.2 + (1.43 \times Milk) + (0.064 \times NaK) + (0.83 \times DM\%) + (0.54 \times TMP) + (0.08 \times DIM)$
Alternative equations		
	Murphy et al. (1983)	$= 16.0 + (1.58 \times DMI) + (0.90 \times Milk) + (0.05 \times NaI) + (1.20 \times mnTMP)$
	Meyer et al. (2004)	$= -26.1 + (1.30 \times Milk) + (0.406 \times NaI) + (1.516 \times TMP) + (0.058 \times BW)$
	Murphy et al. (1983)	$= 23.0 + (2.38 \times DMI) + (0.64 \times Milk)$
	Holter and Urban (1992)	$= -32.4 + (2.47 \times DMI) + (0.60 \times Milk) + (0.62 \times DM\%) + (0.091 \times JD) - (0.00026 \times JD^2)$
	Khelil-Arfa et al. (2012)	$= -77.6 + (3.22 \times DMI) + (0.92 \times Milk) - (0.28 \times CONC\%) + (0.83 \times DM\%) + (0.037 \times BW)$
	Appuhamy et al. (2014)	$= -34.6 + (2.75 \times DMI) + (0.84 \times Milk) + (2.32 \times Ash\%) + (0.27 \times DM\%)$
	Little and Shaw (1978)	$= 12.3 + (2.15 \times DMI) + (0.73 \times Milk)$
	Stockdale and King (1983)	$= -9.37 + (2.30 \times DMI) + (0.53 \times DM\%)$
	Castle and Thomas (1975)	$= -15.3 + (2.53 \times Milk) + (0.45 \times DM\%)$
	Khelil-Arfa et al. (2012)	$= -41.1 + (1.54 \times Milk) - (0.29 \times CONC\%) + (0.97 \times DM\%) + (0.039 \times BW)$
	Dahlborn et al. (1998)	$= 14.3 + (1.28 \times Milk) + (0.32 \times DM\%)$
Recommended equation		
Dry Cows		
Equation 9-3	Appuhamy et al. (2016)	$= (1.16 \times DMI) + (0.23 \times DM\%) + (0.44 \times TMP) + (0.061 \times TMPC^2)$
Alternative equations		
	Appuhamy et al. (2016)	$= (0.69 \times DMI) + (0.28 \times DM\%) + (0.85 \times TMP)$
	Holter and Urban (1992)	$= 10.34 + (0.230 \times DM\%) + (2.21 \times DMI) + (0.0394 \times (CP\%)^2)$

[a] Adapted from Appuhamy et al. (2016).
[b] DMI (kg/d), BW (kg), Milk = milk yield (kg/d), DM% = dry matter percentage of the diet, CONC% = concentrate content of the diet (% of DM), CP% = dietary CP content (% of DM), Ash% = dietary total ash content (% DM), NaI = sodium intake (g/d), TMP = daily average ambient temperature (°C), mnTMP = daily minimum ambient temperature (°C), JD = Julian day, TMP = daily mean ambient temperature (°C), TMPC^2 = (TMP − 16.4)^2, and NaK = sum concentration of Na and K in the diet, milliequivalent/DM kg, (% Na/0.023) + (% K/0.039) × 10).

general, measures to increase consumption of water should be encouraged, but water intake alone should not be used to evaluate the effects of water quality. One cannot assume underconsumption is a result of poor water quality as it may be reduced in response to other factors such as poor health or production as well as access to watering devices (Kononoff et al., 2017).

Dry Cows

Fewer equations exist to predict FWI in dry cows (see Table 9-1), and further research in this area is needed to test current predictive equations. Equations for dry cows are based mostly on DMI, dry matter (DM) concentration of the diet, and ambient temperature; however, factors such as K likely affect water intake, but data are lacking. For dry cows, Equation 9-3 (see Table 9-1) is recommended (Appuhamy et al., 2016), but published studies measuring FWI in dry cows are lacking, and this prediction will likely be improved with more data. Because an independent data set was not available, Appuhamy et al. (2016) did not compare Equation 9-3 to the alternative equation of Holter and Urban (1992) also listed in Table 9-1. Equation 9-3 is recommended because data used to develop it represented a greater range of DMI as well as environmental and diet conditions, but it is possible that the

alternative equation of Holter and Urban (1992) could predict FWI as well as or better than Equation 9-3.

Calves and Heifers

Water should be provided free choice to calves, including those being fed a liquid diet (Drackley, 2008). Kertz et al. (1984) observed that weight gain was reduced by 38 percent and starter intake was reduced by 31 percent when calves had restricted access to water. Currently, models to predict FWI in young calves are not available, and published data are scarce, thereby precluding development of a model. Some studies have reported low FWI during the period before weaning (de Passille et al., 2011), but most observe that in early life, FWI is approximately 0.75 to 1 kg/d (Thomas et al., 2007; Wickramasinghe et al., 2019) and increases with age (Wenge et al., 2014). By 20 days of age, FWI increases dramatically (Kertz et al., 1984), and this increase in FWI occurs in parallel with reductions in feeding of milk replacer and increasing starter intake. DMI (in the form of calf starter) is likely directly related to FWI in young calves, and calves may require four times greater FWI than DMI or an FWI to DMI ratio of 4:1 (kg basis) (Kertz, 2014). Quigley et al. (2006) reported that prior to weaning, FWI/DMI was 2:1 but that this increased to 4:1 after full weaning. The amount

of water consumed prior to weaning is also a function of liquid feed consumption, and when liquid feeding rates are high, FWI/DMI may be less than 2:1 (Wickramasinghe et al., 2019). In addition, these investigators determined that withholding water until 17 days of age reduced milk intake, as well as BW and heart girth at 5 months of age compared to calves given access to water at birth. Increases in FWI in calves are also associated with increased environmental temperatures, feed restriction, increased water temperature in cold environments (Huuskonen et al., 2011), increased starter intake (Kertz, 1984; Wenge et al., 2014), and development of ruminal fermentation (Abe et al., 1999a,b). Providing fresh warm water is important in calves suffering from diarrhea (McGuirk, 2008) because calves increase FWI by 25 to 50 percent when suffering from diarrhea (Jenny et al., 1978). Data on FWI in older growing heifers are limited, and no equations have been developed to predict their FWI. Equations outlined by NASEM (2016) to predict FWI in growing feedlot cattle do not appear to be accurate based on limited data such as that published by Zanton and Heinrichs (2016).

Ambient Temperatures and Free Water Intake

The increase in FWI with increasing temperatures is well known (NRC, 1981), but the response is variable across individual animals and locations (Arias and Mader, 2011). During hot weather, the increase in water consumption is believed to be a response to the need to support evaporative and respiratory heat losses (Pereira et al., 2014). If not properly restored, water located within the vascular and extracellular compartments may be disrupted, leading to interference with osmotic pressure and blood pressure. Such physiological changes can ultimately threaten thermoregulation and cardiovascular function (Silanikove, 1994). McDowell et al. (1969) observed that FWI is 29 percent greater when a Holstein cow is housed at 32°C compared to being housed in temperatures between 15°C and 24°C. In a temperature-controlled study (temperature mean $= 8.6 \pm 7.1$; minimum $= -5.6$°C; maximum $= 23.3$°C), for each degree Celsius increase in ambient temperature, FWI increased by 1.5 kg (Meyer et al., 2004). These investigators concluded that daily mean and minimum or maximum environmental temperatures are highly correlated with FWI, and each may influence FWI. In another controlled study, increasing ambient temperature from 15°C to 28°C increased FWI by 10 and 42 percent for lactating and dry cows, respectively (Khelil-Arfa et al., 2014). Evaporative losses were estimated as a proportion of DMI, and those losses were compensated for by an increase in FWI.

Other Factors Affecting Free Water Intake

In general, and even in warm environmental temperatures, cattle likely prefer warm over cool water (30°C versus <14°C) and cattle prefer water between 20°C and 28°C

(Lanham et al., 1986). In hot, arid climates, a preference for cool water exists (Challis et al., 1987). In a study conducted in Canada and over four seasons, water was offered at either ambient temperature (7°C to 10°C) or warmed (30°C to 33°C), and FWI increased with water temperature. The greatest change in intake response was observed in the winter (5.9 percent) while the lowest change in response (2.8 percent) was observed in the spring (Osborne et al., 2002). Cows in this study were not under heat stress. In a similar study using bull calves, water was offered at either cool (6°C to 8°C) or warmed (16°C to 18°C) temperature, and FWI increased with water temperature during both the preweaning and postweaning stages (Huuskonen et al., 2011). In the summer months, chilling water has increased FWI of lactating dairy cattle and reduced respiration rates and body temperature (Lanham et al., 1986; Milam et al., 1986). In grazing cattle, warm environmental conditions play a major role on both drinking behaviors and FWI. The number of drinking bouts increases with THI, but at very high THI, the number of bouts decreases, possibly indicating an inability to thermoregulate in these conditions (Pereyra et al., 2010). Estrus can decrease FWI in lactating cows (Reith et al., 2014); however, flavoring agents (orange or vanilla) did not affect fluid water intake (Thomas et al., 2007).

WATER LOSSES

Milk Losses

Holter and Urban (1992) summarized four energy balance trials with 329 lactating Holstein cows housed at 18°C and observed that water losses through milk averaged 34 percent and ranged from 19 to 52 percent of total water intake (TWI; fluid plus feed water). Cows housed in a climatic chamber at 15°C had water losses through milk that averaged 24 percent of TWI, but this was reduced to 21 percent when the animals were housed in high temperatures (Khelil-Arfa et al., 2014; see Figure 9-1).

Fecal and Urinary Losses

For cows producing 23 kg of milk, fecal water contributed 61 percent of the total manure water (Appuhamy et al., 2014). The proportion of water lost in the feces when expressed as the percentage of TWI can be as low as 30 percent in lactating cows (McDowell et al., 1969) and as high as 44 percent (Khelil-Arfa et al., 2014) in thermoneutral conditions but decreased to 35 percent in warmer temperatures (see Figure 9-1).

The pituitary hormone, antidiuretic hormone (ADH), also known as vasopressin, largely regulates the excretion of water by the kidney. The release of ADH is likely governed by plasma osmoconcentration, but it may also be stimulated by pain, exercise, or psychological stress. When the animal is deprived of water, the concentration of ADH in
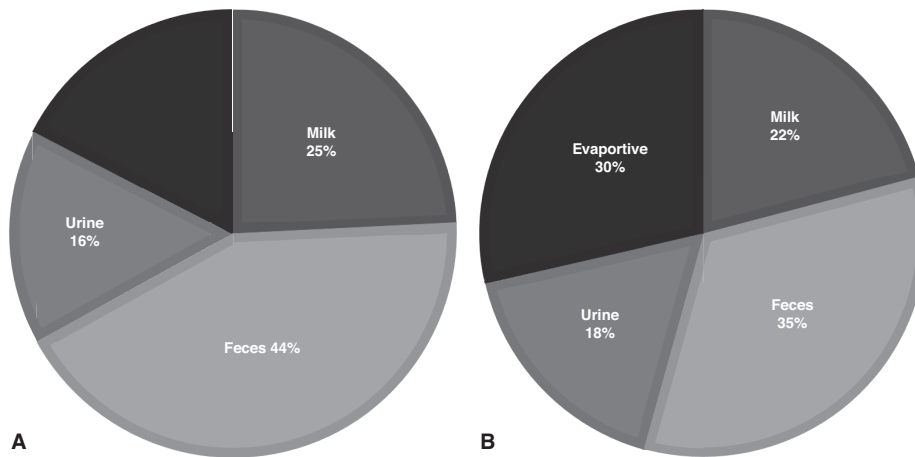
FIGURE 9-1   Water losses as reported by percentage of TWI (water acquired through drinking and feed consumption) by lactating dairy cows housed in (A) thermoneutral (15°C, TWI = 108 kg) and (B) high temperatures (28°C, TWI = 113 kg) as reported by Khelil-Arfa et al. (2014).
NOTE: Due to imbalance in metabolic water, retained water, and analytical error, values do not sum to 100 percent.

the blood increases, resulting in a reduction in urine volume. Conversely, when the animal is in excessive positive fluid balance, the concentration of ADH is reduced in the blood, and water excreted in the urine is increased until it is similar in concentration to that of plasma (Reece, 2004). Urinary losses of water have been reported to range between 11 and 21 percent of TWI (McDowell et al., 1969; Holter and Urban, 1992; Dahlborn et al., 1998). In a study evaluating the effect of increasing ambient temperature (15°C to 28°C) and sodium bicarbonate (0.20 percent DM and 0.50 percent DM), Khelil-Arfa et al. (2014) observed urine losses increased from 15 to 21 percent in lactating cows as temperature and sodium bicarbonate increased. These effects did not occur with dry cows.

Evaporative Loss

Water lost through evaporation increased from 18 to 30 percent of TWI when lactating cows moved from thermoneutral to higher-temperature conditions (Khelil-Arfa et al., 2014; see Figure 9-1). In dry cows, the response was 28 to 44 percent. Differences between lactating and dry cows may be due to a change in fractionation of the body water pool (Abeni et al., 2015). The efficiency of evaporative losses from the skin is also affected by the thickness, length, and color of the haircoat (Gebremedhin et al., 2008).

Sweat Losses

Sweating is an active process, which is triggered by an increase in body core temperatures and involves the secretion of fluid by the sweat glands (NRC, 2007). During this process, heat along with water is lost from the surface of the

skin (Gebremedhin and Wu, 2002). To dissipate heat, dairy cattle sweat in two different ways (Bernabucci et al., 2010). The first is insensible sweating, in which, unless relative humidity is 100 percent, sweat leaves the body constantly. The second is thermal sweating and serves as the principal mechanism of cooling with increasing temperatures. The vaporization of 1 L of water or sweat requires 0.58 Mcal (2.42 MJ) (Bernabucci et al., 2010). Jersey cows have a sweating rate of 189 ± 84.6 g/m^2-h, while mostly black or mostly white Holsteins have a sweating rate of 414 ± 158.7 g/m^2-h or 281 ± 119.4 g/m^2-h, respectively (Gebremedhin et al., 2008). These observations support the suggestion that Jersey cattle are more heat tolerant than Holstein cattle and that mostly black Holstein cattle possess higher solar absorption characteristics than mostly white Holstein cattle.

Respiratory Losses

Cattle also lose water through respiration. This type of loss is enhanced and facilitated through polypnea or the behavior known as panting (Gaughan and Mader, 2014). Research conducted in Missouri (Kibler and Brody, 1949, 1950, 1952, 1954) attempted to quantify heat loss through different routes. In summarizing these observations, Brouk et al. (2003) noted that at temperatures above 21°C, heat was primarily lost through moisture evaporation from the skin and lungs. However, in animals that were not cooled and as temperatures exceeded 32°C, over 85 percent of the total heat dissipation occurred through vaporization of water from the body surface and lungs. Respiratory water loss (RWL) at different air temperatures (Ta) and relative humidity (RH) increases with rising Ta but declines with increasing RH with no interaction (Berman, 2006). Using data from climate-

controlled chambers, Berman (2006) developed the following model to predict RWL (g h^{-1}) at different Ta (°C) and RH (%):

$$RWL = 0.41 - 0.02 \times Ta + 0.0005 \times Ta^2 - 0.004 \times RH + 0.00004 \times RH^2 \quad \text{(Equation 9-4)}$$

Water Restriction and Dehydration

The metabolic use and loss of body water is a continuous process, but consumption is not. Because of this all animals, but especially lactating cows housed in confinement, should have almost continuous access to clean water. Dehydration of as little as 10 percent of TBW may have serious implications on health, while the loss of 15 to 20 percent may be fatal (Beede, 2012). When water intake was restricted to 50 percent expected voluntary intake for 4 days, milk yield dropped by 74 percent, but when cows were allowed to consume 90 percent of expected water intake for 14 days, milk yield only decreased 3 percent (Little et al., 1980). In both situations, water restriction caused significant changes in blood composition, with all analytes increasing in concentration. These findings suggest a reduction in blood volume and hemoconcentration.

WATER QUALITY

Water can contain dissolved minerals, organic compounds, and microorganisms that may affect milk production and animal health. In 2005, NRC (2005) published guidelines on mineral tolerances from many sources, including water. The committee acknowledged that although there have been substantial advancements of analytical methods to measure minerals in water, information is limited on how many of these minerals affect animals. Studies evaluating the effects of water quality on dairy cattle are limited, and as a result, guidelines are usually extrapolated across species. Notably, guidelines for water quality for humans are often included that are particularly conservative. Because the quality of water may change over time and season, water should be sampled periodically during different seasons and assayed. Maintaining historical data can be useful in identifying subtle changes in water quality. Specific sampling protocols have been developed for water because often it contains only trace amounts of some minerals, and microbiological testing requires aseptic sampling. Commercial testing laboratories can provide accepted sampling protocols, and usually they will provide proper sample containers. To evaluate water for dairy cows, the water needs to be sampled at the point of consumption; however, sampling at different points in the water supply change can help identify sources of contamination (Dege, 2011). Table 9-2 lists thresholds of what can be considered upper potentially concernable concentrations for drinking water in cattle. Information in this table should be used cautiously as there is a general lack of research information around many components (Beede, 2012). Nonethe-

less, when water samples contain constituents greater than what is listed in the table, the taste and odor of water may be affected. In addition, diet modifications may need to be made to avoid mineral problems or toxicities described in Chapter 7.

Total Dissolved Solids and Salinity

Total dissolved solids (TDS) are an inexact measure of inorganic constituents dissolved in a water sample because it may also include organic compounds. The term "salinity" is sometimes used to refer to TDS; however, in this usage, salinity refers to all dissolved salts (e.g., magnesium sulfate, sodium bicarbonate, and sodium chloride). TDS are the concentration of total ions present in water but do not identify and quantify individual components of that water sample. Consequently, its value as a quality indicator for water is limited and should be interpreted with caution. The nature of TDS is influenced by the local geology, but the primary ions usually found in water are carbonate, bicarbonate, Cl$^-$, fluoride, sulfate, phosphate, nitrate, calcium (Ca), magnesium (Mg), Na, and K (NRC, 1974). Cl$^-$ is the ionized form of chlorine (Cl), and those two elemental forms have very different effects both in the rumen and on animal tissues.

Although the effect of varying TDS on milk production has been investigated, effects are likely influenced by the ions used to alter TDS. Therefore, effects of TDS on dairy cattle across studies are variable (Challis et al., 1987; Bahman et al., 1993; Valtorta et al., 2008; Shapasand et al., 2010). Nonetheless, general guidelines have been established (see Table 9-3).

In a study with grazing Holstein cows producing about 24 kg/d of milk, drinking water with 1,000, 5,000, or 10,000 mg/L TDS did not affect milk production or composition (Valtorta et al., 2008). In that study, TDS were increased by adding Na and calcium chloride, Mg and sodium sulfate, and sodium bicarbonate. Bahman et al. (1993) reported no difference in milk yields (averaged about 22 kg) between cows fed water with about 450 or 3,600 mg/L TDS (difference was mostly sulfate, Cl$^-$, Na, Ca, and Mg). Conversely, "high-producing" cows (based on 1988 standards; actual production not given) had about 25 percent lower lactation persistency when consuming water with 4,100 mg/L TDS compared to cows consuming water with 450 mg/L TDS; however, water intake was actually greater for the high-TDS water (Wegner and Schuh, 1988). Similarly, Challis et al. (1987) reported that reducing TDS from approximately 4,400 mg/L to 441 mg/L increased milk production from about 25 kg/d to 34 kg/d. In that study, TDS were elevated mostly by sulfate, but the high-TDS water also had more Ca, Mg, Na, and Cl$^-$ than the low-TDS water.

Effects of drinking saline water (i.e., water with high TDS from predominantly sodium chloride [NaCl]) are more consistent. When TDS were increased from about 200 to

TABLE 9-2   Drinking Water Standards for Humans and Upper Potentially Concernable Concentrations for Cattle[a,b]

	U.S. EPA		This Publication
	Enforceable or Secondary[c]	Human MCL[d]	Potentially Concernable Concentrations for Cattle
TDSs,[e] mg/L	Secondary	500	See Table 9-3
pH	Secondary	6.5–8.5	—
Nitrate − N (NO_3-N), mg/L	Enforceable	10[f]	See Table 9-5
Nitrite − N (NO_2-N), mg/L	Enforceable	1.0	—
Sulfate (SO_4^{2-}), mg/L	Secondary	250[g]	Calves = 500
			Adult cattle = 1,000
Chemical, mg/L unless otherwise listed			
Aluminum	Secondary	0.05–0.20	5.0–10.0 (Beede, 2012)
Arsenic	Enforceable	0.01	0.20 (NRC, 2005)
Barium	Enforceable	2.0	>10 (Beede, 2012)
Boron	—	—	150 (NRC, 2005)
Cadmium	Enforceable	0.005	0.01–0.05 (NRC, 2005; Beede, 2012)
Calcium	—	—	—[h]
Chloride	Secondary	250	300 (Beede, 2012)
Chlorine (Cl_2)	Enforceable	4.0[i]	—
Chromium	Enforceable	0.1	0.1–1.0 (NRC, 2005; Beede, 2012)
Cobalt	—	—	1.0 (NRC, 2005)
Copper	Enforceable	1.3	1.3 (EPA, 2009)
Copper	Secondary	1.0	—
Fluoride	Secondary	2.0	2.0 (NRC, 2005)
Iron	Secondary	0.3	0.40 (Beede, 2012)
Lead	Enforceable	0.015	0.05–0.10 (NRC, 2005)
Magnesium	—	—	—[h]
Manganese	Secondary	0.05	0.50 (Beede, 2012)
Mercury	Enforceable	0.002	0.01 (Beede, 2012
Molybdenum	—	—	0.06 (Beede, 2012)
Nickel	—	—	1.0 (Beede, 2012)
Phosphorus	—	—	—
Potassium	—	—	—[j]
Selenium	Enforceable	0.05	0.05 (Beede, 2012)
Silver	Secondary	0.10	0.05 (Beede, 2012)
Sodium	—	—	300 (Beede, 2012)
Vanadium	—	—	0.10 (NRC, 2005; Beede, 2012)
Zinc	Secondary	5.0	5.0–25.0 (NRC, 2005; Beede, 2012)

NOTES: There is a general lack of research information around many components; caution for use of this table should be exercised, when water samples contain constituents greater than what is listed in the table, the taste and odor of water may be affected and/or diet modifications may need to be made to avoid problems or toxicities.

[a] Ranges listed reflect a lack of information.

[b] Problems may occur when the following are observed (Beede, 2012): fluoride >2.4 mg/L may result in mottling, manganese >0.05 mg/L may affect taste, and sodium >20 mg/L may affect veal calves.

[c] Enforceable standard (EPA, 2009). Secondary are nonenforceable guidelines regarding contaminants that may cause cosmetic or aesthetic effects in drinking water for humans. The EPA recommends secondary standards to water systems but does not require systems to comply (EPA, 2009).

[d] MCL = Maximum contaminant level for humans, the highest concentration of a contaminant that is allowed in drinking water. MCL is only associated with enforceable standards (EPA, 2009).

[e] TDS = total dissolved solids.

[f] Equivalent to 44 mg/L of nitrate (NO_3).

[g] Sulfate sulfur ($SO_4^2 − S$) = sulfate (SO_4^{2-}) × 0.333.

[h] Potentially concernable concentrations for cattle for calcium and magnesium are unknown, but these may affect total dissolved solids (TDS); calcium >500 mg/L and/or magnesium >125 mg/L have been suggested to be concentrations worthy of further evaluation (Beede, 2012).

[i] Maximum residual disinfectant level for humans, the highest level of a disinfectant allowed in drinking water.

[j] Potentially concernable concentrations for cattle for potassium are unknown but concentrations >20 mg/L in drinking water fed to dry cows may warrant further evaluation because of its impact on dietary cation–anion difference.

2,500 mg/L via addition of NaCl, milk yield decreased from 34.8 to 32.9 kg/d (Jaster et al., 1978). Solomon et al. (1995) found that Holstein cows that consumed water with TDS of about 440 mg/L produced more fat-corrected milk (31.6 versus 29.8 kg/d) than cows that consumed water with TDS of about 1,500 mg/L (NaCl was mostly increased, but water also differed in sulfate, Ca, and Mg). Conversely, Arjomandfar et al. (2010) observed no effect on milk yield (averaged 35 kg/d) when TDS were reduced from 1,400 to 570 mg/kg through desalination (the high-TDS water contained predominantly NaCl but also contained higher concentrations of Ca, Mg, and sulfate).

TABLE 9-3   Guidelines for TDS in Water for Dairy Cattle Consumption[a]

TDS (mg/L)	Comments
<1,000	Safe and should pose no health problems.
1,000–2,999	Generally safe but may cause a mild, temporary diarrhea in animals not accustomed to the water.
3,000–4,999	Water may be refused when first offered to animals or cause temporary diarrhea. Animal performance may be less than optimum because water intake is not maximized.
5,000–6,999	Avoid these waters as a source of drinking water, may result in reductions in milk production.
>7,000	These waters should not be fed to cattle. Health problems and/or poor production will result.

[a]In general, TDS alone are not adequate to characterize drinking water of cattle, and it is further suggested that specific salt components and bacteriological measures are also needed.
SOURCE: NRC (1974).

A likely reason for the mixed responses to reducing TDS in water is the ionic makeup of the water. For example, high intakes of sulfate and Cl^- are detrimental to milk production during summer months (Sanchez et al., 1994). Furthermore, high concentrations of these minerals in water will likely decrease the dietary cation–anion difference (DCAD) consumed by the cow, which can reduce intake and milk yield (see Chapter 7). The DCAD is usually calculated as $(Na + K) - (Cl + S)$, where mineral concentrations are expressed as mEq/kg. Including Na or K supplied by water into that equation generally does not alter DCAD because the counterion of the cation is usually Cl^-. However, water with high concentrations of sulfate can reduce DCAD because the counterion is often Mg or Ca. This may be problematic when aiming for DCAD targets in prefresh diets. For example, assuming water did not provide additional Na or K, if a dry cow consumed 11 kg DM and drank 35 L water per day that contained 500 mg S/L, the S in the water would decrease DCAD by about 90 mEq/kg.

Hardness

Water hardness is usually described as the total cationic effects of Ca and Mg within water, but other cations may exist in water and include zinc (Zn), iron (Fe), strontium, aluminum, and manganese (Mn). Categories of hardness as described by NRC (1980) are soft (0 to 60 mg/L), moderately hard (61 to 120 mg/L), hard (121 to 180 mg/L), and very hard (>180 mg/L). Based on tests of up to 290 mg/L, hardness of water has been observed to have no effect on water intake of lactating cows (Graf and Holdaway, 1952; Blosser and Soni, 1957) but has been observed to be negatively associated with FWI in weaned calves (Senevirathne et al., 2018). However, water hardness may affect water handling systems because it may increase the accumulation of scale and may negatively affect water delivery systems (NRC, 2012). In addition, in-

creasing hardness may reduce cleaning efficiency of milking equipment, and hardness poses a risk factor for bacteriological quality of bulk tank milk (Elmoslemany et al., 2009).

pH

Currently, no guidelines for pH exist for drinking water for dairy cattle; however, the U.S. Environmental Protection Agency (EPA, 2009) recommends that for human consumption, water pH should be between 6.5 and 8.5. No information in the literature was found on the effects of varying the pH of drinking water on water intake, animal health, animal production, or the microbial environment in the rumen. However, pH likely has an influence on the survival of some microorganisms found in water (Szewzyk et al., 2000).

Minerals and Ionic Constituents of Water

Water may contain minerals, which can help meet the mineral requirements of animals, but if concentrations are excessive, these minerals can reduce water intake and have other detrimental effects on health and production. Water can supply absorbable minerals to cows, but generally this does not need to be included in supply calculations because the mineral content of water in most studies that evaluated mineral nutrition was not measured or considered in supply calculations. Users may consider adjusting dietary mineral supply downward when mineral concentrations in the water being consumed are high. However, including water minerals in total supply usually has a trivial effect on total supply (Castillo et al., 2013).

Speciation refers to the form of any given element in water. Elements may appear as a hydrated ion, as a neutral molecule, as a complex with an additional ion, or as some other molecule. Ground water commonly contains mineral species as hydroxo and carbonate complexes. The reactivity, toxicity, and bioavailability of mineral elements found in water are dependent on the form in which they exist; consequently, simply knowing the concentration of a particular mineral in drinking water yields limited information (NRC, 2005). Table 9-4 lists the major and minor ionic species commonly found in ground water. These species are usually present in water due to contact between water and nearby mineral deposits, while the minor constituents, ammonium, carbonate, and sulfide, may be present because of microbial and algal activity (Tchobanoglous and Schroeder, 1985). Traditionally, water analysis focuses on the total concentration of a mineral in a water sample and usually does not report data related to speciation. Such results can be evaluated for completeness and accuracy by determining if the sum of cations (eq/L) equals the sum of anions (eq/L). This is because, by the principle of electroneutrality, they must be equal in a solution. NRC (2005) notes that the difference of up to 2 percent may be due to uncontrollable error, but a difference

TABLE 9-4 Elements and Major and Minor Ionic Species That Are Common of Ground Waters[a]

Major Ionic Species	
Cations	Anions
Calcium (Ca^{2+})	Bicarbonate (HCO_3^-)
Magnesium (Mg^{2+})	Sulfate (SO_4^{2-})
Sodium (Na^+)	Chloride (Cl^-)
Potassium (K^+)	Nitrate (NO_3^-)
Minor Ionic Species	
Cations	Anions
Aluminum (Al^{3+})	Bisulfate (HSO_4^-)
Ammonium (NH_4^+)	Bisulfite (HSO_3^-)
Arsenic (As^+)	Carbonate (CO_3^{2-})
Barium (Ba^{2+})	Fluoride (F^-)
Boron (BO_4^{3-})	Hydroxide (OH^-)
Copper (Cu^{2+})	Phosphate, mono ($H_2PO_4^-$)
Iron, ferrous (Fe^{2+})	Phosphate, di (HPO_4^{2-})
Iron, ferric (Fe^{3+})	Phosphate, tri (PO_4^{3-})
Manganese (Mn^{2+})	Sulfide (S^{2-})
	Sulfite (SO_3^{2-})

[a]Adapted from Tchobanoglous and Schroeder (1985).

of 5 percent or greater suggests error in either sampling or analysis or that one or more ionic species were not reported.

Sulfate

Sulfates in drinking water usually originate from the dissolution of sulfate-bearing minerals located in both soils and rocks. Another source of sulfate contamination in water may be household or industrial wastes and detergents that contain sulfates (Veenhuizen and Shurson, 1992). Laboratories can report either sulfate or sulfate–sulfur and to convert sulfate into sulfate–sulfur multiply by 0.33. High concentrations of sulfate (SO_4^{2-}) ions in drinking water may negatively affect both feed and water intake (Loneragan et al., 2001). Weeth and Hunter (1971) observed that when sulfate in drinking water was increased to 3,493 mg sulfate/L (by adding sodium sulfate), water intake by Hereford heifers was reduced by 35 percent. Hereford heifers consuming water with 2,814 mg/L sulfate (from sodium sulfate) reduced feed intake and weight gain (Weeth and Capps, 1972). Although Digesti and Weeth (1976) concluded the safe maximum concentration for sulfate in drinking water is 2,500 mg sulfate/L, the current consensus recommendation is that water sulfate should not exceed 500 mg/L and 1,000 mg/L for calves and adult cows, respectively. NRC (2005) suggests that water for cattle fed high-concentrate diets should contain less than 600 mg sulfate/L while also noting that when consuming a high-forage diet, cattle can safely drink water containing 2,500 mg sulfate/L. Deep well water in some areas may contain 3,000 mg/L or more sulfate (Patterson and

Johnson, 2003). The rumen is a reducing environment; thus, most sulfur (S) originating from salts is reduced to sulfide. This can become so abundant that the combined S from feed and water will together tie up many trace minerals, making them unavailable to the animal. Depending on dietary S concentration, water with 1,000 to 1,500 mg sulfate/L may cause antagonism of copper (Cu) and selenium (Se) (see Chapter 7 for more detail).

Common forms of sulfate in water include Ca, Fe, Mg, Mn, and Na salts. Although the animal's response to increasing sulfate in water would depend on the specific form of sulfate present, little research exists in comparing these forms. In a study using Angus heifers, Grout et al. (2006) observed that the extent of aversion to water high in sulfate is, in part, dependent on the associated cation. Specifically, they found that increasing the concentrations of sulfate at 1,500, 3,000, and 4,500 mg/L in the form of magnesium sulfate reduced water intake, but reductions were not observed when cattle consumed sodium sulfate.

Iron

Waters containing high concentrations of Fe are often easy to recognize, as the water appears rusty in color, contains sediment, and possesses a metallic taste. Consumption of excessive amounts of Fe can antagonize cobalt (Co), Cu, Mn, Se, and Zn (Olkowski, 2009). Some experimental evidence suggests that oxidative stress may be spurred by high concentrations of Fe in drinking water. Free Fe catalyzes reactions via the Haber–Weiss reaction (Kehrer, 2000). This condition may be brought about when the consumption of Fe exceeds requirements, and as a result, the concentration of reactive oxygen and nitrogen (N) species increases. For example, abomasal infusions of ferrous lactate have been shown to negatively affect milk protein composition and overall stability of milk (Wang et al., 2016). Additional oxidative stress may be of concern in periparturient cows with a compromised immune system (Celi, 2010; Konvičná et al., 2015). Dietary Fe supplementation is rarely needed for adult cattle, and if water contains Fe, dietary supplementation should usually be avoided. The maximum contaminant level of Fe in drinking water for humans is 0.30 mg/L (EPA, 2009), and this concentration is often listed as a caution level for dairy cattle (Genther and Beede, 2013). In a study with sheep, no differences in FWI were observed when the concentration of Fe (from ferric sulfate [$Fe_2(SO_4)_3$]) was increased from 75 to 145 mg/L (Horvath, 1985). The effect of different Fe concentrations, different valances (ferrous [Fe^{+2}] or ferric [Fe^{+3}]), and different Fe sources (salts) in drinking water on FWI by lactating dairy cows were tested by Genther and Beede (2013). When water contained added ferrous lactate ($Fe(C_3H_5O_3)_2$), cows reduced FWI and spent less time drinking with 8 mg/L, compared with 4 or 0 mg/L. Valence of Fe source, namely ferrous sulfate ($FeSO_4$) or fer-

ric sulfate ($Fe_2(SO_4)_3$), did not affect FWI when offered at 0 or 8 mg/L, despite some visual differences in the appearance of the water. When FWI was compared with 0, 8, or 12.5 mg/L from different salts of Fe (ferrous chloride [$FeCl_2$] or ferric chloride [$FeCl_3$]), no differences in FWI were observed. When water with 0 or 8 mg/L of ferrous lactate ($Fe(C_3H_5O_3)_2$), ferrous sulfate ($FeSO_4$), or ferrous chloride ($FeCl_2$) was offered, cows drank more water without added Fe, but FWI was not affected by the different ferrous salts. These authors also noted that analytical method had a major effect on assayed Fe concentrations. A direct metal analysis of the raw water sample, without acidification, yielded values that were only 7 to 25 percent of the concentrations obtained when nitric acidification was conducted prior to analysis. Hence, when evaluating data, it is important to know which method was used.

Nitrate

Nitrate (NO_3^-) in drinking water may be a result of industrial pollution or heavy fertilization of fields, or it may be associated with shallow wells (Wang et al., 1999; Wright, 2007). There are currently no documented needs of dietary NO_3^- or nitrite (NO_2^-) by animals (NRC, 2005); however, it has been used to reduce ruminal methane production. Due to their caustic action, NO_3^- consumed in high concentrations may cause gastroenteritis. In addition, when consumed by cattle, NO_3^- can be used as a source of N for bacteria in the rumen (Russell, 2002). Most critically, the rumen is also the site of reduction of NO_3^- to NO_2^-. In the case of acute toxicosis, NO_2^- is absorbed into the bloodstream, which triggers oxidation of the ferrous Fe in hemoglobin to form methemoglobin. This reaction reduces the oxygen-carrying capacity of blood and may cause asphyxiation. Symptoms of NO_3^- poisoning include excessive salivation, abdominal pain, diarrhea and vomiting, and brown-colored mucous membranes (Radostits and Done, 2007). NO_2^- poisoning will result in impaired breathing, gasping, and rapid respiration. Signs may also include muscle tremor, weakness, stumbling gait, cyanosis, and a weak pulse. Abortion in ruminants is believed to follow NO_3^- poisoning (Bruning-Fann and Kaneene, 1993) and has been observed in both dairy and beef herds (Yeruham et al., 1997). In dairy cows, NO_3^- concentrations up to 180 mg/L in drinking water did not increase the concentration of NO_3^- in milk (Kammerer et al., 1992). In a field study with 54 cows, with half consuming water of 19 mg/L NO_3^- and the other half consuming water of 374 mg/L NO_3^- with the addition of potassium nitrate for 35 months, the first 20 months resulted in no effects on reproductive performance, but in the last 15 months, services per conception increased and first service conception rate decreased in cows drinking the high NO_3^- water, but no differences were observed in blood hemoglobin and methemoglobin (Kahler et al., 1974).

TABLE 9-5   Guidelines for NO_3^- and NO_3-N in Drinking Water[a] for Dairy Cattle

Nitrate (NO_3^-), mg/L	Nitrate Nitrogen (NO_3-N), mg/L	Guidelines
0–44	0–10	Safe for consumption by cattle
45–132	10–20	Generally safe when offered with balanced diets with low nitrate feeds
133–220	20–40	May be harmful if consumed for long periods of time
221–660	40–100	Cattle at risk and possible death
>660	>100	Unsafe—possible death; do not use as water source

[a] Nitrate nitrogen (NO_3-N) $\times 4.43 =$ nitrate (NO_3^-).
SOURCE: NRC (1974).

In a survey of 128 Iowa dairy farms, an elevation in the NO_3^- concentration of drinking water was correlated with increasing calving intervals (Ensley, 2000). By increasing the number of NO_3^- metabolizing rumen microbes, ruminants can adapt to diets high in NO_3^- (Allison and Reddy, 1984; Lin et al., 2013).

As in the last publication, NO_3-N in water is recommended not to exceed 10 mg/L, which is equivalent to 44 mg/L NO_3^-. Cattle are usually more at risk of NO_2^- poisoning because of high levels of NO_3^- in feeds, but the concentration in water likely has an additive effect on the animal (ANZECC, 2000). Water testing results, which include NO_3^- and NO_2^- in mg/L, can be converted to N values by dividing these values by 4.43 and 3.29, respectively (NRC, 2005). Table 9-5 lists guidelines for NO_3^- in drinking water of cattle.

Minerals and Potentially Toxic Substances in Water

The tolerable and toxic concentrations of minerals in domestic animals have been reviewed (NRC, 2005). The publication lists guidelines for drinking water for both humans and livestock. The guidelines for humans are listed in Table 9-2. Upper concentration guidelines for cattle are based on those of NRC (2001, 2005), Beede (2012), and Socha et al. (2003) but overall are unique to this publication. The values included in the table were not developed and reported in attempt to define toxic concentrations or even recommended ranges, but they are intended to be used as a reference when evaluating water samples. The publication notes that although conservative, the EPA enforceable and secondary water quality guidelines can act as safe guidelines for livestock. Enforceable standards are defined as concentrations that cannot be exceeded and set a mark for beyond which action to achieve lower levels must be taken. Secondary standards are concentrations that beyond which cosmetic or aesthetic effects may occur.

Additional points made by the NRC (2005) report that are relevant to feeding dairy cattle include a listing of minerals that fall into five categories. These include minerals that

1. Can be found naturally and at toxic levels in water or may contribute to the overall toxicity of the mineral: most commonly arsenic, barium, Fe, Mn, NaCl, sulfur, and nitrate fall into this category.
2. Can be found naturally and presence is rare but significant risks of toxicities: namely lithium, strontium, and uranium.
3. Usually are found at low levels with toxicity occurring due to the contamination from other sources: aluminum, bismuth, boron, bromine, cadmium, chromium, Co, Cu, lead, mercury, molybdenum, nickel, silicon, tin, and other rare earth elements.
4. Are macroelements unlikely to be found at toxic levels in water but may result in aesthetic secondary effects: Ca, Mg, phosphorus (P), and K.
5. Are trace minerals that may be found in water and may contribute to both a toxic concentration and secondary aesthetic effects: Co, Cu, Fe, Mn, and Se along with water containing a high concentration of NaCl.

Microbiological Considerations of Water

Determination of microorganisms in water is difficult, but drinking water is the largest and most direct source of microbial contaminants and potential pathogens (LeJeune and Gay, 2002). Water may be contaminated by runoff or may be a result of the water distribution and delivery systems. These may enhance bacterial conditions through coatings of biofilms that act as microbial habitats (Van Eenige, 2013). Surfaces of water troughs may also be contaminated by bacteria from cud, fecal matter, dust, feed, or bedding (LeJeune et al., 2001a,b). Water is commonly evaluated for total coliform bacteria and total fecal coliforms. Total coliforms are a generic group of Gram-negative bacteria. Fecal coliforms are not defined taxonomically and are, as the name suggests, often present in the water because of fecal contamination but may originate from other sources. Fecal coliforms are also known as thermotolerant coliforms (Alonso et al., 1999). Common bacteria found in contaminated water include enteric bacteria *Escherichia coli* and *Salmonella* but may also include other microorganisms such as *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Leptospira*, *Burkholderia pseudomallei*, *Clostridium botulinum*, *Mycobacteria* (pulmonary disease), *Pseudomonas cyanobacteria*, *Cryptosporidum*, and *Giardia* (ANZECC, 2000). As in the last edition of this report, no quality standards are set for water contaminated with microorganisms, as evidence to support them is lacking (Van Eenige, 2013). Water is frequently tested for the presence of

thermotolerant (fecal) coliforms, but this test provides no indication of the presence of microbial pathogens (ANZECC, 2000). In addition, in a study involving feedlot cattle, Sanderson et al. (2005) observed no relationship between water coliform count and fecal prevalence of *E. coli* O157, but suggested that water coliform count is a measure of *E. coli* O157 exposure. Despite these limitations, a median threshold for thermotolerant (fecal) coliforms for livestock has been recommended to be 1,000 thermotolerant (fecal) coliforms/L (ANZECC, 2000).

Cows or young stock on pasture may be provided surface water to drink. In these cases, animals may be at risk from toxic cyanobacteria (or blue-green algae). The poisoning of livestock by toxic cyanobacteria was first scientifically reported in the late 1800s when animals consumed water from a freshwater lake at the mouth of the Murray River in South Australia (Francis, 1878). Such mortalities have also been reported in grazing adult dairy cows (Galey et al., 1987; Kerr et al., 1987) and in grazing dairy heifers (Fitzgerald and Poppenga, 1993). It is estimated that 40 of the 2,000 species of cyanobacteria that have been identified are capable of being toxigenic (Briand et al., 2003) and may produce hepatotoxins, neurotoxins, dermatotoxins/irritant toxins, cytotoxins, and toxins that may cause gastrointestinal disturbance (Olkowski, 2009). Colonizing both terrestrial and aquatic biotopes and in both marine and freshwater ecosystems, cyanobacteria are photosynthetic prokaryotes, with growth commonly occurring in late summer to autumn (Briand et al., 2003). Risk factors include shallow waters that are neutral to alkaline (Carvalho et al., 2011) and contain high concentrations of N and P. In many livestock operations, the concentrations of N and P are commonly increased in bodies of water when evaporative losses occur along with manure or fertilizer contaminations (Radostits and Done, 2007). The presence of cyanobacteria is typically determined through microscopic examination. If drinking water is suspected to contain cyanobacteria, an alternative source of drinking water should be made available to cattle until it is treated or determined to be safe (Olkowski, 2009).

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10

Nutrient Requirements of the Young Calf

INTRODUCTION

From birth until weaning to solid feed, calves undergo tremendous physiologic and metabolic changes (Toullec and Guilloteau, 1989; Meale et al., 2017). During the preruminant stage, digestion and metabolism are similar to those of nonruminant animals, and dietary requirements must be met with high-quality liquid diets containing sources of carbohydrates, proteins, and fats that are digested efficiently. The most critical period is the first 2 to 3 weeks of life when the calf's digestive system is immature but developing rapidly with regard to digestive secretions and enzymatic activity (Toullec and Guilloteau, 1989; Davis and Drackley, 1998).

Except for calves raised for veal production, calves should be encouraged to consume solid feed at an early age to stimulate development of a functional rumen. Development of the ruminal epithelial tissue that is responsible for absorption of volatile fatty acids (VFAs) is stimulated by the VFAs, particularly butyrate, produced by ruminal microbes (Sander et al., 1959). A starter concentrate high in readily fermentable carbohydrates supports development of the microbiota and its fermentation necessary for proper ruminal tissue growth (Brownlee, 1956; Flatt et al., 1958; Williams and Frost, 1992; Greenwood et al., 1997). The functioning rumen epithelium can absorb and metabolize the VFAs, which aids in raising ruminal pH to levels suitable for fiber fermentation.

The rumen and its microbial population are immature at this stage (Anderson et al., 1987a,b), and ruminal cellulose digestibility is limited (Williams and Frost, 1992). Consequently, forages other than fresh grass that is high in sugars (Ohtani et al., 1976) are not effective in developing a functional rumen and may limit metabolizable energy (ME) intake (MEI) in young calves (Stobo et al., 1966). Calves have limited ability to use forages until well after weaning (Quigley, 1996a; Davis and Drackley, 1998). Nevertheless, adequate particle size of starter feed—whether pelleted, ground, or texturized—is important to prevent abnormal development and keratinization of ruminal papillae and to prevent impaction of fine particles between papillae (McGavin and Morrill, 1976; Greenwood et al., 1997; Beharka et al., 1998).

With respect to the nutrient requirements of the calf, three phases for development of digestive function are recognized (Davis and Clark, 1981):

- Liquid-feeding phase. Essentially all of the nutrient requirements are met by milk or milk replacer (MR). The functional reticular (esophageal) groove shunts liquid feeds directly to the omasum to avoid microbial breakdown in the reticulorumen (Orskov, 1972).
- Transition phase. Liquid feed and starter contribute to meeting the nutrient requirements. Starter enables development of the reticulorumen.
- Ruminant phase. The calf derives its nutrients from solid feeds, primarily through microbial fermentation in the reticulorumen. Ruminal fermentation and microbial protein synthesis are not yet mature during the early stages of this phase.

Similar to the previous edition (NRC, 2001), this chapter discusses the nutrient requirements of calves in each of these phases, but in this edition, the committee made the following changes: (1) empty body weight (EBW) was used for all calculations; (2) an equation to estimate starter intake was added; (3) energy requirements were updated and include different maintenance requirements for different classes of animals, breeds, and environmental conditions, and estimating composition of body gain was included and efficiency of ME use for gain was updated; (4) calculation of feed ME values was revised; (5) a new metabolizable protein system (MP) was used; and (6) mineral requirement system was changed and some vitamin recommendations were revised. For purposes of estimating nutrient requirements, it is assumed that cattle less than 18 percent of mature body weight (MatBW; 125 kg for Holsteins with mature weight of 700 kg) are calves and those that are >18 percent are heifers. Growth requirements for the latter are discussed in Chapter 11.

BODY WEIGHT CONVERSIONS

All calculations in this edition were made on an EBW basis unless otherwise noted. Factors for converting body weight (BW) into EBW for various calves and diets are in Table 10-1 and range from 0.85 to 0.96 (Diaz et al., 2001; Tikofsky et al., 2001; Blome et al., 2003; Meyer, 2005; Bartlett et al., 2006; Hill et al., 2008a; Mills et al., 2010; Stamey Lanier et al., 2021). Mathematically, if EBW as a proportion of BW is unchanged, then EBW gain (EBG) should be the same proportion to BW gain as the ratio of EBW/BW. However, because the ratio of EBW to BW decreases as the rumen develops, the ratio of EBG to average daily gain (ADG) will be less than the ratio of EBW to BW. Factors for converting ADG into EBG are in Table 10-1 (Meyer, 2005; Hill et al., 2008a; Stamey Lanier, 2021). For heavy veal calves (>125 kg), EBW was calculated as 0.93 BW based on data from Gerrits et al. (1996). The decrease in proportion of BW as EBW likely occurs because of the rapid change in solid feed intake and expansion of rumen and gut size. At any point during this changeover to solid feed, the change in EBW is less than the change in BW. Consequently, during weaning transition to solid food only, EBW/BW will be somewhere between 0.93 and 0.85, and as a result, EBG will be in the range of 0.5 to 0.6 ADG. The time course and implications in these changes in body composition during the weaning transition deserve further research.

DRY MATTER INTAKE

Historically, most dairy calves have not been fed milk or MR for ad libitum intake. Maximal dry matter intake (DMI) from milk or MR is about 2.25 percent of BW, with calves achieving DMI of 2 percent of BW during the first week of life (Jasper and Weary, 2002). Near ad libitum intake of MR dry matter (DM) increased to approximately 2.5 percent of BW for calves >65 kg (Diaz et al., 2001). For veal calves, voluntary intake of MR DM declines to less than 2 percent of BW by 120 kg BW (Gerrits et al., 1996).

For most herd replacements, calves are offered a fixed and limited quantity of milk or MR DM daily with ad libitum access to starter and, perhaps, limited amounts of forage. Total DMI during this period will increase as starter intake increases. In an analysis of 219 treatment means from 64 published studies (see later section on model evaluation), mean DMI for calves (<8 weeks old, BW = 54.5 ± 8.8 kg, mean ± SD) was 1.93 ± 0.33 percent of BW, with a range of 1.17 to 3.06 percent of BW. During the weaning process, DMI increases rapidly. From the same data summary (79 treatment means from 27 studies), intake of DM from solid feeds for weaned calves (>8 weeks old, BW = 95.6 ± 19.0 kg, mean ± SD) averaged 3.06 ± 0.31 percent of BW, with a range of 2.16 to 4.45 percent of BW.

Many factors affect starter intake in young calves, the most important of which are milk or MR DMI and age (Kertz et al., 1979). Other factors include initial BW, water availability, starter quality and physical form, and quality of MR (summarized by Davis and Drackley, 1998). Starter intake is highly variable, with coefficients of variation among published studies of 90 percent and 19 to 38 percent among similar studies conducted at the same location (Kertz et al., 1979). Much of this variability may be from individual calf differences in how quickly rumen pH is stabilized as solid feed begins to be consumed (Williams and Frost, 1992).

The committee developed models to predict starter intake for use with this edition. BW, daily MEI from the liquid diet (MEiLD), ADG, and time relative to first offer of starter (FPstarter) were used as independent variables. For calves in temperate climates, a model was developed using individual animal data (n = 26,952 observations from 1,356 calves) from 28 studies carried out in 4 U.S. states and the Netherlands (Georgia, n = 168; Illinois, n = 1,925; Minnesota, n = 6,052; Ohio, n = 16,457; and the Netherlands, n = 2,350). An external data set (n = 8,891 individual observations, nine studies) was developed to evaluate the models using data from four U.S. states (Iowa, n = 6,332; New Hampshire, n = 1,519; New York, n = 892; Virginia, n = 148). Intake of milk or MR ranged from 0.11 to 1.99 kg/d and starter intake from 0.00 to 2.85 kg/d. For calves in subtropical environments, equations to predict starter intake were developed using individual animal data (n = 3,491 observations from 853 calves) from 15 studies carried out in the United States and Brazil (Florida, n = 1,127; Georgia, n = 179; Brazil, n = 2,185). An independent data set (n = 479 individual observations, five studies) was used to evaluate the models using data from the United States and Brazil (Georgia, n = 96; Brazil, n = 383). Thus, 25 percent of studies (5 of 20 studies) were used for model evaluation. Intake of milk or MR DM ranged from 0.21 to 2.07 kg/d and starter intake from 0.00 to 1.93 kg/d.

For each environmental condition, two model-fitting approaches were used. First, a set of linear mixed models was developed using an automated model selection approach ("MuMIn" and "lme4" packages) and parallel computation in R (version 4.0.1). The linear mixed models included the random effect of study. Interactions and squared terms were tested. Then, the linear mixed models with the lowest Akaike information criterion (AIC) values were selected and evaluated. Second, for nonlinear models (exponential and logistic), the initial parameters were fitted using the "nls" function (nonlinear least squares) and plots from the "easynls" package. Then, the initial parameters were used to fit nonlinear mixed-effects regression using the "nlme" package in R. The random effect of study was added to the A coefficient. Nonlinear models with or without the random effect of study were evaluated, and adjustments with different fixed effects were tested. Finally, the best models from the external validation were selected based on small AIC values from derivation, high concordance correlation coefficient (CCC), minimal slope and mean biases, and low root mean squared prediction error (RMSE). The best models from

the external evaluation were reevaluated using a repeated K-fold cross-validation (n = 500, k = 10) using both development and external databases to have more information about model performance.

The equation selected for calves in temperate conditions is as follows:

$$\text{Starter DMI (g/d)} = -652.525 + (\text{BW} \times 14.734) \\ + (\text{MeiLD} \times 18.896) + (\text{FPstarter} \times 73.303) \\ + (\text{FPstarter}^2 \times 13.496) - (29.614 \times \text{FPstarter} \times \text{MEiLD})$$

(Equation 10-1)

where BW is in kg, MEiLD is in Mcal/d, and FPstarter is in weeks. In the model, age is used as a proxy for week when starter is first offered, assuming starter was offered during first week of life. This model had an RMSE of 262 g/d, with a CCC of 0.71. The average R^2 using the development database in repeated K-fold cross-validation was 0.66 ± 0.01 and from the evaluation database was 0.67 ± 0.02.

The equation for calves in semitropical conditions is as follows:

$$\text{Starter DMI (g/d)} = 600.053 \times (1 + 14863.651 \\ \times (\exp(-1.553 \times \text{FPstarter})))^{-1} + (9.951 \times \text{BW}) \\ - (130.434 \times \text{MEiLD})$$

(Equation 10-2)

This model had an RMSE of 222 g/d and a CCC of 0.78. When users enter an environmental temperature >35°C, Equation 10-2 is used. Otherwise Equation 10-1 is used.

ENERGY REQUIREMENTS OF CALVES

Energy Units and Classes of Calves

The current revision expresses energy requirements and dietary energy supply for calves in terms of ME, although derivation of these requirements is based in part on determination of net energy (NE) values. Data on energy requirements were derived for classes of calves fed only milk or MR, calves fed milk or MR plus starter feed (without or with forage), and weaned calves to 125 kg of BW fed starter or grower diets and limited forage. Requirements for veal calves fed only milk or MR also were evaluated.

Description of the Database

The previous edition (NRC, 2001) adopted the summary equation of Toullec (1989), which was derived from a number of studies with veal calves, for prediction of ME requirements of all classes of calves. Because this equation was derived from older studies with heavier calves of greater relative maturity and with the goal of fattening, the previous committee recognized the need for data on the composition of live weight gain for dairy calves of current genetics. Since that publication, a number of studies have provided data on

body composition and composition of EBG of Holstein (Diaz et al., 2001; Tikofsky et al., 2001; Blome et al., 2003; Brown et al., 2005b; Meyer, 2005; Bartlett et al., 2006; Hill et al., 2008a; Mills et al., 2010; Stamey Lanier et al., 2021), Jersey (Bascom et al., 2007), and Holstein × Gyr crossbred (Silva et al., 2017) calves. The present committee derived equations for energy and protein requirements from a subset of these body composition studies (Diaz et al., 2001; Tikofsky et al., 2001; Meyer, 2005; Bartlett et al., 2006; Bascom et al., 2007; Mills et al., 2010; Stamey Lanier et al., 2021).

Individual data were available for 255 calves from seven comparative slaughter studies with appropriate baseline groups to measure changes in body composition as calves grew. Six of the studies used Holstein calves (Diaz et al., 2001; Tikofsky et al., 2001; Meyer, 2005; Bartlett et al., 2006; Mills et al., 2010; Stamey Lanier et al., 2021) and one used Jersey calves (Bascom et al., 2007). In two of the studies, calves were fed both MR and solid feeds (Meyer, 2005; Stamey Lanier et al., 2021), while calves in the remaining studies were fed only milk or MR. Data were combined into a common database for analysis.

Maintenance Energy Requirements

Conceptually, maintenance is a simple idea, but its determination is fraught with difficulties, including whether to define it as the point of zero weight change or zero change in body energy. Methodological considerations also complicate measurement of maintenance, whether by calorimetric or comparative slaughter procedures. In previous NRC systems, net energy for maintenance (NEm) was defined as heat production (HP) at zero MEI, by extrapolation of the regression of HP on MEI to the y-intercept, plus an activity allowance. Labussiere et al. (2009, 2011) proposed a method to calculate maintenance that considered increases in apparent maintenance HP with increasing MEI prior to determination of fasting heat production. Others (ARC, 1980; Moe, 1981) have argued against the use of measured fasting HP as a baseline for maintenance. Regardless of methodology, reliable estimates of maintenance are necessary for defining energy use by growing calves.

The second component of maintenance has been an allowance for activity, typically 10 percent of NEm. Thus, the NEm value adopted by NRC (2001) was 0.086 Mcal/kg $\text{BW}^{0.75}$, which was similar to calorimetric data from the USDA Beltsville station, where activity was embedded in the total estimate of NEm. Others have argued that the activity allowance is too high for growing cattle, which would inflate estimates of maintenance (Ainslie et al., 1992; Van Amburgh et al., 1998). Labussiere et al. (2008b, 2009, 2011) measured the energy cost of standing activity in veal calves by calorimetry and found that it accounted for about 3.5 to 8 percent of MEI, supporting the idea that a maintenance activity allowance of 10 percent is too large. The NEm value determined by extrapolation of regression equations to zero

MEI in comparative slaughter experiments would include the components of basal metabolism and activity and thus may be more reliable.

Maintenance energy requirements historically have been expressed relative to $BW^{0.75}$, but this relationship has not been evaluated experimentally in dairy calves until recently (Labussière et al., 2008b). Those researchers found that expressing BW raised to a power of 0.85 best fit the data and minimized variation for fasting heat production measured by calorimetry in Holstein veal calves over a range of BW from 73 to 221 kg. Furthermore, use of the coefficient 0.75 resulted in significantly different estimates compared with the exponents 0.80, 0.85, or 0.90. However, use of 0.85 as the exponent to calculate metabolic BW in the current database increased variation in estimates of energy relationships, and thus the exponent 0.75 continues to be used in this revision.

Heat production was calculated as the difference between MEI and retained energy (RE), where RE was calculated from the increase in body energy content between baseline calves slaughtered at the start of the feeding period and calves at the end of the feeding period (Lofgreen and Garrett, 1968). The NEm was calculated as the intercept (a) of the equation of exponential regression ($NEm = a \times e^{b \times MEI}$) when $MEI = 0$, as described by Ferrell and Jenkins (1998a,b). In this equation, NEm = heat production in units of $Mcal/EBW^{0.75}$ per day, MEI = metabolizable energy intake ($Mcal/EBW^{0.75}$ per day), and a and b are equation parameters. Regressions explored data from all calves, Holstein calves fed milk or MR only, Holstein calves fed milk or MR plus solid feed, and Jersey calves fed MR or milk only. Study was included as a random effect in a nonlinear mixed model using R software. Treatments that were known to be protein deficient in Bartlett et al. (2006) were removed as were data for two extreme outliers, leaving 235 observations for the analysis. From multiple regression analyses, the committee concluded that separate values for maintenance could not be justified among those groups. Therefore, for small or large breed calves fed milk or MR without or with starter, the final nonlinear equation was as follows (see Figure 10-1a):

$$HP, Mcal/EBW^{0.75} = 0.077 \times e^{(3.3426 \times MEI, Mcal/EBW^{0.75})}$$
$$(RMSE = 0.011, CCC = 0.950)$$

(Equation 10-3)

Analysis of regressions and residuals indicated no mean or slope bias (see Figure 10-1b). The NEm derived from the intercept ($0.077\ Mcal/EBW^{0.75}$) is considerably lower than the value established by previous NRC committees ($0.086\ Mcal/BW^{0.75}$; NRC 1989, 2001) but is in the range of estimates by others (ARC, 1980; NRC, 1978; Silva et al., 2017) and the value used by the beef NASEM (2016).

By the iterative method, the ME requirement for maintenance (MEm, $Mcal/EBW^{0.75}$ per day) was determined as the point where MEI and heat production are equal

(i.e., the point at which there is no energy retention in the body) (Lofgreen and Garrett, 1968). MEm was determined to be $0.107\ Mcal/kg\ EBW^{0.75}$, or $0.101\ Mcal/kg\ BW^{0.75}$, which is similar to the value used by NRC (2001) as well as others (ARC, 1980; Labussiere et al., 2007). The efficiency of use of ME for maintenance (k_m) was calculated as the ratio between the NE and MEm. The resulting value (0.719) also is lower than the value (0.86) used previously by NRC (2001) but is similar to the efficiency values of 0.726 and 0.706 from INRA (2019) and Silva et al. (2017), respectively.

Based on the studies in the database with calves fed both starter and liquid feed (Meyer, 2005; Stamey Lanier et al., 2021), their maintenance energy requirements were about 2 percent higher than calves fed milk only, but these were not statistically different. Greater requirements as calves consume solid feeds might be predicted, as the size of metabolically active organs, such as the gastrointestinal tract and liver, increases along with rumen development.

The NEm for weaned heifers up to 125 kg BW was set to $0.097\ Mcal/kg\ EBW^{0.75}$ ($0.0825\ Mcal/kg\ BW^{0.75}$), based on estimates from Stamey Lanier (2021) and Meyer (2005). This value is intermediate between the preweaned and growing heifer values (see Chapter 11). The corresponding MEm values are $0.138\ Mcal\ ME/kg\ EBW^{0.75}$ or $0.117\ Mcal\ ME/kg\ BW^{0.75}$. These values are higher than NRC (2001) but lower than those in other systems (ARC, 1980; INRA, 2019). The National Academies Beef Model (NASEM, 2016) set maintenance NEm for growing dairy breed calves to $0.095\ Mcal/kg\ BW^{0.75}$, or $0.112\ Mcal/kg\ EBW^{0.75}$. Assuming an efficiency of ME use for maintenance of about 0.70 implicit in the beef guidelines (NASEM, 2016), these equate to $0.137\ Mcal\ ME/kg\ BW^{0.75}$ and $0.160\ Mcal\ ME/kg\ EBW^{0.75}$. Because of the paucity of body composition studies with weaned calves in this weight range, additional data are needed to more accurately model requirements for maintenance.

Based on the very limited data available for estimation of energy requirements for milk-fed Jersey calves (Bascom et al., 2007), the determined coefficient for NEm was about 6.6 percent greater and the coefficient for MEm about 15.1 percent greater than that for Holstein calves fed milk (not statistically significant). The comparative slaughter data from Bascom et al. (2007) are directionally consistent with observations by others that maintenance for Jersey calves may be up to 20 percent greater than Holsteins (Ballou and DePeters, 2008; Ballou, 2012; Van Amburgh et al., 2019), even when corrected by use of metabolic BW. The discrepancy may be attributable to the ratios of surface area to body mass. Brody (1945) found that surface area in Holstein cattle from 41 to 617 kg BW was described by the equation $0.14 \times BW^{0.57}$. Consequently, surface area to mass ratios are greater in calves that are smaller than the average Holstein calf, so rate of heat loss would increase more than predicted by $BW^{0.75}$ alone. In turn, the metabolic rate and hence maintenance HP would be greater in smaller Jersey calves. However, in the absence of
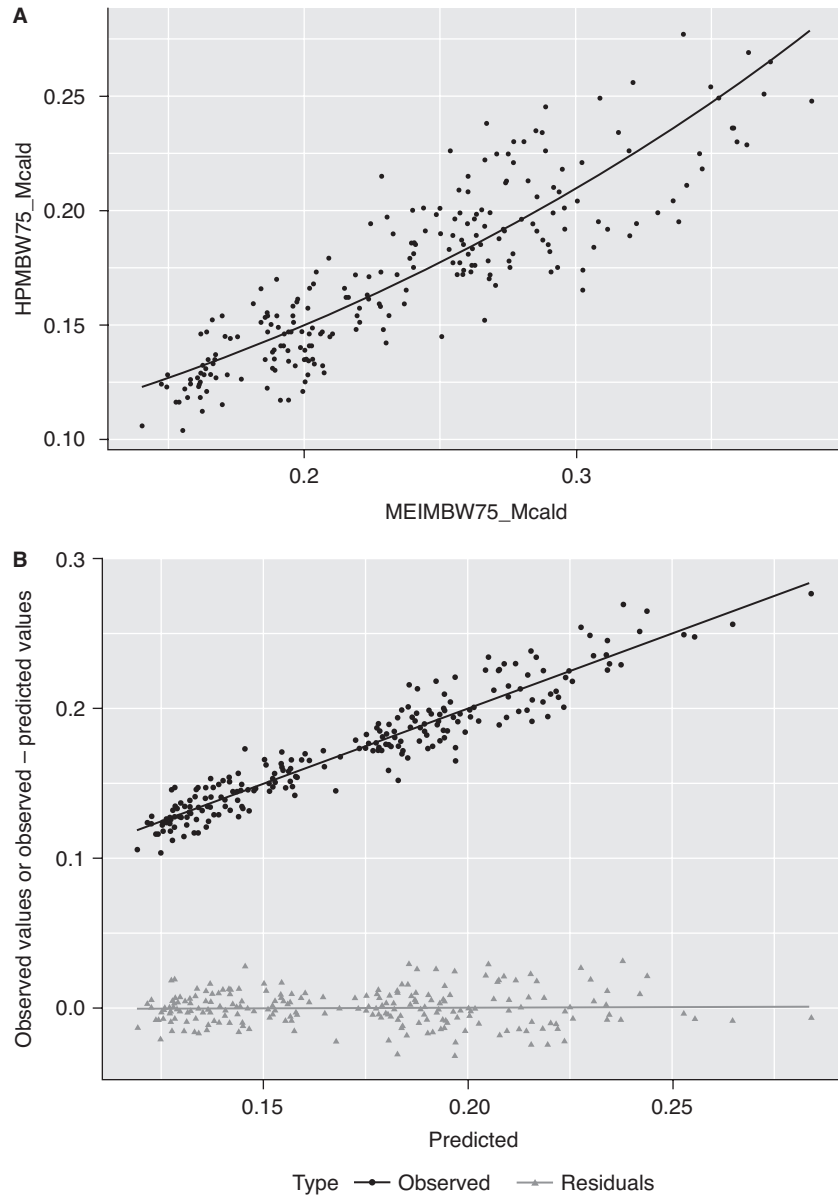
FIGURE 10-1   Relationship between daily HP and MEI (both as Mcal/kg BW$^{0.75}$) and plots of observed − predicted versus predicted and residuals.

TABLE 10-1   Ratios of EBW to BW and Energy Requirements for Maintenance[a]

Item	Milk or Milk Replacer Only		Milk or Milk Replacer Plus Solid Feed		Weaned, Solid Feeds Only	
	Holstein/ Large Breed	Jersey/ Small Breed	Holstein/ Large Breed	Jersey/ Small Breed	Holstein/ Large Breed	Jersey/ Small Breed
EBW/BW	0.94	0.94	0.93	0.93	0.85	0.85
EBG/ADG	0.94	0.94	0.91	0.91	0.85	0.85
NEm, kcal/kg EBW$^{0.75}$	76.9	76.9	76.9	76.9	97.0	97.0
NEm, kcal/kg BW$^{0.75}$	72.3	72.3	72.3	72.3	82.5	82.5
MEm, kcal/kg EBW$^{0.75}$	107.0	107.0	113.8	113.8	Var[b]	Var[b]
MEm, kcal/kg BW$^{0.75}$	100.0	100.0	105.8	105.8	Var[b]	Var[b]
Efficiency of ME use for NEm, k_m	0.72	0.72	0.69	0.69	Var[b]	Var[b]

[a] Expressed as a function of metabolic EBW or live BW and efficiency of ME use for NEm (k_m).
[b] Determined as efficiency of solid feed use (as determined from ME density by Equation 10-4).

sufficient data, the committee left the values the same as for large-breed calves. Additional research is needed to better define the requirements of small-breed calves.

The efficiency of use of ME from solid feeds is calculated from the ratio of NEm to ME using the equation from Galyean et al. (2016), as follows:

$$NEm, Mcal/kg \ DM = (1.1104 \times ME) - (0.0946 \times ME^2) + (0.0065 \times ME^3) - 0.7783$$

(Equation 10-4)

where ME is expressed in terms of Mcal/kg DM. Over the range of ME values encountered for starters (i.e., 2.5 to 3.5 Mcal/kg DM), efficiencies would vary only from 0.60 to 0.64. The overall dietary efficiency of ME use is calculated as the weighted mean of ME provided by liquid and solid feeds as in the previous edition (NRC, 2001).

Effects of Environment on Maintenance Requirements

Calves are born with limited body energy reserves and only modest insulation afforded by hair coat and body fat. Without feed, a newborn calf probably has enough body energy stores in the form of fat and glycogen to last no more than about 1 day in temperatures below its lower critical temperature (Alexander et al., 1975; Okamoto et al., 1986; Rowan, 1992).

Energy standards are based on the premise that the animal is in a thermoneutral environment. In such an environment, the animal is not required to elicit specific heat-conserving or heat-dissipating mechanisms to maintain core body temperature (NRC, 1981). The thermoneutral zone shifts depending on many factors, the more important being age, amount of feed intake, amount of subcutaneous fat, and length and thickness of hair coat. The thermoneutral zone in very young calves ranges from 15°C to 25°C (NRC, 2001). Thus, when the environmental temperature drops below 15°C, which is referred to as the lower critical temperature, the calf must expend energy to maintain body temperature. In practical terms, the maintenance energy requirement is increased. For older calves and calves at greater feed intakes, cold tolerance is greater and the lower critical temperature may be as low as –5°C to –10°C (Webster et al., 1978). Data in Table 10-2 illustrate the effects of a decrease in environmental temperature above the upper critical temperature or below the lower critical temperature on energy requirements for maintenance. For cold stress, the values were calculated from research data of Schrama et al. (1992a). In the example given in Table 10-2, if the lower critical temperature is 15°C and the effective ambient temperature is 0°C, the maintenance energy requirement for calves <3 weeks old is increased by 38 percent. Scibilia et al. (1987) reported that maintenance ME requirement was increased by 32 percent for calves housed at –4°C compared with calves housed at 10°C. Table 10-2 suggests that calves at this temperature (–5°C) would have a 30 percent greater maintenance requirement. These estimates agree at least qualitatively with other reports

TABLE 10-2 Effect of Environmental Temperature on Energy Requirement of Young Calves[a]

Environmental Temperature		Increase in Maintenance Energy Requirement (kcal of NEm/d)		Maintenance Energy Requirement (kcal of ME/d)		Increase in ME Required for Maintenance (%)	
°F	°C	Birth to 3 Weeks of Age[b]	>3 Weeks of Age[c]	Birth to 3 Weeks of Age[b]	>3 Weeks of Age[c]	Birth to 3 Weeks of Age[b]	>3 Weeks of Age[c]
113	45	698	698	2,557	2,675	38	40
104	40	524	524	2,383	2,501	28	30
95	35	349	349	2,208	2,326	19	20
86	30	175	175	2,034	2,152	9	10
77	25	0	0	1,859	1,977	0	0
68	20	0	0	1,859	1,977	0	0
59	15	175	0	2,034	1,977	9	0
50	10	349	0	2,208	1,977	19	0
41	5	524	175	2,383	2,152	28	9
32	0	698	349	2,557	2,326	38	18
23	–5	873	524	2,732	2,501	47	26
14	–10	1,048	698	2,907	2,675	56	35
5	–15	1,222	873	3,081	2,850	66	44
–4	–20	1,397	1,048	3,256	3,025	75	53
–13	–25	1,572	1,222	3,431	3,199	85	62
–22	–30	1,746	1,397	3,605	3,374	94	71

[a] Calculated for a calf with EBW of 45 kg. Extra heat production = 2.01 kcal/kg$^{0.75}$ per day for each degree decrease in environmental temperature (°C) below lower critical temperature (Schrama et al., 1992a) or for each degree increase above upper critical temperature. Heat production is in terms of net energy (NE), but metabolizable energy (ME) is assumed to be used with 100 percent efficiency for HP.

[b] Maintenance energy requirement 107 kcal/kg$^{0.75}$ EBW per day. Calves from birth to 3 weeks of age have lower critical temperature in the range of 15°C to 25°C. Data above were calculated on the basis of a lower critical temperature of 15°C and an upper critical temperature of 25°C.

[c] Maintenance energy requirement 113.8 kcal/kg$^{0.75}$ EBW per day. Data for calves older than 3 weeks of age were calculated on the basis of a lower critical temperature of 5°C and an upper critical temperature of 25°C.

(Soberon and Van Amburgh, 2012). Effects of cold stress in increasing maintenance requirements have been incorporated into the model provided with this publication as 2.01 kcal/$kg^{0.75}$ per day for each degree decrease in environmental temperature (°C) below the lower critical temperature (Schrama et al., 1992a).

Calves, especially very young calves, must be fed extra energy during cold weather to satisfy the increase in maintenance energy requirements. That can be accomplished by increasing the amount of liquid diet being fed, by adding an additional feeding daily, by adding additional milk solids to the liquid diet (Schingoethe et al., 1986), by incorporating additional fat into the liquid diet (Scibilia et al., 1987; Jaster et al., 1990), or by addition of fat to calf starter (Johnson et al., 1956; Araujo et al., 2014; Hill et al., 2015; Ghasemi et al., 2017; Berends et al., 2018; Doolatabad et al., 2020; Ghorbani et al., 2020). Additional fat in MR or starter decreased starter intake in one study (Kuehn et al., 1994), which negated at least a portion of the increased energy density from fat supplementation. However, in more recent studies in which milk or MR was fed in larger amounts, fat in starter did not decrease starter intake so that energy intake was increased (Araujo et al., 2014; Hill et al., 2015; Berends et al., 2018; Doolatabad et al., 2020; Ghorbani et al., 2020). If additional solids are fed, the DM concentration of MR should not exceed 20 percent to avoid problems with excessive mineral intake (Jenny et al., 1978; Ternouth et al., 1985) that can lead to osmotic imbalances in the gut, and supplemental water should always be provided. The availability of free water is critically important to starter intake (Kertz et al., 1984); provision of warm water two to three times daily during cold weather may help to stimulate starter feed intake, which would help to counteract cold stress.

Heat stress also increases maintenance energy requirements for panting and heat dissipation. Unlike cold stress, however, heat stress decreases DMI in cattle (West, 2003). Little research has been conducted to quantify the increased requirements resulting from heat stress in calves (Roland et al., 2016). Spain and Spiers (1996) found that calves began to pant at 26°C, which is similar to older cattle (Spain and Spiers, 1996). The committee adopted this temperature (26°C) as the upper critical temperature for calves. At environmental temperatures above this, increasing amounts of ME are used to cool the calf, thereby increasing maintenance ME requirements. In the absence of data, the model assumes that the increase of maintenance requirement per degree of temperature above the upper critical temperature occurs in the same proportion as the response to cold temperatures. Empirically, linearizing the qualitative recommendations for heat stress in older heifers in the previous version (NRC, 2001) approximates the approach adopted. Conditions such as proper heat abatement and nighttime cooling would reduce the effect of heat stress on maintenance requirement. Calves will not eat more to meet the greater maintenance requirement for heat dissipation; rather, the calf will decrease voluntary intake,

particularly for starter, in response to heat stress (Roland et al., 2016). The result is that growth may decrease during periods of heat stress. In addition, heat stress is detrimental to the immune system, thereby increasing potential for morbidity, which further increases maintenance requirements due to activity of the immune system and decreases growth (Bagath et al., 2019).

Energy Requirements for Growth

Retained Energy and Empty Body Weight Gain

The current system for calf growth is based on ME-allowable growth and MP-allowable growth. Establishment of the energy requirements for growth relies on accurate estimation of the amount of RE per unit of growing tissue, as determined by relative amounts of fat and protein deposited. The amount of protein deposited per unit of BW gain generally is quite invariable if MP is sufficient, whereas tissue fat deposition is variable depending on total MEI or limitation of growth by MP supply (Van Amburgh et al., 2019).

The database of individual calves from comparative slaughter studies described above was used to derive equations to predict RE from EBW and EBG. Three equation forms were evaluated using R software to relate RE to EBW and EBG. Each equation included the random effect of study. The first form was that used by NRC (2001): RE (Mcal/d) $= a \times (EBG^b$, kg/d$) \times (EBW^c$, kg), where a, b, and c are equation parameters. The second form was the same but $EBW^{0.75}$ was used rather than a model-derived exponent. Finally, the committee used the previous NRC equation form but without the a coefficient.

The first form had an unacceptably high (>964) variance inflation function as a result of the substantial correlations between the a and c parameters, which made this model highly unstable to changes in inputs, and it was removed from further consideration. Of the other two forms, the form without the a coefficient had a lower RMSE and less mean bias and slope bias and was selected to estimate RE:

$$RE = (EBG^{1.100}, kg/d) \times (EBW, kg^{0.205})$$
$$\text{(Equation 10-5)}$$

Mean bias (0.004) and slope bias (−0.012) were not significantly different from zero (RMSE = 0.186, CCC = 0.966). Validation of the model against literature values is presented in a later section.

Use of Metabolizable Energy for Retained Energy

To predict RE and growth from a quantity of ME available to the animal, the efficiency of ME use for RE must be known. The reported efficiency of ME use for RE varies from approximately 0.40 to more than 0.70, largely due to the age of the calf and whether it is accreting protein rapidly with

minimal fat deposition or is actively fattening, as well as the dietary fat content relative to total ME and protein. Protein deposition is energetically expensive because of the adenosine triphosphate (ATP) costs for peptide bond formation but also because of concurrent protein turnover. In contrast, fat deposition is energetically much more efficient. Van den Borne et al. (2007) demonstrated that body fat deposition does not originate from dietary carbohydrate in milk-fed calves but rather from dietary fat. This finding in preruminant calves means that they are similar to adult cattle since ruminants use little glucose carbon to form the carbon chains of fatty acids (FAs). Instead, glucose arising from lactose digestion is largely used to fuel protein synthesis (Roy et al., 1970; Donnelly, 1983; Tikofsky et al., 2001). The committee concluded that insufficient data were available to model the effects of the interrelationships of dietary fat content, total MEI, and dietary MP intake on the efficiency of ME use for RE because the resulting partitioning of RE into fat or lean tissue growth was not always predictable.

Because HP was modeled with a curvilinear relationship to MEI, by default, the relationship between RE and ME must also be curvilinear. However, the difference in RE/ME across the range of MEI encountered in practice is small, and this relationship is usually approximated as linear. Therefore, the use of ME for RE was calculated by regressing RE on MEI using Proc Mixed in SAS (v 9.4): $RE = a + b \times MEI$, where RE and MEI are in Mcal/EBW$^{0.75}$ per day, and a and b are equation parameters. Study was included as a random effect. The efficiency of use of the ME for weight gain (k_g) was assumed to be the slope coefficient (b) of the regression of the RE as a function of MEI, according to Ferrell and Jenkins (1998b). The resulting efficiency for use of milk or MR ME was 0.46, which is much lower than the NRC (2001) value (0.69) and on the low end of previous literature estimates, most from heavier calves in veal-type settings. The equation from Toullec (1989) used by NRC (2001) was derived from veal calves at heavier BW where fat deposition would be greater, in contrast to the young and rapidly growing, leaner calves making up the current database. Labussiere et al. (2007) calculated an average efficiency of 0.64 from 12 previous studies that measured RE by comparative slaughter or calorimetry. In an extensive analysis of growth data, INRA (2019) determined that the efficiency of use of ME for RE was 0.55. Van Amburgh et al. (2019) adopted the value of 0.55 in an analysis of growth data. The current committee adopted the value of 0.55 for k_g, which is also the average of the current database value and the summary of previous literature estimates (Labussiere et al., 2007).

The efficiency of ME use from starter is less than for milk components. Multiple regression analysis indicated that although a common intercept could be used for calves fed milk only or milk plus starter, the interaction of diet type and MEI was significant, indicating that efficiency of ME use for RE was different between milk only and milk plus starter. The committee adopted the modified equations from Galyean et al. (2016) to calculate RE/ME as the ratio of NE (i.e., RE) to dietary ME. The NEg was calculated as follows:

$$NEg, Mcal/kg\ DM = (1.1376 \times ME) - (0.1198 \times ME^2) + (0.0076 \times ME^3) - 1.2979$$
$$\text{(Equation 10-6)}$$

where ME is in Mcal/kg DM. Over the range of starter ME concentrations encountered in practice (i.e., 2.5 to 3.5 Mcal/kg), RE/ME varies from 0.378 to 0.441. These efficiencies also are lower than the 0.69 used by NRC (2001) but are consistent with calves depositing a greater proportion of RE as protein at this growth stage (INRA, 2019). Silva et al. (2017) reported results of a comparative slaughter study with Holstein or Holstein × Gyr crossbred calves in Brazil that were fed whole milk without or with starter. They found that the apparent RE/ME was 0.574 for calves fed whole milk and 0.516 for calves fed whole milk plus starter. Silva et al. (2017) calculated that the k_g for starter alone would be 0.393, which is in the range of efficiencies predicted by the present model.

Equations to predict proportions of fat and protein in EBW gain were derived from the six Holstein studies in the data set according to NASEM (2016) methodology with study as a random effect:

$$\text{Proportion of fat in EBG} = 0.0786 + 0.0370 \times RE, Mcal/d$$
$$\text{(Equation 10-7)}$$

$$\text{Proportion of protein in EBG} = 0.1910 - 0.0071 \times RE, Mcal/d$$
$$\text{(Equation 10-8)}$$

At the mean RE for the data set (1.456 Mcal/d), predicted proportions of fat and protein in EBG are 0.132 and 0.181.

EFFECTS OF SOURCES OF ENERGY

Energy requirements are calculated as ME regardless of whether the source of ME is fat, carbohydrate, or protein. Nevertheless, source of energy may alter partitioning of nutrients, growth, and health.

Fats and Fatty Acids

Compared to adult dairy cows, surprisingly little published research has addressed digestibility of different fatty acid (FA) sources in MR, despite fat supplying 20 to >40 percent of total energy in MR. Typically, milk fat has been replaced with tallow, lard, and coconut oil, but restrictions on animal fat usage in animal feeds in many countries have shifted use to various blends of palm oil, rapeseed oil, and other hydrogenated vegetable oils. Huuskonen et al. (2005) replaced lard in MR with blends of 75 percent palm, 20 percent coconut, and 5 percent rapeseed oils or 75 percent palm and 25 percent coconut oils. Fat digestibility and calf gains were similar among all three diets. Overall, the paucity of data on intestinal

digestibility of different FAs in young calves precludes efforts to model it. Given the importance of FAs to the ME of calf diets, continued research into FA digestibility and effects of dietary FA profile is warranted.

More studies have determined production and immune responses to altered dietary FA profiles in MR or starter. Bowen Yoho et al. (2013) compared pasteurized Jersey milk and MR containing fat that was 100:0, 80:20, or 60:40 lard/coconut oil fed to Jersey calves, and the MR with 20 percent coconut oil increased measures of stature but ADG did not differ. MR fortified with increased concentrations of butyrate (C4:0), medium-chain FAs (C8:0, C10:0, C12:0, C14:0), and essential polyunsaturated FAs (PUFAs; C18:3) increased ADG and improved gain/feed in calves less than 2 months of age (Hill et al., 2007a, 2009a). Subsequent studies confirmed these effects and showed that the FA blend enhanced some aspects of immune response when added to MR (Hill et al., 2011c; Esselburn et al., 2013). Given the complexity of the blend that was added, the specific FA or acids responsible for the enhanced growth and immune responses could not be determined. Ballou et al. (2008) and Ballou and DePeters (2008) fed Jersey calves MR in which 5 or 10 percent of the lipid was replaced with fish oil. Fish oil attenuated the acute phase response and modified several other indicators of immune function in a linear response to dose but did not affect growth or health of calves. Karcher et al. (2014) fed MR with 17 percent fat from lard, 15 percent lard plus 2 percent flax oil, or 15 percent lard plus 2 percent fish oil. The flax oil–supplemented MR resulted in greater ADG and feed efficiency, whereas fish oil had no effect. The flax oil diet also modulated some aspects of immune function in a beneficial direction.

Fat is often included in starters, usually in amounts less than 5 percent of DM. Several studies evaluated addition of fats to calf starters (discussed in Ghasemi et al., 2017) with variable results. Hill et al. (2007c) found that the same mixture of butyrate, medium-chain FAs, and C18:3 as used in MR (Hill et al., 2007a) improved growth and efficiency when added to the starter. Hill et al. (2009a) fed starter with calcium (Ca) salts of either flax oil (rich in C18:3) or fish oil (rich in long-chain *n*-3 FAs), and the Ca salts of flax oil, but not fish oil, increased ADG and feed efficiency in a dose-dependent manner in preweaned calves; the Ca salts of flax oil increased ADG and feed efficiency in postweaned calves.

Dairy cattle have tissue requirements for dietary essential (those that cannot be synthesized de novo) PUFAs, which are linoleic (C18:2) and linoleic (C18:3) acids. Despite extensive biohydrogenation in the rumen, enough of these FAs escape from the rumen so that dairy cows are not overtly deficient (Palmquist, 2009). Nevertheless, there is interest in optimizing supply of essential PUFAs and the ratio of the omega-6 (C18:2 and its elongation–desaturation products) to omega-3 (C18:3 and its elongation–desaturation products). While no requirements for C18:2 and C18:3 have been established for young calves, comparing MR with typical milk fat can be a starting point for adequacy.

Standard milk fat contains 1 to 3 percent C18:2 and 0.5 to 2 percent C18:3 (Jensen et al., 1991), yielding typical C18:2 to C18:3 ratios of 4:1 to 6:1. Assuming a milk fat content of 3.8 percent and contents in fat of 2 percent C18:2 and 0.6 percent C18:3, a calf consuming 1 kg of milk solids daily takes in 5.1 g/d of C18:2 and 1.5 g/d of 18:3. MR containing 20 percent fat based on lard (~8.5 percent C18:2, 1.1 percent C18:3) or tallow (~4.5 percent C18:2, 0.8 percent C18:3) would supply similar amounts of these PUFAs. Based on the results summarized above, supplementation of sources of C18:3 to MR may have merit by narrowing the ratio of C18:2 to C18:3 as well as ensuring adequate daily intakes of these FAs.

Carbohydrates

The young calf lacks the digestive enzymes necessary to digest starch but has a high capacity to digest lactose. Although the capacity for lactose digestion has long been the subject of controversy (e.g., Roy, 1969), Gilbert et al. (2015) demonstrated that more than 97 percent of lactose disappears by the end of the ileum. Some of this could be attributable to fermentation (Tanan, 2005), but under most circumstances, enzymatic capacity does not limit lactose utilization in calves (Gilbert et al., 2015).

A substantial body of research has examined the ability of starch to replace some lactose in calf MR. Gilbert et al. (2015) substituted gelatinized starch, maltodextrins, branched maltodextrins, or maltose for lactose in increasing amounts. Apparent ileal disappearance was 61.6 percent and total tract disappearance over 99 percent. However, fermentation accounted for an amount equivalent to 89 percent of starch intake, with half of that fermentation occurring before the terminal ileum regardless of the starch product. Maltase activity may be limiting in vivo starch digestion (Gilbert et al., 2015). Thus, while small amounts of starch can replace lactose, much of its disappearance will be attributable to fermentation rather than enzymatic digestion (Tanan, 2005).

Other alternatives to lactose have been explored in young calves, including glucose, galactose, fructose, glycerol, and dextrins. Gilbert et al. (2016) replaced one-third of the lactose content of MR fed to male Holstein veal calves averaging 114 kg BW with glucose, fructose, or glycerol. The control MR contained 46 percent lactose. Energy and nitrogen (N) retention did not differ among treatments, although greater fecal losses were measured for fructose, and fructose was oxidized more slowly than glucose or glycerol.

Carbohydrates Versus Fats as Energy Source

The goal of early nutrition and growth may be different depending on the class of calf under consideration. For herd replacements, lean growth of frame (bone and muscle) is the primary concern, whereas for veal calves, early fattening is key. For male calves destined for feedlots, frame growth

also is important in the calf stage. High rates of lean growth depend on nonlimiting quantities of protein as a source of essential amino acids (AAs) and sufficient available energy to drive protein synthesis. Gain of BW is affected most markedly by deposition of protein, which brings with it water in a 4:1 ratio. Roy (1980) described the relationship between BW gain and protein and fat deposition in equation form as follows:

$$\text{BW gain, kg/d} = 0.175 + 3.92 \times \text{protein gain, kg/d} \\ + 0.618 \times \text{fat gain, kg/d} \quad \text{(Equation 10-9)}$$

In turn, body protein deposition responds in a linear fashion to increasing dietary protein over the range of practical feed intakes in calves (Gerrits et al., 1996; Bartlett et al., 2006). Gerrits et al. (1996) detected a plateau in body protein deposition with increasing dietary crude protein (CP) only in heavy veal calves (160–240 kg BW) at a digestible CP intake of 498 g/d when body protein deposition reached 244 g/d.

The optimal fuel to drive high rates of protein deposition has been the subject of debate. Tikofsky et al. (2001) found that increasing dietary fat intake in isonitrogenous and isocaloric diets increased fat deposition but did not change EBW gain or EB protein gain. Similar results were obtained by Roy et al. (1970) when fat was increased from 20 percent to 30 percent of the diet. Body fat does not originate from dietary carbohydrate in milk-fed calves (van den Borne et al., 2007), so if dietary protein is not limiting, extra energy from lactose will fuel more body protein deposition. In contrast, extra energy as fat may increase body fat deposition. Measurement of BW gain does not necessarily reflect all aspects of energy utilization. If the calf partitions dietary energy preferentially to body fat storage, an increase in RE might not appear as a corresponding increase in EBW gain. Overall, it is clear that carbohydrate oxidation rather than FA oxidation drives the majority of protein synthesis in young calves.

PROTEIN REQUIREMENTS

Similar to the previous edition, the model is driven on the basis of ME-allowable gain, with protein requirements calculated for maintenance and the predicted growth rate. In this edition, the committee adopted use of MP instead of apparently digestible protein (ADP) used in the previous edition (NRC, 2001).

Maintenance

Maintenance uses of MP constitute those losses that do not contribute to structural growth. Calculation of maintenance requirements was modified slightly from the previous version. An allowance for scurf (hair, skin, secretions) was added, calculated as follows:

$$\text{Scurf CP, g/d} = 0.22 \times \text{BW}^{0.60} \quad \text{(Equation 10-10)}$$

where BW is in kg (Swanson, 1977). The calculation of endogenous urinary CP loss (EUCP) was adjusted slightly from the previous edition because of incongruities associated with use of $\text{BW}^{0.75}$ according to Swanson (1977):

$$\text{EUCP, g/d} = 2.75 \times \text{BW}^{0.50} \quad \text{(Equation 10-11)}$$

Calculation of metabolic fecal CP (MFP) remained the same as the previous edition:

$$\text{MFP, g/d} = (11.9 \times \text{LFDMI, kg/d}) \\ + (20.6 \times \text{SFDMI, kg/d}) \\ \text{(Equation 10-12)}$$

where LFDMI is liquid feed DMI, and SFDMI is solid feed DMI.

Total maintenance net protein (NP) is the sum of EUCP, MFP, and scurf CP. To convert NP to MP, an assumed efficiency of 0.68 was adopted for scurf and MFP and 1.0 for EUCP, consistent with calculations for other classes of cattle (see Chapter 6).

$$\text{MPmaintenance, g/d} = \text{EUCP, g/d} + ((\text{Scurf CP, g/d} \\ + \text{MFP, g/d}) / 0.68) \quad \text{(Equation 10-13)}$$

Growth

NP for growth (NPg, g/d) as in the previous edition was calculated as the CP retained in the EBG but now is calculated as a function of the rate of gain and energy content of the gain as in the beef report (NASEM, 2016). The equation derived from the database of 255 individual calves from seven studies (Diaz et al., 2001; Tikofsky et al., 2001; Meyer, 2005; Bartlett et al., 2006; Bascom et al., 2007; Mills et al., 2010; Stamey Lanier et al., 2021) described earlier is the following:

$$\text{NPg} = (166.2 \times \text{EBW gain, kg/d}) + (6.1276 \\ \times (\text{RE, Mcal/d} / \text{EBW gain, kg/d})) \\ \text{(Equation 10-14)}$$

In NRC (2001), the amount of N in gain (G) was constant at 30 g N/kg BW gain, which was roughly the average of a range of values (Blaxter and Wood, 1951; Roy, 1970; Donnelly and Hutton, 1976b; NRC, 1978; Davis and Drackley, 1998). However, this value is not constant but should be calculated based on the rate and composition of BW gain.

Efficiency of converting MPg to NPg decreases with age of calves (Labussiere et al., 2007). Rather than use a fixed correction, the committee adopted an empirical equation that decreased the efficiency of use from 0.70 at birth (6 percent of MatBW) to 0.55 at 200 kg BW (28 percent of MatBW):

$$\text{Efficiency of MP for gain} = 0.70 - 0.532 \\ \times \text{proportion of MatBW} \\ \text{(Equation 10-15)}$$

This approach is based on literature estimates (summarized by Labussiere et al., 2007) showing that in young calves, the efficiency of MP or ADP use for BW gain was approximately 0.70, and data from heavy veal calves (Gerrits et al., 1996; Labussiere et al., 2008a) showed the efficiency was lower. Basing the adjustment on a proportion of MatBW allows use for both large-breed and small-breed calves. The equation is not intended to be used for animals greater than 200 kg and is not applied to ruminant heifers >125 kg BW (see Chapter 11).

In NRC (2001), efficiency of protein use was based on the biological value (BV) concept as used in the original calculations of ADP by Mitchell (1943). The BV of milk proteins, equated to the efficiency of N use for growth above maintenance, was assigned a value of 0.80 (Donnelly and Hutton, 1976a). The same factor was assumed to apply for efficiency of use of dietary protein for maintenance functions. This value was determined at limiting protein intakes and assumes that the diet being fed is properly balanced for all essential nutrients and that energy intake is sufficient to support protein synthesis. Protein intake must not be in excess of that required for the targeted gain allowed by energy intake. The BV decreased as protein intake was increased in the studies of Donnelly and Hutton (1976a). A value of 0.77 was used by NRC (1978). Studies by Terosky et al. (1997) found that apparent BV for MR containing 21 percent CP from skim milk protein, whey protein concentrate, or mixtures of the two ranged from 0.692 to 0.765. Estimates of true biological value (corrected for endogenous N loss and metabolic fecal N [MFN]) from that study are in excess of 0.80.

However, BV is meant to describe the relative protein quality of different sources when the protein is fed at limiting concentrations (Blaxter and Mitchell, 1948). Use of the efficiency values in the present edition more accurately reflects the utilization of AAs for growth at production intakes where energy and protein are designed to be approximately in balance.

Metabolizable Protein

Conversion of CP to MP uses a factor of 0.95 for milk or milk-derived ingredients, 0.75 for dietary proteins digested postruminally in the young calf fed both milk and starter, and a value of 0.70 for conversion of CP to MP for calves with a functioning rumen. For calves fed a combination of both milk or MR and starter, the conversion of CP to MP is an average of the efficiencies for the liquid diet (0.95) and for liquid plus starter (0.75) weighted by the amounts of protein provided from each source. The conversion of CP to MP at 0.95 for milk proteins agrees with literature data and other requirement systems (ARC, 1980) but is slightly higher than the value for conversion of dietary CP to absorbable AAs (0.91) used by NRC (1978). The value of 0.95 represents true digestibility of milk proteins in young calves. Because digestion of even high-quality milk proteins is immature during the first 2 to 3 weeks of age (Arieli et al., 1995; Terosky et al., 1997),

the value of milk proteins may be overestimated during the early liquid-feeding period and may be underestimated for older calves. The committee concluded that information was insufficient to model age-related CP digestibility in the young calf. The value of 0.75 for calves fed milk or MR plus starter is retained from NRC (2001).

Requirements for MP and CP have been established on the basis of diets containing milk proteins with high digestibility and high BV; calves might not use alternative, nonmilk proteins in MR at these high efficiencies. When using nonmilk protein sources, the AA profile should be considered, and the AA most likely to be limiting (lysine [Lys], methionine [Met], threonine [Thr]; Williams and Hewitt, 1979) should be supplemented to the levels found in milk proteins (Hill et al., 2008c). In addition, vegetable proteins may increase endogenous CP flows in the intestine that would decrease apparent fecal or intestinal digestibilities of CP (Lallès, 1993). Montagne et al. (2001) measured endogenous flows of CP at the ileum and found that nonmilk proteins increased ileal CP flow with resulting decreases in apparent digestibility relative to a skim milk–based diet. Apparent digestibilities of CP at the ileum were 0.85 with soy protein concentrate, 0.73 with soy protein isolate, and 0.81 with potato concentrate compared with 0.91 with skim milk powder. After adjustment for the increased endogenous losses, real digestibilities (i.e., after correction for both specific and nonspecific endogenous losses) of the proteins were 0.96, 0.95, 0.94, and 0.99, respectively. Such endogenous losses necessitate recalculation of the endogenous N loss to 4 to 7 g N per kilogram of DMI from MR to provide a more correct estimate of true protein digestibility. Greater MFN loss places an additional maintenance cost on energy as well, although the value for this additional energetic cost is not easily estimated. In the model, users have the option to specify that an MR contains vegetable proteins, which changes the calculation of MFP (but not energy) to 34.4 g/kg of DMI.

Rumen Microbial Protein

Developing rumen function has profound effects on the supply of nutrients to the calf. Resident bacteria ferment ingested starter and forage and produce VFAs and microbial crude protein (MCP), which becomes a source of AAs for the calf. Increasing fermentation occurs with increasing intake of fermentable carbohydrate, so that the flow of microbial N becomes a greater proportion of the total N reaching the intestine (Leibholz, 1975, 1978; Quigley et al., 1985; Lallès and Poncet, 1990; Obitsu et al., 1995). Quigley and Schwab (1988) reported a high correlation ($r = 0.92$) between calf starter intake and percentage of N as microbial N in abomasal contents of calves from 2 to 11 weeks of age, suggesting that the key driver to changing the nature of abomasal N was intake of dry feed.

A meta-analysis was conducted using studies that reported the ratio of microbial N to total N in abomasal or duodenal

TABLE 10-3 Studies Comprising the Data Used to Relate DMI to Microbial N Flow

Reference	N	Forage (%)	Age (Weeks)	DMI (kg/d)	MN[a] (%)
Lallès and Poncet, 1990	6	20	9–20	0.8–2.4	60–68
Leibholz, 1975	8	15	6–13	0.6–2.5	32–74
Leibholz, 1978	5	15	6–10	0.8–1.8	28–70
Obitsu et al., 1995	8	30–40	10–15	1.5–2.4	48–64
Quigley et al., 1985	39	0/ad libitum[b]	2–11	0.1–3.1	6–83

[a] Microbial N flow as percentage of total N flow at the abomasum or duodenum.
[b] Half of calves had ad libitum access to long grass hay.
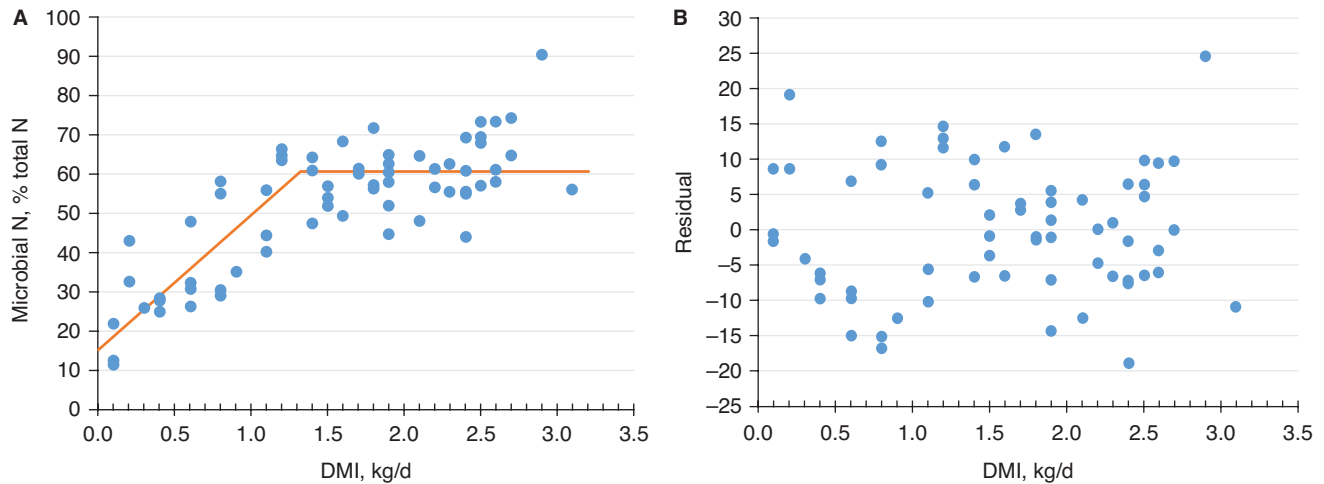
FIGURE 10-2 Broken-line regression (A) and residuals (B) of contribution of microbial N to total N to dry feed DMI in calves from 0 to 20 weeks of age. Data derived from meta-analysis of four published studies. The breakpoint occurred at 1.32 kg/d (SE = 0.118), resulting in 60.6 (SE = 1.43) percent of total N flow as microbial N. Adjusted $R^2 = 0.70$ calculated according to Robbins et al. (2006).

content of calves from 2 to 20 weeks of age (see Table 10-3). A total of 66 observations were used in the analysis using the techniques outlined by St-Pierre (2001). A subsequent broken-line regression analysis was conducted using methods by Robbins et al. (2006) to determine the DMI at which no further increase in microbial contribution as a proportion of total N occurred. Microbial N as a proportion of total N increased with increasing DMI to 1.3 kg/d; thereafter, microbial N contribution was constant, as defined by the broken-line regression (see Figure 10-2). Thus, once starter intake reaches 1.3 kg/d, the proportion of total CP reaching the intestine for digestion that is of microbial origin will be maximized.

Based on these data, conversion efficiency of CP to MP for ruminating calves consuming starter is set at 0.70 (NRC, 1978). Insufficient data were available to allow calculations of the amounts of rumen-degradable protein or rumen-undegradable protein (RUP) supplied with any degree of confidence. However, assuming that N flow to the abomasum approximates N intake, that microbial CP is 80 percent true protein that is 80 percent digestible (see Chapter 6), and that undegraded feed proteins are 0.80 digestible (NRC, 1989) leads to a conversion of CP to MP of about 0.71. The efficiency used in this edition (0.70) is slightly lower than the value of 0.75 from ARC (1980)

used in the last version of this publication (NRC, 2001). Use of the lower value provides more consistent predictions of literature values. Conversions of CP to MP for calves fed starter and milk or starter and MR are assumed to be additive based on the relative amounts of CP supplied by starter and milk (or MR).

CALCULATION OF METABOLIZABLE ENERGY VALUES OF FEEDS

Milk, MR, and ingredients used in MR use different coefficients for digestibility and a different method for calculating ME than solid feeds. When users select "calf" as the target animal, ingredients listed under "calf feeds" must be used for the liquid feeds in the diet. Starter feeds and ingredients used to make starter feeds follow the protocols set out for feeds for other classes of cattle as described in Chapters 3 and 19. Composition data are shown for the most common ingredients used in manufacture of MR in Table 10-4.

The ME values of liquid feeds are calculated similarly to the previous edition with modifications. First, the gross energy (GE) of the feed is calculated by multiplying the percentage composition on a DM basis by the respective heats of combustion, according to the following formula:

TABLE 10-4   Composition of Some Common Ingredients Used in the Manufacture of MRs

Feed or Ingredient	DM (%)	DE (Mcal/kg DM)	ME (Mcal/kg DM)	Ash (% of DM)	CP (% of DM)	CF (% of DM)	Lactose (% of DM)
Whole milk	12.5	5.59	5.37	6.3	25.4	30.8	39.2
Skim milk, fresh	10	4.19	4.02	6.9	35.5	0.3	56
Skim milk, powder	94	4.25	4.08	6.9	37.4	1.0	54
Whey, dried	93	3.80	3.65	8.1	13.5	1.0	76
Whey protein concentrate	93	4.35	4.17	6.0	37.1	2.2	54
Whey, fresh	7	3.78	3.62	8.7	14.2	0.7	76
Whey, delactosed	93	3.54	3.40	16.5	23.0	1.5	55
Whey permeate	98	3.55	3.41	9.0	3.7	0	87
Casein	91	5.29	5.08	4.0	92.7	0.7	—
Caseinate, sodium	96			2.5	85	0.5	—
Soy protein concentrate	96			7.0	67	0.3	—
Soy protein isolate	95			4.5	86	0.5	—
Soy flour	96			6.3	53	0.2	—
Modified wheat protein	96			3.0	82	2.0	—
Porcine plasma protein	97				67	0.5	—
Bovine plasma protein	97				68	0.5	—

$$GE, Mcal/kg\ DM = ((FA \times 9.4) + (Protein \times 5.65) + (100 - Protein - FA - Ash \times 4)) / 100$$

(Equation 10-16)

where values are on a DM basis. FA concentration is better than crude fat (CF) for nutritional characterization (see Chapter 4) of feeds, and it is used in Equation 10-16; however, many feed labels such as those on MR are based on CF. CF from ingredients commonly used in MRs can be converted to FA by multiplying CF by 0.945 (Paul and Southgate, 1978). Feeds can contain other organic compounds such as partially hydrolyzed starch, dextrins, glucose, or glycerol that may be incorporated in small amounts (usually less than 10 percent of DM in aggregate) into MR. This fraction is assumed to have the same heat of combustion as lactose (4 Mcal/kg). Values for whole milk are determined similarly after converting the composition to a DM basis.

Ash content normally is not listed on feed tags but generally will be 6 to 12 percent of total MR DM. Because ash has no energy, it affects the ME value and should always be determined analytically. Users are cautioned that feed tag values for MR components are given on an "as fed" or air-dry basis, which for MR is usually 95 to 97 percent DM. Failure to account for this residual moisture will introduce error into the calculation of ME.

The ME values for MR then are derived by multiplying the gross energy content by 0.91, which is the product of the average digestibility (0.95) and metabolizability of the digestible energy (DE) (0.96) for MR (Gerrits et al., 1996; Diaz et al., 2001; Blome et al., 2003; Labussiere et al., 2007, 2008a). For whole milk, the GE is multiplied by 0.93 because of the slightly higher digestibility for milk (0.97; NRC, 2001).

The DE values for solid feeds are calculated as in Chapter 3 with the exception that the digestibility coefficient for fat is assumed to be 0.81 rather than 0.74 as for older

TABLE 10-5   Summary of Studies in Which CF Digestibility Was Measured in Weaned Calves (Seven Studies, 37 Treatment Means)[a]

	Mean	Range	SD
BW, kg	98.7	63–135	24.6
Age, days	76	51–112	24.8
DMI, kg/d	2.5	1.3–4.3	0.76
Dietary fat, % of DM	4.0	2.2–5.1	0.69
Fat digestibility	0.81	0.70–0.91	0.05

[a] Sources of data: Chapman et al. (2016); Dennis et al. (2018); Hill et al. (2010, 2016b, 2016c); Hu et al. (2019); Stewart and Schingoethe (1984).

cattle. The coefficient of 0.81 for fat digestibility represents the average of studies that measured digestibilities for CF in weaned calves (see Table 10-5). The DE was calculated without discounting for intake or starch concentration (i.e., intake was set at 3.5 percent of BW and dietary starch was assumed to be 25 percent in equations). The efficiency of converting DE to ME by young calves fed MR and various starters (Pattanaik et al., 2003) varied from 0.91 to 0.95; therefore, the ME of dry feeds was set at DE×0.93. To derive accurate estimates, starter should be analyzed as described in Chapter 3.

For calves to achieve the calculated ME values, the rumen must be sufficiently developed to support near-mature rumen fermentation, both in terms of microbiota and rumen epithelial (papillae) development. In calves in which the development is not complete, digestibilities will be lower, particularly for neutral detergent fiber (NDF) (Terre et al., 2007; Hill et al., 2010; Chapman et al., 2016). This situation may be a problem when calves are fed large amounts of milk or MR early in life, which limits the intake of calf starter. If such calves are weaned too early, rumen development may be incomplete so that the ME obtained by the calf is less than estimated. While rumen

development depends on roughly 3 weeks of starter consumption, Quigley et al. (2019b) found that the cumulative intake of nonstructural carbohydrates was most highly related to digestion and achievement of predicted ME values. Because this variable will be difficult to determine on farm, the committee has incorporated a somewhat arbitrary discount of 10 percent of calculated ME of starter for preweaned calves consuming ≥1.5 percent of BW as milk or MR solids. The option to use this adjustment can be turned on or off by the user within the computer software.

VALIDATION OF MODEL WITH EXPERIMENTAL DATA

To evaluate the ability of the model to predict values from the literature, the committee assembled a database of 416 treatment means from 94 published studies that provided enough information to estimate MEIs, BW, and BW gains (see Table 10-6). Studies included some that were conducted before the previous NRC (2001) document as well as those conducted since and represented a range of milk or MR intakes, starter intakes, and ADG.

Studies included calves fed milk or MR only (21 studies), calves fed milk or MR plus starter without or with forage (64 studies), and weaned calves (23 studies). The ME densities of liquid and solid feeds were calculated according to the methods described in this chapter, including discounted ME for starter when early milk intakes were greater than 1.5 percent of BW. Mean BW was calculated as the average of BW at the beginning of the growth period and BW at the end of the period, and ADG was calculated for that period. Maintenance ME was calculated, including any requirements for thermoregulation, which was subtracted from total MEI to yield ME for gain. The MEg was multiplied by the efficiency of ME use for gain, resulting in NE (i.e., RE). Rearrangement of Equation 10-6 as shown in Equation 10-17 allowed calculation of both ME-allowable and MP-allowable EBW gain and then ADG:

$$\text{EBW gain (kg/d)} = \text{RE, Mcal/d} / (\text{EBW}^{0.205}, \text{kg})^{1/1.1}$$
(Equation 10-17)

The more limiting of ME-allowable or MP-allowable ADG was compared with the actual ADG reported in the studies. The regression of observed study ADG on model predicted ADG (pADG), with study as a random effect, resulted in the following equation:

$$\text{ADG, kg/d} = 0.073 + 0.867 \times \text{pADG, kg/d}$$
(Equation 10-18)

This model resulted in a mean predicted value of 0.679 kg/d compared with an observed mean of 0.689 kg/d, with an RMSE of 0.110 kg/d (15.9 percent of the mean). The plots of observed versus predicted values and residuals are shown

TABLE 10-6   Studies Used to Validate Prediction Models

Milk or MR Only

Blome et al., 2003	Lammers et al., 1998
Chagas et al., 2018	Marshall and Smith, 1970
Donnelly, 1983	Morrison et al., 2017
Donnelly and Hutton, 1976a,b	Quigley, 2002
Drackley et al., 2006	Quigley et al., 1997a
Gerrits et al., 1996	Quigley et al., 2002
Jenkins and Emmons, 1983	Roy et al., 1970
Johnson and Elliott, 1972a,b	Silva et al., 2017
Khouri and Pickering, 1968	Van den Borne et al., 2006
Labussierre et al., 2008a	Vasquez et al., 2017

Milk or MR Plus Starter (without or with forage)

Abdelgadir et al., 1996a	Jaster et al., 1990
Amado et al., 2019	Jaster et al., 1992
Bach et al., 2013	Kiezebrink et al., 2015
Brown et al., 2005b	Kmicikewycz et al., 2013
Byrne et al., 2017	Korst et al., 2017
Castro et al., 2016a,b,c	Lammers et al., 1998
Chapman et al., 2016	Lee et al., 2009
Chapman et al., 2017	Lesmeister and Heinrichs, 2004
Coverdale et al., 2004	Lesmeister and Heinrichs, 2005
Cowles et al., 2006	MacPherson et al., 2016
Cruywagen et al., 1996	Meale et al., 2015
Curtis et al., 2018	Moallem et al., 2010
Davis-Rincker et al., 2011	Morrison et al., 2009
Dennis et al., 2018	Osorio et al., 2012
Eckert et al., 2015	Porter et al., 2007
Frieten et al., 2017	Quigley, 1996b
Geiger et al., 2014	Quigley, 2002
Geiger et al., 2016	Quigley and Wolfe, 2003
Guindon et al., 2015	Quigley et al., 1995
Hepola et al., 2008	Quigley et al., 2006
Hill et al., 2006a	Quigley et al., 2018
Hill et al., 2006b	Raeth-Knight et al., 2009
Hill et al., 2007a	Richard et al., 1988
Hill et al., 2007b	Rosenberger et al., 2017
Hill et al., 2007d	Schaff et al., 2016
Hill et al., 2007e	Silva et al., 2017
Hill et al., 2008b	Stamey et al., 2012
Hill et al., 2010	Suárez et al., 2006
Hill et al., 2013	Terre et al., 2009
Hill et al., 2016c	Terre et al., 2015
Hu et al., 2019	Terui et al., 1996
Huber et al., 1984	Yunta et al., 2015

Weaned

Abdelgadir et al., 1996a	Lee et al., 2009
Brown et al., 2005b	Meyer, 2005
Chester-Jones et al., 1991	Neathery et al., 1991
Dennis et al., 2018	Osorio et al., 2012
Hill et al., 2007e	Rosenberger et al., 2017
Hill et al., 2012	Stewart and Schingoethe, 1984
Hill et al., 2013	Stobo et al., 1966
Hill et al., 2016a	Stobo et al., 1967
Hill et al., 2016b	Terre et al., 2009
Hu et al., 2019	Terre et al., 2013
Klotz and Heitmann, 2006	Terui et al., 1996
Korst et al., 2017	
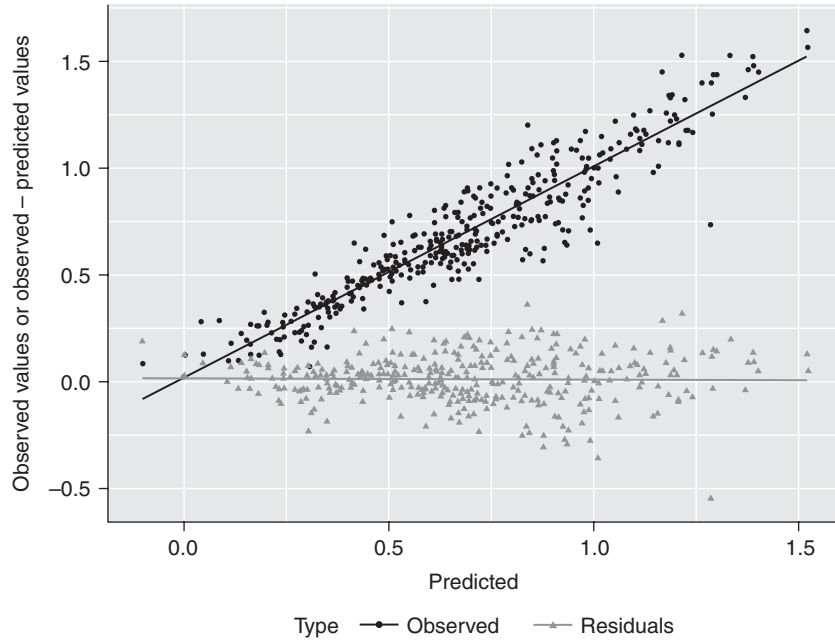
FIGURE 10-3   Observed minus predicted values for ADG from 397 literature treatment means, with residuals plotted. The ADGs were predicted with Equation 10-18, and the studies used (see Table 10-6) represented a wide range of years published, amounts of milk or MR fed, ADG, studies with or without starter, and weaned calves.

in Figure 10-3. The model RMSEP was 0.110. The CCC was 0.95. The model, therefore, was robust in predicting calf growth.

A comparison of ADG predicted by Equation 10-18 with ADG predicted by the equation from the previous edition (NRC, 2001) was made for a randomly chosen subset of 111 means from the studies listed in Table 10-6. Predictions of observed ADG by the current model and by the model from NRC (2001) are shown in Figure 10-4. For this subset of studies, the regression of observed versus predicted values using Equation 10-18 was $0.98x + 0.01$, with an RMSE of 0.12, an AIC of -171.0, and a CCC of 0.93. For the NRC (2001) prediction, the equation was $1.01x - 0.09$, with an RMSE of 0.16, an AIC of -120, and a CCC of 0.85. The NRC (2001) model showed significant mean bias (21.6 percent of MSE). Therefore, the model fit and predictions in the current edition are an improvement over the previous model (NRC, 2001), particularly for calves at low ADG and for those receiving both milk or MR and starter.

ENERGY AND PROTEIN REQUIREMENTS FOR CALVES

Examples of requirements for ME and MP for various classes of calves are in Tables 10-7 through 10-11. The DMI listed in the tables has been computed as the amount of DM necessary to meet the ME requirement. Consequently, these should not be construed as predictions of voluntary feed intake, which was discussed in a previous section.
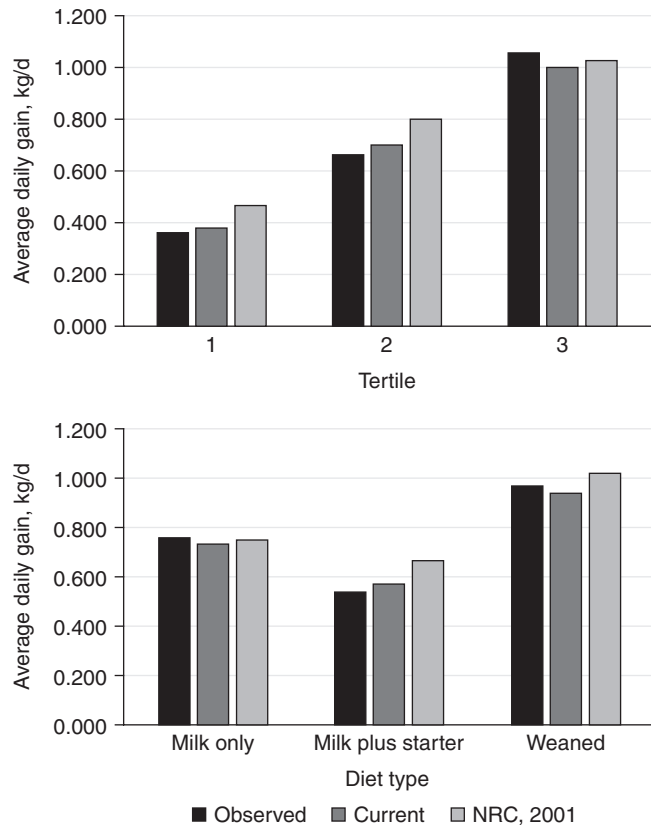
FIGURE 10-4   Comparison of actual mean ADG from 111 treatment means from the literature with values predicted by the current model or the previous (NRC, 2001) model.

TABLE 10-7   Daily Energy and Protein Requirements of Young Replacement Calves Fed Only Milk or MR

BW (kg)	Breed[a]	ADG (g/d)	DMI[b] (kg/d)	ME (Mcal/d)	NEm (Mcal/d)	MP (g/d)	CP[c] (g/d)	CP (% of DMI)
25	SB	200	0.36	1.69	0.82	83	87	24.2
		400	0.49	2.33	0.82	132	139	28.2
30	SB	200	0.40	1.88	0.94	86	91	22.7
		400	0.54	2.54	0.94	136	143	26.6
		600	0.69	3.23	0.94	186	196	28.5
35	SB	200	0.44	2.06	1.06	89	94	21.4
		400	0.58	2.74	1.06	140	147	25.2
		600	0.73	3.45	1.06	190	200	27.2
		800	0.89	4.19	1.06	240	253	28.3
40	LB	200	0.48	2.23	1.17	91	96	19.8
		400	0.64	2.93	1.17	142	149	23.4
		600	0.80	3.66	1.17	192	202	25.4
		800	0.96	4.42	1.17	242	254	26.5
45	LB	200	0.52	2.40	1.28	94	99	19.0
		400	0.68	3.11	1.28	145	152	22.5
		600	0.84	3.86	1.28	195	205	24.5
		800	1.01	4.64	1.28	245	258	25.6
50	LB	200	0.56	2.56	1.38	97	102	18.3
		400	0.71	3.29	1.38	148	155	21.8
		600	0.88	4.05	1.38	198	209	23.7
		800	1.05	4.85	1.38	249	262	24.9
		1,000	1.23	5.66	1.38	299	315	25.6

[a] SB = small breed (based on Jersey, MatBW = 530 kg) and LB = large breed (based on Holstein, MatBW = 700 kg).
[b] DMI necessary to meet requirement for ME when fed milk replacer containing 4.7 Mcal ME/kg of DM (SB calves) or 4.6 ME/kg of DM (LB calves).
[c] Assumes all milk protein with MP/CP of 0.95.

Young Replacement Calves Fed Milk or Milk Replacer Only

The energy requirements of young large-breed and small-breed calves fed only milk or MR and weighing 25 to 50 kg are given in Table 10-7. Users who desire requirements for higher rates of gain should refer to Table 10-11. The energy content of BW gain predicted by Equation 10-6 is 1.62 Mcal/kg for a 40-kg calf gaining 0.20 kg/d and 2.11 Mcal/kg for a 75-kg calf gaining 0.80 kg/d. Values predicted by this equation are similar to those predicted by NRC (2001) for the smaller calf (1.56 Mcal/kg) but are 18 percent lower for the larger calf gaining more rapidly (2.57 Mcal/kg BW gain). The ME requirements for the 40-kg calf gaining 0.20 kg/d (2.20 Mcal/d) and the 75-kg calf gaining 0.80 kg/d (5.66 Mcal/d) predicted by the current equations compare with 2.04 Mcal/d and 5.52 Mcal/d, respectively, predicted by NRC (2001). The current edition predicts lower ADG for a given intake than the previous edition. In the database of literature studies used to validate the current models, there were 103 treatment means from 21 studies. The regression (St-Pierre, 2001) of observed values on predicted values (kg/d) for ADG was as follows: Observed ADG = 0.070 + 0.903 × Predicted ADG.

Users should be aware that ME requirements for maintenance may be underestimated for calves during the first week of life because of the high and variable basal metabolic rate observed during this time (Roy et al., 1957; Gonzalez-Jimenez and Blaxter, 1962; Vermorel et al., 1983; Okamoto et al., 1986; Schrama et al., 1992b; Ortigues et al., 1994; Arieli et al., 1995). Furthermore, because the digestive tract is immature and developing rapidly, the digestibility of diets may be lower during this time (Schrama et al., 1992b; Arieli et al., 1995; Liang et al., 2016), thereby overestimating dietary energy supply. The net result of these effects is that ADG of calves during the first week of life may be considerably less than the predicted energy-allowable gains shown in Table 10-7.

Young Calves Fed Milk and Starter Feed or Milk Replacer and Starter Feed

Under good management, calves should be consuming appreciable nutrients from starter feed by the second week of life. To encourage early consumption of calf starter, calves should be given free access to water and a nutritious, highly palatable starter from the first week of life until they are weaned. Consumption of starter is critical to development of an active, functioning rumen. Fermentation products, principally butyrate, from fermentation of solid feeds in the developing rumen are responsible for development of functional ruminal epithelial tissue (Sander et al., 1959).

TABLE 10-8   Daily Energy and Protein Requirements of Small-Breed Calves Fed Milk or MR and Starter at Two Different Ratios[a]

BW (kg)	ADG (g/d)	Diet	DMI[b] (kg/d)	ME (Mcal/d)	NEm (Mcal/d)	MP (g/d)	CP[c] (g/d)	CP (% of DMI)
30	200	80:20[d]	0.43	1.96	0.93	86	94	21.8
	200	40:60[e]	0.53	2.02	0.93	91	109	20.6
	400	80:20	0.58	2.62	0.93	135	148	25.6
	400	40:60	0.72	2.75	0.93	142	170	23.6
	600	80:20	0.73	3.32	0.93	184	202	27.6
	600	40:60	0.93	3.52	0.93	192	232	25.0
40	200	80:20	0.51	2.33	1.16	92	101	19.7
	200	40:60	0.63	2.39	1.16	98	118	18.7
	400	80:20	0.67	3.03	1.16	142	156	23.4
	400	40:60	0.83	3.16	1.16	150	180	21.7
	600	80:20	0.83	3.77	1.16	192	211	25.4
	600	40:60	1.05	3.98	1.16	202	243	23.2
	800	80:20	1.00	4.54	1.16	241	265	26.5
	800	40:60	1.27	4.82	1.16	253	305	24.0
50	400	80:20	0.75	3.41	1.37	149	163	21.8
	400	40:60	0.93	3.54	1.37	157	189	20.3
	600	80:20	0.92	4.18	1.37	199	219	23.8
	600	40:60	1.16	4.40	1.37	210	253	21.9
	800	80:20	1.10	4.98	1.37	250	274	25.0
	800	40:60	1.39	5.28	1.37	263	317	22.8
	1,000	80:20	1.28	5.80	1.37	300	330	25.8
	1,000	40:60	1.63	6.19	1.37	316	380	23.4
60	400	80:20	0.83	3.75	1.57	155	170	20.6
	400	40:60	1.03	3.90	1.57	164	198	19.3
	600	80:20	1.00	4.56	1.57	206	227	22.6
	600	40:60	1.26	4.79	1.57	218	263	20.9
	800	80:20	1.19	5.39	1.57	258	283	23.8
	800	40:60	1.50	5.70	1.57	272	328	21.8
	1,000	80:20	1.38	6.25	1.57	309	340	24.7
	1,000	40:60	1.75	6.64	1.57	326	392	22.4

[a] Expressed as proportion of DM from MR to proportion of DM from starter.
[b] Total DMI with mean ME density needed to meet ME requirements.
[c] Total dietary CP needed, assuming all-milk protein MR.
[d] Assumes MR contains 4.9 Mcal ME/kg DM and starter contains 3.1 Mcal ME/kg DM.
[e] Assumes MR contains 4.7 Mcal ME/kg DM and starter contains 3.2 Mcal ME/kg DM.

Efficiencies of utilization of ME for maintenance and gain will be somewhat lower for starter feeds than for milk or MR (NRC, 1978). In the current edition, the committee has returned to the use of the equations of Garrett (1980), as updated by Galyean et al. (2016), to derive the efficiencies of utilization of ME from starter for maintenance (k_m) and gain (k_g). The efficiency of use of ME from the total diet is the average of individual efficiencies for milk or MR and starter, weighted according to their contribution to the total ME in the diet (see Tables 10-8 and 10-9). The computer model included with this edition calculates these values for varied proportions of DMI from milk and starter or MR and starter.

The ME requirement (Mcal/d) of a 50-kg large-breed calf gaining 0.60 kg/d when fed only milk or MR (see Table 10-7) is 3.89 compared to 4.18 and 4.40 for the same calf obtaining 80 and 40 percent of her DM from MR (see Table 10-8). The ME requirements for calves consuming both starter and MR

are higher than those in the 2001 edition (NRC, 2001), but the relationship between the current model predictions and literature data is robust. From the literature database, with 219 treatment means from 64 studies, the equation was as follows: Observed ADG $= 0.112 + 0.773 \times$ Predicted ADG. In most cases, ME, and not MP, limited growth.

Calves from Weaning to Body Weight of 125 kg

Since the publication of NRC (2001), a few studies have provided information about body composition of weaned calves (Brown et al., 2005b; Meyer, 2005; Stamey Lanier et al., 2021), and many more have provided data on intake and growth rates. Requirements have been derived using the same methodology as described for younger calves (see Table 10-10). Comparison of literature data for ADG (79 treatment means from 23 published studies) with model

TABLE 10-9   Daily Energy and Protein Requirements of Large-Breed Calves Fed Milk or MR and Starter at Two Different Ratios[a]

BW (kg)	ADG (g/d)	Diet	DMI[b] (kg/d)	ME (Mcal/d)	NEm (Mcal/d)	MP (g/d)	CP[c] (g/d)	CP (% of DMI)
50	400	80:20[d]	0.79	3.40	1.37	147	162	20.6
	400	40:60[e]	0.93	3.54	1.37	155	187	20.0
	600	80:20	0.97	4.18	1.37	197	217	22.4
	600	40:60	1.16	4.40	1.37	207	250	21.6
	800	80:20	1.15	4.98	1.37	247	271	23.6
	800	40:60	1.39	5.28	1.37	259	312	22.5
60	400	80:20	0.87	3.75	1.57	153	168	19.4
	400	40:60	1.03	3.90	1.57	162	195	19.0
	600	80:20	1.05	4.55	1.57	204	224	21.2
	600	40:60	1.26	4.79	1.57	214	258	20.5
	800	80:20	1.25	5.38	1.57	254	279	22.4
	800	40:60	1.50	5.70	1.57	267	322	21.4
	1,000	80:20	1.44	6.24	1.57	305	335	23.2
	1,000	40:60	1.75	6.64	1.57	320	386	22.0
70	400	80:20	0.94	4.08	1.76	159	174	18.4
	400	40:60	1.11	4.24	1.76	168	202	18.1
	600	80:20	1.14	4.91	1.76	210	231	20.3
	600	40:60	1.35	5.15	1.76	221	267	19.7
	800	80:20	1.33	5.77	1.76	261	287	21.5
	800	40:60	1.60	6.10	1.76	275	332	20.6
	1,000	80:20	1.54	6.65	1.76	312	343	22.3
	1,000	40:60	1.86	7.07	1.76	328	396	21.3
80	600	80:20	1.21	5.25	1.90	216	237	19.5
	600	40:60	1.45	5.50	1.90	228	275	19.0
	800	80:20	1.42	6.13	1.90	268	294	20.7
	800	40:60	1.70	6.47	1.90	282	340	20.0
	1,000	80:20	1.63	7.03	1.90	320	351	21.6
	1,000	40:60	1.96	7.47	1.90	337	406	20.6

[a] Expressed as proportion of DM from MR to proportion of DM from starter.
[b] Total DMI with mean ME density needed to meet ME requirements.
[c] Total dietary CP needed, assuming all-milk protein MR.
[d] Assumes MR contains 4.6 Mcal ME/kg DM and starter contains 3.2 Mcal ME/kg DM.
[e] Assumes MR contains 4.7 Mcal ME/kg DM and starter contains 3.2 Mcal ME/kg DM.

predictions yielded the following relationship: Observed ADG = 0.366 + 0.662 × Predicted ADG. The large intercept of the equation indicates that the model underpredicts growth at low growth rates and overpredicts growth at high growth rates. In the absence of more comparative slaughter studies with calves of this weight range, however, the committee was not able to derive an equation with less slope bias.

Veal Calves

The calculations used to derive the ME requirements for veal calves (see Table 10-11) are the same as those for milk-fed replacement calves (see Table 10-7) with the exception that EBW/ADG gain is set at 0.91, as described in an earlier section. Predicted requirements agree closely with experimental data for veal calf growth, even at BW greater than those in the database. Veal calves are fed at rates approaching ad libitum intake, so rates of gain will be higher than those of limit-fed replacement calves. The ME, MP, and

DM requirements given here agree closely with data in the literature (Gerrits et al., 1996; van den Borne et al., 2006; Labussiere et al., 2011). Current systems of veal production in many areas provide small amounts of solid feed in addition to high intakes of milk (Suárez et al., 2006; Labussiere et al., 2009). Requirements for these calves can be estimated by using the principles established for calves fed both milk and solid feeds.

MINERAL AND VITAMIN REQUIREMENTS OF CALVES

Minerals

Detailed information on the roles of minerals is given in Chapter 7. Mineral absorption and factors affecting mineral absorption are also discussed in Chapter 7; however, that discussion emphasizes absorption by functioning ruminants. Clear differences in mineral absorption between preruminant calves and older cattle have been shown for many minerals

TABLE 10-10 Daily Energy and Protein Requirements of Weaned Large- or Small-Breed Calves Fed Only Solid Feeds

BW (kg)	ADG (g/d)	DMI[a] (kg/d)	ME (Mcal/d)	NEm (Mcal/d)	MP (g/d)	CP (g/d)	CP (% of DMI)
55	400	1.465	4.394	1.734	174.3	249.0	17.0
	600	1.768	5.305	1.734	227.9	325.6	18.4
	800	2.083	6.248	1.734	281.6	402.3	19.3
65	400	1.607	4.820	1.966	183.1	261.6	16.3
	600	1.921	5.763	1.966	237.7	339.6	17.7
	800	2.246	6.739	1.966	292.5	417.9	18.6
75	400	1.742	5.226	2.188	191.5	273.6	15.7
	600	2.066	6.197	2.188	247.2	353.1	17.1
	800	2.401	7.202	2.188	303.0	432.9	18.0
85	600	2.204	6.612	2.404	251.2	358.8	16.3
	800	2.548	7.643	2.404	306.5	437.9	17.2
	1,000	2.900	8.700	2.404	362.0	517.1	17.8
95	600	2.337	7.010	2.613	259.4	370.5	15.9
	800	2.688	8.065	2.613	315.5	450.7	16.8
	1,000	3.049	9.146	2.613	371.7	531.0	17.4
105	600	2.465	7.394	2.817	267.3	381.9	15.5
	800	2.824	8.471	2.817	324.2	463.1	16.4
	1,000	3.192	9.575	2.817	381.2	544.6	17.1
	1,200	3.567	10.701	2.817	438.4	626.3	17.6
115	600	2.589	7.766	3.015	275.1	393.0	15.2
	800	2.954	8.863	3.015	332.8	475.4	16.1
	1,000	3.329	9.988	3.015	390.6	558.0	16.8
	1,200	3.712	11.136	3.015	448.6	640.8	17.3
125	600	2.709	8.128	3.210	282.7	403.9	14.9
	800	3.081	9.244	3.210	341.2	487.4	15.8
	1,000	3.463	10.388	3.210	399.8	571.1	16.5
	1,200	3.852	11.556	3.210	458.6	655.1	17.0
	1,400	4.248	12.743	3.210	517.4	739.2	17.4

[a] Assumes starter contains 3.0 Mcal ME/kg DM.

likely because of both diet and physiology. In the previous edition (NRC, 2001), mineral recommendations were presented on a dietary concentration basis, and those recommendations were largely unchanged from NRC (1989).

The committee took a more quantitative approach to establishing minerals recommendations than in the past; however, these recommendations should be considered as Adequate Intakes (AIs) rather than requirements. AIs for calcium (Ca), phosphorus (P), and magnesium (Mg) in g/d of total diet were calculated using equations from Castro et al. (2019). Their equations were derived from data from multiple studies with preweaned calves (Holstein and Holstein × Gyr cross) that used the comparative slaughter technique. Calves in four of those studies were fed milk and starter, and in one study, calves were fed only MR. Breed generally did not affect results. In the equations below, the value in the denominator is the experimentally derived retention coefficient that converts retained mineral to dietary mineral. Those coefficients likely will be influenced by source of mineral, but the committee assumed that those coefficients will be reasonably accurate for mixed diets of liquid and solid feed for preweaned calves. Absorption coefficients (ACs) are not used for macrominerals for young calves.

Dietary AIs are calculated as follows:

$$Ca, g/d = [0.0127 \times EBW + (14.4 \times EBW^{-0.139} \times EBG)] / 0.73$$
$$\text{(Equation 10-19)}$$

$$P, g/d = [0.0118 \times EBW + (5.85 \times EBW^{-0.027} \times EBG)] / 0.65$$
$$\text{(Equation 10-20)}$$

$$Mg, g/d = [0.0035 \times EBW + (0.60 \times EBW^{-0.036} \times EBG)] / 0.30$$
$$\text{(Equation 10-21)}$$

$$K, g/d = [0.0203 \times EBW + (1.14 \times EBW^{-0.048} \times EBG)] / 0.13$$
$$\text{(Equation 10-22)}$$

$$Na, g/d = [0.00637 \times EBW + (1.508 \times EBW^{-0.045} \times EBG)] / 0.24$$
$$\text{(Equation 10-23)}$$

For these equations, empty BW (EBW) and daily empty body gain (EBG) equal $0.94 \times BW$ and $0.91 \times ADG$ in kilograms. K is potassium and Na is sodium.

Essentially no information is available on the chloride (Cl⁻) requirement of young calves; therefore, the committee decided to use the ratio of Cl⁻ to Na requirement calculated

TABLE 10-11   Daily Energy and Protein Requirements of Large-Breed Veal Calves Fed Only Milk or MR

BW (kg)	ADG (g/d)	DMI[a] (kg/d)	ME (Mcal/d)	NEm (Mcal/d)	MP (g/d)	CP[b] (g/d)	CP (% of DMI)
40	300	0.55	2.54	1.17	114	120	21.8
	600	0.78	3.59	1.17	187	197	25.2
50	300	0.63	2.88	1.38	120	126	20.1
	600	0.86	3.98	1.38	193	204	23.6
	900	1.12	5.13	1.38	267	281	25.2
75	300	0.79	3.65	1.87	132	139	17.6
	600	1.05	4.84	1.87	208	219	20.8
	900	1.32	6.10	1.87	284	299	22.6
	1,200	1.61	7.40	1.87	360	379	23.6
100	600	1.22	5.60	2.32	222	234	19.2
	900	1.51	6.93	2.32	300	316	21.0
	1,200	1.81	8.31	2.32	379	399	22.0
	1,500	2.11	9.73	2.32	457	480	22.8
125	600	1.37	6.30	2.75	235	247	18.1
	900	1.67	7.70	2.75	316	333	19.9
	1,200	1.99	9.14	2.75	397	418	21.0
	1,500	2.31	10.62	2.75	478	503	21.8
150	600	1.51	6.95	3.15	248	261	17.2
	900	1.83	8.40	3.15	331	349	19.1
	1,200	2.15	9.90	3.15	415	437	20.3
	1,500	2.49	11.40	3.15	498	525	21.1
175	600	1.65	7.57	3.54	260	274	16.6
	900	1.97	9.07	3.54	349	365	18.5
	1,200	2.31	10.62	3.54	433	456	19.8
	1,500	2.65	12.20	3.54	519	547	20.6
200	600	1.77	8.17	3.91	273	287	16.2
	900	2.11	9.70	3.91	362	381	18.1
	1,200	2.45	11.29	3.91	452	475	19.4
	1,500	2.81	12.92	3.91	541	569	20.3
225	600	1.90	8.73	4.27	286	301	15.8
	900	2.24	10.31	4.27	378	398	17.8
	1,200	2.59	11.94	4.27	471	496	19.1
	1,500	2.96	13.61	4.27	563	593	20.0

[a] DMI necessary to meet requirement for ME when fed MR containing 4.6 ME/kg of DM.
[b] Assumes MP/CP of 0.95.

for lactating cows (i.e., 0.8) and multiply that by the Na requirement as calculated using Equation 10-23.

For the trace minerals, copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), equations developed for older cattle were applied to calves, but different ACs were used. No AI is given for cobalt (Co) in MR because young calves lack a functioning rumen and cannot convert Co into vitamin B_{12}. The AIs for trace minerals are calculated as follows:

$$Cu, mg/d = (0.0145 \times BW + 2.5 \times ADG) / 0.5$$
(Equation 10-24)

$$Fe, mg/d = (34 \times ADG) / 0.25$$
(Equation 10-25)

$$Mn, mg/d = [(0.0026 \times BW + 0.7 \times ADG)] / 0.01$$
(Equation 10-26)

$$Zn, mg/d = (2 \times DMI + 24 \times ADG) / 0.25$$
(Equation 10-27)

For Cu, 2.5 mg/kg ADG was used for young calves, rather than 2.0 used for growing heifers, which reflects greater concentrations of Cu in organs than in muscle (see Chapter 7). For Zn, 24 mg/kg ADG was used for young calves, which increased to 30 mg/kg ADG for growing heifers, reflecting the greater concentration of Zn in muscle than in organs (Watson et al., 2018). The denominators in the above equations are calf-specific AC (see Table 10-12). Inadequate data are available to generate feed-specific AC for calves; therefore, all diets fed to young calves have the same AC. The calf ACs were derived from experiments conducted on young calves, and when data on absorption of minerals by preruminant calves could not be found, nonruminant data (e.g., human, swine, rodents) were used (see Chapter 7). When multiple ACs were available, the committee used the lower value to reduce the risk of deficiencies.

In the model, when users select calf as animal type and the diet does not include any liquid feed (i.e., a weaned calf), requirement equations used for older animals (see Chapter 7) are used for all minerals along with the ACs in Table 10-12 under the weaned calf column. A single AC for each mineral is used for the total diet; individual feed ACs are not used if calf is selected.

TABLE 10-12   ACs for Minerals Used for Young Calves

Mineral	Milk Replacer and Starter[a]	Weaned Diet[b]
Ca	NA[c]	0.60
P	NA	0.75
Mg	NA	0.26
K	NA	1.0
Na	NA	1.0
Cu	0.5	0.10
Fe	0.25	0.10
Mn	0.01	0.005
Zn	0.20	0.20

[a] ACs in this column are appropriate for preweaned calves (i.e., nonmature rumen).

[b] ACs in this column are used for postweaned calves. The ACs for macrominerals and most trace minerals are typical for diets fed to adult cattle. The AC for Cu is derived from newly weaned lambs (Suttle, 1975) and will continue to decrease to the value used for older cattle (0.05) as dietary forage increases.

[c] For preweaned calves, retention coefficients (Castro et al., 2019) rather than AC are used for macrominerals.

TABLE 10-13   Recommended Concentrations of Minerals in MR and Starter (DM Basis) to Provide AIs for Calves Between 35 and 125 kg of BW and Growing Between 0.5 and 1.2 kg/d

Mineral	Milk Replacer	Starter	Grower
Ca, %	0.80	0.75	0.65
P, %	0.60	0.37	0.33
Mg, %	0.15	0.15	0.16
K, %	1.10	0.60	0.60
Na, %	0.40	0.22	0.20
Cl, %	0.32	0.17	0.15
Co, mg/kg	NA	0.2	0.2
Cu, mg/kg	5	12	12
I, mg/kg	0.8	0.8	0.5
Fe, mg/kg	85	60	55
Mn, mg/kg	60	40	60
Se, mg/kg	0.3	0.3	0.3
Zn, mg/kg	65	55	50

To derive recommended concentrations of minerals in MR, daily dietary requirements were calculated for different-sized calves (35 to 85 kg) with different rates of gain (0.5 to 1.0 kg/d), and appropriate DMI values based on different amounts of MR and starter were assigned so that total dietary concentrations could be calculated. This was done for 20 different situations. The concentrations of mineral needed to meet AI were averaged across the different situations to obtain recommended dietary concentrations of minerals. Because calves consume liquid feed as their sole diet for at least several days, recommended concentrations in MR (see Table 10-13) were the same concentrations as recommended for total diet. This approach resulted in macromineral concentrations that were quite similar to that of milk on a DM basis (see Table 10-14).

TABLE 10-14   Concentrations of Minerals and Fat-Soluble Vitamins in Whole Milk (per Liter)[a]

Mineral	Range	Mean[b]
Ca, g	0.93–1.47	1.0
P, g	0.8–1.0	0.9
Mg, g	0.10–0.13	0.11
K, g	1.27–1.71	1.49
Na, g	0.33–0.48	0.41
Cl⁻, g	0.85–1.09	0.97
S, g	0.18–0.31	0.25
Cu, mg	0.03–0.06[c]	0.04
I, mg	0.1–0.4[d]	0.2
Fe, mg	0.1–0.4	0.2
Mn, mg	0.012–0.05	0.03
Se, mg	0.018–0.04[e]	0.02
Zn, mg	3–6	4.0

Vitamins[f]	Range	Mean[b]
Vitamin A, IU	300–1,300	1,000
Vitamin D, IU	14–40	27
Vitamin E, IU	0.7–1.8	1.2

[a] Sources of data can be found in Chapter 7.

[b] Mean values are for Holstein cows; however, breed can affect concentrations of some minerals. Limited data are available for other breeds (Cerbulis and Farrell, 1976; Carroll et al., 2006; Stocco et al., 2019).

[c] Concentrations can be greater if cows are fed very high amounts of Cu.

[d] Concentrations in milk have a positive linear relationship to iodine (I) intake. These ranges reflect feeding at approximate I requirements.

[e] Lower concentrations reflect milk from cows fed inorganic selenium (Se) at approximate requirements. The higher concentrations reflect milk from cows fed Se yeast at approximate requirements.

[f] Concentrations of vitamins A, D, and E have a positive relationship to intake of the vitamin by the cow and with the fat concentration of the milk.

NOTE: S = sulfur.

Starter was assumed to be the primary nutrient source for calves immediately postweaning. Recommended concentrations of minerals in starter were determined by calculating mineral requirements using the equations above for calves weighing 110 and 60 kg (representing Holsteins and Jerseys) gaining between 0.5 and 1.2 kg/d with appropriate intakes (only fed dry feeds). Nutrient concentrations to meet the requirements were calculated and averaged. However, Equations 10-25 to 10-28 were developed with data from young calves with limited rumen function, but after weaning, calves are becoming functional ruminants. Therefore, requirements and dietary concentrations were also calculated using mineral equations and AC developed for functioning ruminants (see Chapter 7), except for Cu. The recommended Cu concentrations for starter calculated using calf and functioning ruminant equations differed markedly (sometimes by more than a factor of 2). These animals are transitioning into functioning ruminants, and no data are available on mineral absorption by this type of animal; therefore, the recommended Cu concentrations in starter and grower (see Table 10-14) reflect Cu absorption measured in newly weaned lambs (Suttle, 1975). The recommended dietary concentrations of other minerals in the grower were calculated using the same method as used

for starter except the committee used calves that weighed between 80 and 125 kg to calculate AIs.

For iodine (I), the AI for nonruminating calves was set based on the AI established for human infants (0.8 mg I/kg DMI; see Chapter 7), and no AC is used. The AI for supplemental selenium (Se) is set at 0.3 mg Se/kg DMI, which is the same as that used for older cattle and NRC (2001). No AC is used. Based on all available data, Se supplementation at that rate should prevent white muscle disease. In most cases, whole milk will not provide adequate Se and will need to be supplemented.

Compared to recommended concentrations in the previous edition (NRC, 2001), recommended Ca concentrations are lower for MR but similar for starter and grower. Recommended concentrations of P are about 15 percent lower for MR, starter and grower. Recommended K concentration in MR is about 70 percent higher but similar for starter and grower, and recommended Na concentrations are similar to the previous edition. Recommended concentrations of Cu are about half the previous value, and Fe is about 15 percent lower for MR but similar for starter and grower. Recommended concentration of Mn is higher for MR but similar to the previous edition for starter and grower. Zn concentrations are about 40 percent greater than those in the previous edition.

In the computer model, when growing heifer is selected, the ACs change to those described in Chapter 7. Therefore, the growing calf submodel should be used until calves are greater than 125 kg of BW.

Vitamins

Detailed information on the roles of vitamins is given in Chapter 8. No new information is available on the responses by calves to supplemental water-soluble vitamins; therefore, the previous recommendations were retained (see Table 10-15). The B-vitamins (including vitamin B_{12}) and choline should be added to MR, but once calves are weaned and consuming dry feed, the basal diet and ruminal synthesis appear adequate to meet the needs for water-soluble vitamins by the growing calf. New data are also not available regarding vitamin K supplementation to calves. Based on limited data (Nestor and Conrad, 1990), supplemental vitamin K is not needed by young calves if not fed moldy sweet clover.

Vitamin A

Because of limited placental transfer, calves are born with very low stores of retinol and β-carotene and are dependent on an adequate and timely intake of colostrum that contains adequate concentrations of retinol. A calf fed 3 L of first milking colostrum could ingest more than 30,000 IU of retinol (ca. 0.9 mg of retinol), which will elevate hepatic concentrations of retinol into the acceptable range (ca. >20 mg/kg of liver wet weight). However, vitamin A nutrition of the dam during the prepartum period and time after birth when

the calf is fed (Zanker et al., 2000; Puvogel et al., 2008) affect vitamin A status of the very young calf, which likely will affect the calf's response to vitamin A supplementation during the first several weeks of life. Holstein calves that received adequate retinol via colostrum and were fed about 5,400 IU of vitamin A/d maintained hepatic concentration of retinol at approximately 20 mg/kg wet weight during the first 4 weeks of life (Swanson et al., 2000). However, hepatic retinol decreased when calves were fed 3,800 IU of vitamin A/d (indicative of inadequate consumption). Liver concentrations increased over time to about 40 mg/kg wet weight when calves consumed 10,600 IU/d and to about 100 mg/kg wet weight when fed 26,600 IU/d (indicative of excess consumption). Based on this study, Holstein calves fed MR need to consume about 5,400 IU/d of vitamin A. That is contingent on calves receiving adequate colostral retinol to elevate hepatic retinol to about 20 mg/kg wet weight. The AI of vitamin A for older cattle was set at 110 IU/kg BW; if that value was applied to young Holstein calves, the AI would be approximately 6,600 IU/d. Because of the uncertainty associated with colostral retinol, the AI of vitamin A was set at 110 IU/kg BW for young calves, which is approximately equivalent to 11,000 IU/kg of MR solids when fed at 0.6 kg/d. At high rates of MR intake, that concentration may lead to excessive intake of vitamin A. Higher rates of MR intake lead to greater growth rates, but increasing growth rates (0 to 1.2 kg/d) in young calves did not affect serum concentrations of retinol, although all calves were fed excess vitamin A (Nonnecke et al., 2010). Vitamin A intakes of approximately 17,000 to 39,000 IU/d reduce plasma α-tocopherol concentrations substantially in young calves (Franklin et al., 1998; Ametaj et al., 2000). This suggests that at high rates of MR intake (e.g., >1 kg of solid/d), the concentration of vitamin A in the MR should be less than 9,900 IU/kg of MR solids. No available data show any benefit of feeding more than the current recommended AI (i.e., ca. 7,000 IU/d) of vitamin A, whereas data are available showing negative effects of higher intakes of vitamin A on vitamin E status.

Vitamin D

Although vitamin D is receiving renewed research for adult cows, new data are still very limited for young calves. Vitamin D status (as measured by plasma concentrations of 25-OH vitamin D) was depressed when young calves were infected with bovine diarrhea virus (Nonnecke et al., 2014). Young calves fed 5,000 IU/d of vitamin D_3 tended to have fewer health issues than calves fed no supplemental vitamin D (Krueger et al., 2016). Calves fed no supplemental vitamin D had plasma 25-OH vitamin D concentrations <20 ng/mL by 14 days of age, and intake of supplemental vitamin D by calves is linearly related to plasma or serum concentrations of 25-OH vitamin D. To obtain a serum concentration of 30 ng/mL, calves need to consume approximately 2,100 IU of vitamin D_3/d (Nelson et al., 2016). Concentrations of

serum 25-OH vitamin D <30 ng/mL are associated with increased health problems in humans (Norman, 2008). The optimal serum concentration of 25-OH vitamin D for dairy calves is not known. The committee set the AI of vitamin D_3 at 32 IU/kg BW (i.e., 2,100 IU/d for a 65-kg calf) based on maintaining serum 25-OH vitamin D at 30 ng/mL, which is close to the recommended AI for older cattle. At an intake of 0.6 kg/d, MR would need to contain 3,500 IU of vitamin D_3/kg solids to meet the AI of vitamin D (see Table 10-15). This represents a substantial increase from NRC (2001).

Vitamin E

The recommended intake of vitamin E was increased to 50 IU/kg of MR solids (approximately 30 IU/d) for young calves in 2001 (NRC, 2001). Although one study (Reddy et al., 1987) reported improved growth rates when calves were fed 125 or 250 IU of vitamin E/d compared to those fed no supplemental vitamin E, the NRC (2001) committee concluded that inadequate data were available to increase the recommendation further. The ADG in Reddy et al. (1987) was low (<150 g/d), and growth rate appears to affect vitamin E status of young calves. Serum α-tocopherol concentrations were lower in calves gaining 1.2 kg/d compared to calves gaining 0.55 kg/d, even though vitamin E intakes (approximately 300 IU/d) were similar (Nonnecke et al., 2010). Supplemental vitamin E (500 IU/d) increased growth in calves fed adequate energy and protein to grow at 0.5 kg/d but did not affect growth in calves fed to grow at 0.25 kg/d (Krueger et al., 2014). Conversely, Sehested et al. (2004) reported no difference in growth rate (approximately 0.8 kg/d) between young Holstein calves fed 0 or 500 IU of supplemental vitamin E. In nonruminants (and preruminant calves), the vitamin E requirement is a function of intake of PUFA, and based on typical intakes of PUFA by young calves, vitamin E intake needs to be about 30 IU/d just to

prevent oxidative stress caused by PUFA. Infections can significantly deplete plasma stores of vitamin E (Nonnecke et al., 2014), and many studies have shown enhanced immune function when vitamin E is supplemented at rates greater than 30 IU/d to young calves (Reddy et al., 1986; Eicher-Pruiett et al., 1992; Eicher et al., 1994; Samanta et al., 2006; Pekmezci and Cakiroglu, 2009). These data in total strongly suggest that intake of vitamin E should be greater than 30 IU/d. In lieu of perfect data and based on the definition of AI, the committee chose the lowest supplementation rate that has been shown to be beneficial (i.e., 125 IU/d or about 2 IU/kg BW; see Table 10-15) as the AI for vitamin E. The committee acknowledges that this may not be adequate for rapidly growing calves.

PRACTICAL FEEDING CONSIDERATIONS

Rates of Milk or Milk Replacer Feeding

The optimal amount of milk or MR to provide remains controversial. The primary point of contention is that feeding larger amounts of liquid delays increases in starter intake, which, in turn, stimulate development of ruminal fermentation and the absorptive epithelium. A large body of research evidence since NRC (2001) highlights the greater early growth obtained by feeding milk at higher rates (12 to 20 percent of BW) than the "traditional" 8 to 10 percent of BW (Drackley, 2008; Khan et al., 2011a). Like other mammalian neonates, calves given free access to milk will drink large amounts in preference to dry feed. Calves with ad libitum access to milk will consume about 20 percent of their BW daily, which for 50- to 60-kg Holstein calves would be 10 to 12 L of whole milk daily (Jasper and Weary, 2002; Khan et al., 2007; Sweeney et al., 2010). Assuming 12.5 percent solids, this amount equates to 1.3 to 1.6 kg/d of milk solids or about 2.5 percent of BW. Likewise, Holstein calves with ad libitum access to MR will consume 1.2 to 1.4 kg of DM daily (Schaff et al., 2016; Frieten et al., 2017; Korst et al., 2017; Curtis et al., 2018). Because calves respond to greater amounts of milk or MR with greater growth (Khouri and Pickering, 1968; Hodgson, 1971; Huber et al., 1984; Yunta et al., 2015; Rosenberger et al., 2017), defining a requirement for a level of feeding is not possible except to establish the relationship between rates of growth and the amounts of nutrients needed to fuel that growth. Recommendations about how much milk or MR calves should be fed must be made with the understanding that calves willingly drink much more milk or MR than the limited amounts offered in standard practice for decades.

It is useful to establish a standardized framework for feeding rates for the discussion that follows. Consumption of <400 g of milk or MR solids daily for large-breed calves (300 g/d for small-breed calves) will be referred to as "severely restricted" because this amount may not cover maintenance requirements, especially if an immune challenge is present or

TABLE 10-15   Recommended AIs for Fat-Soluble Vitamins[a]

	IU/kg BW	IU/kg DM		
		Milk Replacer[b]	Starter[c]	Grower[d]
Vitamin A	110	11,000	3,700	3,700
Vitamin D	32	3200	1,100	1,100
Vitamin E	2.0	200	67	67

[a]Water-soluble vitamins are needed in MR. Recommended concentrations (per kilogram of DM) are 6.5 mg of DM for thiamin, riboflavin, and pyridoxine; 13 mg pantothenic acid; 10 mg niacin; 0.1 mg biotin; 0.5 mg folic acid; 0.07 B_{12}; and 1,000 mg choline (NRC, 2001). Microbial synthesis of vitamin K within the intestines appears adequate, and supplemental vitamin K is usually not needed (Nestor and Conrad, 1990).

[b]These values assume a 60-kg calf that is consuming 0.6 kg of MR solids. Concentrations should be reduced if calves are fed substantially greater amounts of MR (e.g., ≥1 kg/d of solids).

[c]These values assume an 80-kg calf consuming 2.4 kg of starter DM.

[d]These values assume a 110-kg calf consuming 3.3 kg of grower DM.

in adverse environmental conditions. Feeding rates of 400 to 600 g/d for large-breed calves (300 to 450 g/d for small breeds) will be referred to as "low" rates of milk or MR feeding. Rates between 600 and 900 g/d (451 to 700 g/d for small breeds) are referred to as "moderate" feeding rates, and anything >900 g/d (700 g/d) and less than ad libitum is referred to as "high" rates of feeding. The median (50th percentile) milk or MR intake for preweaned Holstein heifers in the United States was 5.5 L/d (Urie et al., 2018), which at 12.5 percent solids and density of 1.03 g/mL would equate to about 708 g/d of milk solids, or a "moderate" rate of feeding. Put another way, about 50 percent of dairy producers fed Holstein calves only 4 to 5 L of milk or MR daily (515 to 644 g/d of milk solids; USDA, 2016).

The rationale for severely restricted or low rates of feeding for neonatal calves includes seeking to stimulate early intake of starter, which is less expensive per unit of feed (although not necessarily less expensive per unit of BW gain); to encourage early weaning; and to decrease the incidence of digestive disorders such as diarrhea (Khan et al., 2011a). During the first 2 weeks of life, the limited amount of milk provides calves with only enough nutrients in excess of maintenance to grow 0.2 to 0.3 kg/d under thermoneutral and non-immune-challenged conditions. With low rates of milk intake, calves will rapidly increase their intake of starter beginning at around 2 weeks of age (Williams and Frost, 1992). Greater intakes of the fermentable carbohydrates in starter stimulate microbial growth and ruminal fermentation, resulting in a sharp increase in growth rate (Kertz et al., 1979).

Development of early starter intake is inversely proportional to the amount of liquid feed offered (Hodgson, 1971; Jasper and Weary, 2002; Stamey et al., 2012), which should not be surprising since calves have a maximum total DMI or energy intake like other animals. Rumen development takes about 3 weeks of intake of a typical starter (Williams and Frost, 1992), although recent studies have shown that it is the cumulative consumption of nonfiber carbohydrates (starch and sugars) rather than just total starter intake that is more highly related to rumen development (Quigley et al., 2019a). When weaning occurs before the calf has consumed sufficient fermentable carbohydrates, the rumen may not be able to efficiently convert feeds to metabolizable nutrients, and growth rates suffer. Nutrient digestibility, particularly NDF, was decreased after weaning for calves fed larger amounts of MR before weaning (Terre et al., 2007; Hill et al., 2010), which likely was due to inadequate development of the rumen function. No studies have reported effects of different milk feeding levels on digestibility, where the time of measurement relative to achieving a certain starter intake was controlled; thus, at the same age, calves fed less milk will by default have consumed more starter and have more well-developed rumen function than calves fed larger volumes of liquid feed.

Under good management, limited liquid feeding programs have been successful. However, when calves are challenged by infectious or environmental stressors, severely restricted or low nutrient intakes may limit effective immune responses

or not allow sufficient heat production for thermoregulation (Godden et al., 2005; Olivett et al., 2012; Ballou et al., 2015). Considerable evidence indicates that feeding rates that do not achieve maintenance are inadequate to support optimal health and function of the immune system, especially under adverse environmental conditions (Williams et al., 1981; Griebel et al., 1987; Pollock et al., 1993, 1994; Godden et al., 2005; Ollivett et al., 2012; Ballou et al., 2013, 2015). The advantages to calf health of greater amounts of liquid feed likely relate to providing more nutrients to support an immune response (Nonnecke et al., 2003; Foote et al., 2005a,b, 2007; Ballou, 2012; Obeidat et al., 2013). Some aspects of the immune system in isolated immune cells appear to be downregulated by high rates of milk feeding (Nonnecke et al., 2003; Foote et al., 2005a,b, 2007), although the significance of these changes in vivo has not been delineated.

Greater preweaning growth rates from feeding more milk or increased starter intake are associated with greater milk yields in first lactation (see studies summarized in Bach et al., 2012; Soberon et al., 2012; Gelsinger et al., 2016). Heifers that grow more rapidly in early life are not heavier at first parturition but may calve earlier (Van Amburgh et al., 2019). The mechanism(s) responsible for such an effect of early growth remain unclear, although several lines of evidence have emerged from studies where early growth was stimulated by greater amounts of MR. Greater rates of MR feeding increased mammary parenchymal mass and parenchymal DNA and RNA without fat deposition (Brown et al., 2005a). Greater MR intake was associated with greater mass and increased proliferation of mammary epithelial cells in heifers killed at 100 kg BW (Meyer et al., 2006a,b). An enhanced plane of nutrition (1.1 kg versus 0.44 kg of MR) resulted in greater mammary parenchymal tissue growth in response to estrogen stimulation (Geiger et al., 2016), although starter intake was restricted in the low-feeding group. Finally, changes in the mammary gland transcriptome when calves are fed different rates of MR were consistent with greater mammary development in heifers fed greater amounts of MR (Piantoni et al., 2008, 2010, 2012; Vailati-Riboni et al., 2018). Studies on the mechanisms of the effect of greater early ADG achieved by greater starter intake are not available but could be reasoned to occur by similar mechanisms. More research is needed in this area.

Since publication of NRC (2001) guidelines, a large body of behavioral studies has been published. These studies established that calves fed low amounts of milk demonstrate behavioral signs of hunger, including increased vocalization and decreased resting time compared with calves fed at least 8 L/d (Thomas et al., 2001; de Paula Vieira et al., 2008). In a titration study in which calves were fed 6, 8, 10, or 12 L/d of whole milk, calves actually consumed 5.7, 7.2, 8.3, and 9.4 kg/d (Rosenberger et al., 2017). Calves made 11.1, 3.6, 1.7, and 0.4 unrewarded visits to the automated feeder, indicating that calves offered less than 8 L/d displayed clear signs of hunger. The amount of solids consumed by calves consuming 7.2 kg/d was 890 g/d.

Hill and colleagues (Hill et al., 2006b, 2007d,e, 2009b,c) established that feeding Holstein calves 0.68 kg/d of MR solids containing 26 percent CP and 17 percent fat increases ADG relative to lower feeding rates but does not significantly decrease starter intake. A limitation of this body of work is that the calves were males transported to the research facility within 1 to 2 days of birth, and replication by other research groups has been limited. Based on the preponderance of evidence, the committee recommends that the minimum amount of milk or MR solids to be fed under thermoneutral conditions should be 1.5 percent of birth BW, similar to the body of work by Hill and colleagues. The welfare argument for feeding calves more than this (8 L/d or ca. 1,000 g/d of milk solids) to avoid hunger is compelling. The committee encourages adoption of programs that provide greater rates of liquid feeding than this minimum of 1.5 percent of BW as milk solids, based on considerations for calf well-being and enhanced nutrient supply for early growth.

Limited evidence suggests that increased feeding rates can start almost immediately after birth. Knauer et al. (2017) compared a gradual increase of milk offered (from 5–6 L/d to 6–8 L/d over a 7- to 14-day period) with a fixed amount of 6 to 8 L/d from day 1 of life. Offering a fixed milk allowance from day 1 improved calf growth during the first 3 weeks compared with the gradual increase in milk allowance, with no detrimental effect on calf health.

Typical MR contains 10 to 20 percent less energy than comparable volumes of whole milk because of lower fat content (i.e., 15 to 20 percent in MRs compared with 25 to 30 percent in milk). A 45-kg calf fed 0.51 kg of MR solids (9 percent of BW at 12.5 percent solids) that contains ME at 4.7 Mcal/kg of DM would consume enough energy for maintenance and a body weight gain of 0.19 kg/d under thermoneutral conditions. According to the model presented in this edition, feeding the same volume of whole milk would support a gain of 0.29 kg/d. If the same calf is housed at −5°C, 0.51 g/d of MR powder is insufficient even for maintenance and weight loss would ensue.

Starter

Concentrates are more effective than forages in stimulating development of the rumen (Hibbs et al., 1956; Warner et al., 1956). The VFAs produced from carbohydrate fermentation are at least partly responsible for development of ruminal papillae and the corresponding epithelium, with limited studies suggesting that butyrate is the most effective, followed by propionate, and acetate least effective (Flatt et al., 1958; Sander et al., 1959; Tamate et al., 1962). The actual cellular mechanisms responsible for stimulation of rumen epithelial development by fermentable substrates remain unresolved (Baldwin and Connor, 2017). Small amounts of chopped or ground forage can help prevent acidosis and parakeratosis if the concentrate particles are too small (Brownlee, 1956). Forage also stimulates muscle growth and rumen volume (Flatt et al., 1958). Calves raised in pasture systems likely rely on sugars and fructosans in fresh grass as the initial fermentative substrates.

Calves raised as herd replacements should be encouraged to eat starter from the first week of life. Starter should be kept clean and fresh and physically separated from water to avoid cross-contamination. Because starter provides the fermentable substrate for the developing ruminal microbiota, the most important factors are palatability and content of fermentable carbohydrates. Starter formulations have ranged between <20 percent and >40 percent starch, supplied mainly from cereal grains. Corn generally has promoted the greatest intake and resulted in the greatest ADG, with oats, barley, wheat, rice, and sorghum grains also used (Khan et al., 2016). Oats were an acceptable substitute for corn, but molasses and soy hulls resulted in decreased ADG (Hill et al., 2008d). Compared with oats or barley, corn and wheat resulted in greater DMI, higher rumen pH, increased papillae length, and heavier rumens (Khan et al., 2008). Under most situations, starter formulations will contain between 22 percent and 38 percent starch (see Table 10-16). Studies have examined the effectiveness of grain-processing methods, including steam-flaking,

TABLE 10-16 Example Nutrient Specifications for Typical Starter Varying in CP and Starch Content

Variables	Units	16% CP, Low Starch	18% CP, Low Starch	18% CP, High Starch	18% CP, High Starch	22% CP, Moderate Starch	22% CP, High Starch
DM	% As fed	87.7	87.5	88.7	86.3	87.8	89.0
Starch	% DM	15.1	20.7	36.9	39.0	25.5	32.9
CP	% DM	18.8	20.0	18.7	20.2	24.7	25.0
ADF	% DM	10.1	14.2	7.9	7.6	9.4	7.0
NDF	% DM	24.8	29.5	18.9	15.9	16.3	13.7
NDFD48	% NDF	49.1	60.0	53.1	55.4	65.3	59.6
Lignin	% DM	2.91	2.19	2.09	1.83	1.61	1.61
Ash	% DM	8.0	9.1	8.3	7.9	7.0	8.8
Starch	% DM	15.1	20.7	36.9	39.0	25.5	32.9
Water-soluble carbohydrates	% DM	4.2	3.5	8.2	7.2	12.0	9.2
Crude fat	% DM	6.9	5.1	3.9	3.6	3.3	3.2
DE, base	Mcal/kg	2.67	3.21	3.22	3.19	3.62	3.38
ME	Mcal/kg	2.48	2.99	2.99	2.97	3.37	3.14

grinding, cracking, and rolling, but with variable effects on calf performance (Abdelgadir et al., 1996b; Lesmeister and Henrichs, 2004).

The concept of palatability can be extended to address "appetence" or the actual preference to consume a given ingredient or mixed feed. While molasses is generally considered to be a palatable feed ingredient that may promote intake, excessive molasses (12 percent of DM) decreased starter intake compared with a formula containing 5 percent molasses (Lesmeister and Heinrichs, 2005). Miller-Cushon et al. (2014a,b) conducted extensive pairwise preference tests of energy and high-protein ingredients. They found that wheat meal was the highest-ranked feed type for preference to be consumed, followed by sorghum meal. Barley meal and corn meal were equally ranked, falling below wheat meal and sorghum meal but above all other feed types. Corn gluten feed and rice meal were ranked lowest. According to this method of comparison, soybean meal was the highest-ranked high-protein ingredient, followed by dried distillers grains. Corn gluten meal was the lowest ranked, followed by rapeseed meal. The preference for ingredient mixtures followed the rankings of individual ingredients (Miller-Cushon et al., 2014a,b). Montoro et al. (2012) determined that calves consumed similar total DM but different ratios of ingredients when they were offered separately compared with when they were provided as a mixed starter feed. Providing chopped grass hay promoted greater feed intake and digestibilities compared with providing the same amount of the hay in ground form (Montoro and Bach, 2012).

Digestion of NDF is negligible in very young calves. During the first week of life, before initiation of starter intake, ruminal pH ranges between 6.0 and 6.3 but then rapidly falls as starter intake increases (Anderson et al., 1987a). Although high-starch starter formulas (>32 percent) provide the most digestible energy, the resulting rumen pH is often very low (<5.5; Anderson et al., 1987a; Williams and Frost, 1992), in ranges that would be considered detrimental for mature ruminants. The low rumen pH results from the rapid fermentation of starch, the slower VFA absorption rates by the immature epithelium, and the low rate of saliva production in preruminants (Williams and Frost, 1992). Starter formulas with less starch or more slowly fermented starch and more digestible fiber can help to prevent the drastic drop in ruminal pH and help maintain pH greater than 6.0, allowing fibrolytic bacteria to become established and functional and help maintain rumen health. Good sources of readily fermentable fiber include beet pulp, brewers grains, soy hulls, and citrus pulp (Suárez et al., 2006; Porter et al., 2007; Hill et al., 2016a; Oltramari et al., 2018). Small amounts (<15 percent) of forage fiber, such as alfalfa meal, ground grass, or chopped grass hays, can be included to help buffer the rumen and to provide abrasive effects to help keep the keratin layer of epithelium thin so that absorption is maximized (Greenwood et al., 1997).

Starter DE and ME are calculated as described earlier using NDFD48, which as a concept has not been applied previously to calves. The NDFD48 values may not be accurate for calves,

but they serve to provide a relative measure of the fermentation characteristics of various NDF sources. The committee chose a convenience sample of 17 studies that measured NDF digestibility in calves (Spanski et al., 1997; Khuntia and Chaudary, 2002; Terré et al., 2006; Porter et al., 2007; Castells et al., 2012, 2015; Ghassemi Nejad et al., 2012; Montoro et al., 2013; Chapman et al., 2016, 2017; Hill et al., 2016a,b; Ghasemi et al., 2017; Dennis et al., 2018; Mojahedi et al., 2018; Quigley et al., 2018; Hu et al., 2019). Digestibility was determined when calves were consuming both milk or MR and starter before weaning (n = 23 treatment means), immediately after weaning (n = 39 treatment means), or in calves at least 3 weeks postweaning (n = 26 treatment means). Digestibility of NDF averaged 32.0 percent (range, 4.8 to 69.3 percent; coefficient of variation (CV) = 81.2 percent) in preweaned calves, 42.6 percent (range, 4.6 to 71.2 percent; CV = 34.2 percent) in recently weaned calves, and 57.6 percent (range, 42.3 to 70.7 percent; CV = 15.4 percent) in weaned calves. Thus, NDF digestibility increased with age and time after weaning, but the variability of results demonstrates that many factors must affect digestibility in addition to the nature of the NDF source. These may include starter intake, milk or MR intake, environment, and individual animal variability. Quigley et al. (2018) demonstrated that cumulative intake of NDF was a major predictor of NDF digestibility, with digestibility increasing sharply until approximately 2 kg of cumulative NDF intake and then beginning to plateau with additional intake. As described earlier, users can select to discount the ME value of starter for cases where NDF digestibility is expected to be depressed, such as when calves have been fed high amounts of milk or MR.

While a common recommendation is that NDF content of the starter should be >13 percent of DM, the physical form of the starter and its relationships with bedding material and forage provision are also important (Khan et al., 2016). Terre et al. (2013) reported that calves bedded on sawdust and fed a pelleted starter with 18 percent NDF had greater ADG than a pelleted starter with 27 percent NDF, regardless of whether calves were offered chopped forage. The physical characteristics of the starter mix are important to prevent anatomic or physiological abnormalities in the developing rumen. A minimum particle size is necessary to prevent parakeratosis of the rumen epithelium and impaction of fine particles among papillae (McGavin and Morrill, 1976; Greenwood et al., 1997). Studies suggest (Warner, 1991; Hill et al., 2008c; Porter et al., 2007) that at least 80 percent of the particles in a complete starter should be greater than 1,190 μm, and the starter should have a weighted mean particle size of approximately 2,000 μm or greater to prevent parakeratosis and bloat. The guidelines hold true regardless of the physical presentation of the starter (ground, mash, pellet, multiparticle, or texturized) since pellets would dissociate once hydrated in the rumen, depending on pellet hardness. Particle size determined by wet sieving techniques probably represents the true particle size availability in the rumen after consumption by the calf.

The optimal physical form is widely debated in the field, but research studies show few repeatable effects when confounding factors are controlled (Bateman et al., 2009). Porter et al. (2007) reported greater ADG, starter intake, and earlier rumination in calves fed a coarse mash (meal) starter compared with the same ingredients in pelleted form after being ground to fine particle size. Franklin et al. (2003) reported less starter intake and decreased ADG in calves fed a pelleted starter compared with those fed a textured starter, with calves fed the textured starter having greater intakes and ADG than either pelleted or meal forms; unfortunately, the ingredient composition was not identical among starter physical forms. Other studies also reported lower intakes and ADG when starters were fed as finely ground meals rather than as pelleted meals or coarse particles as a mash or textured (Lassiter et al., 1955; Gardner, 1967; Kertz et al., 1979), but experimental details were limited. Beharka et al. (1998) fed a diet of 25 percent alfalfa and 75 percent concentrate, either as coarse particles or finely ground. Ruminal pH was greater and papillae in the dorsal area of the rumen were longer for calves fed the coarse diet. Coverdale et al. (2004) restricted starter intake and reported that ADG was greater for calves fed a textured starter compared with a finely ground starter, but no difference was detected when calves had ad libitum access to the starters. Bach et al. (2007) fed diets of the same composition either as a pellet or a coarse mash and starter intake was lower for the pelleted diet, although this resulted in increased gain to feed ratio. Kertz (2007) questioned this study because no mention of the impact of bedding consumption was provided for calves fed the pelleted diet, which could confound the conclusions about effects of physical form and particle size. In a follow-up study, growth and intake were not different between pelleted and textured starters of the same ingredient composition when fed with forage to ensure a favorable rumen environment in calves bedded on sawdust (Terre et al., 2015). Hill et al. (2012) concluded that high-starch, low-fiber textured starters provided the greatest DMI and ADG for weaned calves between 2 and 4 months of age.

This body of research has tended to encourage the conclusion that calves should not be fed finely ground starters or pelleted starters without some forage when calves are not bedded with straw. However, Castells et al. (2015) reported high intakes of ground starter when provided with free access to chopped hay in calves bedded on wood shavings. Pazoki et al. (2017) found that calves bedded on sand and fed a fine meal with the addition of 10 percent chopped alfalfa outperformed calves fed the same starter as a pellet or as a textured mixture but without forage. Bateman et al. (2009) found that calves fed a pelleted starter with half of the DM provided as a fine meal had decreased intakes and ADG compared with calves fed only the pelleted form. Thus, the inconsistency of the physical form (e.g., pellets with abundant fines or coarse and fine particles together) may be what inhibits intake and performance, as long as sufficient particle size is provided by forage or bedding.

A variety of protein sources are used in starters, including soybean meal, canola meal, cottonseed meal, sunflower seed meal, linseed meal, corn gluten meal, and distillers dried grains (Khan et al., 2016). Soybean meal is the most widely used among any of the common proteins (Drackley, 2008). Attempts to increase MP by supplementing protein sources high in RUP generally have been ineffective in increasing ADG of calves (Drackley, 2008). In contrast, increasing soybean meal increased both MP supply and calf growth around weaning (Stamey et al., 2012).

The required protein content of the starter often has been taken out of context from the calf's growth rate and the amount of milk being fed before weaning. A CP content of 18 percent (DM basis) may be adequate for systems where 0.45 to 0.55 kg/d of milk or MR DM is fed (Akayezu et al., 1994; NRC, 2001), but when feeding 0.9 kg/d or more of milk or MR DM, starter CP content of 22 to 25 percent of DM may result in increased growth (Stamey et al., 2012; Stamey Lanier et al., 2021). Starter CP content should be consistent with requirements calculated elsewhere in this chapter. For example, small-breed calves growing rapidly may need to be fed a starter containing >22 percent CP depending on the protein content and amount of the MR fed (see Table 10-8).

Supplemental fat has been added to starter in an attempt to increase energy density and improve calf growth. Supplementing specific functional FAs was discussed in an earlier section. Fat addition to starter has generally shown few or inconsistent effects, either before or after weaning (Khan et al., 2016). Johnson et al. (1956) fed up to 10 percent tallow in calf starter and reported that DMI and ADG were not affected. Miller (1962) compared starters with 10 percent added fat from tallow, lard, butter, and hydrogenated cottonseed oil. Intake of starter and ADG did not differ among treatments. In contrast, Miller et al. (1959) showed that feeding 10 percent brown grease or hydrogenated cottonseed oil significantly decreased starter intake. Araujo et al. (2014) fed full-fat soybeans to supply 11 percent fat in the starter DM and observed no effects on DMI, in contrast to an earlier study (Kuehn et al., 1994) that reported negative effects of starter containing 7 percent added fat from soybeans. Hill et al. (2015) determined that supplementation of starters with 2 percent tallow or soybean oil decreased ADG. Berends et al. (2015) fed an extruded pellet that contained hydrogenated palm FAs and increased starter fat content to 7 percent. They reported increased starter intake and ADG with the higher-fat starter. Ghasemi et al. (2017) supplemented 3 percent fat from tallow, soybean oil, palm fat, or a mixture of palm, soybean oil, and fish oil. Inclusion of soybean oil increased calf performance, but palm fat and tallow did not. Doolatabad et al. (2020) fed a starter containing 7.5 percent fat from full-fat soybeans and prilled palm fat and noted no improvements in calf growth. Ghorbani et al. (2020) provided 2 percent fat from soybean oil or extruded or heated soybeans. Growth rate of calves was not affected. Overall, the variability in responses among

studies indicates that more research is needed on factors affecting responses to fat.

In summary, starter composition and physical form are areas that still need more careful research attention, particularly for calves at higher milk feeding rates. Researchers must control and report factors such as whether the calves were housed on straw or other organic bedding, the particle size distribution of the starter or of the ingredients before pelleting, whether calves received any forage, and the particle size distribution of that forage.

Forage Provision

For many years, feeding forage prior to weaning was not recommended, and only very limited amounts were recommended after weaning. This recommendation was based on studies showing that ad libitum forage availability decreased concentrate consumption, which was key to rumen development (Tamate et al., 1962). In calves fed a limited amount of concentrate, greater forage intakes decreased EBW gain (Stobo et al., 1966). Forage is not well used by the young calf because of the limited rumen functionality to allow for forage fermentation (Anderson et al., 1987a; Khan et al., 2011b). However, recent research has challenged these assertions and indicates that the blanket recommendation to not feed forage is too simplistic.

When assessing the value of forage, several confounding factors must be considered, including particle size and physical form of the starter, type and amount of forage, whether the forage is offered separately or as a part of a total mixed ration (TMR), and whether the calf is bedded on straw or other organic bedding (Kertz, 2007). A meta-analysis (Imani et al., 2017) that did not consider bedding type found that improvement in overall starter intake was greater for calves offered alfalfa hay compared with those offered other types of forages (ryegrass hay, oat hay, barley straw, triticale silage, or corn silage). This analysis also found that ADG was greater for calves fed >10 percent of DM as forage compared with those fed ≤10 percent of DM as forage. However, the authors stated that the advantages in BW gain at the higher amount of forage provision could be due to increased gut fill. Increases of ADG were less for calves fed forages with textured starter compared with those fed forage with ground starters (Imani et al., 2017).

Calves bedded on straw and fed a textured starter with an adequate particle size had decreased starter intake and ADG when fed grass hay or cottonseed hulls (Hill et al., 2008b). In calves bedded on sawdust and fed large amounts of milk (8 kg/d) and a textured starter of undescribed particle size, providing ad libitum access to grass hay increased rumen pH and empty rumen weight without significant effects on EBW or stature measurements (Khan et al., 2011b). In contrast, for calves bedded on sawdust and fed a pelleted starter, ad libitum access to different forages (except alfalfa hay) increased starter intake and ADG (Castells et al., 2012). In that study, alfalfa hay was consumed in the largest amount

(14 percent of total DMI) and reduced starter intake. Oat hay was consumed at 8 percent of total DMI but stimulated starter intake. The other forages, including barley straw, ryegrass straw, triticale silage, and corn silage, were consumed only in limited amounts (4 to 5 percent of DM) but also stimulated starter intake. For calves bedded on sawdust and fed pelleted starters with either 18 or 27 percent NDF, providing access to chopped oat hay did not affect intake or ADG before weaning but increased both postweaning (Terré et al., 2013). Terré et al. (2015a) demonstrated the interactions between starter physical form and access to chopped oat hay in calves bedded on sawdust.

One reason for not recommending feeding forage was that the low digestibility would increase gut fill, which would be measured as ADG (Stobo et al., 1966). Calves consuming large volumes of milk and offered long grass hay for ad libitum intake showed an increase in gut contents (Khan et al., 2011b). In contrast, calves with ad libitum access to chopped oat hay consumed 4 percent of their total DMI as forage and did not have any change in gut contents, compared with calves in the same study that had ad libitum access to alfalfa hay and consumed 14 percent of their total DMI as forage (Castells et al., 2013). In calves in whom chopped alfalfa hay was provided in the starter mix, no difference in gut contents was observed (Pazoki et al., 2017). Overall, increases in gut fill are more likely when feeding large amounts of forage (about >15 percent of total intake) or when alfalfa is fed rather than grasses.

In summary, current evidence indicates that calves fed textured starters of adequate particle size and bedded on chopped straw likely will obtain little benefit from forage provision, and ADG may be decreased. However, calves fed pelleted starters and not bedded with straw (or bedded with long straw) should be fed some chopped forage to maintain rumen environment and promote starter intake. Alfalfa hay should be limited to no more than 10 percent of total DMI. Other chopped forages should either be provided in small amounts for ad libitum intake or included in the starter or TMR at no more than 5 percent of total DM.

Weaning Management

Recommended weaning time is often based on a set amount of starter consumption on a consistent basis (e.g., large-breed calves can be weaned when consuming at least 0.9 kg of DM from a good-quality starter daily for 3 consecutive days) (Drackley, 2008). These recommendations assume that intake of starter will increase rapidly once milk feeding is reduced or eliminated (Stamey et al., 2012) so that slumps in growth rate are minimal and short-lived. However, the nutritional adaptation that must take place for the calf to wean successfully with minimal challenge to health and well-being is substantial. Table 10-17 shows EBW gains predicted by the equations in this chapter for calves before and after weaning for different amounts of starter intake. The drastic differences between

TABLE 10-17   Predicted EBW Gains for Calves (Small-Breed and Large-Breed Calves Are Similar) of Equal EBW Before and After Weaning[a]

Preweaned Calf					Weaned Calf			
BW, kg	EBW, kg	Milk Replacer DMI, kg/d	Starter DMI,[b] kg/d	EBW Gain, kg/d	BW, kg	EBW, kg	Starter DMI,[b] kg/d	EBW Gain, kg/d
60	55.8	0.25	0.75	0.20	65.6	55.8	0.75	–0.16
60	55.8	0.25	1.25	0.52	65.6	55.8	1.25	0.14
60	55.8	0.25	1.75	0.83	65.6	55.8	1.75	0.45
60	55.8	0.25	2.25	1.14	65.6	55.8	2.25	0.76
80	74.4	0.25	1.0	0.24	87	74.4	1.0	–0.15
80	74.4	0.25	1.5	0.54	87	74.4	1.5	0.14
80	74.4	0.25	2.0	0.84	87	74.4	2.0	0.51
80	74.4	0.25	2.5	1.13	87	74.4	2.5	0.724

[a]Note that because of gut fill, EBW makes up a smaller proportion of BW after weaning than before weaning.
[b]Starter ME assumed to be 3.1 Mcal/kg.

preweaned and weaned calves arise from the differences in calculation of maintenance. These differences would likely be less in practice because the adjustments in gut size will be gradual. Nevertheless, the calculations make clear that for a period of a few days, weaned calves will struggle to gain BW at anywhere near the rate before weaning. Consequently, health and well-being will be put at risk (Williams et al., 1981; Griebel et al., 1987; Pollock et al., 1993, 1994). Based on these reasons, the committee recommends that small-breed calves be consuming at least 1.25 kg/d (assumed 90 percent DM) of starter and large-breed calves at least 1.5 kg/d before complete weaning. These higher thresholds of starter intake for weaning should help ease the weaning transition for calves fed larger amounts of milk and growing faster before weaning.

With restricted milk or MR feeding under good management, successful weaning has been reported as early as 4 weeks of age (Hodgson, 1965; Kertz et al., 1979, 1984). Early weaning (24 days) suppressed some aspects of the innate immune system relative to weaning at 45 days (Hulbert et al., 2011). More aggressive milk-feeding programs will delay development of starter intake and weaning age (Hodgson, 1971; Huber et al., 1984), and weaning at an older age will help ease the transition (Hodgson, 1965; de Pasillé et al., 2011; Eckert et al., 2015; de Pasillé and Rushen, 2016). Gradual weaning over a period of 4 to 10 days is recommended rather than abrupt weaning (Sweeney et al., 2010).

Feeding Frequency

Williams et al. (1986) compared feeding frequencies of one, two, four, or six times daily for calves fed either 0.55 or 0.86 kg of DM daily of MR containing skim milk. Heat production, energy retention, respiratory quotient, and ADG were similar among feeding frequencies. Research conducted during the 1960s and 1970s (Appleman and Owen, 1975; Otterby and Linn, 1981) demonstrated that once-daily feeding resulted in mainly similar performance relative to twice or more daily feedings. More recent studies have confirmed those findings (Kehoe et al., 2007). A concern with once-daily feeding is that observation of calves may be less frequent and early signs of disease might be missed if management is not optimal (Davis and Drackley, 1998).

For calves fed larger amounts of liquid feed, increasing feeding frequency may improve efficiency of nutrient use. Strzetelski et al. (2001) tested feeding frequencies of one, two, and three times daily at either limited feeding or feeding to appetite of an MR containing dried skim milk, whey, buttermilk, and processed soy proteins. Calves fed limited MR two and three times daily and calves fed to appetite three times daily had greater weight gains and lower starter consumption than calves fed once daily. More frequent feedings may prevent abomasal ulcers (Ahmed et al., 2002). Efficiency of both protein and energy use was improved by increasing feeding frequency of an MR containing only whey proteins from two to four times daily, particularly when fed at 2.5 times maintenance compared with 1.5 times maintenance (van den Borne et al., 2006). In contrast, Kmicikewycz et al. (2013) found no benefit to increasing to four feedings daily compared with two feedings daily.

In summary, in calves fed low or moderate amounts of liquid feed, feeding more than twice daily had no repeatable effect on ADG or health. At higher feeding rates (≥2.5 times maintenance), increasing the number of feedings daily may improve efficiency of nutrient use, especially for calves fed nonclotting MR. More frequent feedings also may help abomasal health. Calves may benefit from an extra feeding (i.e., greater total daily intake) when housed outside during cold weather (Schingoethe et al., 1986).

Group Housing and Automated Calf Feeders

For many years, individual housing in hutches or stalls has been the gold standard in the dairy industry (Callan and Garry, 2002), but interest in group housing and automated feeders has increased in recent years (USDA, 2016). Resistance to

group housing arises from concerns about calf health and veterinary recommendations to prevent calf-to-calf contact (Callan and Garry, 2002). Early studies found that calves housed individually had lower morbidity and mortality rates (Waltner-Toews et al., 1986a,b). However, later larger-scale, observational studies found no differences in health between individually housed calves and calves housed in small groups of six to eight (Losinger and Heinrichs, 1997; Svensson et al., 2006). Individual housing is criticized for restricting physical movement and social interaction among calves and faces increasing public opposition (Rushen et al., 2008).

Group housing offers several potential advantages for calf growth and welfare (Costa et al., 2016). Housing calves in groups allows social interaction and more normal behaviors than individual housing (Chua et al., 2002). Group housing can facilitate transition to solid feed, leading to better post-weaning weight gains (de Paula Vieira et al., 2010; Costa et al., 2015; Miller-Cushon and DeVries, 2016). Group housing also decreases labor for feeding and management (Nordlund, 2008). Concern remains, however, that group housing can result in more disease spread among calves, particularly if ventilation is poor (Lago et al., 2006; Nordlund, 2008). Larger group size (>8 calves per pen) may increase risk for mortality and respiratory disease (Losinger and Heinrichs, 1997; Svensson and Liberg, 2006; Svensson et al., 2006). All-in, all-out systems have lower risk for mortality and morbidity than continuous-flow systems (Pedersen et al., 2009).

Automated feeders are becoming widely used because of inherent advantages in labor allocation and the ability to feed more milk or MR to calves in several meals per day. While direct comparisons of the systems are limited, the autofeeder system has resulted in similar performance and was cost-effective (Kung et al., 1997; Kack and Ziemerink, 2010). The computer control systems can simplify daily changes in milk feeding amounts, leading to peak milk consumption and weaning. In addition, the computer collects information about feeding behavior, which can be used to alert the producer to changes than may signal onset of disease (Svensson and Jensen, 2007; Knauer et al., 2017). The systems can reduce mortality to below-national averages (Jorgensen et al., 2017b), but as with any technology, management affects success. This includes fundamental management practices such as care of the newborn calf, excellent colostrum management, and limiting exposure to pathogens, particularly those in the liquid diet (Jorgensen et al., 2017b). Other key issues relate to the facility, because many autofeeders are installed in retrofitted existing structures. Adequate ventilation is critical to success and must be addressed when retrofitting (Nordlund, 2008; Jorgensen et al., 2017c). Air should not be shared with older cattle, which is associated with increased incidence of diarrhea and respiratory disease (Medrano-Galarza et al., 2018b). Bedding must be kept clean and dry, as wet bedding packs were associated with increased incidence of respiratory disease (Medrano-Galarza et al., 2018b), and the areas around the feeders should be cleaned daily (Jorgensen et al., 2017c).

Of particular importance is proper cleaning and sanitation of the feeder, including the mixing chamber, milk lines, and nipples (Jorgensen et al., 2017a,b,c; Medrano-Galarza et al., 2018b).

Milk, pasteurized waste milk, or MR can be fed successfully through autofeeders. The feeding program and feeding management are critical. Early introduction (within 24 hours after birth) to the feeder compared with the more common practice of introducing calves at 5 to 14 days after birth results in both better outcomes for the calf and less total labor per calf (Medrano-Galarza et al., 2018a). Feeding larger volumes of milk per calf (>6 L/d) decreases feeder occupancy and thereby decreases competition in the pen (Jensen, 2006; Borderas et al., 2009). Shortening the time to peak milk intake by the calf has positive effects on growth and health (Jorgensen et al., 2017a; Medrano-Galarza et al., 2018a). Consumption of solid feeds from computer-controlled feeders can be used (de Passillé and Rushen, 2016) to monitor dry feed intake and adjust milk allowance downward for easier weaning (de Passillé and Rushen, 2012).

OTHER ASPECTS OF CALF NUTRITION

Fetal Nutrition

The developing fetal calf requires a balanced supply of nutrients from the mother via the placenta throughout gestation, but quantitatively fetal nutrient demands become significant only during the last trimester. More than 60 percent of total fetal weight gain occurs during the last 60 days of gestation (Eley et al., 1978) and is linear during that period through 270 days of pregnancy (Bell et al., 1995). Most of the carbon and N for fetal growth and energy supply comes from glucose, AAs, and lactate; the latter arises from glycolytic metabolism in the placenta (Reynolds et al., 1986). Glucose supplies approximately half of the energy needs of the conceptus, with 30 to 40 percent of respiratory fuel provided by AAs (Bell, 1995).

Although severe undernutrition can impair normal fetal development (NRC, 1968), the developing fetus is afforded a high priority for maternal nutrients. Moderate underfeeding or overfeeding of either protein or energy during the dry period (last 2 months of gestation) did not result in significant changes in calf birth weight (Nocek et al., 1983; Grum et al., 1996; Dewhurst et al., 2002; Dann et al., 2006; Douglas et al., 2006; Silva-Del Rio et al., 2010; Janovick and Drackley, 2010; Litherland et al., 2012; Mann et al., 2015). Likewise, neither viability nor health of newborn calves were affected by moderate maternal under- or overfeeding during the dry period (Davis and Drackley, 1998; Quigley and Drewry, 1998). Prolonged restriction of protein or energy during gestation decreased thermogenic abilities of beef calves at birth (Carstens et al., 1987; Ridder et al., 1991). Micke et al. (2010) found that overfeeding both energy and protein during the middle trimester of pregnancy in beef cattle resulted in

greater calf birth weight than overfeeding energy with protein limited to 63 to 75 percent of requirement.

Maternal deficiencies of P, Mn, Co, Cu, Zn, or Se can result in deficiencies in the fetus and newborn calf (NRC, 1968). The fetus can concentrate some of these minerals, particularly Cu (Hidiroglou and Knipfel, 1981) and Se (Van Saun et al., 1989a), providing some protection against marginal deficiencies in the mother. Se supplementation of pregnant cows increased Se reserves in the newborn calves (Abdelrahman and Kincaid, 1995). Placental transfer of vitamin E to the developing fetus is low, although the fetal calf appears to have some ability to concentrate vitamin E from the dam (Van Saun et al., 1989b). The calf is born with a low vitamin E status and is highly dependent on intake of colostrum and then milk or MR to obtain needed vitamin E during early postnatal life. This is also true for retinol (Nonnecke et al., 1999) and vitamin D (Nonnecke et al., 2009). Overall, if diets for pregnant cows are balanced to meet recommendations for pregnancy and maternal growth (see Chapters 7 and 8), as well as for optimal transition success (see Chapter 12), nutrient supply should be adequate for normal growth and development of the fetal calf (Davis and Drackley, 1998; Quigley and Drewry, 1998).

Supplemental fats fed to the dam during the dry period may affect the developing fetal calf. Garcia et al. (2014a,b) fed either mostly saturated free FAs or Ca salts of unsaturated FAs enriched in C18:2 *n*-6 during the last 4 weeks of gestation. FA composition of colostrum and calf plasma reflected the composition of the fat supplement. Elongation and transfer of *n*-3 FAs by the placenta were decreased, but elongation and transfer of *n*-6 FA were increased. Differences in calf plasma FA profiles persisted through at least 60 days of age (Garcia et al., 2014b). Birth weight was increased by fat supplements for calves from parous dams but not from nulliparous dams. Apparent efficiency of immunoglobulin G (IgG) absorption from colostrum was greater for calves from dams fed fat, especially those fed saturated FAs (Garcia et al., 2014a). Calves from dams fed saturated FAs before parturition tended to have higher ADG; health or immune measures were not affected (Garcia et al., 2014b). Whether a mixture of FAs can be identified that will benefit newborn calves when fed to the cow in late gestation remains to be determined.

Feeding rumen-protected Met to cows during the last month of gestation increased Met concentration in maternal plasma by 29 percent and increased calf birth weight, perhaps related to increased expression of placental genes encoding transporters for neutral AAs and glucose, as well as increasing mTOR protein abundance (Batistel et al., 2017). Rumen-protected Met fed during the last 28 days of gestation also increased calf birth weight and ADG through 9 weeks of age (Alharthi et al., 2018). Methylation and demethylation of cytosine moieties in DNA modulate expression of many genes; some of these changes may alter the phenotype of the offspring. No measures of DNA methylation were made in these studies, but results are consistent with possible epigenetic effects of methionine (Chavatte-Palmer et al., 2018).

Colostrum

Calves are born with a naive immune system and essentially devoid of circulating immunoglobulins (Ig) due to the synepitheliochorial placenta of ruminants. Ig from colostrum (defined as the first lacteal secretion produced by the cow after calving) consumed prior to cessation of macromolecular transport, at approximately 24 hours after birth, are absorbed into intestinal cells via nonspecific pinocytosis and delivered intact to the circulation. Colostrum contains large, but variable, amounts of Ig, particularly IgG, which are transported from the maternal circulation into the colostrum during the final 3 weeks of gestation. Intake of adequate, high-quality (>50 g IgG per liter), and sanitary colostrum by the newborn calf is one of the most, if not the most, important factors related to reduced calf morbidity and mortality (Nocek et al., 1984; Wells et al., 1996; Godden et al., 2019).

Serum IgG concentration is an indicator of consumption and quality of colostrum. Serum IgG concentrations ≥10 g/L, when measured at 24 to 48 hours of age, are associated with lower risks of morbidity and mortality (Wells et al., 1996; Windeyer et al., 2014; Godden et al., 2019). To achieve a serum IgG concentration of 10 g/L, calves should be fed 150 to 200 g IgG from colostrum or colostrum replacement products in the first 24 hours. Concentration of IgG in the serum of a calf may be estimated using the following formula (Quigley and Drewry, 1998): Serum IgG (g/L) = IgG intake (grams) × AEA / PV, where AEA is apparent efficiency of IgG absorption (%), and PV = plasma volume (liters). The AEA is calculated as AEA (%) = serum IgG (g/L) × PV (liters) / IgG intake (grams).

The AEA of ingested IgG is affected by many factors related to the calf, stress at calving, environmental factors, and characteristics of the IgG source (Quigley and Drewry, 1998; Godden, 2008; Godden et al., 2019). The most important factor affecting AEA is age of the calf. The AEA declines with advancing age, maturation of the gastrointestinal tract, and turnover of intestinal cells capable of pinocytosis of macromolecules, so that by 22 to 24 hours after birth, the calf is no longer able to absorb IgG into the bloodstream (Bush and Staley, 1980; Kruse, 1983). This phenomenon is known as "gut closure." Penhale et al. (1973) concluded that absorption declines gradually and progressively to closure, which occurs independently for each class of Ig (16, 22, and 27 hours for IgM, IgA, and IgG). Stott et al. (1979) estimated time of closure by Joinpoint regression with data from 210 calves. Closure was estimated near 24 hours of age with a normal distribution and standard deviation of approximately 4 hours, and the authors found no significant differences in closure time for IgG, IgM, and IgA. Estimated time for closure was delayed as feeding time was delayed; nevertheless, closure

occurred spontaneously with age at a progressively increased rate after 12 hours postpartum.

Typical AEA ranges from <10 percent to approximately 40 percent. Ig not measured in the circulation may move into extravascular pools (MacDougall and Mulligan, 1969) or migrate into the intestine (Besser et al., 1998), thereby limiting AEA to approximately 50 percent (McEwan et al., 1970). Plasma volume is related to body size and is typically estimated using BW and averages about 8.9 percent of BW for Holstein and Jersey newborn calves (Quigley et al., 1998; Cabral et al., 2015).

Concentration of IgG in colostrum is highly variable (Morrill et al., 2012), and this variation contributes significantly to variation in serum IgG concentration and failure of calves to achieve satisfactory serum IgG concentration. In a survey of calf management practices on U.S. dairy farms, Urie et al. (2018a) reported serum IgG in 2,498 calves fed varying sources and amounts of colostrum or colostrum replacers, and most calves (72.7 percent) had serum IgG >15 g/L but 14.3 percent had serum IgG between 10 and 14 g/L, and 13 percent of calves had serum IgG <10 g/L, which is considered failure of passive transfer of immunity. Management factors associated with greater serum IgG concentrations were feeding a greater mass of IgG from colostrum and feeding colostrum at an early age (Shivley et al., 2018).

Time after parturition has a significant effect on IgG concentrations in colostrum. Concentrations of IgG were 27 percent lower in colostrum collected 10 hours postparturition compared with colostrum collected 2 hours postpartum (Moore et al., 2005). The IgG concentration in second-milking (12 hours later) colostrum from Jersey cows was 44 percent lower than that in first-milking colostrum (Silva-del-Río et al., 2017). Greater yields of colostrum are associated with lower concentrations of IgG (Pritchett et al., 1991; Silva-del-Río et al., 2017). Lesser factors affecting Ig concentrations are parity (increased concentrations with increasing parity; Muller and Ellinger, 1981; Kehoe et al., 2011) and perhaps breed (Muller and Ellinger, 1981; Morrill et al., 2012). Nutrition of the late-gestation cow does not appear to have much effect on Ig concentrations in colostrum (Quigley and Drewry, 1998; Dunn et al., 2017) but research data are limited. Responses to dietary protein fed to the dam on absorption of colostrum Ig by the calf are inconsistent (Quigley and Drewry, 1998). Some studies reported that low dietary protein fed to the late-gestation dam reduced Ig absorption by the calf, but other studies found no effect. Late-gestation heifers that were exposed to heat stress produced colostrum with lower concentrations of IgG (Nardone et al., 1997), whereas heat-stressed late-gestation multiparous cows produced colostrum with about 10 percent greater concentration (not statistically different) of IgG (Tao et al., 2012). Accumulating evidence suggests that maternal heat stress in late gestation reduces the immunocompetence of the neonate partly because of reduced absorption of colostral IgG (Tao and Dahl, 2013). Cabral et al. (2016) found that colostral IgG concentration could be predicted from previous lactation Dairy Herd Improvement Association data and weather data.

Amount of colostrum to feed in the first feeding and first 24 hours of life necessarily depends on concentration of IgG in the colostrum. A reasonable goal is to feed 150 g IgG in the first feeding and 200 g in the first 24 hours (Godden et al., 2019). Approximately 25 percent of first-milking colostrum contains too little IgG to provide >150 g IgG in a reasonable volume (e.g., ≤4 L in the first feeding; Morrill et al., 2012; Godden et al., 2019). To achieve >150 g IgG, colostrum containing greater than 50 g IgG per liter must be fed. A BRIX refractometer may be used to estimate the IgG concentration of colostrum and determine the optimal amount of colostrum to be fed (Bielmann et al., 2010; Quigley et al., 2013). The BRIX refractometer is inexpensive, and the method is reasonably accurate. The refractometer measures the refractive index (nD) of a solution and calculates BRIX percentage, based on the statistical relationship between nD and sugar in the solution. Table 10-18 has equations relating the BRIX reading to IgG; equations differ for colostrum from Holstein and Jersey cows (Morrill et al., 2015). The breakpoint is the BRIX reading at which colostrum contains at least 50 g IgG per liter. Holstein calves should be fed 3 L of colostrum with at least 22 percent BRIX within 1 hour of birth and 2 to 3 L fed at 10 to 12 hours of age. For Jersey calves, 2 L of colostrum with at least 18 percent BRIX should be fed within 1 hour after birth and again at 10 to 12 hours of age. Another approach is to administer 3.8 to 4.0 L of colostrum in the first feeding within 1 hour of age via an esophageal feeder with an optional second feeding of 2 L at 10 to 12 hours of age. Feeding a larger volume of colostrum in the first feeding generally increases absorption of IgG, as the calf is more efficient in absorbing IgG early in life. However, even with this approach, low BRIX colostrum should not be used. Conneely et al. (2014) compared effects on calf serum IgG concentrations when first-milking colostrum was fed within 2 hours of birth at 7.0, 8.5, or 10.0 percent of calf birth BW. Average first-milking colostrum intake for the three treatments was 2.6 L (range, 1.7 to 3.4), 3.2 L (2.0 to 4.2), and 3.8 L (2.4 to 4.9), respectively. Calf serum IgG concentrations (measured from 24 hours to 26 days of life) did not differ between calves fed colostrum at 7.0 or 10 percent of birth weight. However, calves fed colostrum at 8.5 percent of birth weight had significantly greater (approximately 15 to 25 percent) IgG concentrations than the other two groups at all sampling points. This study supports the 3-L feeding rate; however, the authors suggested that feeding 8.5 percent of birth weight rather than a fixed 3 L may enhance Ig absorption by reducing distension of abomasum in small calves and enhancing absomasal emptying.

Transition milk (milk produced during days 2 and 3 after calving) should be fed during days 2 and 3 if possible. Transition milk is higher in solids, protein, fat, and immunoglobulins compared to normal milk (Godden et al., 2019). Additional nutrients and other components of transition milk may promote intestinal development (Hammon and Blum,

TABLE 10-18   Regression Equations to Estimate IgG in Colostrum Using BRIX Readings[a]

Author	N	Breed	Intercept	Slope	R^2	BP[b]
Chigerwe et al., 2008	171	Holstein	−24.7	3.96	0.41	22
Bielman et al., 2010 (optical)	273	Holstein	−188.262	10.730	0.51	22
Bielman et al., 2010 (digital)	273	Holstein	−207.434	11.561	0.53	22
Morrill et al., 2012[c]	823	Mix[d]	−40.509	5.2358	0.53	18
Quigley et al., 2013	183	Holstein	−61.896	5.666	0.75	21
Bartier et al., 2015	460	Not reported	−29.257	3.8393	0.43	23
Morrill et al., 2015	58	Jersey	−49.292	6.0052	0.63	18
Løkke et al., 2016	126	Mix[d]	−58.00	4.82	0.66	22
Silva-del-Río et al., 2017	202	Jersey[e]	−53.3	5.5	0.58	21

[a] Equation: Colostrum IgG (g/L) = Intercept + slope × BRIX (%).

[b] Breakpoint = Recommended BRIX concentration (%) providing a minimum of 50 g/L IgG.

[c] BRIX values were calculated from refractive index, which was measured in the study.

[d] Samples from Holstein, Jersey, unclassified and pooled samples.

[e] First- and second-milking colostrum from multiparous Jersey cows only.

1997; Rauprich et al., 2000; Blättler et al., 2001) and provide local intestinal immunity to reduce the risk of infection (Berge et al., 2009; Chamorro et al., 2017).

The importance of the non-IgG compounds in colostrum is now receiving research attention. Concentration of fat in colostrum decreases as time after parturition increases, and the composition of the lipid fraction (e.g., higher concentrations of cholesterol and very long-chain FAs in colostrum compared to transition milk) also changes (Contarini et al., 2014). Changes in the lipid fraction may be important to calf health and development. Concentrations of several potentially bioactive compounds (e.g., cytokines and lactoferrin) are very high in first-milking colostrum but decrease rapidly after parturition, often by 70 to 80 percent within three milkings after parturition (Sobczuk-Szul et al., 2013). Colostrum contains elevated concentrations of some hormones (e.g., insulin, growth hormone), growth factors such as IGF-1, enzymes, nucleotides, and oligosaccharides (reviewed by McGrath et al., 2016; Ontsouka et al., 2016). High-quality colostrum is a good source of many vitamins (Foley and Otterby, 1978; Godden et al., 2019).

Nutritional manipulations have been investigated as ways to improve colostrum IgG content or absorption. Addition of supranutritional amounts of Se (3 mg per calf) as sodium selenite to colostrum increased IgG absorption in young calves (Kamada et al., 2007; Hall et al., 2014). Feeding dry cows Se-yeast (105 mg/d) tended to increase absorption of IgG by their calves (Hall et al., 2014). Supplementing prepartum cows with 48 g/d of nicotinic acid increased IgG concentrations in colostrum but did not affect absorption of IgG by calves (Aragona et al., 2016, 2019).

Factors affecting yield of first-milking colostrum have not been investigated extensively. Cows fed diets that provided approximately 95, 120, or 130 percent of their energy requirement (based on NRC, 2001) during the last 2 weeks of gestation produced statistically similar yields of first-milking colostrum (5.9, 7.0, and 7.3 kg; Mann et al., 2016). A prospective study on a Jersey farm in Texas determined that month of calving had the greatest effect on colostrum yield (lowest in winter, highest in summer); however, length of previous lactation and length of dry period (longer periods associated with reduced yield) also affected yield (Gavin et al., 2018). Primiparous cows had greater yield of colostrum than multiparous cows but only between late autumn and early spring. Kruse (1970) reported that multiparous cows produced more colostrum than primiparous animals, but variation in yield was extremely large. Colostrum yield may respond to genetic selection (Cabral et al., 2016; Gavin et al., 2018).

Bacterial contamination of colostrum, especially when stored and handled incorrectly, can be substantial (Houser et al., 2008; Morrill et al., 2012; Cummins et al., 2017), and high bacterial counts in colostrum can be detrimental to the calf (Godden et al., 2012; Cummins et al., 2017). Pasteurization of colostrum reduces bacterial counts without affecting Ig concentrations (Godden et al., 2006; Elizondo-Salazar and Heinrichs, 2009). Feeding heat-treated colostrum increases serum concentrations of IgG (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009) and reduces calf morbidity (Godden et al., 2012) compared to feeding raw colostrum.

Colostrum replacer products are widely available and are generally powders that contain a spray-dried source of bovine immunoglobulins, including whey, colostrum, or bovine plasma (Cabral et al., 2013). Some products are composed exclusively of spray-dried bovine colostrum, whereas others contain ingredients such as dry fat, vitamins and minerals, and emulsifiers. The mass of IgG per dose varies widely among products. Results generally suggest that AEA is similar to or less than maternal colostrum. Methods of manufacturing are important to quality of the product and influence the ability of the calf to absorb and use ingested IgG (Chelack et al., 1993; Campbell et al., 2007).

Performance of calves fed a colostrum replacer was improved compared to calves fed poor-quality colostrum (Aly et al., 2013), suggesting that a viable option for colostrum replacer management is to monitor colostrum quality and replace low IgG or contaminated colostrum with a colostrum

product that provides 150 to 200 g IgG. Other studies suggest that colostrum replacement products administered as a substitute for maternal colostrum result in lower levels of calf IgG but can confer adequate transfer of immunity provided that an Ig mass of 150 to 200 g is delivered in a timely manner (Lago et al., 2018; Desjardins-Morrissette et al., 2018; Shivley, 2018). Contribution of nutrients such as fat and non-Ig proteins may influence early life energy metabolism; therefore, compositions providing greater amounts of protein and fat in addition to IgG are more effective.

Water and Electrolytes

The importance of clean drinking water, available from the first few days after birth, cannot be overstated. Approximately 75 to 80 percent of the weight of the animal is water. It functions as a solvent for nutrients, thermoregulator, and osmoregulator (Davis and Drackley, 1998). Calves, due to the greater risk of digestive disorders, experience greater problems with water balance than older animals. Water should be offered to calves beginning in the first week of life. Warm water (16°C to 18°C) stimulates water intake compared to offering cold (6°C to 8°C) water (Huuskonen et al., 2011). A lack of water can impair starter intake and BW gain by >30 percent (Kertz et al., 1984). Water intake and dry feed intake are highly correlated (Kertz et al., 1984; Quigley et al., 2006; Hepola et al., 2008; Eckert et al., 2015); thus, offering water early in life will promote dry feed intake and promote early rumen development. Thickett et al. (1981) calculated that for each liter of water consumed during the first 5 weeks of life, calves consumed an additional 82 g of calf starter and increased BW gain by 56 g/d. Similarly, recent data suggest that offering water from birth resulted in greater starter intake, BW gain, and nutrient digestibility compared to offering water beginning at 17 days of age (Wickramasinghe et al., 2019). Frequent replacement of water will encourage consumption. Postweaning ratio of water consumed per kilogram of dry (ca. 90 percent DM) feed intake was approximately 4:1 (Quigley et al., 2006; Hepola et al., 2008).

Calves <1 week of age that were transported to a research facility consumed an average of about 1.5 L/d of free water within the first week of age and from 1.5 to 3 L/d from weeks 1 to 3 of life (Morrison et al., 2019). Other reports suggest that young calves will consume less than 2 L/d for the first 3 weeks and then increase water intake in a fashion correlated with starter intake and weaning (Thickett et al., 1981; Kertz et al., 1984; Quigley et al., 2006).

Calves fed large volumes of milk preweaning will consume less free water compared to calves fed less milk or MR (Hepola et al., 2008; de Passillé et al., 2011). However, large volumes of high-protein MR increased consumption of water (Guindon et al., 2015; Stamey et al., 2021). Quigley et al. (2006) reported that calves fed limited MR tended to consume more water (4 L/d) than calves that were fed up to 0.9 kg of MR powder per day and weaned at 42 days (3.6 L/d).

Calves that develop diarrhea may lose 1 to 10 percent of BW. Mortality is common when dehydration exceeds about 12 percent (Davis and Drackley, 1998). Replacement of both water and electrolytes is essential in the treatment of diarrhea, and scouring calves require additional water to replace that lost in feces. Additional feedings of reconstituted electrolytes are needed to provide liquid in addition to electrolytes. Mixing electrolytes with milk or MR and feeding according to normal milk feeding program do not provide additional water and do not replace water lost due to diarrhea (Smith and Berchtold, 2014). Addition of electrolytes containing Na and glucose to milk or MR may dramatically increase osmolality of the resulting mixture, decreasing the rate of abomasal emptying and increasing the risk of abomasal bloat (Burgstaller et al., 2017). Smith (2009) suggested that osmolality should be less than 700 to 750 mOsm/L of the final solution.

Smith (2009) identified four requirements for oral rehydration solutions for calves: (1) provide sufficient Na to normalize extracellular fluid volume, (2) provide agents (glucose, citrate, acetate, propionate, or glycine) to facilitate absorption of Na and water from the intestine, (3) provide an alkalinizing or buffering agent (Na salts of acetate, propionate, or bicarbonate) to correct metabolic acidosis, and (4) provide energy, because most calves with diarrhea are in negative energy balance. Milk feeding should be continued during treatment with oral rehydration solution (Garthwaite et al., 1994; Smith, 2009). Electrolytes containing sodium bicarbonate ($NaHCO_3$) may inhibit clot formation if preweaned calves are fed liquid diets containing casein (e.g., whole milk or MR containing skim milk); however, products containing other alkalinizing agents (Na salts of acetate, propionate) do not interfere with clot formation.

Disease

According to a survey of dairy farms in the United States (Urie et al., 2018b), morbidity of preweaned dairy heifer calves was 34 percent (859 calves of 2,545 surveyed). Over half of all cases involved digestive signs (usually diarrhea) and another 31 percent was due to respiratory disease. Thus, it is important to consider effects of diseases common to preweaned calves on nutrient requirements and supply of young calves.

Malnutrition increases susceptibility to infection and severity of infections once they occur (França et al., 2009). The relationship between undernutrition and infection was highlighted by Calder and Jackson (2000). Undernutrition compromises barrier and immune functions, thereby allowing pathogens access to the body and decreasing the ability of the host to eliminate pathogens once they enter the body. Subsequently, infections may alter nutritional status by inducing anorexia, reducing nutrient absorption, increasing nutrient requirements, and increasing losses of endogenous nutrients (Calder and Jackson, 2000). Nutrition may play an important role in the outcome of an infection, potentially by affecting

pathogen virulence and resource availability (Pike et al., 2019). Furthermore, a decline in nutrient availability caused by, for example, anorexia may separately reduce the immune system's capacity to suppress the infection (Pike et al., 2019).

Effects of disease on nutrient requirements have been researched for many years (e.g., Scrimshaw, 1977); however, effects of disease on nutrient requirements and supply in young calves are not well documented. Data suggest that diarrhea increases rate of passage of nutrients; increases fecal excretion of water, solids, and nutrients; and reduces nutrient digestibility. Diarrhea, however, is only a clinical sign of alimentary tract dysfunction. An important mechanism by which the intestinal tract of a young calf reacts to pathogenic bacteria or viruses or indigestible dietary nutrients is hypersecretion and reduced absorption, resulting in loss of fluids, electrolytes, and nutrients, and the net effect is diarrhea (Radostits, 1975).

Fecal excretion in a group of scouring calves was 20 to 40 times the total fecal mass excreted by healthy calves (Blaxter and Wood, 1953). Apparent digestibility of DM fell dramatically, in some cases to approximately 40 percent. Digestion of fat was markedly impaired and fecal excretion of FAs increased more than 20-fold. Excretion of purine N was indicative of extensive microbial fermentation, which reduced fecal pH (6.0 in diarrheic calves compared to 6.8 in normal calves). Consequently, fecal solids excretion increased, as did excretion of fecal water. Increased rate of passage was at least partially responsible, as digesta passage time through the gastrointestinal tract declined from 48 hours in normal calves to 6 hours in scouring calves. Furthermore, excretion of Ca, Mg, and P increased in scouring calves in proportion to excretion of fecal soaps. Excretion of Na and K was much greater and was correlated with excretion of water.

Diarrheic calves had increased fecal excretion of fat, lactate, and acetate compared to healthy calves (Youanes and Herdt, 1987). Due to impaired nutrient digestibility, more than 50 percent of diarrheic calves were in negative energy balance even though intake of ME was above normal maintenance ME requirement. Doll et al. (2004) reported that apparent digestibility of milk lipids declined linearly ($r = 0.8$) with increasing severity of diarrhea in milk-fed calves, and in cases of watery diarrhea (fecal excretion >50 g/kg BW), apparent digestibility of fat was <50 percent. On the other hand, apparent digestibility of crude fiber increased in diarrheic calves experimentally infected with *Eimeria bovis* (Daugschies et al., 1998), which causes coccidiosis primarily in older calves shortly after weaning. Increase in digestibility was attributed to slower rate of ruminal nutrient passage in infected calves.

Morrison et al. (2019) reported effects of diarrhea on intake of MR, free water, electrolytes, and starter and BW gain for 21 days after arrival at the research farm. Calves that developed diarrhea refused MR and consumed less total solids (15.9 versus 16.6 kg) and total water (104 versus 108 L) from MR over the 21-day period compared to healthy calves. Total starter DMI during the first 21 days was decreased about 40 percent in diarrheic calves. As a result, cumulative total DMI from all sources was less when calves had diarrhea. Healthy calves were heavier and had greater hip height and heart girth at 21 days compared to diarrheic calves.

Improving the ability to formulate diets that maximize production and maintain competence of immune function and disease resistance would increase both welfare and profitability (Kogut and Klasing, 2009). Further research is needed in young calves to refine the understanding of the interactions among nutrient requirements, nutrient supply, immune function, and resistance to disease to improve the ability to meet requirements for both optimal growth and disease resistance.

Milk Replacers

According to a recent review of feeding practices in the United States (Urie et al., 2018a), 39 percent of dairy operations used MR alone and another 38.5 percent used MR in combination with milk. Numerous changes have occurred in the formulation of MR in the dairy industry since publication of NRC (2001) gudelines (Kertz and Loften, 2013), primarily related to use of various protein sources in MR formulas. The ability of these protein sources to supply an adequate amount and profile of AAs for growth of preruminant calves depends on the AA profile of the protein, the quality of the manufacturing process, and the ability of the calf to digest the protein. High temperatures during drying can damage proteins and lessen their biologic value (Wilson and Wheelock, 1972). Heat damage can be determined by various methods, including measurement of furosine in the ingredients or MR (Guerra-Hernandez et al., 2002). Furthermore, antinutritional factors present in some protein sources such as soy or pea proteins can decrease AA net absorption and efficiency of AA use (Huisman, 1989; Lallès, 1993).

Proteins in MR are mainly dairy in origin and are mostly from whey proteins and dried skim milk. Skim milk can be replaced by whey protein without change in digestibility or animal performance (Terosky et al., 1997; Lammers et al., 1998; Huuskonen et al., 2017). Although casein-containing ingredients (e.g., skim milk) will generally form a clot in the abomasum after consumption, this does not appear to be an advantage to clot formation in terms of digestibility or animal performance (Davis and Drackley, 1998; Longenbach and Heinrichs, 1998).

Research on and use of nonmilk proteins in MR have advanced significantly in the past 20 years. Vegetable proteins such as hydrolyzed wheat gluten, soy protein concentrate, and pea protein are processed to improve solubility and digestibility. Although older work suggests that incompletely processed ingredients such as soy flour may cause allergic reactions in the intestine of calves (Lallès et al., 1996; Dréau and Lalles, 1999), more recent data using improved protein production methods indicate that vegetable proteins can provide a portion of the MR formula with animal performance similar to all-milk formulas. For example, performance of calves fed MR

in which hydrolyzed wheat protein replaced approximately 50 percent of milk proteins was similar to calves fed all-milk proteins (Terui et al., 1996; Ortigues-Marty et al., 2003; Castro et al., 2016c) although nutrient digestibility was slightly lower (Branco-Pardal et al., 1995). Conversely, Hill et al. (2008d) reported lower growth when calves were fed MR containing hydrolyzed wheat gluten compared to milk protein. More highly processed pea protein isolate had digestibility similar to that of milk protein, whereas pea protein concentrate (still containing starch and oligosaccharides) was poorly used (Bhatty and Christison, 1980).

Other alternative protein sources include spray-dried animal plasma (Morrill et al., 1995; Quigley and Bernard, 1996; Quigley and Drew, 2000; Quigley et al., 2002; Quigley and Wolfe, 2003; Raeth et al., 2016; Vasquez et al., 2017; Morrison et al., 2017) and whole egg or egg yolk (Quigley et al., 2002; Touchette et al., 2003; Santoro et al., 2004; Dennis et al., 2017). Significant variability among reports suggests that quality and processing of raw materials (e.g., heating, spray-drying) may influence digestibility and animal performance.

An important consideration in MR formulation is to meet AA requirements of the calf, especially when nonmilk proteins are incorporated into the MR (Morrison et al., 2017; Vasquez et al., 2017). Hill et al. (2008e) reported that optimal concentrations of Lys and Met in MR containing 26 percent CP were 2.34 and 0.72 percent of DM, respectively. Others (Wang et al., 2012; Margerison et al., 2013) have generally supported this finding, although Castro et al. (2016c) concluded that addition of Met was unnecessary when MR (DM basis) contained >28 percent CP and 0.57 percent Met. Low rates of BW gain due to stressors or ill health may limit expression of improved nutrition associated with AA formulation (da Silva et al., 2018). Addition of arginine (Arg) or histidine (His) to MR containing 27 percent CP, 2.43 percent Lys, 0.76 percent Met, 0.68 percent Arg, and 0.48 percent His (DM basis) had no effect on calf performance when MR were fed at 680 g of solids daily (Hill et al., 2011b).

Fat sources in MR are typically tallow, choice white grease, lard, or palm oil components. Smaller amounts (typically <15 percent of total lipid) of coconut oil are often included to enhance digestibility and for the antimicrobial effects of the medium-chain FAs (López-Colum et al., 2019). The degree of homogenization is critical for high fat digestibility (Raven, 1970). Emulsifiers, such as lecithin and monoglycerides, often are added to enhance mixing characteristics and fat digestibility. In general, vegetable oils and fat sources that contain large amounts of free FAs are poorly digested by calves (Jenkins et al., 1985). The primary carbohydrate in MR is lactose from dried whey. Other carbohydrates such as maltodextrin or starch are poorly digested, particularly in young calves, and are not recommended for calves less than 4 weeks of age.

Increasing the protein to energy ratio is beneficial when increased amounts of MR are offered (Bartlett et al., 2006;

Bascom et al., 2007). Such changes in the composition of MR have achieved greater growth rates and energy retention, along with lower fat and greater lean tissue deposition (Diaz et al., 2001; Blome et al., 2003; Bartlett et al., 2006). For growth rates of 0.2 to 0.4 kg/d, protein/energy ratios (percent CP/ME Mcal/kg) generally should be in the range of 4.2 to 4.9, for growth of 0.4 to 0.7 kg/d in the range of 5 to 5.7, and for growth >0.7 kg/d in the range of 5.8 to 6.2.

Management of MR is critical to ensuring adequate growth rates and health of calves. MRs should be mixed in water at temperatures recommended by the manufacturer. Consistency of the amount of liquid fed daily to calves improves intake and BW gain (Hill et al., 2008b).

Solids concentration of whole milk (Holstein) is about 12.5 percent, and most MRs are formulated to be reconstituted to 12.5 to 15 percent solids. Increasing solids is a strategy to increase nutrient intake when feed management limits volume that can be fed—for example, when feeding from nipple bottles. Increasing solids concentration of MR to 20 percent DM did not influence nutrient digestibility (Ternouth et al., 1985a,b; Azevedo et al., 2016), although free water intake increased (Ternouth et al., 1985a). Jenny et al. (1982) reported that feces became more fluid with increasing solids concentration. Abomasal bloat seems to become more prevalent with MR concentrations over approximately 15 percent (Burgstaller et al., 2017). Availability and management of free water are important if attempting to feed solids concentrations >17 percent.

Whole Milk

Whole milk, whether saleable or unsaleable, remains the most commonly used liquid feed for calves prior to weaning on dairy farms (Urie et al., 2018a). Nonsaleable milk, also called waste milk, is not suitable for commercial sale and may be produced by cows immediately after calving, cows with active mammary infections, or those that have been treated with antibiotics.

Composition

Although bulk saleable milk has a reasonably consistent nutrient concentration, waste milk is by nature more variable (Jorgensen et al., 2006; Moore et al., 2009). Solids and nutrient content of waste milk can vary from that of whole milk, depending on the contribution of colostrum and transition milk (which increases solids, protein, and fat), water (contamination from washing procedures), and milk from sick or treated cows. Moore et al. (2009) reported that waste milk from one calf ranch in California averaged 11.2 percent solids (range, 5.1 to 13.4 percent), which was significantly different from the average solids in saleable milk.

Variation in nutrient content of milk may negatively influence calf performance. For example, Hill et al. (2008b) fed two MRs at either a variable daily rate (0.525 to 0.788 kg

DM/d) or a fixed daily rate (0.653 kg DM/d) to provide an average of 0.653 kg DM/d in four treatments. The MRs were either 27 percent CP and 31 percent fat (to simulate Holstein milk) or 27 percent CP and 17 percent fat. Calves fed a fixed daily rate of MR solids grew faster, ate more calf starter, and were more efficient compared to calves that were fed the variable amount of milk solids daily. Solids content of milk can be standardized with addition of powdered MR or specialized extender products designed for that purpose. Adjusting solids concentration up to 20 percent DM can be done without negative effect on calf performance (Azevedo et al., 2016), if free water is always available. However, increasing solids concentration of milk increases osmolality and may influence abomasal outflow rates if osmolality exceeds approximately 600 mOsm/kg (Burgstaller et al., 2017). In addition, milk contains some imbalances in vitamins (vitamins D and E) and minerals (Fe, Mn, Zn, Cu, I, Se) compared to nutrient requirements, and supplementing whole milk with vitamins and minerals and increasing the protein to energy ratio may improve growth and feed efficiency (Glosson et al., 2015).

Microbial Contamination and Pasteurization

Waste milk may be contaminated due to mastitis and other organisms that may be passed from cow to calf. Waste milk has been implicated in the transmission of numerous disease-causing organisms, including *Cryptosporidium parvum*, *Mycoplasma bovis*, and *Mycobacterium avium* subsp. *paratuberculosis* (Kesler, 1981; Selim and Cullor, 1997; Walz et al., 1997; Butler et al., 2000; Stabel, 2001). Pasteurization reduces bacterial counts in milk (Butler et al., 2000; Stabel, 2001; Stabel et al., 2004; Elizondo-Salazar et al., 2010) and improves health and growth of calves (Jamaluddin et al., 1996; Selim and Cullor, 1997; Armengol and Fraile, 2016). Regrowth of bacteria following pasteurization is possible if the waste milk is not cooled quickly and is stored for extended periods (Elizondo-Salazar et al., 2010); therefore, milk should be fed within an hour of pasteurization. Routine evaluation of pasteurization efficacy is also necessary by monitoring total plate counts and coliform counts. Jorgensen et al. (2006) reported that pasteurization was ineffective in up to 13 percent of operations evaluated in the study. Ruzante et al. (2008) reported survival of *M. avium* subsp. *paratuberculosis* in pasteurized waste milk from commercial dairy farms.

Antibiotic Residues

A significant risk of using waste milk is the transmission of antibiotic residues that may promote development of antibiotic resistance in bacteria in the milk and the animal. Even low levels of antibiotics fed to calves influence the composition of intestinal microflora (Yousif et al., 2018), the presence of antimicrobial-resistant organisms (Langford et al., 2003; Randall et al., 2014; Maynou et al., 2017; Pereira et al., 2014, 2018; Tempini et al., 2018), and calf nutrient

metabolism (Pereira et al., 2018; Li et al., 2019). Maynou et al. (2017) reported changes in composition of both fecal and nasal microbiota, pointing to systemic effects on animal metabolism or direct contact of nasal microbiota to other animals or waste milk. Development of antibiotic-resistant bacteria from the use of waste milk is a significant risk to animal and human health. If possible, only pasteurized waste milk from cows not treated with antimicrobials should be fed (Aust et al., 2013; EFSA Panel on Biological Hazards, 2017), and waste milk from treated cows should be discarded in an environmentally safe manner by incorporation into manure (Payer and Holmes, 1994).

Feed Additives

A wide variety of additives may be included in the diets of young calves. Generally, these additives can be categorized by function: stabilize or modify gastrointestinal function, support gut or systemic immune function, interfere with growth of potential pathogens, or increase digestibility. Some products may fit into multiple categories, depending on dosage and application.

The effectiveness of a feed additive to improve growth, intake, health, or efficiency necessarily depends on the mode of action of the additive and whether an effective dose was administered at the appropriate time. Additives that may influence calf health by reducing exposure to ingested pathogens (e.g., yeast products, plasma, and essential oils [EOs]) may not be effective in reducing diarrhea on farms where pathogen exposure is already low. Maintenance of viability of living organisms (yeasts and bacteria) and functional proteins (immunoglobulins, lactoferrin) intended to alter digestive function is also an important consideration in feed manufacturing, preparation, and storage. Practices such as pelleting calf starters at high temperatures or extended storage of products at temperatures >40°C may degrade effectiveness and economic benefit of additives.

The committee organized feed additives for calves by primary function in the animal. However, the committee recognizes that many additives have multiple functional roles—for example, yeast culture has been shown to modify the rumen environment (Xiao et al., 2016; Ma et al., 2020a,b) but also may influence intestinal immunity (Ma et al., 2020b) and severity of diarrhea (Alugongo et al., 2017), although not all studies have reported significant changes in gut permeability or plasma metabolites in response to yeast culture addition (Pisoni and Relling, 2020). Therefore, the groupings should be considered as a means to facilitate presentation and are not indicative of sole functions of the respective additives.

Selected additives reported for use in calves are intended to support or maintain rumen function (ionophores, rumen buffers, live yeast), are used as antimicrobials (antibiotics, lactoferrin, EOs, immunoglobulins), modulate gut immunity or microflora (oligosaccharides, yeast culture, direct-fed microbials), and stimulate organ growth (butyrate).

Rumen Function

Ionophores include monensin, lasalocid, and decoquinate and are added to feeds to modify rumen fermentation and control coccidiosis. Dietary lasalocid and decoquinate are effective in control of coccidiosis (Hoblet et al., 1989; Heinrichs et al., 1990; Heinrichs and Bush, 1991; Eicher-Pruiett et al., 1992; Quigley et al., 1997a). Supplementation in calf starter requires adequate feed intake to achieve effective dosages, but infection with coccidia often occurs before starter intake is sufficient (Quigley et al., 1997a). To counter this, amprolium is often added to milk or MR to control *Eimeria* sp. (Ghanem et al., 2008), although amprolium can induce thiamin deficiency unless thiamin is supplemented in milk (Dodd et al., 1996). Concentrations of ionophores effective against coccidia may be insufficient to influence rumen VFA profiles (Klotz and Heitmann, 2006).

Addition of $NaHCO_3$ to calf starters helps maintain rumen pH and normal rumen function. Addition of 3 percent bicarbonate in calf starters to young calves increased rumen pH (Quigley et al., 1992), although no performance changes were reported. Others have added 1 to 3 percent of DM as $NaHCO_3$ with no response (Foroozandeh and Shakeri, 2017) or improved growth and starter intake (Curnick et al., 1983). Addition of 2 percent $NaHCO_3$ was shown to increase K excretion by the calf (Tucker et al., 1991).

Live yeast added to calf starter (*Saccharomyces cerevisiae*) or milk (*S. cerevisiae*, spp. *boulardii*) for 42 days increased or tended to increase intake and growth while reducing days with diarrhea (Galvão et al., 2005). Fomenky et al. (2017) reported that *S. cerevisiae* spp. *boulardii* altered intestinal lactobacilli populations and colonic morphology. Improved performance may also be associated with stabilization of rumen fermentation during the rumen development period (Terré et al., 2015a,b). However, other studies reported no significant performance benefit to addition of yeast products (Pinos-Rodriguez et al., 2008; Huuskonen and Pesonen, 2015). Typical inclusion rate for live yeast products is approximately 1.5×10^6 cfu/g of calf starter.

Antimicrobials

Antibiotics have been used as feed additives for young calves since the 1950s (Kertz et al., 2017) and have consistently resulted in improved health and performance of calves (Kiser, 1976; Tomkins and Jaster, 1991; Quigley et al., 1997b; Quigley and Drew, 2000; Heinrichs et al., 2003; Berge et al., 2005; Kehoe and Carlson, 2015). However, because of concerns regarding development and transmission of antibiotic resistance in gut microflora of calves fed antibiotics (e.g., Berge et al., 2006), the addition of antimicrobials such as neomycin, oxytetracycline, and chlortetracycline to diets of young calves as a growth promoter is no longer permitted in the United States, without veterinary prescription (Mzyk et al., 2017).

Supplemental lactoferrin can improve intake (Joslin et al., 2002), decrease fecal scores, and reduce the number of days that calves experience diarrhea (Roblee et al., 2003; Prenner et al., 2007), although it has not always been effective (English et al., 2007). It was effective in reducing the risk of death in calves with diarrhea (Habing et al., 2017) when fed at the onset of clinical signs of diarrhea for 3 days; however, a field trial did not report improved health in calves with diarrhea that were treated with lactoferrin (Pempek et al., 2019). Adding lactoferrin up to 1 g/d to colostrum replacer reduced apparent efficiency of IgG absorption in newborn calves (Shea et al., 2009). Its mode of action may be via anti-inflammatory functions (Kushibiki et al., 2008).

EOs, including garlic, cinnamaldehyde, eugenol, capsicum, and anethole, among others, are considered antimicrobial and have been evaluated as a means of manipulating rumen fermentation. At this writing, data on the use of EOs in calf diets are sparse. Blends of EOs added to calf starter improved DMI, BW gain, and feed efficiency (Kazemi-Bonchenari et al., 2018; Salazar et al., 2019). The addition of EOs to starter appears to influence rumen fermentation patterns (Santos et al., 2015; Saeedi et al., 2017), which may be valuable in promoting rumen development. Froehlich et al. (2017) reported that calves fed a blend of EOs (carvacrol, caryophyllene, cymene, cineole, terpinene, and thymol) at 2.5 g/d had greater BW gain than calves fed EOs at 5.0 or 7.5 g/d, as well as greater BW gain and increased body length and withers heights to 56 days compared with another group of calves fed a yeast cell wall product at 4 g/d. Types of EOs used and dosage in each product affect the likelihood of a positive response, but the mechanism of action remains unknown where positive responses have been observed.

Ig, including colostrum, colostrum replacers, and animal plasma, have been fed to calves in MR. Orally administered Ig are immunologically active in the gut and may provide local protection against potential pathogens. Reduced days with diarrhea occurred when calves were fed MR containing 4 to 5 percent plasma (Quigley and Drew, 2000; Quigley et al., 2002; Quigley and Wolfe, 2003) or bovine colostrum (Berge et al., 2009; Chamorro et al., 2017). Another source of Ig is IgY produced from eggs collected from chickens that have been hyperimmunized against one or more pathogens. Typical inclusion rates are approximately 5 to 10 g/d of egg yolk (Yokoyama et al., 1988; Ikemori et al., 1997; Kuroki et al., 1997).

Modifiers of Gut Immunity or Microflora

Oligosaccharides have been used to manipulate bacterial flora of the intestinal tract of animals, potentially reducing the incidence of disease. These carbohydrates reduce adhesion of certain bacterial species to the intestinal epithelium, most notably *Escherichia coli* (K99+) and *Salmonella* sp. Oligosaccharides may also increase the growth of beneficial intestinal bacteria, including lactobacilli and bifidobacteria.

Oligosaccharides containing mannose and fructose have been fed to calves to improve intestinal health and to reduce the incidence of disease (Morrison et al., 2010; Grand et al., 2013; Heinrichs et al., 2013; Kara et al., 2015), although not all studies have reported significant benefit to the addition of oligosaccharides to MR (Terré et al., 2007; Hill et al., 2008c; Heinrichs et al., 2009; Quezada-Mendoza et al., 2011; da Silva et al., 2012). Heinrichs et al. (2003) reported that the addition of mannose oligosaccharides to MR was equally effective as neomycin plus oxytetracycline in controlling the incidence and severity of diarrhea.

Galactosyl-lactose, a trisaccharide (galactose plus lactose) that is produced by enzymatic treatment of whey using β-galactosidase derived from *Aspergillus oryzae*, was equally effective as antibiotics in reducing diarrhea when added at 1 percent of DM to MR (Quigley et al., 1997b). MR formulated to have about 3 percent galactooligosaccharides (GOSs) produced notable changes in intestinal microflora but did not benefit calf growth or health, perhaps because lactose content was decreased by the GOS production process (Castro et al., 2016a,b).

Yeast culture, when added to high-grain diets, reduces ruminal lactate concentrations (Quigley et al., 1992) and alters rumen bacterial populations and butyrate concentrations (Xiao et al., 2016) in young calves. Improved growth (Leismaster et al., 2004) and health (Magalhães et al., 2008; Fokkink et al., 2009; Alugongo et al., 2017) were reported when yeast culture was included in the diet, although not all studies have reported improved calf performance (Fokkink et al., 2009). Yeast culture in MR and calf starter also reduced effects of challenge with *Salmonella enterica* serotype *Typhimurium* (Brewer et al., 2014; Harris et al., 2017). Depending on the specific formulation, typical inclusion rates for yeast culture are 3.5 to 14 g/d. Hydrolyzed yeast also can improve immune response in young calves when added to MR (Kim et al., 2011). Conversely, preparations primarily of nonviable yeast did not significantly affect calf performance (Saldana et al., 2019).

Direct-fed microbial (DFM) products may be added to calf MR to influence gut microflora and protect against potential pathogenic infection. Some studies have reported positive effects on calf health (Abe et al., 1995; Timmerman et al., 2005; Morrison et al., 2010) or performance (Cruywagen et al., 1996); however, others (Geiger et al., 2014) found no effect of these additives. Inclusion rates of DFM products vary depending on the types of bacteria, yeast, and fungi included in the product.

Butyrate

Adding sodium or calcium butyrate has sometimes (Guilloteau et al., 2009, 2010; Roh et al., 2018) but not always improved calf health, growth, and efficiency when included in milk (Araujo et al., 2015; Frieten et al., 2017), calf starters (McCurdy et al., 2019), or both (Górka et al., 2011a,b, 2014,

2018). Typical inclusion rate for sodium butyrate is 0.3 percent of DM in MR or calf starter. Sodium butyrate, when fed in combination with other FAs, has increased intake, BW gain, digestibility of nutrients, and improved overall immune response (Hill et al., 2007b,c, 2011a,c; Quigley et al., 2019), improved gut morphology (Koch et al., 2019), and altered microbial profiles in the hindgut (O'Hara et al., 2018). Guilloteau et al. (2009) concluded that 0.3 percent of DM as butyrate enhanced production of digestive enzymes and increased absorptive capability in the upper small intestine. Kato et al. (2011) opined that improved performance and digestibility by butyrate was due to improved insulin sensitivity and a better digestive functional development.

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11

Growth

INTRODUCTION

The costs of raising replacement heifers and the impact of heifer growth on lifetime milk production and profits underscore the importance of accurate predictions for heifer nutrient requirements (Tozer and Heinrichs, 2001). The energy and protein requirements for growing heifers are determined primarily by the animals' maintenance requirements, the amount of daily body tissue gain and its composition, and the efficiency of converting feeds to body tissues. Targets for daily gain depend on targets for age and body weight (BW) at breeding and first calving. How heifers are fed can affect not only growth rate and feed costs but also the timing of puberty, the composition of gain, and future milk production.

Improvements Made from the Seventh Revised Edition

The growth requirements in the seventh edition were based on equations developed for beef cattle, which typically have a higher proportion of fat than do dairy breeds. The model contained new terms for its size-scaling approach that made it confusing, and it had mathematical incongruities; for example, gut fill was 14.5 percent of BW but only 4 percent of BW gain. In the past 20 years, new publications have reported body composition for Holstein cattle, and these data were used to develop equations based on Holsteins that are size-scaled for use in other dairy breeds.

Terminology and Relationships for Body Weight and Body Weight Gain

In this edition, the following terms are used to describe growth. BW is the normal live weight of an animal without fasting, and BW gain is the increase in BW over a defined time period such as average daily gain (ADG). Empty BW (EBW) is BW without ingesta, and empty body gain (EBG) is gain without digesta. These cannot be measured easily in a live animal and are estimated as 85 percent of BW for heifers (NRC, 1989) and supported by Waldo et al. (1997).

$$EBW = 0.85 \times BW \qquad \text{(Equation 11-1a)}$$

$$EBG = 0.85 \times ADG \qquad \text{(Equation 11-1b)}$$

The committee recognizes that the mass of ingesta, or gut fill, is not a constant function of BW; rather, gut fill is generally a function of feed intake and digestion kinetics and can be considerably different for heifers fed a poorly digestible diet ad libitum than for heifers fed a highly digestible diet at restricted intake. To date, no solutions seem adequate to accurately predict gut fill. Thus, the committee decided to use a constant value of 15 percent, with the recognition that this value is not adequate for all situations; for example, in Waldo et al. (1997), change in gut fill ranged from 11 percent of ADG for heifers gaining 1,000 g/d on a corn silage–based diet to 19 percent of ADG for heifers gaining 770 g/d on an alfalfa-based diet. Further work is needed.

ENERGY AND PROTEIN REQUIREMENTS FOR GROWING DAIRY HEIFERS

Setting energy and protein requirements for growing heifers requires quantitative estimates for maintenance requirements and composition of gain as heifers mature, for the effects of diet on structural growth and milk production potential, and for the efficiency of metabolic conversions.

Maintenance Requirements

The maintenance energy requirement for heifers was set on a metabolizable energy (ME) basis as follows:

$$\text{ME for maintenance (Mcal/d)} = 0.15 \times (\text{kg BW})^{0.75}$$
$$\text{(Equation 11-2)}$$

This matches the maintenance requirement for cows and is similar to the value used by the beef NASEM (2016) for dairy breeds of $0.095 \times BW^{0.75}$ on a net energy (NE) basis. Activity is assumed to be 10 percent of the requirement; heifers in large dry lots or on pasture may be more active and thus have greater maintenance requirements. Adjustments for compensatory growth, body condition score (BCS), or previous temperature were not included. More data are needed to refine maintenance estimates. Equations to generate the maintenance requirement for metabolizable protein (MP) are the same as for adult cows (see Chapter 6) and include scurf, endogenous urinary loss, and metabolic fecal losses. The efficiency of converting MP to net protein (NP) was assumed to equal the target efficiency of 0.69 for scurf and metabolic fecal protein (MFP) and 1.0 for endogenous urinary nitrogen (N) (see Chapter 6 for details). Although some of these losses are as nitrogen rather than protein or amino acids, all values are put on a crude protein (CP) basis ($N \times 6.25$).

$$MP\text{-scurf (g/d)} = (0.20 \times BW^{0.60}) / 0.69$$
(Equation 11-3a)

$$MP\text{-endogenous urinary (g/d)} = 53 \times 6.25 \times BW \times 0.001$$
(Equation 11-3b)

$$MP\text{-MFP (g/d)} = ((11.62 + 0.134 \times NDF\%DM) \times DMI) / 0.69$$
(Equation 11-3c)

The Composition of Gain and Growth Requirements

The NE required for growth is defined as the energy retained in body tissues during growth and is a function of the proportion of retained fat and protein (Garrett et al., 1959). As animals mature, the percentage of protein diminishes and the percentage of fat increases in the empty body, and chemical maturity is achieved when weight gain contains little protein and is mostly fat. Simpfendorfer (1974) summarized data on the body composition of growing cattle from birth to maturity; within cattle of similar mature size, 96 to 99 percent of the variation in chemical composition was associated with differences in BW.

Previous committees on dairy and beef cattle nutrition (e.g., NRC, 2001; NASEM, 2016) adopted the equation developed by Garrett (1980) to predict the energy content of weight gain. Garrett's data set included 72 comparative slaughter experiments conducted at the University of California between 1960 and 1980 with approximately 3,500 cattle (predominantly British breed beef steers) fed a variety of diets. The Garrett equation describes the relationship between retained energy (RE) and EBG for a given EBW and the composition of EBW gain at a particular stage of growth in cattle. Because the BW at which cattle reach a given chemical composition varies depending on mature size and sex, body composition may differ among animals of similar BW (NRC, 1996). Thus, most systems to predict body composition and nutrient requirements in the past 20 years have used

a size-scaling approach to account for differences in composition at a specific BW that are due to differences in mature BW (MatBW). The size-scaling approach adopted by NRC (2001) involved calculation of the relationship between an animal's current BW, its MatBW, and a standard reference weight. The MatBW of the standard reference animal (NRC, 2001) was 500 kg and defined as the weight at which skeletal development is complete and the empty body contains 25 percent fat corresponding to a BCS of 3 on a 1 to 5 scale.

In the past 20 years, several studies have measured body composition for Holstein cattle. Based on a meta-analysis of 26 studies with 129 treatment means on Holsteins, de Souza and VandeHaar (2018) showed that the equations of the seventh edition generally underestimated the fat content of the empty body and the RE per kilogram of gain in young heifers and overestimated the fat content of the empty body and the RE per kilogram of gain in older heifers. Moreover, the fat content of mature Holsteins at a body condition of 3 on a 5-point scale was 22 percent, not 25 percent. Part of the reason for underestimating the fat content of young heifers was that in NRC (2001), the energy content of gain was assumed to be proportional to the 0.75 power. Reasons were not provided for the use of the 0.75 power function, and it lacks biological support. A natural log function provided a better fit of the relationship of BW to body composition for Holsteins from birth to maturity (de Souza and VandeHaar, 2018).

The text within NRC (2001) implies that the composition of body gain is highly sensitive to the rate of gain, with animals gaining faster depositing a greater proportion of fat than animals growing slower. However, Table 11-1 in NRC (2001) does not bear this out; RE per day was proportional to ADG to the 1.097 power, and thus the RE content of EBG was proportional to ADG to the 0.097 power. Hence, the RE content of EBG was only 7 percent greater for a heifer gaining 1.2 kg/d than for one gaining 0.6 kg/d, which is much less of a response in body composition to rate of gain than in the literature cited (Radcliff et al., 1997; Waldo et al., 1997).

The current committee developed new equations to describe growth of heifers based on 26 publications with 129 treatment means for body composition of Holstein cattle from birth to maturity. Publications and variables used in the model are shown in Table 11-1. The composition of gain had a greater fat content, and thus energy content, in heifers with faster growth rates. However, the committee deemed that setting requirements based on the higher proportion of fat gain during faster growth was not reasonable because the resulting diets would be low in protein relative to energy and might limit proper frame and mammary growth. Instead, fat content of EBW was regressed against EBW for cattle from birth to maturity. For EBW between weaning (~80 kg) and first calving (~570 kg), a linear regression based on EBW fit the data as well as a quadratic function or functions based on log BW or BW to any power. The fat content of gain was derived from the fat content of EBW. To ensure mathematical consistency, the fat content of EBW or EBG was used

TABLE 11-1   Publications Used to Develop Equations for Composition of Gain in Dairy Heifers[a]

Reference	Method for Composition	EBW Measured?	Range in EBW, kg	Range in ADG, g/d
Heifers				
Brown et al., 2005b	Carcass composition	No	51 to 97	379 to 900
Chelikani et al., 2003	Urea dilution	No	239 to 269	520 to 1,040
Davis Rincker et al., 2008b	Rib composition	No	140 to 173	645 to 1,100
Diaz et al., 2001	EBW composition	Yes	40 to 99	590 to 1,210
Meinert et al., 1992	Urea dilution	No	476 to 584	720 to 810
Meyer, 2005	EBW composition	Yes	85 to 310	610 to 963
Moallem et al., 2004	EBW composition	Yes	79 to 241	820 to 961
Radcliff et al., 1997	Carcass composition	No	286 to 349	770 to 1,270
Steen et al., 1992	Urea dilution	No	386 to 401	714 to 769
Waldo et al., 1997	EBW composition	Yes	155 to 289	766 to 1,004
Whitlock et al., 2002	Carcass composition	No	272 to 277	1,130 to 1,180
Calves				
Bartlett et al., 2006	EBW composition	Yes	44 to 68	250 to 700
Blome et al., 2003	EBW composition	Yes	47 to 58	380 to 620
Chapman et al., 2017	D_2O dilution	No	57 to 63	422 to 747
Donnelly and Hutton, 1976	EBW composition	Yes	61 to 70	610 to 830
Hill et al., 2008	EBW composition	Yes	59 to 88	360 to 450
Mills et al., 2010	EBW composition	Yes	~83	880 to 990
Rius et al., 2005	Carcass composition	Yes	63 to 150	1,090 to 1,230
Robelin and Chilliard, 1989	EBW composition	Yes	83 to 111	740 to 800
Swartz et al., 1991	Urea dilution	No	83 to 87	840 to 890
Tikofsky et al., 2001	EBW composition	Yes	~80	760 to 810
Cows				
Agnew et al., 2005	EBW composition	Yes	412	
Andrew et al., 1994	EBW composition	Yes	452 to 480	
Belyea et al., 1978	K40	No	441 to 507	
Chibisa et al., 2008	Urea dilution	Yes	553 to 656	
Komaragiri et al., 1998	D_2O dilution	Yes	408 to 520	
Komaragiri and Erdman, 1997	D_2O dilution	No	450 to 638	
Martin and Ehle, 1986	D_2O dilution	Yes	595 to 689	
McGuffey et al., 1991	EBW composition	Yes	461 to 476	
Soderholm et al., 1988	D_2O dilution	No	491 to 594	
von Soosten et al., 2012	EBW composition	Yes	398 to 447	

[a] If EBW was not measured, EBW was the sum of components parts, or $0.85 \times$ BW for calves and heifers, or $0.82 \times$ BW for cows.

to calculate fat-free matter (FFM), and the composition of FFM was assumed to be a constant of 21.5 percent protein, 5.6 percent ash, and 72.9 percent water, as shown by Waldo et al. (1997) and proposed for use in the seventh edition. The composition of FFM may change slightly as cattle age, but the effect of this change on the protein content of EBG is trivial compared to the effect of changes in FFM content. The RE content of EBW and EBG was calculated as a sum of the energy from retained fat and protein, using RE values of 9.4 Mcal/kg for fat and 5.55 Mcal/kg for protein (NRC, 2001).

Assuming Holsteins in this data set had an average MatBW of 700 kg, a size-scaling approach was developed with the standard reference MatBW at 700 kg and a mature EBW at 574 kg (82 percent of 700). This size-scaling approach enables all equations to be used for other breeds, for which data are lacking. A similar approach was used in NRC (2001), but the current equations are simpler and use Hol-

steins as the reference. Equations were then converted, and composition of BW and BW gain were based on a percentage of an animal's expected MatBW. Gut fill for heifers was set at 15 percent of BW (instead of 18 percent for cows), so that EBG was 85 percent of ADG.

The resulting regressions are in Figure 11-1. As EBW increases, the composition of EBW changes linearly, with protein content decreasing and fat content increasing. The average content of EBW is 20 percent protein and 9 percent fat at 70 kg EBW (12 percent of mature EBW) and 17 percent protein and 22 percent fat at 470 kg EBW (82 percent of mature EBW). The assumption was made that these represent the average body composition for normal growth.

The equations that best described the data of studies from Table 11-1 were as follows:

$$\text{Fat in EBW (Fat\_EBW; kg/kg)} = 0.067 + 0.188 \times (\text{BW / MatBW}) \quad \text{(Equation 11-4a)}$$
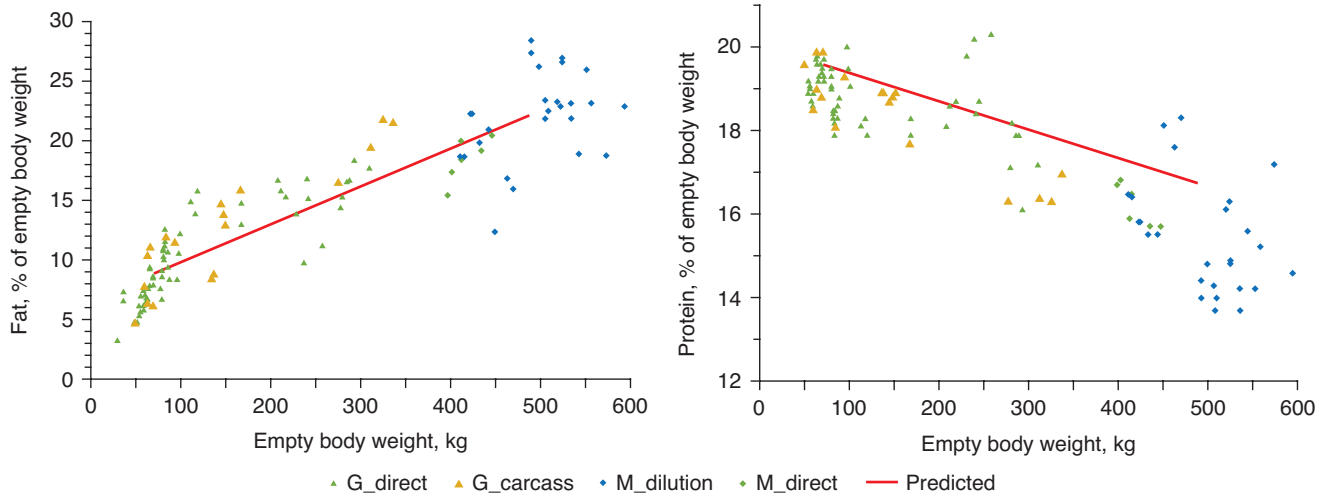
FIGURE 11-1   Fat and protein content of EBW in Holstein cattle. Points are treatment means, after adjustment for study effects, from studies using direct chemical measures of empty body mass (G_Direct), carcass or rib sections (G_Carcass) in growing calves or heifers, or direct chemical measures of empty body mass (M_Direct) or water dilution methodology (M_Dilution) in cows. Prediction lines are considered valid only between 70 and 490 kg EBW.

$$\text{FFM in EBW (FFM\_EBW; kg/kg)} = 1 - \text{Fat\_EBW}$$
(Equation 11-4b)

$$\text{Protein in EBW (Protein\_EBW; kg/kg)} = 0.215 \times \text{FFM\_EBW}$$
(Equation 11-4c)

$$\text{Ash in EBW (Ash\_EBW; kg/kg)} = 0.056 \times \text{FFM\_EBW}$$
(Equation 11-4d)

$$\text{Water in EBW (Water\_EBW; kg/kg)} = 0.729 \times \text{FFM\_EBW}$$
(Equation 11-4e)

Using the above equations to describe the composition of EBW at various ages, the composition of EBG (i.e., true growth) is the following:

$$\text{Fat in EBG (Fat\_EBG; kg/kg)} = 0.067 + 0.375 \\ \times (\text{BW / MatBW}) \quad \text{(Equation 11-5a)}$$

$$\text{FFM in EBG (FFM\_EBG; kg/kg)} = 1 - \text{Fat\_EBG}$$
(Equation 11-5b)

$$\text{Protein in EBG (Protein\_EBG; kg/kg)} = 0.215 \times \text{FFM\_EBG}$$
(Equation 11-5c)

$$\text{RE in EBG (RE\_EBG; Mcal/kg)} = 9.4 \times \text{Fat\_EBG} \\ + 5.55 \times \text{Protein\_EBG} \quad \text{(Equation 11-5d)}$$

$$\text{Ash in EBG (Ash\_EBG; kg/kg)} = 0.056 \times \text{FFM\_EBG}$$
(Equation 11-5e)

$$\text{Water in EBG (Water\_EBG; kg/kg)} = 0.729 \times \text{FFM\_EBG}$$
(Equation 11-5f)

where $\text{EBW} = 0.85 \times \text{BW}$ and $\text{EBG} = 0.85 \times \text{ADG}$ in kg and kg/d, respectively. Estimated ash and water concentration in EBW and EBG are not needed in the model but are shown here for completeness.

Thus, the composition of live BW gain is as follows:

$$\text{Fat in ADG (Fat\_ADG; kg/kg)} = 0.85 \times \text{Fat\_EBG}$$
(Equation 11-6a)

$$\text{RE in ADG (RE\_ADG; Mcal/kg)} = 0.85 \times \text{RE\_EBG}$$
(Equation 11-6b)

$$\text{Protein in ADG (Protein\_ADG; kg/kg)} = 0.85 \times \text{Protein\_EBG}$$
(Equation 11-6c)

The expected composition of gain is shown from weaning to first calving in Figure 11-2, with comparisons to the NRC (2001) system for gains of 0.6 and 1.0 kg/d. The fat and energy contents of gain in the new equations are similar to NRC (2001) for midweight heifers but are greater for young prepubertal heifers and less for older heifers. The protein content of gain is less than NRC (2001) in all cases because that model assumed that body gain was 96 percent tissue (EBG/ADG was 0.96). As an animal approaches maturity (95 percent of MatBW), the composition of ADG as true frame growth approaches 36 percent fat, 10.5 percent protein, and 4.0 Mcal of RE/kg.

After first calving, frame growth will continue but will be only a small portion of total requirements. Assuming that BCS is maintained at 3.0 to 3.5 and that gut fill is constant at 18 percent, Equations 11-5a to 11-5d are used to estimate body composition of the growth; however, Equations 11-6a to 11-6c are modified by replacing 0.85 with 0.82 because
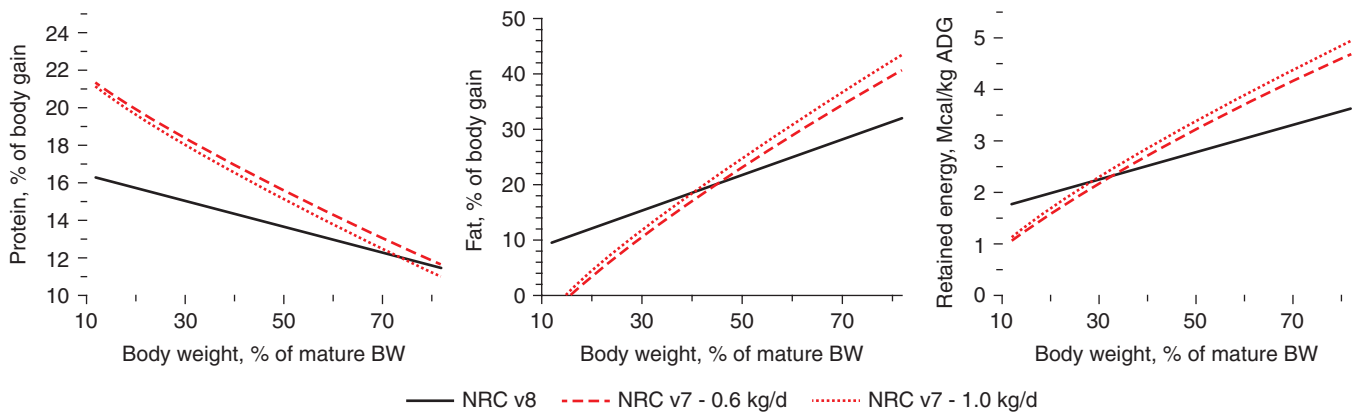
FIGURE 11-2 Expected fat, protein, and energy content of ADG in cattle from weaning to first calving using the new equations of this edition (black solid line) or equations from NRC (2001) for animals gaining 0.6 (red long dashed line) or 1.0 kg BW/d (red short dashed line). These projections are based on the assumptions that EBG/ADG is 96 percent in NRC (2001) and 85 percent in the current version.

of differences in gut fill. In contrast, the composition of BW change when associated with BCS change is 62 percent fat, 8 percent protein, and 6.3 Mcal/kg.

The efficiencies of converting ME to net energy-gain (i.e., retained energy [RE]) and of converting MP to retained protein (equivalent to net protein gain, or NP) were not changed from NRC (2001).

$$\text{ME for growth (Mcal/d)} = \text{RE (Mcal/d)} / 0.40$$
(Equation 11-7)

As animals mature, the efficiency of converting MP to NP is decreased; the equation used by NRC (2001) dropped NP/MP from 0.77 to 0.39 as BW increased from 12 percent to 82 percent of MatBW. That equation yields values for young heifers greater than is reasonable based on Chapter 10. In NASEM (2016), NP/MP is essentially 0.5 for most growing cattle. The current committee set NP/MP at 0.6 for heifers at 12 percent of MatBW and decreased it linearly to 0.39 for heifers at 82 percent:

$$\text{MP to NP efficiency (NP-eff)} = 0.64 - 0.3 \times \text{EBW/Mature EBW}$$
(Equation 11-8)

where EBW cannot exceed Mature EBW and EBW/Mature EBW can be approximated as BW/MatBW.

$$\text{MP for growth (g MP/d)} = \text{Retained protein (g/d)} / \text{NP-eff}$$
(Equation 11-9)

EFFECTS OF PLANE OF NUTRITION ON FUTURE MILK PRODUCTION

Energy Nutrition

How heifers are fed, starting at birth, can have long-term impacts on milk production through effects on the develop-

ing mammary gland and BW and BCS at first calving. Effects of preweaning nutrition are covered in Chapter 10. Recent reviews on heifer-rearing programs include Le Cozler et al. (2008), Lohakare et al. (2012), and Heinrichs et al. (2017).

First calving at 22 to 24 months is considered to best balance the cost of growing heifers with their production and lifetime income potential (Ettema and Santos, 2004; Heinrichs et al., 2017). Calving at <22 months usually is associated with smaller BW at first calving or requires rapid growth from birth to calving. Both inadequate size at first calving and rapid growth rates may limit subsequent milk production (Heinrichs et al., 2017).

The idea that smaller heifers at calving produce less milk is based almost entirely on correlations, often with all heifers fed and managed the same; these correlations are useful for formulating hypotheses, but they are not causal. A notable exception is a study of 500 dairy heifers of mixed breeds by Lin et al. (1986). Heifers were randomly assigned to two groups for breeding eligibility at 11.5 or 15 months, and those heifers bred early, compared to those bred late, calved 3 months earlier (23 versus 26 months), weighed 50 kg less at calving, and produced 300 kg less milk in their first lactation. Abeni et al. (2000) fed 42 Holstein–Friesian heifers and set breeding eligibility at 370 or 420 kg; those that calved early weighed 50 kg less at calving and produced 550 kg (7 percent) less energy-corrected milk in their first lactation. Assuming the lower milk yield of early bred heifers in both studies was due to their lower BW at calving, each kilogram decrease in BW could be expected to decrease milk production about 10 kg during the first lactation.

Target BWs for milestones in the life of a heifer are the same as those in NRC (2001) and shown in Table 11-2. As in NRC (2001), these targets are set as a percentage of MatBW to enable use for breeds other than Holstein or Jersey. Target BW for individual Holstein or Jersey heifers also might be adjusted as genomic predictions of MatBW become more available. The target BWs after the first and second calvings are 82 percent

and 92 percent of MatBW, or 570 kg and 640 kg for Holsteins. The target BW at conception is 55 percent of MatBW, or about 380 kg for Holsteins. Target BCS at all milestones (breeding and calving) is 3.0 to 3.5. If the target age at first calving is 22 months, conception must occur at 13 months. Given that larger BW (with proper BCS) at calving results in more milk, the target at first calving may in some situations be >82 percent of MatBW. Given that conception often requires more than one insemination, optimal BW at first breeding may be 50 percent of MatBW or even less. Lighter BW at breeding may be acceptable if heifers are fed in confined systems for optimal growth once pregnant. If they are in systems where they will grow more slowly during gestation, they should be heavier at breeding.

To attain a BW of 350 to 380 kg (50 to 55 percent of 700 kg) by 13 months, Holsteins must gain ~0.85 kg/d on average from birth to 13 months, and because gains this fast are unlikely in the first 2 months, gains of ~0.90 kg/d are needed during much of the rest of the first year to attain first calving at 22 months. Based on the seminal article by Sinha and Tucker (1969), the mammary gland grows at an allometric rate between 3 and 9 months of age. However, on a fractional growth basis, the mass and DNA content of the mammary gland per 100 kg BW increased 50-fold from 1 to 3 months of age but only 3-fold from 3 to 5 months. Brown et al. (2005a) reported that the mass of mammary parenchyma per 100 kg BW increased 4- to 8-fold between 8 and 14 wk of age; in their study, they were unable to detect parenchymal tissue at 2 d of age. Thus, mammary growth is allometric from birth to about the time of puberty, when it slows down to the rate of other body tissues; during this time, the milk production potential of a heifer may be responsive to nutrition. An increased plane of nutrition before weaning enhances later milk production, but an increased plane of nutrition between the time of weaning and puberty may impair it.

Retrospective correlation analyses often find that high rates of gain in heifers do not impair and may even enhance subsequent milk production. For example, Krpálková et al. (2014) examined relationships of BW, BCS, and ADG of 780 heifers to their milk yield as cows; heifers were divided retrospectively into the three groups based on BW at 14 months. They found that heifers with the greatest BW at 14 months (ADG > 0.95 kg/d from 5 to 14 months) produced the most milk over their first three lactations. The larger heifers had slightly higher BCS at 14 months (3.5 versus 3.2) and calved 1 month earlier (24 versus 25 months) than the smallest heifers. Volkmann et al. (2019) found that rapid growth rates from birth to 1 year did not impair milk production of 2,300 Holsteins. These data suggest that rapid growth rates in heifers can be consistent with high milk production as cows. However, correlations of gain with milk production in animals that are all fed and managed the same are not causal and can be misleading. Some of the endogenous controls of lean growth also control milk production (such as somatotropin); hence, faster growth should be associated with greater milk

yield. In setting targets for ADG when formulating diets, the question is whether average gains >0.95 kg/d would promote more milk than average gains <0.85 kg/d.

Based on studies where heifers were randomly assigned to diets that promoted fast or slow growth, ADG >0.9 kg/d during the period between weaning and breeding generally decreased milk yield in the first lactation (Zanton and Heinrichs, 2005). However, diets that promoted gains between 0.9 and 1.0 kg/d caused only small drops in future milk yield (5 percent or less), and only diets promoting gains >1.0 kg/d decreased milk production by greater than 10 percent. Two additional studies not cited by Zanton and Heinrichs (2005) also support that diet-induced gains >1.0 kg/d decrease subsequent milk yield by >10 percent (Gardner et al., 1977; Peri et al., 1993). Van Amburgh et al. (2019) proposed that this decrease in milk yield from feeding high energy in the first year of life was due to excess body condition carried over to first calving; however, this was not the case for all studies. For example, Radcliff et al. (2000) fed diets promoting gains of 0.8 or 1.1 kg/d from 4 months of age until confirmed pregnant and found that heifers fed for faster growth had greater BCS at first insemination (4.2 versus 3.5) but the same BCS at calving (3.5 versus 3.7) and same postpartum BW (515 versus 539 kg); they calved 3 months earlier (631 versus 719 days) and produced 10 percent less energy-corrected milk in the first lactation.

The etiology for the effect of high-energy diets fed to prepuberty heifers on milk production potential is not clear. Ever since Sinha and Tucker (1969) showed that the mammary gland slowed from allometric to isometric growth at puberty, one mechanism considered for the decreased mass of mammary parenchyma per unit of BW at puberty was that high-energy diets hasten the age of puberty and thus truncate the period of allometric mammary growth. This was demonstrated by Meyer et al. (2006), who killed heifers at 50-kg increments of BW that were fed high- or low-energy diets; heifers fed high-energy diets gained 0.95 kg/d, compared to 0.65 kg/d for low-energy diets, but the rate of mammary parenchymal accretion was the same in each. Heifers fed high-energy diets attained puberty earlier and had less parenchymal mass at puberty. Van Amburgh et al. (2019) further demonstrated that early puberty with truncated mammary growth likely could explain results of several other studies. However, the question remains whether mammary growth would have been constant outside the bounds of 0.65 to 0.95 kg/d and why diet might alter mammary development in heifers before 2 months of age but not between 2 months and puberty.

Possibly more important than rate of gain per se is the extent of fat deposition in body and mammary tissues, as proposed by Swanson (1960) 60 years ago. Silva et al. (2002) examined the relationships of ADG and body fat content of individual heifers fed for rapid gains in several studies. They found that for heifers around the time of puberty, those with the greatest amount of body fat had the least amount of mammary parenchyma, and for heifers that were bred and followed through lactation, those with higher BCS at breed-

ing produced less milk as cows (Silva et al., 2002). Thus, although a high rate of gain is often considered the cause of impaired milk production potential, the problem may actually be that diets that promote rapid gains also promote excessive fat accretion. The idea that fat could interfere with mammary development is supported by studies showing negative effects of leptin on insulin-like growth factor-I (IGF-I)–stimulated mammary cell proliferation in heifers (Silva et al., 2008) and that diet-induced obesity impairs normal mammary development in other species, such as mice (Kamikawa et al., 2009). If excess fattening is the cause of impaired mammary development, then feeding programs should focus on sound growth without fattening. In Meyer et al. (2006), heifers fed high energy to gain 0.95 kg/d had greater mammary fat pad mass but not greater body fat content per kilogram BW during the prepubertal period; this rate of gain seems reasonable for modern Holsteins.

Whether the decrease in mammary parenchymal mass at puberty is important is not clear. Perhaps the mammary gland compensates after puberty for the potential development that was not realized prior to puberty. However, as discussed earlier, multiple studies have demonstrated that prepubertal diets that promote rapid gains in the prepubertal period decrease first-lactation milk yield. Rapid growth often increases BCS, and in some of these studies, increased BCS might have been carried over to the time of calving; however, in some studies, heifers calved at similar BCS, which indicates that postpubertal development may not be able to compensate for decreased prepubertal development. If heifers grow rapidly without fattening, then perhaps gains >1.0 kg/d are acceptable for Holsteins, but no studies to date have shown that gains greater than 1.0 kg/d during the prepubertal period result in as much milk once heifers have calved. Optimal rates of gain should be adjusted for expected MatBW and are shown in Table 11-2; the upper threshold for prepubertal Jersey heifers is ~750 g/d.

TABLE 11-2   Target Weights (kg), Ages, and ADGs (kg/d) for Growing Dairy Cattle

	Percent of Mature BW	Holstein	Jersey
Mature BW	100	700	520
Birth BW	6	42	31
Weaning BW	12	84	62
Conception BW	55	385	286
First calving prepartum BW	91	638	426
First calving postpartum BW	82	574	474
Second calving postpartum BW	92	644	478
Conception age, months		13	13
First calving age, months		22	22
Prepubertal ADG	0.13	0.90	0.67
Postpubertal ADG	0.10	0.69	0.51
Postpubertal gain + pregnancy	0.13	0.92	0.69
First-lactation ADG	0.027	0.19	0.14
Second-lactation ADG	0.022	0.15	0.11

Protein Nutrition

The requirement for dietary protein in NRC (2001) was calculated based on the rate of protein accretion, the efficiency with which MP is converted to retained protein, and the digestibility, rumen degradability, rumen kinetics, and microbial metabolism of dietary CP. Because the major target for protein deposition is muscle, muscle accretion was the major determinant of the final dietary protein requirements. However, optimal heifer-rearing programs must consider effects of protein nutrition not only on muscle accretion but also on structural growth, feed efficiency, and future milk production potential. Dietary protein supplies amino acids, which are the building blocks for protein synthesis in all of these functions, but amino acids also can alter hormonal signals and cellular machinery in ways that are not easily quantified (Rezaei et al., 2016). Setting protein requirements based solely on relationships between dietary protein and protein accretion does not account for effects of protein on physiological regulation. Several studies in the past 20 years have examined protein requirements for dairy heifers as a function of ME intake, and most have shown that the required ratios of protein to energy in NRC (2001) were reasonable. From NRC (2001), the required protein to energy ratio in diets for heifers at 150 kg BW gaining 900 g/d was 72 g CP/Mcal ME. The required ratio was sensitive to level of maturity and to rate of gain. The required ratio decreased from 72 to 64 g/Mcal for 150-kg heifers as gain decreased from 900 to 600 g/d and decreased from 72 to 52 for heifers gaining 900 g/d as BW increased from 150 to 350 kg BW. However, these requirements were based on equations where the protein content of EBG for young heifers and EBG as a percentage of ADG of all heifers were too high (EBG was 96 percent of ADG). Correcting these equations results in lower requirements for protein relative to energy, and these new requirements may be inconsistent with recommendations for optimal mammary development.

Studies in which excess energy intake depressed mammary development or subsequent milk production the most were those with the lowest protein content per unit energy in the diet of the heifers fed for rapid growth (Whitlock et al., 2002). Whitlock et al. (2002) directly tested the effect of protein in Holstein heifers by feeding ad libitum high-energy diets containing 48, 57, and 66 g CP per Mcal ME (corresponding to estimates of 37, 41, and 44 g MP) from 130 to 320 kg BW. The diets had no effect on carcass gain or carcass composition and no significant effect overall on mammary development. However, for heifers that naturally grew the fastest and achieved puberty earliest, those fed the high-protein diet had greater mammary development. Lammers and Heinrichs (2000) fed prepubertal heifers from 200 to 340 kg BW diets containing 46, 54, and 61 g CP/Mcal ME with dry matter intake (DMI) restricted to 2.45 percent of BW per day; heifers grew 1,000 to 1,100 g/d. The rate of gain, feed efficiency, structural growth, and teat elongation were

greater in heifers fed 61 than 46 or 54 g CP/Mcal ME. Even in heifers growing at slower rates, dietary protein may affect mammary development. Pirlo et al. (1997) fed prepubertal Friesian heifers diets with high or low energy and high or low protein. The high-energy diets promoted gains of 820 g/d and contained 62 or 50 g CP/Mcal ME from 100 to 200 kg of BW and 49 or 40 g CP/Mcal ME from 200 to 300 kg. Compared to groups fed low-energy diets, heifers fed high energy with low protein tended to produce 15 percent less milk protein as cows, but those fed high energy with high protein produced as much as those fed low energy. Gabler and Heinrichs (2003) fed 60 prepubertal Holstein heifers between 125 and 234 kg BW diets with varying protein content at restricted intakes to gain 0.8 kg/d; they found that diets with a CP/ME ratio of 59 or 68 outperformed diets containing 48 or 77 g/Mcal when considering structural growth, feed efficiency, and protein efficiency.

On the basis of the above studies, the current committee determined that diets for heifers from weaning to first calving should contain a minimum amount of MP per Mcal of ME to meet desired rates of gain, minimize the risk of excessive fat deposition, optimize lean tissue and structural growth, and maximize mammary development and subsequent milk production:

$$\text{Minimum MP (g/Mcal ME)} = (53 - 25 \times \text{BW/MatBW})$$
(Equation 11-10)

TOTAL REQUIREMENTS FOR METABOLIZABLE ENERGY AND METABOLIZABLE PROTEIN

$$\text{Total ME requirement (Mcal/d)} = \text{ME for maintenance} + \text{ME for growth} + \text{ME for pregnancy}$$

$$\text{Total MP requirement (g/d)} = \text{MP for maintenance} + \text{MP for growth} + \text{MP for pregnancy}$$

Requirements for maintenance and growth were discussed above and requirements for pregnancy are discussed in Chapters 3 and 6.

Because of effects on future milk yield (discussed above), the total MP requirement should be increased if below the minimum threshold of MP/ME for optimal development.

If MP (g/d) < $(53 - 25 \times \text{BW/MatBW}) \times \text{ME (Mcal/d)}$, then

$$\text{MP (g/d)} = (53 - 25 \times \text{BW/MatBW}) \times \text{ME (Mcal/d)}$$
(Equation 11-11)

Using these equations, requirements for ME and MP are shown in Tables 11-3 and 11-4 for cattle with a mature BW of 700 kg and 520 kg, the typical MatBW for Holsteins and Jerseys. For these examples, the minimum MP to ME ratio was the determining factor for setting the requirement for MP. The committee suggests that the minimal dietary protein for

optimal milk production potential may be higher than that for structural growth. Further research is needed to refine MP requirements for heifers that support both growth and future milk production potential.

TARGET BODY WEIGHTS AT BREEDING AND CALVING

Targets in Table 11-2 are set for heifers reared in intensive or dry lot environments. Heifers grown on pasture or in situations where poor-quality feedstuffs are cost-effective should use similar targets for BW but should consider later targets for age at each BW target. These targets assume that heifers will have BCS consistent with normal, sound growth.

PREDICTING GAIN FROM AVAILABLE METABOLIZABLE ENERGY AND METABOLIZABLE PROTEIN

The above equations can be reversed to predict gain. Although the composition of gain was not adjusted with changes in rate of gain when setting requirements, the change in composition is used to adjust the predicted gain based on available energy. In NRC (2001), RE per day was proportional to ADG to the 1.097 power, so the RE content of gain was proportional to ADG to the 0.097 power. Both the current calf and heifer models will continue to use this value, albeit rounded to 0.10, for predicting gains. In addition, in the heifer model, the prediction is adjusted so that the base RE content of gain is set for a heifer of MatBW at 700 kg gaining 840 g/d, or 0.12 percent of MatBW per day. For heifers of 700 kg MatBW, gains greater than 840 g/d will result in greater RE per kilogram of gain, or less gain than predicted without the adjustment. For small breeds, 840 g/d would be a fast rate of gain; the base for a heifer with 520 kg MatBW would be at 620 g/d gain. Because the minimum MP to ME ratio was the determining factor for setting the requirement for MP in most cases, allowable gains based on protein were not predicted. If the MP to ME ratio of a diet is less than that shown in Table 11-3 or 11-4 for a heifer of a given BW, the diet has insufficient protein for optimal growth and development. The equations to predict gain from ME intake are as follows:

$$\text{RE (Mcal/d)} = (\text{ME intake} - \text{ME for maintenance}) \times 0.40$$
(Equation 11-12a)

$$\text{ADG (kg/d)} = [\text{RE} / (0.85 \times (1.74 + 3.08 \times (\text{BW/MatBW}))) \times (\text{MatBW}/700)^{0.1}]^{(1.0/1.1)}$$
(Equation 11-12b)

$$\text{RE of ADG (Mcal/kg)} = \text{RE (Mcal/d)} / \text{ADG (kg)}$$
(Equation 11-12c)

$$\text{Fat in ADG (kg/kg)} = 0.85 \times ((\text{RE of ADG} / 0.85 - 1.19) / 8.21)$$
(Equation 11-12d)

TABLE 11-3   Requirements for Energy and Protein in Heifers with MatBW of 700 kg[a]

Live BW	112	224	336	420	560
BW as % of mature BW	16	32	48	60	80
Estimated DMI, kg/d	3.3	6.0	8.0	9.3	10.9
Maintenance					
ME, Mcal/d	5.2	8.7	11.8	13.9	17.3
MP, g/d	121	228	325	389	486
Body composition					
Body fat, %	8.2	10.8	13.3	15.3	18.4
Body protein, %	16.5	16.0	15.4	15.0	14.3
Body energy, Mcal/kg	1.69	1.90	2.11	2.27	2.53
Composition of gain					
Fat in ADG, %	10.8	15.9	21.0	24.8	31.2
Protein in ADG, %	16.0	14.9	13.8	12.9	11.6
Energy in ADG, Mcal/kg	1.90	2.32	2.74	3.05	3.58
Retained energy, Mcal/d					
ADG = 700 g/d	1.33	1.62	1.92	2.14	2.50
ADG = 840 g/d	1.60	1.95	2.30	2.56	3.00
ADG = 980 g/d	1.86	2.27	2.68	2.99	3.50
Protein gain, g/d					
ADG = 700 g/d	112	104	96	91	81
ADG = 840 g/d	134	125	116	109	97
ADG = 980 g/d	156	146	135	127	113
ME for pregnancy, Mcal/d					4.16
MP for pregnancy, g/d					250
ME requirement, Mcal/d					
ADG = 700 g/d	8.5	12.7	16.6	19.3	27.7
ADG = 840 g/d	9.2	13.6	17.5	20.3	28.9
ADG = 980 g/d	9.8	14.4	18.5	21.4	30.2
MP to NP conversion	0.59	0.54	0.50	0.46	0.40
Minimum MP/ME	49	45	41	38	33
MP requirement, g/d					
ADG = 700 g/d	416	573	679	732	939
ADG = 840 g/d	448	610	718	772	979
ADG = 980 g/d	481	646	758	813	1,020
ME/kg diet DM					
ADG = 700 g/d	2.5	2.1	2.1	2.1	2.5
ADG = 840 g/d	2.7	2.3	2.2	2.2	2.7
ADG = 980 g/d	2.9	2.4	2.3	2.3	2.8
CP, % of diet DM					
ADG = 700 g/d	19.9	15.4	13.6	12.7	13.5
ADG = 840 g/d	21.5	16.4	14.4	13.4	14.1
ADG = 980 g/d	23.1	17.4	15.2	14.1	14.7

[a]Predicted DMI and the resulting ME and CP densities were based on the DMI prediction from Chapter 2 and using a conversion of CP to MP of 0.62. Heifer for last column is 40 days prepartum.

Protein in ADG (kg/kg) = 0.85 × (1 − (Fat content of ADG / 0.85) × 0.215)   (Equation 11-12e)

Reported diet-induced changes in composition of gain vary widely. Waldo et al. (1997) measured EBG to be 21 percent fat for 330-kg heifers that had gained 780 g/d but 25 percent fat for those gaining 990 g/d. Radcliff et al. (1997) observed 17 percent fat in carcasses of 340-kg heifers gaining 770 g/d for 4 months and 25 percent fat in those gaining 1,200 g/d; BCS was 2.9 and 3.9, respectively. Davis Rincker et al. (2008b) estimated the RE content of ADG to be 1.6 Mcal/kg (8 percent fat) in young heifers gaining 640 g/d for

12 weeks and 3.0 Mcal/kg (25 percent fat) for those gaining 1.08 kg/d. Meyer et al. (2006) found little difference in the composition of ADG in heifers fed low- or high-energy diets from birth to 250 kg BW and gaining ~660 versus ~930 g/d. As BW increased from 100 to 250 kg, the RE content of ADG increased from ~1.4 to 2.7 Mcal/kg (from 10 to 21 percent fat) with little difference between diets. However, for heifers killed at 300 or 350 kg, the RE of ADG was ~2.1 Mcal/kg (20 percent fat) on the low-energy diet and nearly double that on the high-energy diet. Thus, neither the previous nor current NRC models adequately account for changes in the composition of gain that occur when heifers are fed diets

TABLE 11-4   Requirements for Energy and Protein in Heifers with MatBW of 520 kg[a]

Live BW	83	166	250	312	416
BW as % of mature BW	16	32	48	60	80
Estimated DMI, kg/d	2.5	4.5	6.0	6.9	8.1
Maintenance					
ME, Mcal/d	4.1	6.9	9.4	11.1	13.8
MP, g/d	90	170	242	290	362
Body composition					
Body fat, %	8.2	10.8	13.3	15.3	18.4
Body protein, %	16.5	16.0	15.4	15.0	14.3
Body energy, Mcal/kg	1.69	1.90	2.11	2.27	2.53
Composition of gain					
Fat in ADG, %	10.8	15.9	21.0	24.8	31.2
Protein in ADG, %	16.0	14.9	13.8	12.9	11.6
RE in ADG, Mcal/kg	1.90	2.32	2.74	3.05	3.58
Retained energy, Mcal/d					
ADG = 520 g/d	0.99	1.21	1.42	1.59	1.86
ADG = 624 g/d	1.19	1.45	1.71	1.90	2.23
ADG = 728 g/d	1.38	1.69	1.99	2.22	2.60
Protein gain, g/d					
ADG = 520 g/d	83	77	72	67	60
ADG = 624 g/d	100	93	86	81	72
ADG = 728 g/d	116	108	100	94	84
ME for pregnancy, Mcal/d					3.04
MP for pregnancy, g/d					183
ME requirement, Mcal/d					
ADG = 520 g/d	6.6	10.0	13.0	15.1	21.5
ADG = 624 g/d	7.1	10.6	13.7	15.9	22.4
ADG = 728 g/d	7.6	11.2	14.4	16.7	23.4
MP to NP conversion	0.59	0.54	0.50	0.46	0.40
Minimum MP/ME	49	45	41	38	33
MP requirement, g/d					
ADG = 520 g/d	323	448	532	574	710
ADG = 624 g/d	348	475	561	604	740
ADG = 728 g/d	372	503	590	634	771
ME, Mcal/kg diet DM					
ADG = 520 g/d	2.6	2.2	2.2	2.2	2.7
ADG = 624 g/d	2.8	2.4	2.3	2.3	2.8
ADG = 728 g/d	3.0	2.5	2.4	2.4	2.9
CP, % of diet DM					
ADG = 520 g/d	20.9	16.2	14.4	13.4	14.1
ADG = 624 g/d	22.4	17.2	15.1	14.1	14.7
ADG = 728 g/d	24.0	18.2	15.9	14.8	15.3

[a] Predicted DMI and the resulting ME and CP densities were based on the DMI prediction from Chapter 2 and using a conversion of CP to MP of 0.62. Heifer for last column is 40 days prepartum.

supporting different rates of gain. The current committee decided that data were lacking to change the assumptions from NRC (2001) or NASEM (2016); thus, the RE content of ADG is proportional to the 0.1 power of ADG. In the future, a reasonable approach for predicting composition of gain would be to predict the composition of frame gain and then to use changes in body condition for significant deviations from the standard growth rates.

HEIFER GROWTH PROGRAMS

Between birth and first calving, feed quality and availability may vary due to environmental and price constraints. Peri-

ods of slow growth on low-energy diets may be followed by periods of rapid growth on high-energy diets, and in the end, the development of the heifer may be similar. Such "stairstep" programs may improve lifetime productively (Ford and Park, 2001). Periods of alternating slow and rapid growth might also provide advantages in efficiency due to compensatory gain (NASEM, 2016) and allow use of poor-quality feeds or pastures. At the least, periods of alternating fast and slow growth seem to cause no disadvantage to heifers in the long term. Based on the potential impacts on mammary development discussed earlier, one period to promote slower growth would be between 3 months of age and puberty. Another possibility would be to feed for slower growth when heifers

are being managed for breeding, so that those taking longer to conceive are less likely to gain excess body condition. In addition, if grain is relatively cheap, the most cost-effective way to feed heifers may be to feed high-energy diets with more grain at a restricted intake (Zanton and Heinrichs, 2007, 2008). Restricted feeding of higher-energy diets can promote optimal growth provided feed is uniformly provided so all animals maintain proper body condition. In addition, increased grain and decreased fiber in heifer diets fed at restricted intake would decrease methane and manure output.

MODEL LIMITATIONS AND RESEARCH NEEDS

The current model assumes that the digestibility of feeds and the conversion of DE to ME are the same in heifers as in cows and that the conversion of ME to RE is 0.40, as in NRC 2001. These assumptions need validation via research. The committee recommends that future studies monitor and report BCS, along with diets, intake, and rates and composition of gain. When feeding heifers, BW gain and BCS should be closely monitored to ensure optimal skeletal growth. In addition to diet, activity, environment, genetics, and health can alter the expected gain for a given energy intake. Data to include these factors in determining requirements or predicting gains are lacking. Therefore, when feeding heifers, BW gain and BCS should be closely monitored to ensure that target growth rates are achieved.

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12

Dry and Transition Cows

METABOLIC AND PHYSIOLOGIC STATUS OF THE TRANSITION COW

Hormonal and Metabolite Changes

The transition period in dairy cows is generally defined as the last 3 weeks of gestation and the first 3 weeks of lactation. The prepartum phase of this period is characterized by rapid fetal growth, mammary gland growth and development, colostrum synthesis, and dramatic changes in endocrine status. Many of the hormonal changes during the peripartum period are to prepare the cow for the substantial increase in energy needs postpartum (Ehrhardt et al., 2016). Plasma concentrations of insulin, insulin-like growth factor 1 (IGF1), and leptin decrease and growth hormone increases as the cow progresses from late gestation to early lactation, with acute changes in plasma concentrations at parturition (Kunz et al., 1985; VandeHaar et al., 1999; Block et al., 2001; Doepel et al., 2002; Rhoads et al., 2004). Insulin-sensitive tissues in periparturient cows display insulin resistance so that glucose can be directed to the developing fetus and to the mammary gland. Plasma thyroid hormone (T4 and T3) concentrations gradually increase during late gestation, decrease approximately 50 percent at calving, and then begin to increase (Kunz et al., 1985; Pethes et al., 1985). These hormonal responses are designed to increase energy mobilization, reduce basal metabolic rate, and partition nutrients to the mammary gland.

The changes in endocrine status and the decline in dry matter intake (DMI) that usually occurs around parturition influence metabolism and lead to mobilization of fat from adipose tissue and glycogen from the liver. In healthy dairy cows, from about 2 weeks prepartum until 3 days prepartum, plasma fatty acids (FAs) (commonly referred to as nonesterified FAs or NEFAs) increase from <0.2 mEq/L to about 0.3 mEq/L, and then 1 or 2 days before calving, concentrations increase abruptly and usually reach their peak by the second or third day of lactation (often between 0.8 and 1.0 mEq/L). Concentrations then decrease slowly over the next 2 to 3 weeks (Doepel et al., 2002; LeBlanc et al., 2005; Garverick et al., 2013). How much of the initial increase in plasma FAs can be accounted for by changing endocrine status compared with energy restriction resulting from decreased DMI is not known. Force feeding cows during the prefresh period reduced the magnitude of NEFA increase but did not eliminate it (Bertics et al., 1992), meaning that at least part of the prepartum increase is hormonally induced. The rapid rise in NEFA the day of calving is presumably due to stress, but hormonal changes (e.g., elevated growth hormone) could also be involved. In healthy cows, plasma NEFA concentrations decrease rapidly after calving but remain higher than they were before calving for several weeks. In cows that develop health disorders such as a displaced abomasum, prepartum plasma NEFA concentrations often exceed 0.5 mEq/L and can exceed 1.0 mEq/L during the immediate postpartum period (LeBlanc et al., 2005). High rates of lipolysis and the subsequent elevated NEFAs are associated with inflammation and immune system dysfunction (Bradford et al., 2015; Contreras et al., 2018) and can be a risk factor for ketosis (discussed below).

Plasma glucose concentrations remain stable or increase slightly during the prefresh period, increase dramatically at calving, and then decrease immediately postpartum (Kunz et al., 1985; Vázquez-Añón et al., 1994). The transient increase at calving may result from increased glucagon and glucocorticoid concentrations that promote depletion of hepatic glycogen stores. Although the demand for glucose for lactose synthesis continues after calving, under normal conditions, hepatic glycogen stores begin to replete and are increased by day 14 postpartum (Vázquez-Añón et al., 1994), likely reflecting an increase in gluconeogenic capacity to support lactation. Ketosis can result if this system is dysfunctional (see ketosis section below).

Because of low DMI relative to milk production in the early postpartum period, cows can mobilize substantial amounts of body protein (Bell et al., 2000). Plasma concentrations of 3-methyl histidine (a marker of protein breakdown) can be

high the first few weeks of lactation (van der Drift et al., 2012). Estimates of the quantity of body protein mobilized during early lactation vary widely, but most studies show that by about 4 weeks of lactation, body protein mobilization ceases (Komaragiri and Erdman, 1997; Tebbe and Weiss, 2021).

Blood calcium (Ca) decreases the last few days prior to calving due to transfer to colostrum (Goff and Horst, 1997b). Adaptation of the intestine, kidney, and bone to higher demands for Ca takes several days so that blood Ca typically does not return to normal concentrations until several days postpartum. Ca metabolism is discussed in more detail in the hypocalcemia section below.

Ruminal Changes

As a cow transitions from gestation into lactation, the rumen wall undergoes significant changes in size, morphology, and functionality. Because a substantial diet change almost always occurs at the time of calving, diet and physiological changes are confounded. These changes may be caused by metabolic factors, dietary factors, or, more likely, by both. Rumen tissue mass increased about 5 percent between 1 week prepartum and 10 days postpartum (Reynolds et al., 2004) and continued to increase through at least 120 days postcalving (Baldwin et al., 2004; Reynolds et al., 2004). Small intestinal mass followed a similar pattern but reached a peak by 90 days postcalving (Baldwin et al., 2004; Reynolds et al., 2004). Although rumen tissue mass was greater at 10 days postpartum than 7 days prepartum, total rumen volume did not differ (Reynolds et al., 2004). Steele et al. (2015) described several histological differences in rumen epithelial cells collected 3 weeks before compared with 1 week after calving. Postcalving, cells demonstrated accelerated differentiation and desquamation. Morphological differences on the rumen surface are less consistent possibly because of measurement difficulties. In general, rumen papillae size, surface area, and mass increase between late gestation and lactation (Dirksen et al., 1985; Reynolds et al., 2004; Penner et al., 2006; Steele et al., 2015). Expression of genes related to cell growth regulation and differentiation followed patterns that agreed with the histological and morphological changes observed during the transition period (Steele et al., 2015). These changes indicate increasing absorptive capacity within the rumen during the early lactation period.

The immediate postpartum period usually involves a major diet change of reduced concentrations of forage and fiber and increased concentrations of starch and protein, which in addition to increasing DMI will increase concentrations of ruminal volatile FAs and alter their profile. These changes could cause the observed changes in rumen epithelial cells. However, dietary effects on cellular changes occurring at this time are not consistent, and more research is needed. Dirksen et al. (1985) reported increased length and development of ruminal papillae in cows fed a high-concentrate diet postpartum compared with cows fed high-forage diets prepartum; how-

ever, diet was completely confounded with all other changes that occur at parturition. Penner et al. (2006) observed that a step-up program in which the amount of concentrate in the diet increased prepartum in a stepwise fashion did not affect papillae growth compared to a high-forage diet prepartum. Reynolds et al. (2004) reported that supplementing 800 g of barley grain to a high-forage diet prepartum increased the number of ruminal papillae per square centimeter of rumen wall but greatly reduced their width and surface area. Andersen et al. (1999) reported no difference in rumen epithelium morphology and development between transition cows fed a high (approximately 85 percent) or low (approximately 45 percent) forage diet. Based on available data, benefits of feeding a diet of moderate starch and fiber to transition ruminal cells and rumen tissue morphology from a high-forage gestation diet to a higher-starch lactation diet are not evident. More research is needed evaluating dietary effects on ruminal morphology and epithelial cell physiology during this critical phase.

Immune Status

During the transition period, cows experience varying degrees of immunosuppression (Goff and Horst, 1997b; Hansen, 2013). Neutrophil and lymphocyte function is depressed (Kehrli et al., 1989a,b; Dosogne et al., 1999; Rinaldi et al., 2008), concentrations of circulating immunoglobulins are reduced at least in part because of transfer to colostrum (Herr et al., 2011), and types and numbers of circulating immune cells are changed (see review by Hansen, 2013). Immunosuppression is one cause for increased prevalence of infectious diseases such as mastitis and metritis that can occur around parturition. Estrogen, progesterone, and cortisol concentrations are high shortly before parturition and can suppress immune function. Nutrient consumption is reduced around parturition, which may also contribute to immunosuppression. Because Ca is integral to immune cell activation (Kimura et al., 2006), hypocalcemia increases the degree of immunosuppression (Martinez et al., 2012). Plasma concentrations of α-tocopherol, retinol, and β-carotene decrease during the periparturient period, and these nutrients can influence immune function (see Chapter 8). Decreases in some of those nutrients can contribute to oxidative stress, which can also contribute to immunosuppression (Sordillo and Aitken, 2009; Sordillo, 2013). Various measures of immune function improved when peripartum cows were fed supplemental rumen-protected methionine (Osorio et al., 2013; Vailati-Riboni et al., 2017). The mode of action is unclear at this time but could involve reduced oxidative stress and inflammation (Han et al., 2018).

Oxidative Stress

Oxidative stress occurs when the relationship between oxidants and antioxidants within a cell or tissue is not appropriate for a specific physiological state. In biological

systems, the primary oxidants are reactive oxygen metabolites or species (ROS), but reactive nitrogen species are also important biological oxidants. Free radicals (e.g., superoxide, hydroxyl radical, FA radicals), singlet oxygen, and hydrogen peroxide are major biological ROS. During normal oxidative phosphorylation, a small proportion of the oxygen is not completely reduced to water, resulting in the production of superoxide. Activated phagocytes generate massive quantities of ROS, which are essential for the cells to kill bacteria (Johnston et al., 1975) but contribute to oxidative stress. Cellular antioxidant systems react with ROS and, under normal conditions, maintain proper ROS concentrations. The antioxidant system is composed of enzymes (e.g., superoxide dismutase, catalase, and various glutathione peroxidases), glutathione, α-tocopherol, β-carotene, and ascorbic acid. Superoxide dismutase exists in two forms; the mitochondrial form requires manganese as a cofactor, and the cytosolic form requires copper (Cu) and zinc (Zn). Iron (Fe) is a cofactor for catalase and selenium is a cofactor for glutathione peroxidases. Ascorbic acid is a primary water-soluble antioxidant but can be synthesized by cows and is not considered an essential nutrient for dairy cattle. Adequate dietary supply of antioxidant nutrients can reduce oxidative stress, but in excess, many antioxidant nutrients can act as pro-oxidants and increase oxidative stress (Halliwell, 1996; Lykkesfeldt and Svendsen, 2007).

Oxidative stress can occur under certain disease states (Sordillo and Aitken, 2009; Politis et al., 2012) and is common and may even be normal during the peripartum period (Bernabucci et al., 2005; Castillo et al., 2005; Pedernera et al., 2010; Abuelo et al., 2013). Peripartum oxidative stress is higher in cows that are in excess body condition at calving and that undergo greater body condition losses postcalving possibly because of the production of FA radicals from mobilized fat (Bernabucci et al., 2005; Bradford et al., 2015).

Oxidative stress can be measured using various biomarkers such as thiobarbituric acid reactive substances (TBARS) and F2-isoprostanes (Celi, 2011; Abuelo et al., 2013) or by measuring concentrations of various antioxidants and ROS (Castillo et al., 2005), but no universally accepted measure or index has been developed. Increased oxidative stress in cattle is associated with mastitis (Politis, 2012; Politis et al., 2012), metritis (Baithalu et al., 2017), edema (Miller et al., 1993), retained placenta (Miller et al., 1993), displaced abomasum (Mudron et al., 1997; Qu et al., 2013), and insulin resistance (Abuelo et al., 2016). Many of these are discussed in more detail in specific sections within this chapter and in Chapters 7 (Minerals) and 8 (Vitamins).

NUTRIENT REQUIREMENTS FOR PREGNANCY AND TRANSITION

Nutrient requirements for pregnancy can be found in the individual nutrient chapters. Colostrum synthesis, although not a gestational requirement, can represent a significant draw on some nutrients during the last several days of gestation. Average first-milking colostrum yields are about 4 kg (first lactation) to 8 kg (older cows), and first-day colostrum yields range from 8 to 14 kg for Holsteins (Bobe et al., 2009; Kessler et al., 2014). Average first-milking colostrum yield by Jerseys was about 4 kg, but season (approximately 2.5 kg in winter and 6.6 kg in summer) had a large effect (Gavin et al., 2018). Average concentrations of some important nutrients in colostrum are in Table 12-1 (Cu, manganese, and Fe are in trace concentrations and not shown).

Assuming a yield of 10 kg of colostrum and average concentrations of nutrients (see Table 12-1), the average Holstein cow would secrete 14 Mcal of energy, 1.35 kg of protein, 21 g of Ca, 30 mg of retinol, and 60 mg of tocopherol in her

TABLE 12-1 Average Composition of Colostrum[a]

Nutrient	Concentration	Reference
Crude protein, g/kg	145 (193)[b]	Nocek et al., 1984; Quigley et al., 1994; Hammon et al., 2000; Bobe et al., 2008
Fat, g/kg	65 (50)	Hammon et al., 2000; Bobe et al., 2008
Lactose, g/kg	25 (25)	Bobe et al., 2008
Gross energy, Mcal/kg	1.4 (1.5)	Hammon et al., 2000
Retinol, mg/kg	3.0	Johnston and Chew, 1984; Puvogel et al., 2008
α-Tocopherol, mg/kg	6.0	Weiss et al., 1990b, 1997; Rajaraman et al., 1997; Kumagal and Chaipan, 2004
Vitamin B_{12}, μg/kg	19	Stemme et al., 2006
Calcium, mg/kg	2.1 (2.4)	Foley and Otterby, 1978; Roux et al., 1979; Salih et al., 1987; Shappell et al., 1987; Kume and Tanabe, 1993; Tsioulpas et al., 2007
Phosphorus, mg/kg	1.8	Salih et al., 1987; Shappell et al., 1987; Kume and Tanabe, 1993; Tsioulpas et al., 2007
Magnesium, mg/kg	0.3	Foley and Otterby, 1978; Salih et al., 1987; Shappell et al., 1987; Kume and Tanabe, 1993; Tsioulpas et al., 2007
Potassium, mg/kg	1.3	Salih et al., 1987; Kume and Tanabe, 1993; Tsioulpas et al., 2007
Sodium, mg/kg	0.9	Foley and Otterby, 1978; Shappell et al., 1987; Kume and Tanabe, 1993; Tsioulpas et al., 2007
Copper, mg/kg	0.5	Moeini et al., 2011
Selenium, mg/kg	0.04 to 0.17[c]	Weiss and Hogan, 2005; Salman et al., 2013
Zinc, mg/kg	15	Foley and Otterby, 1978; Vaillancourt and Allen, 1991; Moeini et al., 2011

[a] Data are from Holstein cows unless otherwise noted.
[b] Values in parentheses are for Jersey cows.
[c] Low value represents colostrum from cows fed inorganic Se and high value is from cows fed selenium–yeast.

colostrum the first day postpartum. The impact of Ca secretion is discussed in the hypocalcemia section. The secretion of protein into colostrum is not included in the requirements for transition cows because it is short term, and the preponderance of data does not show any beneficial effects of increased protein supply during the prepartum period (discussed below). However, data on the effects of prepartum protein supply on colostrum yield, nutrient composition, and overall quality are essentially nonexistent. The secretion of retinol and tocopherol into colostrum clearly affects vitamin A and vitamin E status of the cow. Plasma concentrations of retinol and tocopherol drop abruptly about 1 week before parturition (Goff and Stabel, 1990; Weiss et al., 1990a), and a large portion of that decrease is because of transfer to colostrum (Goff et al., 2002). Increasing the intake of vitamin E from 1,000 IU/d (approximate requirement) to 4,000 IU/d during the last 2 weeks of gestation prevented the decrease in plasma concentrations of tocopherol and reduced clinical mastitis (Weiss et al., 1997). Feeding 2,000 IU/d of supplemental vitamin E, but not 1,000 IU/d during the last 2 weeks of gestation, attenuated the decrease in plasma tocopherol concentrations around parturition and reduced somatic cell count (Baldi et al., 2000). Conversely, the decrease in plasma retinol occurs even when cows are fed very high concentrations of vitamin A. Cows fed 550,000 IU/d of supplemental vitamin A (approximately seven times the 2001 NRC requirement) during the dry period still exhibited a decrease in plasma retinol around calving. Assuming a first-day colostrum yield of 10 kg, a cow will lose about 100,000 IU of vitamin A (30 mg of retinol) and about 135 IU of vitamin E (50 mg of *RRR*-tocopherol) via colostrum the first day after parturition. Colostrum synthesis occurs during the last few days of gestation; therefore, during most of the dry or prefresh period, no increased demand for colostral vitamins A and E exists. Hence, the loss of nutrients via colostrum is not included in requirement calculations, but users should recognize the potential impact of colostrum synthesis on overall nutrient requirements of the very late-gestating cow or heifer.

Dry Matter Intake During the Dry Period

Factors Affecting Dry Matter Intake

Feed intake is relatively constant during the initial phase of the dry period (days 60 to 21 prepartum) but can decline quite dramatically thereafter, especially during the 7 to 10 days prior to calving (Hayirli et al., 2003). The major animal factors that influence DMI during this time are body weight (BW), day of gestation, parity, body condition, and health (Grummer et al., 2004; Hayirli and Grummer, 2004). Prefresh cows with excess body condition (>4 on a 5-point scale) consumed about 8 percent less dry matter (DM) than cows at similar BW but with lower body condition scores (Hayirli et al., 2002). Cows with developing metabolic or health problems, including hypocalcemia, have lower DMI prepartum than healthy cows (Goff and Horst, 1997b; Huzzey et al., 2007).

Increasing dietary energy (Coppock et al., 1972; Hernandez-Urdaneta et al., 1976; Minor et al., 1998) or dietary energy and protein (VandeHaar et al., 1999) concentrations during the prefresh period resulted in higher DM and energy intake. However, concentration of dietary crude protein (CP) within the range of about 10 to 16 percent generally does not affect intake in dry and prefresh cows (Hayirli et al., 2002). Concentrations of rumen-undegradable protein (RUP) were negatively associated with prepartum intake, but that may be more related to source of protein (i.e., most of the studies with high RUP fed animal-based proteins) rather than RUP per se. Increasing dietary fat concentration may reduce DMI, but that effect was only observed in prepartum heifers (Hayirli et al., 2002).

Similar to lactating cows (see Chapter 2), diet digestibility, as reflected by source and concentration of neutral detergent fiber (NDF) or estimated net energy for lactation (NEL), is probably the most important dietary factor related to DMI by dry cows. Increasing the NDF concentration of dry cow or prepartum heifer diets by the addition of lower-quality grass (Holcomb et al., 2001), cottonseed hulls (VandeHaar et al., 1999), or straw (Dann et al., 2006; Janovick and Drackley, 2010; Mann et al., 2015) usually reduces DMI. Hayirli et al. (2002) classified diets from multiple studies into three groups: low NDF (28 to 32 percent), medium NDF (35 to 50 percent), and high NDF (50 to 62 percent). Average (across parities) daily DMI for those three classes of diets by animals during the last 3 weeks of gestation was approximately 2.0, 1.7, and 1.6 kg per 100 kg of BW (Hayirli et al., 2002). If the NDF is more digestible (e.g., from soyhulls), DMI can be much higher than those values (Holcomb et al., 2001). An interaction between day of gestation and NDF on DMI may also occur (Janovick et al., 2011). In most studies cited above, as cows approached parturition, the decrease in DMI was less when cows were fed diets that included lower-quality feeds such as straw or mature grass than the control diets. The effect of parity (independent of BW) on DMI during the last 1 to 2 months of gestation is not clear. Using data from multiple studies, on a BW basis, nulliparous animals consumed about 12 percent less DM than multiparous cows during that period (Hayirli et al., 2003). However, Janovick et al. (2011) reported that from about 35 to 21 days prepartum, daily DMI of heifers and cows when fed a higher digestible diet (39 percent NDF) was similar (approximately 2 kg of DMI/100 kg of BW). When animals were fed a straw-based diet (51 percent total NDF), DMI by heifers was about 25 percent less on a BW basis than intake by cows (Janovick et al., 2011).

Predicting Dry Matter Intake in Transition Cows and Dry Cows

In the previous edition, daily DMI (as kg/100 kg of BW) during the last 3 weeks of gestation was estimated using loga-
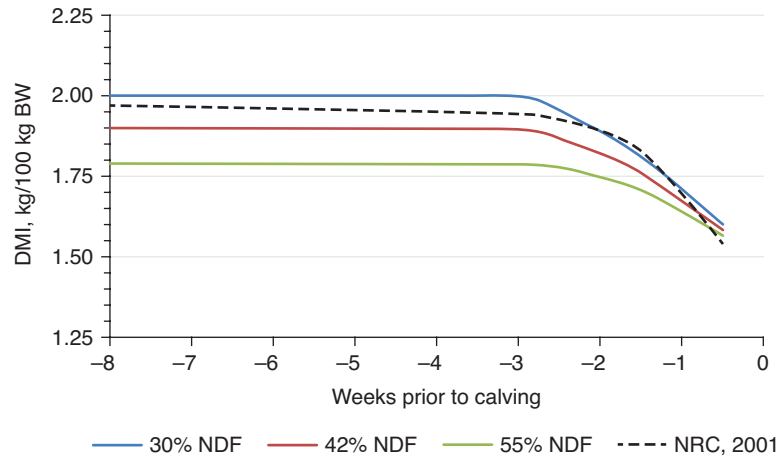
FIGURE 12-1   Estimated (Equation 12-1 and NRC, 2001) daily DMI by cows (>1 parturition) fed diets with 30 to 55 percent NDF (mostly from forages) during the dry period.

rithmic decay functions (one for heifers and one for cows) based solely on day relative to calving (Hayirli et al., 2003). The estimated daily DMIs between 60 and 22 days prepartum using those equations were 1.7 and 2.0 kg DM/100 kg BW for heifers and cows, respectively. Based on those equations, DMI started to decrease sooner (relative to calving) for cows than for heifers so that by calving, DMI per unit of BW was similar between parities. Based on limited data, the model was accurate for heifers but was inaccurate (mean square prediction error was 77 percent of mean intake) and biased (mean overestimation of 1.1 kg) for cows. The low accuracy is at least partially attributed to the lack of any dietary factors in the equation (Hayirli et al., 2002).

Inadequate data were available to conduct a true meta-analysis. Data used by Hayirli et al. (2003) plus recently published data (Holcomb et al., 2001; Dann et al., 2006; Janovick and Drackley, 2010; Mann et al., 2015) were combined to estimate DMI based on diet NDF and stage of gestation using multiple regression. The concentration of forage or roughage NDF was not reported in most studies, and only studies in which forage was the predominant source of NDF were used. Predicted intake is likely inaccurate when cows are fed diets that contain substantial amounts of by-products with more digestible NDF (e.g., soyhulls or distillers grains). The following equation is valid only for diets between 30 and 55 percent NDF. The equation to estimate DMI during the last 3 weeks of gestation for cows is as follows:

$$\text{Daily DMI, kg/100 kg BW} = 1.47 - [(0.365 - 0.0028 \times \text{NDF}) \times \text{Week}] - 0.035 \times \text{Week}^2$$

$$\text{(Equation 12-1)}$$

where NDF is percentage of diet DM (assumed to be mostly from forage). If NDF <30 percent, then NDF = 30, and if NDF >55 percent, then NDF = 55. Week is week prior to calving entered as a negative number. Limited data suggest

that for cows with body condition score (BCS) >4, estimated DMI should be reduced by 8 percent (Hayirli et al., 2003).

DMI during the early phase of the dry period was set at the same value as for week 3 (e.g., 2.0, 1.9, and 1.8 kg/100 kg of BW for cows fed diets with 30, 40, or 50 percent NDF, respectively). Based on Equation 12-1, during the early dry period, reducing dietary NDF increases DMI, but as cows approach parturition, the effect of NDF becomes less (see Figure 12-1).

Because of inadequate data, no interactions with parity, NDF, and stage of gestation could be modeled. Therefore, the same DMI equation for cows (see Equation 12-1) is used for heifers, except DMI is reduced by 12 percent, which is the difference in intake between heifers and cows in the early phase of the dry period (60 to 22 days prepartum) observed by Hayirli et al. (2003).

$$\text{Heifer DMI, kg/100 kg BW} = (1.47 - [(0.365 - 0.0028 \times \text{NDF}) \times \text{Week}] - 0.035 \times \text{Week}^2) \times 0.88$$

$$\text{(Equation 12-2)}$$

Rather than estimating DMI based on day before calving, which will not be known until after the animal has calved, time is expressed in weeks to introduce a degree of uncertainty (3 weeks before calving encompasses the time between 21 and 15 days before calving, 2 weeks is 14 to 8 days prepartum, and 1 week is between 7 days and 1 day prepartum). These equations and values were derived using data from Holstein cattle and are assumed to work for other breeds.

Effect of Prepartum Diet on Postpartum Production

Protein

Based on estimated protein use (i.e., maintenance, fetal growth, mammary gland development), cows and heifers

may have to mobilize body tissue during the immediate pre- and postpartum period to meet those needs. However, direct measurements of changes in maternal protein reserves during late gestation are lacking. Putnam and Varga (1998) measured apparent nitrogen balance in multiparous cows at approximately 10 days prepartum. Cows were fed diets containing 10.6, 12.7, and 14.5 percent CP; DMI was not affected (11.3 kg/d), but as dietary CP concentration increased, apparent nitrogen retention increased from 36 to 49 g/d. Those values include nitrogen retained in the fetus. After correcting for estimated fetal protein deposition, maternal nitrogen balance was still positive for all treatments, suggesting that even a diet containing 10.6 percent CP was adequate to maintain maternal protein stores. Comparable data for heifers are lacking. Because heifers consume less feed and have greater demands for mammary gland development and growth than cows, changes in maternal protein reserves may differ between prepartum cows and heifers.

Because of the potential for losses of maternal protein stores in late gestation, several studies (see Lean et al., 2013) have evaluated the effects of increasing the concentration of CP in prepartum diets on postpartum production. Across those studies, CP concentration of the low-protein diets averaged approximately 12 percent (range from 10 to 13), and the average concentration of the high-protein diets averaged approximately 16 percent (range from 12 to 23 percent). Some individual studies reported increased yields of milk or milk protein when higher CP diets were fed prepartum (Santos et al., 2001), but others reported negative effects of increased prepartum CP (Greenfield et al., 2000). A meta-analysis determined that on average, dietary CP concentration prepartum had no effect on milk yield (Lean et al., 2013). Likewise, providing additional RUP prepartum has not consistently affected milk production postpartum. Studies to determine effects of supplemental amino acids during the prepartum period independent of postpartum supplementation on postpartum production are lacking. Feeding diets with more than about 12 percent CP to cows during the immediate prepartum period will likely not increase milk production in the subsequent lactation. Because of lower DMI and potentially greater requirements, heifers may benefit from higher dietary concentrations of CP prepartum, but data are lacking. A meta-analysis found that postpartum production by cows was not affected by prepartum metabolizable protein (MP) supply, but increasing prepartum MP supply to heifers was beneficial (Husnain and Santos, 2019). The effect of prepartum protein on colostrum quality and yield has not been evaluated. In addition, data are lacking on the effect of varying dietary CP or MP concentration when prepartum cows are fed low-energy (e.g., high-straw) diets.

Protein requirements for mammary growth were not included in the computer model mainly because insufficient data for mammary parenchymal growth rates and the composition of that growth are available. VandeHaar and Donkin (1999) estimated that the additional CP for mammary growth during the last few weeks of gestation would be approximately 120 to 130 g/d, which is equivalent to about 1 percentage unit of dietary CP, assuming mammary parenchymal mass increased by 430 to 460 g/d during the transition period (NRC, 2001). The meta-analysis by Husnain and Santos (2019) supports feeding higher dietary protein prepartum to heifers.

Energy and Carbohydrates

Energy density and concentrations of NDF and starch in prepartum diets are highly correlated (e.g., increasing NDF concentration usually reduces starch and the NEL concentrations). Therefore, effects of changing one of those components cannot be isolated, and all three will be discussed concurrently.

Several studies have examined how energy intake during the prepartum period affects postpartum performance and health (Kunz et al., 1985; Holter et al., 1990; Olsson et al., 1998; Mashek and Beede, 2001; Agenas et al., 2003; McNamara et al., 2003; Dann et al., 2006; Silva-del-Río et al., 2010; Mann et al., 2015). Generally, one treatment consisted of prepartum energy intake at approximately the cow's requirement, and the other treatment or treatments consisted of substantially greater energy intake (e.g., 25 to 50 percent excess energy intake). Energy and DMI was usually increased by replacing lower-quality forage NDF with starch or higher-quality forages. Postpartum intakes usually were not affected by prepartum treatment (cows fed a common diet after calving). In some studies (Janovick and Drackley, 2010; Silva-del-Río et al., 2010; Richards, 2011), milk production was lowered by reducing energy intake prepartum, but in most of the studies, milk production was not affected by prepartum energy intake.

A common practice is to formulate higher-energy diets for closeup or prefresh cows (i.e., cows during the last 2 to 3 weeks prepartum) by increasing the concentration of starch and reducing the amount of forage and fiber in the diet. Potential benefits are to aid in the adaptation of rumen microbes to the higher-starch diet that will be fed after calving and to maintain DM and energy intake during the immediate prepartum period. The rumen microbial population changes as a cow transitions from prepartum to postpartum (Minuti et al., 2015) likely because of dietary changes. Transitioning growing beef cattle from a forage-based diet (ca. 50 percent forage) to a high-concentrate diet (ca. 90 percent) over a period of 2 weeks compared with an abrupt change resulted in greater average daily gains or better feed efficiency, and the ruminal microbial populations required days to weeks to stabilize during the diet change (Brown et al., 2006). The dietary changes a typical dairy cow undergoes during the transition period are substantially less dramatic, but data are lacking on how transition diets may affect ruminal microbial populations.

Increasing starch and reducing fiber concentrations in prefresh diets usually increases DM and energy intakes during the prefresh period, but this usually does not translate into greater postpartum intakes or milk yields (Minor et al., 1998;

Holcomb et al., 2001; Keady et al., 2001; Doepel et al., 2002; Rabelo et al., 2003, 2005; Smith et al., 2005; Vickers et al., 2013). Even when prefresh feed intake was restricted by almost 50 percent, postpartum intake and milk production were not affected (Dann et al., 2006). Evidence suggests that prefresh diets with lower-energy concentrations reduce the risk of subclinical and clinical ketosis during the subsequent lactation (Doepel et al., 2004; Smith et al., 2008; Vickers et al., 2013).

ETIOLOGY AND NUTRITIONAL PREVENTION OF METABOLIC DISORDERS

Fatty Liver

Fat accumulation in the liver occurs when uptake of FAs exceeds the capacity of the liver to oxidize or secrete the FAs, which happens when blood concentrations of NEFAs are elevated (Bobe et al., 2004; Grummer, 2008). Ketosis almost always occurs when cows have moderate (5 to 10 percent of liver wet weight as triacylglyceride) to severe (greater than 10 percent fat) fatty liver. In healthy cows, plasma NEFA concentrations are low (less than about 0.2 mEq/L) until a few days before parturition and then reach concentrations as high as 0.8 mEq/L at calving, remain high for a few days, and then start slowly decreasing (Bertics et al., 1992; Grum et al., 1996; LeBlanc et al., 2005). Cows that are at greater risk to develop metabolic problems postpartum often have plasma NEFA concentrations greater than 0.5 mEq/L prepartum and can be much greater than 1.0 mEq/L postpartum (LeBlanc et al., 2005; Roberts et al., 2012). Plasma NEFAs are elevated when cows are in negative energy balance, which can occur when DMI drops during the immediate prepartum period and almost always occurs during the first few weeks of lactation because intake is low and energy needs are high, causing mobilization of body reserves. Because most cows have elevated NEFAs during the peripartum period, most early lactation cows have some degree of hepatic fat accumulation (Jorritsma et al., 2001; Bobe et al., 2004).

Extensive reviews of the regulation of hepatic lipid metabolism and its relation to fatty liver and ketosis have been published (Emery et al., 1992; Grummer, 1993; Drackley, 1999; Hocquette and Bauchart, 1999; White, 2015). Uptake of NEFAs by the liver is proportional to NEFA concentrations in blood (Emery et al., 1992), and NEFAs taken up by the liver can be esterified or oxidized (Drackley, 1999). The primary esterification product is triglyceride (TG), which can be exported as part of a very low-density lipoprotein or be stored. In ruminants, export of TG occurs at a very slow rate relative to other species (Kleppe et al., 1988; Pullen et al., 1990). Therefore, under conditions of elevated hepatic NEFA uptake, FA esterification and TG accumulation occur.

Fatty liver is a major risk factor for displaced abomasum, ketosis, and immune dysfunction (Bobe et al., 2004). Conversely, these disorders may be a risk factor for fatty liver if they reduce DMI, causing a more severe negative energy balance. In addition to clinical abnormalities, fatty liver is associated with reduced function by hepatocytes (e.g., reduced gluconeogenesis and reduced ureagenesis), increased measures of oxidative stress (e.g., lower concentrations of plasma α-tocopherol and higher concentrations of malondialdehyde), and increased inflammation (Bobe et al., 2004; Bradford et al., 2015).

A major risk factor for development of fatty liver is obesity (Bobe et al., 2004; Roche et al., 2013). During the early postpartum period, fat cows usually have lower DMI than cows in proper body condition, resulting in greater mobilization of body fat (Stockdale, 2001). More severe negative energy balances in early lactation were associated with greater concentrations of hepatic lipid and plasma NEFA (Weber et al., 2013) concentrations of blood NEFAs. Prepartum intake per se does not appear to be related to fatty liver; however, Grummer (2008) hypothesized that prepartum change in intake or, more specifically, change in energy balance may be a cause of hepatic fat accumulation. Increasing dietary starch concentration during the prefresh period usually does not reduce liver fat accumulation postpartum, even though it often increases DMI in the prepartum period and may reduce NEFA concentrations around calving (Overton and Waldron, 2004; Grummer, 2008). Increased dietary starch concentrations in the immediate postpartum period have reduced hepatic fat accumulation (Rabelo et al., 2005). Increasing the concentration of dietary fat during the peripartum period has not consistently affected liver fat accumulation (Skaar et al., 1989; Bertics and Grummer, 1999; Andersen et al., 2008). Responses may be affected by concentration of added fat and type of FAs (e.g., saturated FAs may decrease hepatic lipid concentrations [Andersen et al., 2008]). Monensin often reduces blood ketones, but it has not been shown to reduce hepatic fat accumulation (Zahra et al., 2006; Duffield et al., 2008). Supplementing rumen-protected choline during the peripartum period can reduce liver fat concentrations (Cooke et al., 2007; Zom et al., 2011; Lima et al., 2012; Elek et al., 2013; Zenobi et al., 2018). Niacin has antilipolytic properties, but unless supplemented at very high rates, it usually does not affect plasma NEFA concentrations (reviewed by Grummer, 2008). Supplementing peripartum cows with rumen-protected niacin has reduced plasma NEFAs but has not markedly affected liver lipid concentrations (Yuan et al., 2012; Morey et al., 2011).

Ketosis

Ketosis or hyperketonemia occurs when excessive amounts of long-chain FAs are oxidized via β-oxidation. Excessive amounts of long-chain FAs are released in cows undergoing severe negative energy balance after parturition. Ketone bodies (β-hydroxybutyric acid and acetoacetate) are end products of β-oxidation, and when these accumulate in the blood, clinical signs can be observed. Release of FAs from adipose tissue followed by β-oxidation is stimulated when plasma insulin is low and glucagon is high (Holtenius

and Holtenius, 1996). Many of the clinical signs of ketosis such as reduced DMI and milk production and lethargy are nonspecific. Some cows will show abnormal behavior such as aggression, incoordination, and chewing on nonfood objects. A definitive diagnosis requires some measure of ketones in blood, urine, or milk. The accuracy and value of various tests have been reviewed (Tatone et al., 2016) and will not be discussed. For this discussion, β-hydroxybutyric acid (BHBA) in blood will be used as the standard diagnostic for ketosis. Elevated blood BHBA has been associated with increased risk of numerous health problems, reduced milk yield, and reduced reproductive efficiency (Walsh et al., 2007; Ospina et al., 2010; Chapinal et al., 2012; Suthar et al., 2013; Raboisson et al., 2014). The cutoff for separating healthy lactating cows from cows with subclinical ketosis has varied between approximately 1.0 and 1.4 mmol/L (Duffield, 2000; Raboisson et al., 2014), but a value of >1.2 mmol/L of BHBA is commonly used to define subclinical ketosis. Incidence rates for subclinical ketosis will depend on which cutoff value is used, timing of blood sampling, and so on, but herd-level rates of 20 to 40 percent have been reported (Duffield et al., 2009; McArt et al., 2012; Suthar et al., 2013).

Holtenius and Holtenius (1996) classified ketosis as either type 1 or type 2. Type 1 ketosis generally occurs a few weeks after parturition when milk production and glucose demand by the mammary gland are high and is usually not associated with excessive hepatic fat concentrations. Type 2 occurs at or very near parturition and is usually associated with fatty liver. Type 2 ketosis is often more refractory to treatment than type 1 (Herdt, 2000). This classification scheme illustrates the two major causes of ketosis. Risk factors and causes of type 2 ketosis are largely the same as those for fatty liver (discussed above). With type 1, blood glucose and insulin concentrations are lower and ketone concentrations are higher compared to healthy cows. Low insulin probably enhances FA oxidation by decreasing hepatocyte malonyl-CoA concentrations and sensitivity of carnitine palmitoyltransferase 1 to malonyl-CoA concentrations (Emery et al., 1992). Carnitine palmitoyltransferase 1 is responsible for translocating FAs from the cytosol to the mitochondria for oxidation, and its activity is high with type 1 ketosis. This suggests that for type 1, the supply of precursors for gluconeogenesis is not adequate. Limited availability of substrate could be caused by low DMI. Increasing dietary starch postpartum reduces blood BHBA and increases glucose (Rabelo et al., 2005; McCarthy et al., 2015). Supplementing monensin postpartum reduces blood BHBA and increases glucose (Sauer et al., 1989; McCarthy et al., 2015). Administration of propylene glycol as either a drench or a bolus consumption can reduce blood BHBA (Nielsen and Ingvartsen, 2004).

Udder Edema

Udder edema is characterized by excessive accumulation of fluids in the intercellular tissue spaces of the mammary gland usually during the peripartum period. Edema and congestion occur in the udder and umbilical area and may be prominent in the vulva and brisket. Incidence rate in peri-parturient Holstein heifers was about 12 percent in one herd in Florida (Melendez et al., 2006); however, Morrison et al. (2018) reported a 66 percent incidence rate in three herds in Ontario. Case descriptions were not the same for both studies. Typically, the incidence and severity of udder edema are greater in pregnant heifers than in cows (Zamet et al., 1979; Erb and Grohn, 1988) and tend to be more severe in older than in younger heifers and in heifers with male calves rather than female calves (Melendez et al., 2006). Obese cows (Vigue, 1963) and cows that had udder edema previously (Melendez et al., 2006) are at increased risk for udder edema. Udder edema is moderately heritable (Dentine and McDaniel, 1983). Edema can be a major discomfort to the animal and causes difficulty with milking machine attachment, increased risk of teat and udder injury, and mastitis. Severe udder edema may reduce milk production and cause a pendulous udder (Dentine and McDaniel, 1983). The exact cause(s) of udder edema is unknown; more likely, it is a multifactorial condition. Restriction or stasis of venous and lymph flow from the udder in late pregnancy due to fetal pressure in the pelvic cavity causing increased venous pressure may be a contributing factor (Vestweber and Al-Ani, 1983, 1984; Al-Ani and Vestweber, 1986). Changes in amounts and relative proportions of steroid hormones during late pregnancy may also be involved. Reduced concentrations of proteins, especially globulins, in blood suggest an increase in vascular permeability as animals approach calving and have been associated with greater incidences of udder edema (Vestweber and Al-Ani, 1984).

Emery et al. (1969) reported increased udder edema in heifers fed high-concentrate diets, but that may have been caused by the approximate 75-g/d increase in sodium chloride (NaCl) intake rather than the concentrate per se. Excessive intakes of sodium (Na) and potassium (K) have been implicated as causative agents in udder edema (Randall et al., 1974; Sanders and Sanders, 1981; Vestweber and Al-Ani, 1983; Al-Ani and Vestweber, 1986). Restriction of NaCl and water intakes reduced the severity and incidence of udder edema in pregnant heifers (Hemken et al., 1969). Lower incidence and severity of udder edema were found when diets contained no supplemental salts of Na or K (Randall et al., 1974). In a field study with two commercial dairy herds, K fertilization of alfalfa was implicated as the cause of increased udder edema (Sanders and Sanders, 1981). Cows consumed about 450 g of K/head per day. In an earlier controlled study, consumption of 454 g of a combination of NaCl and potassium chloride (KCl) increased the incidence and severity of udder edema (Randall et al., 1974). In a second study, the incidence and severity of udder edema in pregnant heifers fed a grain mix containing 1 percent NaCl or a grain mix with 4 percent supplemental KCl plus 1 percent NaCl for 20 days did not differ (Randall et al., 1974). Nestor et al. (1988) reported that the severity of udder edema was greater when pregnant heifers were fed

sodium bicarbonate (0 versus 272 g/head per day) or NaCl (23 versus 136 g/head per day) separately but not when both salts were fed together. Excessive intake of the chloride salts of Na or K probably increases the severity of udder edema, especially in late-pregnant heifers. Using forages with low concentrations of K and limiting supplemental Na would be prudent if udder edema is prevalent.

Lema et al. (1992) and Tucker et al. (1992) studied the effects of prepartum calcium chloride ($CaCl_2$), an anionic salt, on the incidence and severity of udder edema. Results were mixed, but supplementation of $CaCl_2$ at approximately 1.5 percent of diet DM often reduced the prevalence and severity of edema in the peripartum period. Oxidative stress may play a role in udder edema (Miller et al., 1993; Mueller et al., 1998). Mueller et al. (1998) reviewed two studies on the effects of antioxidant and pro-oxidants on udder edema. In one study, udder edema during the first week after calving was less in heifers supplemented for 6 weeks before calving with 1,000 IU vitamin E/head per day versus none. In the other study, late-pregnant heifers were fed factorial combinations of vitamin E (0 or 1,000 IU/d), Zn (0 or 800 mg/d), and Fe from iron sulfate (0 or 12 g/d, which is equal to about 1,300 mg/kg of diet DM). Fe can be a pro-oxidant and increases the formation of ROS. Without supplemental Fe, vitamin E reduced severity of udder edema, but Zn did not. When Fe was excessive, vitamin E was ineffective in reducing the severity of udder edema, but Zn was somewhat effective, perhaps by reducing absorption of Fe. This suggests that when ROS are extremely high (e.g., high concentrations of reduced Fe in the diet), antioxidants may not be able to overcome their effects.

Retained Placenta and Metritis

Retained placenta (retained fetal membranes) is defined as failure of the fetal membranes to be expelled within 24 hours after parturition (Kelton et al., 1998). Metritis is defined as postpartum cows with abnormally enlarged uterus with fetid red-brown watery or purulent vaginal discharge within the first 21 days after calving with or without systemic signs of illness (e.g., fever). Most cows (60 to 80 percent) with retained placenta will have metritis, but the incidence of metritis is usually much greater than the incidence of retained placenta (Gilbert et al., 2005; Han and Kim, 2005). Retained placenta and metritis impair various measures of reproductive efficiency (Erb et al., 1985; Opsomer et al., 2000; Giuliodori et al., 2013). Cows that had a retained placenta usually have reduced milk yields (Joosten et al., 1988; Rajala and Gröhn, 1998; Gilbert et al., 2005), but effects of metritis on subsequent milk production are less clear (Giuliodori et al., 2013) possibly because of the varied definitions and severity of metritis.

Multiple physiologic and nutritional factors have been implicated as causes of retained placenta and metritis. Dystocia, twinning, stillbirth, and caesarean section increase the risk for retained placenta (Erb et al., 1985; Han and Kim, 2005).

However, cows that had a retained placenta had elevated serum concentrations of several proinflammatory cytokines and lactate as early as 8 weeks prepartum, indicating calving events are not the only cause of retained placenta (Dervishi et al., 2016). Older cows generally are at greater risk than first-parity cows, and a short gestation period increases risk of retained placenta (Bendixen et al., 1987; Gröhn et al., 1990; Han and Kim, 2005).

Nutritional Factors

Most studies evaluating nutritional influences on retained placenta evaluate supplementation during the entire dry period or during the last 2 or 3 weeks of gestation. Inadequate supply of selenium (Se), vitamin E, vitamin A, and β-carotene is related to increased prevalence of retained placenta. Lower concentrations of serum Zn are associated with increased retained placenta (Sheetal et al., 2014), but data showing that supplementation of Zn reduces retained placenta are lacking. Many or perhaps all of these effects could be mediated via improved immune function. Immune system dysfunction, specifically reduced neutrophil function, prior to parturition was associated with increased prevalence of retained placenta (Kimura et al., 2002). Supplementing Cu to Cu-deficient cows improved the function of neutrophils (Torre et al., 1996); however, clinical data on effects of Cu supplementation on retained placenta are lacking. These nutrients are also involved in cellular antioxidant systems, and maintaining proper concentrations of ROS within cells and tissue can affect production of various prostaglandins and eicosanoids, which can affect placental retention. Oxidative stress increases around parturition, and cows that demonstrate increases in oxidative stress earlier in the prepartum period, to a greater extent, have more severe metritis postpartum (Baithalu et al., 2017) and are more likely to have a retained placenta (Miller et al., 1993).

An excess of ROS can cause peroxidative damage of cell membranes and interfere with normal metabolic function, including normal steroidogenesis (Miller et al., 1993) and arachidonic acid metabolism (Sordillo, 2013). Supplementing diets with antioxidants to meet requirements is crucial during the periparturient period (Weiss et al., 1990a) when blood α-tocopherol concentrations are the lowest of the entire lactation cycle (Goff and Stabel, 1990; Weiss et al., 1990a), and expression of several antioxidant enzymes (Aitken et al., 2009) and total antioxidant capacity (Castillo et al., 2005; Baithalu et al., 2017) is low. Based on the preponderance of data, when basal diets with <0.1 mg of Se/kg of diet DM are supplemented with an additional 0.1 to 0.3 mg/kg Se or cows are injected with Se approximately 2 to 3 weeks prepartum, prevalence of retained placenta is decreased (Trinder et al., 1969; Julien et al., 1976a,b; Segerson et al., 1981; Harrison et al., 1984; Jovanovic et al., 2013). Se supplementation did not always reduce retained placenta, but in most of those instances, basal diets contained more than 0.1 mg Se/kg of DM (Gwazdauskas et al., 1979;

Schingoethe et al., 1982; Hidiroglou et al., 1987; Stowe et al., 1988). Vitamin E supplementation also significantly reduces the risk of retained placenta (Bourne et al., 2007), and the concentration of serum α-tocopherol prepartum was lower in cows that went on to have a retained placenta compared with healthy cows (Qu et al., 2014). The recommended intakes for Se (see Chapter 7) and vitamin E (see Chapter 8) were derived in part from experiments evaluating their effects on retained placenta, and increasing intakes above recommended values will likely not affect prevalence of retained placenta.

Cows showing clinical signs of vitamin A deficiency had increased incidence of retained placenta (Nicholson and Cunningham, 1965). However, in cows with better vitamin A status, no relationship was observed between serum retinol concentrations and retained placenta (LeBlanc et al., 2004). Supplemental β-carotene at 600 or 1,200 mg/d has reduced incidence of retained placenta (Michal et al., 1994; Oliveira et al., 2015). However, in the Oliveira et al. (2015) study, supplementation only reduced prevalence in multiparous cows. LeBlanc et al. (2004) found no difference in serum β-carotene concentrations between late-gestation cows that eventually developed retained placenta and those that did not. Based on available data, after meeting vitamin A recommendations (see Chapter 8), additional supplementation is not expected to affect prevalence of retained placenta. Although β-carotene supplementation can reduce prevalence of retained placenta, inadequate data are currently available to derive an Adequate Intake value.

Cows with hypocalcemia have a higher risk for retained placenta than cows with normal blood Ca (Curtis et al., 1985; Rodríguez et al., 2017), and factors related to hypocalcemia are discussed below. Supplementing late-gestation cows with calcidiol (25-OH vitamin D_3) rather than cholecalciferol (vitamin D_3) greatly reduced the incidence of retained placenta and metritis (Martinez et al., 2018). Vitamin D is related to immune function; however, in that experiment, calcidiol supplementation did not affect neutrophil function prepartum.

Extreme deficiencies of energy, protein, or both can result in retained placenta because cows are weak and, coupled with the stress of parturition, lack strength to expel the placenta (Maas, 1982). Cows fed diets low in CP (8 percent) for the entire dry period had a higher incidence of retained placenta compared with cows fed 15 percent CP (50 versus 20 percent incidence) (Julien et al., 1976a). Fat cows (Morrow, 1976) and cows with elevated plasma NEFAs or ketones prepartum have a higher risk of retained placenta and metritis (Qu et al., 2014; Raboisson et al., 2014).

Milk Fever (Hypocalcemia)

Plasma concentrations of Ca should be 9 to 10 mg/dL (2.25 to 2.5 mM). However, an acute and severe form of hypocalcemia known as milk fever occurs in nearly 5 percent of multiparous dairy cows as a result of the large and sudden secretion of Ca in milk that occurs at the onset of lactation (NAHMS-USDA, 2018). Blood Ca concentrations are often below 4.5 mg/dL (1.12 mM) in these recumbent cows exhibiting muscle paresis. About 50 percent of multiparous dairy cows and 25 percent of heifers experience a subclinical hypocalcemia around the time of calving (Reinhardt et al., 2011). Cows with plasma concentrations less than 8.0 (Reinhardt et al., 2011) to 8.6 mg/dL (Martinez et al., 2012) are considered subclinically hypocalcemic. Cows with subclinical hypocalcemia are at increased risk for immune dysfunction, metritis, displacement of the abomasum, retained placenta, mastitis, and ketosis (Daniel, 1983; Massey et al., 1993; Kimura et al., 2006; Martinez et al., 2012; Chamberlin et al., 2013; Neves et al., 2018; McArt and Neves, 2020). However, time of sampling relative to calving has a marked effect on plasma concentrations of Ca, and the health risks associated with low plasma Ca depend on when (relative to calving) the sample was taken (Neves et al., 2018; McArt et al., 2020). For example, low plasma Ca 1 day postpartum was not related to increased risk of cows developing health problems, but cows with low plasma Ca on day 2, 3, or 4 postpartum had increased risk for metritis and displaced abomasum (Neves et al., 2018). Furthermore, the concentration of plasma Ca at which a statistically increased risk of other health disorders occurs differs between primiparous and multiparous cows (Neves et al., 2018).

Hypophosphatemia and hypomagnesemia can also be present in cows with hypocalcemia and can complicate response to treatment.

Calcium Dynamics of the Periparturient Cow

During the last weeks of gestation, based on the requirements outlined in Chapter 7, a 650-kg dairy cow consuming 12 kg of DM needs to absorb about 24 g Ca each day for body maintenance (11 g) and for fetal development (13 g Ca/d). The average Holstein cow produces about 7 kg of first-milking colostrum (Mann et al., 2016) with 2.1 g Ca/kg (see Table 12-1), representing 16 g Ca removed from the plasma. The Ca concentration in colostrum and transition milk exceeds 2 g/L for at least the first five milkings postpartum (Abd El-Fattah et al., 2012), which, combined with a first five milking yield of about 44 kg (Andrée O'Hara et al., 2019), represents a removal of about 88 g of Ca or 35 g/d with twice-daily milking. This equals a 4-fold increase in Ca requirements compared to the immediate prepartum cow. The exchangeable plasma pool of Ca in an early lactation cow is about 10 mg/kg BW (Ramberg et al., 1970). In a 650-kg BW cow, that pool must be turned over more than five times daily during the first 2 or 3 days of lactation to meet the increased demand created by the mammary gland. As a result, most cows will experience a decline in blood Ca at the onset of lactation, but most cows successfully activate Ca homeostatic mechanisms to return blood Ca concentration back to normal levels shortly after calving.

Ca homeostasis is mediated primarily by the parathyroid gland, which secretes parathyroid hormone (PTH) in response to any reduction in blood Ca concentration. The PTH stimulates release of Ca from bone stores, reduces the amount of Ca lost via urine, and activates the renal enzyme that produces the vitamin D hormone, 1,25-dihydroxyvitamin D. That hormone stimulates transcellular absorption of Ca across the intestinal epithelium to greatly increase uptake of dietary Ca (Goff, 2018). Serotonin is also involved with Ca metabolism perhaps via effects on PTH-related peptide (Hernandez et al., 2012). Infusing serotonin intravenously into prepartum cows increased concentrations of blood Ca postpartum and improved some other measures of Ca status (Weaver et al., 2016). A reduced ability of bone and kidney cells to respond to PTH stimulation has been implicated as the defect in Ca homeostasis that results in prolonged or severe hypocalcemia (Martig and Mayer, 1973). Several factors can interfere with Ca homeostasis, causing more drastic and longer-lasting declines in blood Ca, including age of the cow and breed (Lean et al., 2006; Roche and Berry, 2006; Chiwome et al., 2017).

Heifers rarely develop clinical milk fever, although 25 percent may experience subclinical hypocalcemia. The incidence of clinical and subclinical hypocalcemia increases with each subsequent lactation (Reinhardt et al., 2011; Venjakob et al., 2017). Ca homeostasis in heifers is more robust than in older cows because they are still growing and their bones contain more osteoclasts; hence, PTH only needs to activate the cells. This provides them a larger pool of exchangeable bone Ca on the first day of lactation (Ramberg, 1995). In older cows, the PTH must first induce osteoclast production and then activate the cells to resorb bone Ca. Also, as cows age, there is a reduction in the number of intestinal receptors for 1,25-dihydroxyvitamin D, which may translate into slower activation of intestinal Ca transcellular absorption (Horst et al., 1990).

The Channel Island breeds (Jersey, Guernsey) and, to a lesser extent, Swedish Red and White and Norwegian Red breeds have a higher incidence of milk fever than Holsteins (Lean et al., 2006; Chiwome et al., 2017). The reasons remain unclear, but Jerseys may have greater Ca stress because their colostrum has about 20 percent greater Ca concentration than that from Holsteins.

Diet Cation–Anion Difference and Acid-Base Status

Dietary cations, such as K^+, Na^+, Ca^{++}, and Mg^{++}, will raise blood pH when they are absorbed into the blood, and anions, such as chloride (Cl^-), sulfate (SO_4^{2-}), and phosphate (PO_4^{3-}), have the opposite effect. The difference in the number of milliequivalents of cations and anions absorbed from the diet helps determine blood pH (Stewart, 1983; Goff, 2018). Cows are typically in a state of compensated metabolic alkalosis as their diet consists of forages that are typically high in K. K is absorbed from the diet with nearly 100 percent efficiency, and because forage K often has an organic acid

as the counterion, it is strongly alkalinizing. Grasses and legumes, especially those grown on soils where manure or potash has been applied, are usually major sources of dietary K (Pehrson et al., 1999). Na from compounds that lack an inorganic counterion (e.g., sodium bicarbonate) is also highly alkalinizing as it is absorbed with nearly 100 percent efficiency. Dietary concentrations of Ca and magnesium (Mg) can be high, but they are absorbed with much lower efficiency than K and Na (see Chapter 7) and therefore are less alkalinizing.

Adding Cl^- and SO_4^{2-} without Na or K to the precalving diet can greatly reduce the degree of hypocalcemia at calving (Ender et al., 1971; Block, 1984; Oetzel et al., 1988). Cows in a state of compensated metabolic alkalosis do not respond to PTH stimulation as well as cows placed in a state of compensated metabolic acidosis (Goff and Horst, 1997b; Goff et al., 2014). Metabolic alkalosis impairs bone Ca resorption (Abu Damir et al., 1994; Block, 1994) and the ability of PTH to stimulate timely production of 1,25-dihydroxyvitamin D (Goff et al., 1991; Phillippo et al., 1994).

Dietary Cl^- is absorbed with nearly 100 percent efficiency. Sulfate anions can also acidify the blood but, because of lower absorption SO_4^{2-}, has just 60 percent of the acidifying activity of Cl^- (Spears et al., 1985; Tucker et al., 1991; Goff et al., 2004). When sufficient anions are added to the precalving diet, they induce a compensated metabolic acidosis (Ender et al., 1971; Block, 1984), improving tissue sensitivity to PTH. This restores the competency of Ca homeostatic mechanisms and facilitates a rapid return to normocalcemia after the onset of lactation. Blood pH is difficult and expensive to measure, but urine pH generally reflects blood pH and can be measured on farm to determine the degree of compensated metabolic acidosis experienced by the cow. Diets that reduce urine pH values below 7.0 and usually closer to 6.0 generally improve Ca status (Charbonneau et al., 2006).

Several diet cation–anion difference (DCAD) equations (units of milliequivalents per kilogram of diet DM) have been developed (see Chapter 7). Although SO_4^{2-} is the acidifying anion, labs usually measure sulfur (S) so equations were developed using S. One meta-analysis found that the equation, $DCAD = (Na + K) - (Cl + 0.6 S)$, was best to predict urine pH and had the strongest association with milk fever incidence (Charbonneau et al., 2006). However, the equation, $DCAD = (Na + K) - (Cl + S)$, is probably the most widely used (DeGaris and Lean, 2008). Based on the meta-analysis of Charbonneau et al. (2006), a DCAD of about −200 mEq/kg (expressed as $(Na + K) - (Cl + S)$) is needed to achieve urinary pH of 6.5.

As DCAD decreases, the degree of hypocalcemia will also generally decrease (Moore et al., 2000; Charbonneau et al., 2006; Lean et al., 2006). If the addition of anions fails to acidify the blood enough to cause urine pH to be <7.0, there will be no improvement in periparturient Ca status (Moore et al., 2000; DeGaris and Lean, 2008; Leno et al., 2017; Goff and Koszewski, 2018). If the DCAD is too low, the cow can enter a state of uncompensated metabolic acidosis. As DCAD

is reduced, the net amount of acid excreted into the urine will also increase until urine pH reaches about 6.3, but when urine pH is below 6.3, the net acid excretion in urine is no longer well correlated to DCAD (Constable et al., 2009). When urine pH falls below 6.3, the kidney begins to excrete NH_4^+ into the urine, slowing a further decline in urine and blood pH. The NH_4^+ arises from the combining of H^+ with ammonia that diffuses into the tubular fluid as urine pH is reduced below 5.9. This ability of the kidney to neutralize the excess H^+ allows the cow to remain in a state of compensated metabolic acidosis until urine pH reaches approximately 5.5 (Teloh et al., 2017). Urine pH below 5.3 is indicative of uncompensated metabolic acidosis (Berend, 2017), which will greatly reduce DMI (Goff, 2014). There is evidence that dry cows with urine pH of 7.3 will be less hypocalcemic than cows with urine pH of 7.9 but will exhibit more hypocalcemia than cows with urine pH of 6.0 (Moore et al., 2000). There is little practical difference in the degree of hypocalcemia experienced by cows with urine pH of 5.5 versus 6.7 (Charbonneau et al., 2006; Lean et al., 2006; Melendez and Poock, 2017).

The proper concentration of dietary Ca when cows are fed negative DCAD diets is not known. Successful anionic diets have contained between 0.65 and 1.7 percent Ca (Ender et al., 1971; Block, 1984; Gaynor et al., 1989; Joyce et al., 1997). Once the dietary Ca requirement of the cow has been met, the addition of Ca to precalving diets has little effect on periparturient Ca status if blood has been acidified to the same extent. Dietary Ca, especially when added as calcium carbonate, has a mild alkalinizing effect that will necessitate addition of more anions to achieve the same degree of acidification, which may decrease DMI (Goff and Horst, 1997a; Goff and Koszewski, 2018). DMI is often reduced by low DCAD because of palatability (Oetzel et al., 1991) or by metabolic effects (Zimpel et al., 2018). Commercial anion supplements have been developed that are more palatable than traditional chloride or sulfate salts (Strydom et al., 2016).

Low-Calcium Diets to Prevent Hypocalcemia

This strategy involves limiting absorbed Ca so that the cow is in negative Ca balance for at least 7 to 14 days before calving. Negative Ca balance stimulates secretion of PTH within 3 to 4 days of the dietary Ca reduction, and PTH concentrations will remain elevated until after calving (Goings et al., 1974). Prolonged exposure to high concentrations of PTH overcomes any tissue resistance to PTH caused by metabolic alkalosis (Goff et al., 1986; Liesegang et al., 1998). Ca conservation and Ca mobilization mechanisms, such as renal production of 1,25-dihydroxyvitamin D and bone Ca resorption, are activated prior to the onset of lactation (Boda and Cole, 1954; Goings et al., 1974; Green et al., 1981) so that homeostatic mechanisms are primed and ready to respond to the Ca demands of lactation.

Based on current Ca requirements and an availability coefficient of 0.44 for a high-forage diet (see Chapter 7), to meet the needs of a late-gestation 650-kg cow, the diet would have to contain about 52 g of Ca or 0.43 percent Ca (based on an intake of 12 kg). The studies in which milk fever was effectively prevented using the low dietary Ca approach had dietary Ca below 18 g/d (Boda and Cole, 1954; Goings et al., 1974; Green et al., 1981; Kichura et al., 1982) and likely supplied just 5 to 9 g of absorbable Ca each day. Based on feedstuffs typically available, feeding prepartum diets that are truly deficient in Ca is extremely difficult.

Substances that bind dietary Ca preventing absorption can cause a Ca deficiency and reduce periparturient hypocalcemia. Zeolite A, a sodium–aluminum silicate, binds Ca preventing absorption and can prevent hypocalcemia in cows fed diets with 0.6 to 0.7 percent Ca during the late dry period (Thilsing-Hansen et al., 2002; Pallesen et al., 2008; Kerwin et al., 2019). It also binds phosphate (PO_4^{-3}) (Pallesen et al., 2008), which may help prevent hypocalcemia (see below). Zeolite A also may bind Mg in the diet, resulting in lower plasma Mg concentrations (Thilsing-Hansen et al., 2002). However, when the prepartum diet contained more than 0.21 percent Mg, zeolite did not affect plasma Mg (Pallesen et al., 2008). Phytic acid in rice bran treated with formaldehyde is able to escape the rumen and bind Ca within the lumen of the small intestine, preventing it from being absorbed and improving periparturient Ca status (Martín-Tereso et al., 2016). However, average blood Ca concentrations the first 12 hours postcalving were still generally less than about 2.1 mmol/L.

Effect of Dietary Phosphorus on Hypocalcemia

Because it is an anion, absorbed dietary phosphate (PO_4^{-3}) will acidify the blood; however, excess dietary phosphorus (P) in the prepartum period increases the degree of hypocalcemia (Kichura et al., 1982; Barton et al., 1987). Serum PO_4^{-3} generally increases as dietary P increases (Lopez et al., 2004), and this causes bone cells to secrete a PO_4^{-3} regulating hormone, fibroblast growth factor 23 (FGF23), to reduce blood PO_4^{-3}. The FGF23 circulates in the blood and binds to its receptor on kidney cells and inhibits renal synthesis of 1,25-dihydroxyvitamin D, which then reduces intestinal PO_4^{-3} absorption, causing blood PO_4^{-3} to decline. Unfortunately, lesser production of 1,25-dihydroxyvitamin D also reduces diet Ca absorption, impairing Ca homeostasis (Martin and Quarles, 2012). Restricting P intake to just meet requirement can aid Ca homeostasis. Cows fed an anionic precalving diet that was 0.21 percent P (approximately equal to requirement) had a lower incidence of hypocalcemia than cows fed a 0.44 percent P diet, and the low P diet maintained serum P concentrations that were within the normal range for cows (4 to 6 mg P/dL or 1.23 to 1.86 mmol/L) (Peterson et al., 2005). In a limited study, dairy cows fed P-deficient diets (i.e., plasma inorganic P concentrations were <1.0 mmol/L) the last 4 weeks of gestation had less clinical hypocalcemia than cows fed adequate P (Cohrs et al., 2018).

Dairy cows can develop a condition known as the hypophosphatemic downer cow. These cows exhibit a very

low serum PO_4^{-3} concentration—often below 1.5 mg/dL (0.46 mmol/L). It is generally a complication associated with milk fever and is not a consequence of low dietary P concentrations. The mechanism is not well understood. It could involve reduced P intake because of low DMI that occurs during clinical hypocalcemia or perhaps sequestration of salivary phosphate in the rumen because of the reduced rumen motility that occurs during hypocalcemia. Secretion of PTH caused by low blood Ca increases loss of P via urine but can also increase P mobilization from bone and enhance gut absorption, so normally elevated PTH does not markedly reduce blood P concentrations. Steps taken to reduce milk fever incidence reduce incidence of the hypophosphatemic downer cow as well (Goff, 2014).

Dietary Magnesium and Hypocalcemia

Hypomagnesemia can contribute to hypocalcemia (Allen and Davies, 1981; Van de Braak et al., 1987). Blood Mg concentration is normally 1.9 to 2.4 mg/dL (0.8 to 1 mmol/L), but if blood Mg concentration falls below 1.25 mg/dL (0.5 mmol/L), the ability of the parathyroid gland to secrete PTH is compromised and blood Ca concentration rapidly decreases (Littledike and Goff, 1987). The effect of low Mg on PTH secretion is likely via Mg effects on guanosine diphosphate (GDP) dissociation from receptor proteins (Vetter and Lohse, 2002). This is most common in lactating dairy and beef cattle on pasture and is often referred to as lactation tetany or grass tetany. A less severe decline in blood Mg below 1.7 mg/dL (0.7 mmol/L) can alter the responsiveness of tissues to PTH (Contreras et al., 1982; Littledike and Goff, 1987; Rude et al., 2009). Cows fed adequate dietary Mg in the prefresh period will be slightly hypermagnesemic the day after parturition because of the actions of PTH on reabsorption of Mg from renal tubular fluid. Blood Mg concentration within 24 hours after calving that is equal to or less than 2.0 mg/dL (0.83 mmol/L) suggests inadequate dietary Mg (Goff, 2014). Based on a meta-analysis, an increase in dietary Mg concentration from 0.3 to 0.4 percent of DM, while maintaining DCAD and Ca constant, could result in an approximate 62 percent decrease in milk fever risk (Lean et al., 2006).

Vitamin D and Its Metabolites and Hypocalcemia

Meeting recommendations for dietary vitamin D (see Chapter 8) provides all of the substrate needed for adequate renal synthesis of 1,25-dihydroxyvitamin D. Feeding or injecting up to 10 million units of vitamin D between 4 and 14 days prior to calving can have a pharmacologic effect on Ca and P metabolism and prevent milk fever (Yamagishi et al., 2000). The bulk of the vitamin D is converted to 25-hydroxyvitamin D and other 24-hydroxylated vitamin D metabolites. The greatly elevated concentrations of 25-hydroxyvitamin D allow it to enter target cells. The 25-hydroxyvitamin D has much lower affinity for the vitamin D receptor than does 1,25-dihydroxyvitamin

D; however, at greatly elevated concentrations, it will activate the receptor. The elevated 25-hydroxyvitamin D concentrations also displace 1,25-dihydroxyvitamin D from plasma vitamin D binding protein, raising the concentration of free 1,25-dihydroxyvitamin D (Jones, 2008). This will increase intestinal Ca absorption and can help prevent milk fever. Unfortunately, the dose of vitamin D that effectively prevents milk fever is very close to the toxicity level causing metastatic calcification of soft tissues. Lower doses may induce milk fever because the high levels of 25-hydroxyvitamin D and resulting hypercalcemia and hyperphosphatemia suppress renal synthesis of endogenous 1,25-dihydroxyvitamin D (Littledike and Horst, 1982). Several attempts have been made to feed or inject 25-hydroxyvitamin D prior to calving to prevent hypocalcemia, but they have not been consistently effective (Olson et al., 1973; Taylor et al., 2008; Weiss et al., 2015; Martinez et al., 2018; Rodney et al., 2018).

Experimental treatment with 1,25-dihydroxyvitamin D and its synthetic analogues can be more effective than using the less active vitamin D metabolites, but problems with timing of administration make these treatments impractical (Gast et al., 1977; Hove and Kristiansen, 1982). For effective prevention of milk fever, 1,25-dihydroxyvitamin D had to be administered between 7 and 3 days before calving. However, recently, a single dose of 1,25-dihydroxyvitamin D given within a few hours of calving improved periparturient Ca status when cows were fed an acidifying diet prior to calving (Viera-Neto, 2017). Exogenous 1,25-dihydroxyvitamin D also may have inhibited endogenous production of 1,25-dihydroxyvitamin D, so some cows developed hypocalcemia 5 to 12 days after calving (Horst et al., 2003). Continuous parenteral or oral administration of smaller doses of 1,25-dihydroxyvitamin D analogues prior to calving and for several days after calving can effectively prevent milk fever (Goff and Horst, 1990; Junichiro et al., 2015; Bachmann et al., 2017). A key to the success of these studies was that the daily hormone dose was reduced slowly after calving, allowing the cow to begin to make her own 1,25-dihydroxyvitamin D.

Displaced Abomasum

Displacement of the abomasum (DA) is a costly (Liang et al., 2017) multifactorial disorder and is diagnosed almost exclusively in adult dairy cattle but may occur in calves and young cattle (Zerbin et al., 2015; Biggs and Harvey, 2016; Caixeta et al., 2018). Average herd incidence rate is 2.2 percent (NAHMS-USDA, 2018), but individual herds can have rates as high as 20 percent (Doll et al., 2009). The transition period and several weeks subsequent to calving is the major risk period for development of displaced abomasum (Stengärde et al., 2010). About 80 percent of cases involve displacement to the left side of the cow and generally occur within the first 4 weeks following parturition (Radostits and Done, 2007). Only about 50 percent of the right DA occurs during this time (Zerbin et al., 2015). The causes of left

and right DA are thought to be similar (Doll et al., 2009). Although the nature of relationships and associations is not well defined, twins, dystocia, milk fever, retained placenta, metritis, ketosis, and fatty liver are among the risk factors commonly identified for DA (Geishauser et al., 2000; Van Winden et al., 2003; LeBlanc et al., 2005).

Abomasal Physiology

In the nonpregnant cow, the abomasum occupies the ventral portion of the abdomen, very nearly on the midline, with the pylorus extending to the right side of the cow caudal to the omasum (Dyce et al., 1987; Radostits and Done, 2007). As pregnancy progresses, the growing uterus occupies an increasing volume of the abdominal cavity. The uterus begins to slide under the caudal aspect of the rumen, reducing rumen volume by one-third by the end of gestation. This forces the abomasum forward and slightly to the left side of the cow, although the pylorus continues to extend across the abdomen to the right side of the cow. After calving, the uterus retracts back toward the pelvic inlet, which, under normal conditions, allows the abomasum to return to its original position. In the case of a left DA, the pyloric end of the abomasum slides completely under the rumen to the left side of the cow (Van Winden et al., 2002). Three major factors thought to be responsible for DA are (1) the rumen fails to take up the void left by the retracting uterus, and if the rumen moves into its normal position on the left ventral floor of the abdomen, the abomasum is not able to slide under it; (2) the omentum attached to the abomasum is stretched, permitting movement of the abomasum to the left side; and (3) abomasal atony. Normally, gases produced in the abomasum (mostly carbon dioxide released when bicarbonate from the rumen meets the hydrochloric acid of the abomasum) are expelled back into the rumen as a result of abomasal contractions. These contractions are thought to be impaired in cows developing DA (Breukink and de Ruyter, 1976; Goff and Horst, 1997b; Doll et al., 2009). The exact causes of abomasal atony have not been fully established. Overconditioned cows at dry-off are at greater risk of DA likely because of low DMI around parturition (Cameron et al., 1998). In addition, excess loss of body condition from calving to 4 weeks postpartum is a risk factor for DA (Hoedemaker et al., 2009).

Nutrition and Abomasal Displacement

Reduced DMI before and after calving is likely a cause of DA, although direct evidence is limited (Shaver, 1997). Cows that eventually develop a DA have lower DMI a few days prior to clinical diagnosis (Van Winden et al., 2003). Poor feed bunk management (defined as <30 cm of bunk space per cow, feed refusal not removed daily, and empty feed bunks for a portion of the day) prepartum increased the risk of DA and also likely reduced DMI, but that was not measured (Cameron et al., 1998). Shaver (1997) outlined several

factors that can increase the risk of DA and also likely limit or reduce DMI. These include limited feed availability, crowded pens and feed bunks, improperly mixed total mixed ration (TMR), sorting, and inclusion of unpalatable ingredients in the diet, among other factors.

Ca is needed for proper smooth muscle contractility and neuromuscular transmission (Caixeta et al., 2018); therefore, hypocalcemia is a risk factor for DA (Curtis et al., 1983; Rodríguez et al., 2017). Experimental induction of hypocalcemia reduced the rate of abomasal contractions, which may lead to atony and distension of the abomasum (Daniel, 1983). When plasma Ca levels were reduced from about 9.5 to 7.5 mg/dL, abomasal motility was reduced by 30 percent and strength of contractions was reduced by 35 percent, and when plasma Ca was reduced to 5 mg/dL, these responses were reduced to 70 percent and 50 percent, respectively (Daniel, 1983). Subclinical hypocalcemia increased the likelihood of DA 3.7 times (Rodríguez et al., 2017). The administration of oral $CaCl_2$ at calving to reduce subclinical hypocalcemia decreased the incidence of DA (Oetzel, 1996). However, the association between hypocalcemia and DA has not always been observed (LeBlanc et al., 2005; Chamberlin et al., 2013).

Increasing the proportion of grain in the diet fed to cows in late gestation and early lactation may increase the incidence of displaced abomasum (Coppock, 1974; Van Winden et al., 2004). This may be caused by increased volatile FAs within the abomasum, which can reduce abomasal contractility (Lester and Bolton, 1994). Elevated osmolality of rumen contents when high-grain diets are fed may contribute to paralysis and ultimately displacement of the abomasum (Van Winden et al., 2004). In addition, higher-grain diets may not supply adequate effective fiber needed to stimulate rumination activity, maintain the consistency and depth of the rumen mat, and illicit rumen contractions. Inadequate supply of effective fiber is likely a risk factor for DA. In an observational study, reduced rumination time both prior to and after calving was associated with greater incidence of DA (Stangaferro et al., 2016); however, controlled research with adequate statistical power evaluating the influence of effective fiber on incidence of DA is lacking. Direct research on the influence of TMR particle size, forage fiber, and fiber concentrations on rumen contractility and DA, especially during the transition period, is needed (Caixeta et al., 2018). For further information on effective fiber, see Chapter 5.

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13

Dairy Production Systems

GROUP HOUSED WITH TOTAL MIXED RATIONS

Group housing with total mixed ration (TMR) feeding is the predominant production system used on commercial dairy farms in the United States (Schingoethe, 2017). In addition, most nutrition research with dairy cattle uses TMRs; therefore, no adjustments are needed to the nutrient requirements discussed in individual chapters when cows are fed a TMR. However, cow grouping strategies need to be considered when setting ration formulation parameters. Advantages of a TMR system over component feeding (e.g., concentrate separate from forage or hay separate for silage) include (Schingoethe, 2017) the following: (1) increased, but not absolute, control of what cows consume, making feeding balanced diets easier; (2) ability to include wet ingredients such as brewers grains into the diet; (3) reduced negative effect when including less palatable ingredients in diets; (4) ability to increase energy intake while reducing the risk of rumen upsets such as acidosis; (5) greater feed efficiency (Holter et al., 1977); and (6) increased mechanization and reduced labor costs.

Ideally, in a well-mixed TMR, every mouthful of the ration should provide the exact blend of nutrients that was formulated; however, in reality, cows sort diets and in most situations appear to select against longer particles (Miller-Cushon and DeVries, 2017). Dry matter (DM) concentration of the TMR over a range of about 45 to 65 percent does not consistently affect sorting (Felton and DeVries, 2010; Fish and DeVries, 2012), but including liquid molasses in the TMR (approximately 4 percent of diet DM) reduced sorting (DeVries and Gill, 2012). Perhaps the diet factor that has the greatest effect on cow sorting is particle size. Diets with a larger proportion of longer particles are more easily sorted than diets with more uniform particle size (Kononoff et al., 2003; Leonardi and Armentano, 2003; Onetti et al., 2004; Leonardi et al., 2005). Because large particles are usually forage with high-fiber concentrations, selecting against large particles can increase energy intake, but it also increases the risk for acidosis and rumen upset (see Chapter 5). A cow's desire or ability to sort may be associated with that cow's risk for acidosis (Coon et al., 2019).

Diet Formulation for Groups of Cows

Most confinement dairy farms and some grazing farms have multiple pens (or paddocks). With multiple pens, decisions must be made regarding how cows will be grouped within pens and what the different groups will be fed. Numerous factors go into these decisions such as herd demographics (i.e., distribution among parities, stage of lactation, milk yield distributions, reproductive stage, etc.), size of pens, size of mixer wagon, feed and forage inventories, and feed and milk prices. It is beyond the scope of this section to discuss all of these; readers are referred to a review by Cabrera and Kalantari (2015). The necessity of having a dry cow group and feeding them a specific diet has been known for decades and will not be discussed. The value of transition groups is discussed in Chapter 12.

Independent of any diet differences, separating first-lactation cows from more mature cows often improves milk production and increases behaviors that likely will improve health (e.g., increased lying time) (Krohn and Konggaard, 1979; Phillips and Rind, 2001). Some data (Krohn and Konggaard, 1979) suggest that the benefits of separating first-lactation cows diminish as group size gets larger (>70 cows per pen); however, in that experiment, multiple factors were confounded with group size. The benefits of separating first-lactation cows likely are related to social rank because first-lactation cows often have low rank and cannot compete effectively for resources when housed with older cows.

Based mostly on computer simulations, grouping cows according to nutritional needs (within parity) usually increases income over feed costs (Williams and Oltenacu, 1992; St-Pierre and Thraen, 1999; Kalantari et al., 2015). Two major questions arise with respect to grouping: (1) what criteria should be used to group cows, and (2) what diet formulation

specifications should be used for a group of cows as contrasted to a single cow? Grouping to reduce variation in requirements for metabolizable protein (MP) and net energy within a group is usually economically optimal (Cabrera and Kalantari, 2015; Bach et al., 2020). To realize the saving in feed cost caused by grouping, diets must be formulated correctly for each group. Direct experimental evidence is lacking regarding optimal formulation strategies for different groups, but computer simulation models have been used. Formulating a diet to meet the requirements of an average cow in a pen will likely result in a loss of production from the pen. This is because cows that have lower requirements than the average will likely not increase milk yield in response to additional nutrients (i.e., production is limited by something other than nutrition), but cows that have requirements substantially greater than the average cow will consume inadequate nutrients and production will decrease. The degree of overformulation (i.e., the excess supply of a nutrient relative to the requirement for the average cow in the group) depends on variation in requirements within the pen, feed costs, environmental regulations, and the degree to which intake and production are correlated (Cabrera and Kalantari, 2015). Milk yield and dry matter intake (DMI) have a moderate positive correlation (Hristov et al., 2004); however, the correlation is much weaker in early lactation than in later lactation (Kramer et al., 2008). A strong correlation between DMI and milk yield implies that supply of nutrients will be greater by high-producing cows than low-producing cows when fed the same diet because of differences in DMI. The typical range in marginal response in milk yield to increased DMI is about 2 kg of milk per 1-kg increase in DMI (Bach et al., 2020) when very early lactation cows are excluded. Depending on the variation in milk yields within a pen, expected differences in DMI likely will not be enough to provide adequate nutrients, especially MP, to high-producing cows when diets are formulated for group-average milk yield. A high-producing cow will produce more milk than the average cow, but milk yield will be less than if a more nutrient-dense diet were fed. Stallings and McGilliard (1984) were among the first to propose factors (i.e., lead factors) that could be used to formulate diets for groups of cows. They concluded that diets for a pen of cows should be formulated to meet the energy and protein needs of the average cows plus 1 standard deviation in milk yield. The majority of cows within a pen would be consuming excess protein and energy, but high-producing cows would be fed adequately to maintain high production. Overfeeding protein has an environmental cost because excess nitrogen (N) is excreted by cows; however, excess consumed energy is retained by cows as body fat. This problem was identified using simulation models and resulted in the development of different lead factors for energy and protein (Kalantari et al., 2015). Kalantari et al. (2015) confirmed that for MP formulating for pen, mean milk yield plus 1 within-pen standard deviation is optimal; however, for net energy for lactation (NEL), diets should be formulated for pen mean production. This will result in fewer obese cows. Optimal formulation strategies need to be evaluated with actual data.

Last, grouping cows by stage of lactation rather than production can be useful in managing body condition. Because of the interaction between diet composition and stage of lactation on feed intake (see Chapter 2), diet formulation can be used to modulate energy intake and partitioning of energy between milk and body reserves.

PASTURE-BASED SYSTEMS

Nutrient Supply

Nutrient supply often differs between cows managed under grazing and confinement systems. When no supplemental feed is offered, DMI by grazing cows is almost always lower than for cows fed a TMR in confinement (Bargo et al., 2003). Several factors affect DMI in both confinement and grazing cattle (discussed in Chapter 2), but chewing fatigue and time available to graze can be additional intake constraints for grazing cows. In addition to dietary factors that affect intake under all management systems, pasture allowance (PA), which is the amount of consumable herbage offered per cow per unit of land area; density and height of the sward; and DM concentration of the herbage can affect intake by grazing cattle (Bargo et al., 2003). Taller plants and denser swards will increase DMI assuming no change in forage quality (Rook et al., 1994; Gibb et al., 1999). However, for grazing cows fed no supplemental feed, PA has the greatest effect on DMI. As PA increased, intake of herbage DM increased quadratically, reaching a plateau when PA was approximately 110 kg of DM/cow/d (Bargo et al., 2003) for average Holstein cows grazing high-quality herbage. The recommended equation to estimate DMI when cows are not given supplemental feed is as follows:

If pasture allowance (PA) $\leq$ 108 kg DM/cow, Pasture DMI, kg/d $= 7.79 + 0.26 \times PA - 0.0012 \times PA^2$; otherwise, DMI $= 21.9$ (Equation 13-1)

The average body weight (BW) of cows included in that meta-analysis (Bargo et al., 2003) was not presented, but it likely is based mostly on Holstein data; therefore, Equation 13-1 will overestimate DMI for Jersey cows. The above equation is grazing cattle consuming only herbage; however, supplemental concentrates are often fed to grazing cows. Bargo et al. (2003) evaluated the accuracy of different equations to estimate total DMI by grazing dairy cows fed supplemental concentrate and concluded the DMI equation developed by NRC (2001) for confinement dairy cows was acceptable. Equation 2-1 (Chapter 2) is recommended to estimate DMI by grazing cows fed supplemental concentrates. A more complex equation (Caird and Holmes, 1986) that required more inputs (including sward height, pasture allowance, and amount of concentrate fed) was also accurate.

For this discussion, concentrates include starchy, fibrous, and proteinaceous feedstuffs derived from the seed portion

of plants. The type of concentrate, especially starchy versus fibrous, can affect responses as discussed below. Providing supplemental concentrates increases DMI and milk yield, but DMI is often still less than that for cows fed a TMR (Bargo et al., 2003; Roche et al., 2006; Golder et al., 2014; Auldist et al., 2016). Supplementing concentrate twice daily at milking times increases total DMI but usually reduces consumption of herbage DM. Across numerous studies, substitution rates for concentrates (i.e., kilograms of reduced herbage DMI/kg of consumed concentrate DM) range from about 0.2 to 0.7 (Bargo et al., 2003). The substitution rate tends to increase as PA increases and is greater when starchy concentrates are fed compared to fibrous concentrates. If concentrates are blended with forages, the substitution rate for the blend appears similar to that of concentrate alone (Bargo et al., 2002; Auldist et al., 2012, 2016).

Providing concentrates, especially starchy concentrates, usually (Bargo et al., 2003; Doyle et al., 2005) but not always (Reis and Combs, 2001) reduces neutral detergent fiber (NDF) digestibility. The negative effect on NDF digestibility when supplemental concentrates are fed often results in little or no improvement in energy digestibility of the total diet; however, intake of digestible energy usually increases. Based on rumen pH, volatile fatty acid (FA) patterns, and other measures, feeding concentrate blended with forage results in a more stable rumen (Golder et al., 2014; Greenwood et al., 2014; Auldist et al., 2016). This has not, however, resulted in consistently improved digestibility of DM or fiber compared with supplementing concentrates separate from forage (Bargo et al., 2002; Greenwood et al., 2014). The effect of increasing supplemental concentrate on intake of metabolizable energy is likely not linear and probably follows a diminishing return function. Doyle et al. (2005) calculated that the increase in metabolizable energy intake becomes marginal when more than about 8.5 kg of DM from starchy supplements was fed.

Grazing cattle often have lower N use efficiency (grams of milk N/g of N intake) than cows fed a TMR. When grazing cows are fed starchy concentrates, urinary excretion of N decreases, and milk protein yield and concentration and N use efficiency increase (Stockdale, 2004; Sairanen et al., 2005; Roche et al., 2013). However, these data should not be interpreted to imply efficiency of nutrient use differs because of management system. It likely reflects nutrient composition of diets under the different systems. Indirect measures (e.g., urine allantoin) have been used to estimate microbial protein synthesis by grazing cattle (Carruthers et al., 1996; Carruthers and Neil, 1997), and efficiency of microbial protein synthesis (g/g of digested organic matter) was similar to values obtained with cows fed a TMR. Silva et al. (2014) measured lower efficiency for microbial protein synthesis for grazing cows fed supplemental concentrate compared with the efficiency used by the previous NRC (2001), but that study did not include a treatment with cattle fed a TMR to allow direct comparison.

Fresh forages usually have high concentrations of β-carotene and α-tocopherol (see Chapter 8); therefore, supply of those vitamins from the basal diet can be high for grazing cattle. This should reduce the need for supplemental vitamin E, β-carotene, and vitamin A. Grazing cattle can also have greater exposure to sunlight than confined cattle, which likely reduces the need for supplemental vitamin D. Some data with sheep have shown that soil ingestion reduces copper (Cu) absorption (Suttle et al., 1984). However, in another study with sheep, soil ingestion did not affect liver Cu concentrations (Grace et al., 1996). Sheep tend to graze herbage closer to the ground than cattle, and soil ingestion may be less an issue with cattle than with sheep. Grazing cattle can have low magnesium absorption, but this is likely a function of high potassium rather than any unique aspect of grazing (see Chapter 7).

Nutrient Requirements

The requirements for grazing cattle do not differ from confinement cattle for any nutrient except energy and perhaps protein. Grazing cows expend more energy harvesting feed (walking to collect herbage, prehension, and chewing) than do cows fed a TMR. Because of topography and location of the paddocks, grazing cows also may expend more energy walking to and from the milking center than do cows in confinement. However, the distance between pens and the milking system can be substantial in some confinement systems. Although these energy costs are real, they are currently difficult to quantify, and many necessary inputs will not be known under most situations. However, with pedometers, global positioning devices, and topography maps, these inputs can be known with high accuracy. Energy expended walking within a paddock depends on size of the paddock, topography, and allowance of pasture. Many of these effects have not been quantified or modeled; therefore, energy expended walking within a paddock was assumed to equal the energy expended within a pen. That energy expenditure is incorporated into the maintenance term. Energy expended by walking to and from the milking center is a function of distance, topographical elevation changes, and BW of the cow. Reasonable estimates of BW and distance traveled to and from the milking center can be obtained under field conditions; therefore, that expenditure of energy is calculated as a separate component (i.e., activity). In the seventh revised edition (NRC, 2001), the energetic cost of horizontal locomotion was set at 0.00045 Mcal NEL/kg BW per kilometer. Based on newer data derived from beef cows (Brosh et al., 2006; Aharoni et al., 2009; Brosh et al., 2010), the energy cost for horizontal locomotion for cattle was set at 0.00035 Mcal NEL/kg of BW per kilometer of total distance walked between the paddock and milking center (approximate range in measured values was 0.0003 to 0.0004 Mcal/kg BW per kilometer). This represents a 22 percent decrease in energy required for horizontal walking from NRC (2001); however,

this may still be an overestimation of the cost. D'Hour et al. (1994) reported no difference in milk production or blood nonesterified FA concentrations when grazing cows were forced to walk an additional 6.4 km/d over flat ground. Milk yields started to decrease when cows were forced to walk an additional 10 km/d. Vertical distance traveled is more difficult to estimate, and measuring its energetic cost is less precise than for horizontal distance. For cattle, estimates for energetic cost of vertical locomotion have ranged from about 9 to 19 times the cost of horizontal locomotion (Di Marco and Aello, 1998; Aharoni et al., 2009; Brosh et al., 2010). Because of all of the uncertainties related to measurement, the committee chose the highest value and estimated the cost of vertical locomotion as 0.0067 Mcal NEL/kg of BW per kilometer. Because of newer data, this is a substantial reduction in energy expenditure for vertical locomotion compared with the previous NRC (2001). For a 650-kg cow walking 0.2 km of vertical distance, activity requirement is currently 0.9 Mcal of NEL compared with 3.9 Mcal NEL/d based on NRC (2001). However, NRC (2001) used an incorrect efficiency value for work associated with vertical distance. The value in the NRC (2001) example should only be 1.4 Mcal NEL/d. The value used in this edition is based on a broader set of experimental data and is likely more accurate than both the previous incorrectly calculated value and the corrected value. In the model, energy associated with vertical distance can be calculated from user-entered vertical distance (if known) or, to better reflect the qualitative nature of the estimated requirements for vertical travel, qualitative descriptors can be selected: mild (0.05 km of total vertical distance per day), moderate (approximately 0.2 km of vertical distance), and severe (approximately 0.5 km of vertical distance). These three classes result in 0.2, 0.9, and 2.2 Mcal of NEL expended per day for a 650-kg cow.

The amount of energy expended by the animal harvesting pasture depends on amount of herbage consumed and on PA. When PA is reduced, cows expend more energy to gather food. Angus steers (BW = 259 kg) expended 3.3 times more energy grazing pasture that contained 148 g of DM/m^2 compared with pasture at 228 g DM/m^2 (Di Marco et al., 1996). No supplemental feed was provided in that study. Estimating the energy required for grazing (prehension, mastication, and walking while grazing) is difficult, and data are both limited and highly variable. Estimated energy expended for grazing ranged from about 0.003 to 0.025 Mcal/kg BW$^{0.75}$ per day when cattle were fed no supplemental concentrate (Di Marco et al., 1996; Aharoni et al., 2009; Brosh et al., 2010). The average was 0.0075 Mcal/kg BW$^{0.75}$, which would be the cost of food gathering (in excess of that in confinement) when no supplemental concentrate was fed. Cattle in those studies grazed about 10 hours per day. Dairy cattle fed no supplemental concentrate also graze about 10 hours per day, and on average, that is reduced by 12 minutes for every kilogram of concentrate DM fed (Bargo et al., 2003). The equation in the model adjusts grazing time based on supplemental

feeds (which could include corn silage, hay, concentrate, etc.). Changing the amount of nonpasture intake from 2 to 12 kg/d reduces energy expenditure by about 0.2 Mcal for a 650-kg cow. The model calculates daily NEL required for grazing as follows:

$$(0.0075 \text{ Mcal} \times \text{BW}^{0.75})$$
$$\times (600 - (12 \times \text{kg nonpasture DMI})) / 600$$
$$\text{(Equation 13-2)}$$

Therefore, the total activity requirement for grazing cattle will include horizontal locomotion between the paddock and milking center adjusted for positive vertical distance traveled plus activity associated with gathering food. As an example, a 650-kg cow fed 6 kg of concentrate daily grazing a pasture located 0.6 km from the milking center with a total of 0.2 km change in elevation (i.e., moderate) that makes four one-way trips daily will have an estimated grazing activity requirement of the following:

Horizontal locomotion: $(0.6 \text{ km} \times 4 \text{ trips}) \times 0.00035$
$\times 650 \text{ kg} = 0.54 \text{ Mcal NEL/d}$

Positive vertical locomotion: $(0.2 \text{ km of vertical distance})$
$\times 0.0067 \times 650 \text{ kg} = 0.87 \text{ Mcal NEL/d}$

Grazing activity: $0.0075 \times 650^{0.75} \times 0.88$
$= 0.91 \text{ Mcal NEL/d}$

Total activity energy requirement $= 2.32 \text{ Mcal/d}$

The energetic cost of grazing for heifers is not known. During a 9-hour period (0700 to 1600 h), Holstein and Holstein × Jersey heifers only walked about 2 km after they had adapted to grazing, which takes 5 to 8 days after being first introduced to a grazing system (Lopes et al., 2013). In that study, for the first 8 days after being put on pasture, heifers walked 2 to 5.5 km per 9 hours. Assuming reasonably flat ground, this walking would not be a major energy expenditure. On hilly ground and on sparse pasture, energy expended to graze would be higher.

The equations used to estimate grazing energy requirements are based on the best available data; however, accurate inputs will limit the overall accuracy of the equations. Users should know the amount of concentrate consumed and the approximate distance between the paddock and milking parlor, but vertical distance traveled will usually not be known with accuracy. The additional work expended by grazing cattle may increase protein requirement. Relative to maintenance, strenuous exercise by humans can increase energy expenditure by a factor of 10, but the protein requirement only doubled, and some of the increased protein requirement was to replace amino acids that were oxidized to provide energy (Lemon, 1998). In most situations, the work associated with grazing is not strenuous, and effects on protein requirements are probably small.

FEEDING AND AUTOMATIC MILKING SYSTEMS

Automatic milking systems (AMSs) can be used to conduct daily milking routines (Jacobs and Siegford, 2012). In 2016, as many as 15,000 commercial dairy farms worldwide were using AMSs (Rodenburg et al., 2017). By design, cow movement or traffic differs in facilities equipped with AMSs. Free-flow traffic design refers to facilities that allow cows unrestricted access to all animal areas of the barn. Guided-flow traffic refers to facilities that are equipped with one-way and selection gates. These gates are used to manage traffic by guiding cows to milking, feeding, and resting areas of the barn. Guided-flow designs may be further distinguished by two different flow patterns, namely, "milk first" and "feed first." In milk-first designs, cows exiting the resting area pass through a selection gate. If she is eligible for milking, the gate will operate and guide her to the AMS, but if she is not eligible, it will guide her to the area where feed is located, and she can only reenter the resting area through a one-way gate. The flow of cows through feed-first designs is reversed; cows exiting the feeding area pass through a selection gate. If she is eligible for milking, the gate will operate to guide her to the AMS, but if she is not eligible, it will guide her to the resting area (Endres and Salfer, 2017).

Regardless of the design, a portion of the nutrients supplied to the cow is usually offered during milking times when the cow enters the AMS. This is usually offered in the form of a pellet and intended to supply her with nutrients but also as a reward for visiting the AMS. The mixed feed fed to cows in this system is often referred to as a partially mixed ration or PMR (Bach and Cabrera, 2017). The feeding strategies of free-flowing and feed-first guided designs are similar, while in milk-first guided designs, the amount of feed offered as a reward by the AMS is low, and a greater portion of the nutrients is supplied in the mixed ration available in the feed bunk. Offering more nutrients in the PMR is often more economical than feeding more pellets in the AMS. In general, managers of AMSs strive to have all cows reach a set minimum of visits to an AMS and that these visits be spaced out across the day. The number of visits to the AMS is influenced by the nature of the reward and by other management, environmental, and animal factors that may work to dampen the cows' urge to reach the offering or impede visit to the AMS itself (Bach and Cabrera, 2017). Research manipulating the amount or concentration of nutrients offered by the AMS is lacking. Survey data have indicated that on average, North American producers offer 15.9 kg of concentrate for every 100 kg of milk (Tremblay et al., 2016). As mentioned above, cows are often offered a pelleted feed in the AMS. The pelleting process results in a feed that is easier to handle and also of higher density, but very little research has been conducted on the effects of the pelleting process on digestibility and rumen fermentation. In one study, cows consuming pelleted oats had greater fiber digestion than those consuming rolled or flaked oats (Tosta et al., 2019). Conversely, in one study, pelleting a TMR reduced fiber digestibility (Bofante

et al., 2016). Additional research should be conducted on the impacts of the pelleting process on nutrient availability of feeds when they are pelleted and offered in an AMS. Data are also lacking on factors that affect feed preference, but because this may affect frequency of visits to the AMS, then it should be considered in formulation procedures.

Amount of Reward

Because the nutritional needs of a lactating cow are influenced by milk yield, it logically follows that increasing the amount of concentrate offered would increase milk yield, but this is not always observed in an AMS (Bach et al., 2007; Tremblay et al., 2016; Paddick et al., 2019). This may be because not all feed offered is consumed; a positive but non-linear relationship between the amount of concentrate offered to the cow in an AMS and that refused exists. When more than 4 kg of concentrate is offered at a milking event, the amount of unconsumed concentrate often increases (Bach and Cabrera, 2017). Increasing the concentrate offered may also reduce the amount of PMR consumed and reduce overall DMI but not affect milk yield (Bach et al., 2007). In a feed-first guided-traffic flow barn, providing greater proportions of nutrients in the PMR and not in the AMS may be beneficial and stimulate feed intake. Hare et al. (2018) observed that for every 1 kg of concentrate provided in the AMS, the PMR intake was reduced by 1.58 kg, and in this system, large quantities of feed offered in the AMS were likely not needed. The relationship in substitution ratio is not consistent across studies and ranges from 0.84 to 1.58 (Paddick et al., 2019). Within the same system, these investigators also observed that the forage to concentrate ratio in the PMR and the amount of concentrate offered in the AMS may work as independent factors influencing feed intake and production. Specifically, increasing concentrate offered from 2 to 6 kg/d in the AMS reduced intake of PMR with marginal effects on production but increased variability of AMS concentrate consumption. In contrast, increasing concentrate contained in the PMR increased milk production but did not affect variability of feed intake from either the PMR or AMS (Menajovsky et al., 2018).

Even less research has been conducted in pasture-based systems, and observed effects on visitation to the AMS from increasing the amount of the reward are conflicting (Jago et al., 2007; Lessire et al., 2017). In addition to the reward itself, pasture allocation may have significant effects on intervals of time between milking and milking frequencies (Lyons et al., 2013). Additional research is needed to determine the nature of reward amount and the effect on rumen fermentation, as well as milk yield and composition.

Reward Composition

Effects on number of visits to the AMS by manipulation of the composition of the concentrate feed reward have been evaluated in several studies. Manipulations include starch

content, grain type, and flavoring. Increasing the concentration of starch in the concentrate did not affect the frequency of visits to the AMS (Miron et al., 2004; Halachmi et al., 2006, 2009), and milk production increased in only one study (Halachmi et al., 2009). A study designed to test the effect of preference for a range of different ingredients (barley, wheat, barley–oat mix, corn, grass, fat) on the frequency of visits to the AMS showed that the barley–oat mixture was most preferred so the frequency of visits increased when the feed offered was barley–oat mixture (Madsen et al., 2010). In addition, pellets containing grass and fat resulted in the greater proportion of fetch cows (cows that had to be brought manually to the AMS), suggesting that these ingredients were least preferred. Flavoring the concentrate in the AMS increased the frequency of visits in one study (Migliorati et al., 2009) but not in another (Migliorati et al., 2005). In a study evaluating a molasses-based liquid feed supplement, no differences were observed in either milk production or visits to the AMS, but some measures of metabolic and overall health such as β-hydroxybutyrate and body condition score (BCS) were improved (Moore et al., 2020). The composition of concentrate may have some effect on cows visiting the AMS, but research is too limited to make broad recommendations.

With AMS, cows usually consume nutrients from more than one location (i.e., the concentrate offered by the AMS and a PMR). The supply of minerals and vitamins from both sources should be considered. In addition, by changing the amount of concentrate provided to individual cows, AMS offers the potential for users to adjust diets for individual animal factors such as milk yield, body condition, pregnancy status, health status, age, and growth (André et al., 2009; Bach and Cabrera, 2017; King et al., 2018). Given the rapid adoption of AMS by the dairy industry, there is an urgent need to determine how manipulation of feeding practices and nutritional manipulations may improve production, health, and welfare of dairy cattle.

ORGANIC DAIRY SYSTEMS

All available evidence indicates that nutrient requirements do not differ between dairy cattle managed under an organic-certified system or a conventional system; however, because of economics and regulations, nutrient supply can differ between systems. At least during a portion of the year, organically managed dairy cows must graze, which affects nutrient requirements (discussed above); however, those effects would be the same under conventional grazing systems. In reality, energy expenditure for grazing likely will be greater for organic herds simply because on average, less supplemental feed is given.

Direct comparisons of nutrient composition between feeds grown under organic conditions and those grown conventionally are limited, but most data indicate that at the macronutrient level, organic feedstuffs and conventional feedstuffs are essentially equal (e.g., Kyntäjä et al., 2014). Most studies find little difference in macronutrients between organically grown and conventionally grown human foods, but concentrations of some minerals are often greater in organically grown foods (Bourn and Prescott, 2002). However, mineral concentrations in organically grown hay crop forages and barley grain did not differ substantially from their conventionally grown counterparts, perhaps because manure may have been used as a fertilizer under both systems, and factors other than type of farming system (e.g., year variation) were more important (Gustafson et al., 2007). Although nutrient composition of organic feeds generally does not differ greatly from their conventional counterparts, because of cost and availability, feedstuff choice and diet (not feed) composition can differ between systems. Organic by-product feeds such as distillers grains, brewers grains, and cottonseed are not readily available and not commonly fed to organic herds (Sorge et al., 2016). Forages are usually the primary fiber source. Because of cost (and, in some countries, organic regulations), concentrate inclusion rates are typically lower for organic herds than conventional herds. However, increasing supplementation of concentrates on organic farms is associated with greater milk production (Sehested et al., 2003; Hardie et al., 2014) and greater income over feed costs (Hardie et al., 2014). This likely is related to increased DM and energy intake that often occurs when forage-based diets are supplemented with increasing amounts of concentrates. Similar to what is observed with conventional herds, type of forage (corn silage versus hay crop silage) did not affect milk yields in organic farms, but feed costs were significantly greater for those fed corn silage because of the need to purchase organic protein supplements (Marston et al., 2011). Cows fed organically grown rapeseed meal had similar yields of milk and milk components as cows fed conventionally grown rapeseed meal (Khalili et al., 1999).

Similar to what would be expected with conventionally fed dairy cows, milk and milk component yields are increased when cows are fed organic diets that are properly formulated to meet nutrient requirements (e.g., fiber, protein, and energy) rather than when a single-ingredient concentrate such as barley or beets (Mogensen and Kristensen, 2003) is supplemented. With conventional diets, substituting about 6 percent molasses for ground corn can increase milk and milk component yields (Broderick and Radloff, 2004). However, in a study with organically fed cows, replacing cornmeal with molasses resulted in linear decreases in milk and milk component yields (Ghedini et al., 2018). This may be a result of very different dietary starch concentrations between the two studies. In the study with organically managed cows, diets were low in starch (decreased from 10 to 2 percent as molasses was added) but ranged from 23 to 31 percent with the conventional diets. Because of cost, organic diets are often lower in starch than conventional diets, and that may limit the value of molasses.

Mineral nutrition of organically managed dairy herds is essentially the same as conventionally managed cows because in

the United States, many of the mineral supplements commonly fed are approved for organic farms. Mineral concentrations in milk from organically managed herds are generally similar to milk from conventional herds and depend more on total diet concentrations of minerals rather than production system (Schwendel et al., 2015). In other countries that are more restrictive relative to supplemental minerals, milk from organic herds can have lower concentrations of several trace minerals (Cu, iodine, selenium, and zinc) because dietary concentrations are less (Rey-Crespo et al., 2013). Mineral requirements include minerals secreted into milk; however, the differences obtained, although statistically different, are quantitatively small and would have little impact on overall mineral requirements. Vitamin requirements are likely not different between organically managed herds and conventional herds. However, organic programs require cows to graze a portion of the year, which can affect the need for supplemental vitamins (see above section on grazing systems). Based on blood concentrations of retinol, β-carotene, and α-tocopherol, cows managed organically with a diet based on pasture or high-quality hay crop silage (no corn silage) were in adequate vitamin status without any supplemental synthetic vitamins during most of the lactation cycle (Johansson et al., 2014). However, cows not fed supplemental vitamin E had less than recommended concentrations of α -tocopherol in blood at calving.

Organic dairy producers frequently feed cattle a number of ingredients that are less frequently used in conventional systems. One of these ingredients is brown seaweed, *Ascophyllum nodosum* (kelp meal) (Sorge et al., 2016). It is commonly used as a mineral supplement and is rich in several macrominerals and iodine. The high iodine concentration (and possibly other components) is thought to improve the health of cows; however, this has not been observed in experimental conditions (Antaya et al., 2015, 2019).

GENETICALLY ENGINEERED CROPS AND DAIRY CATTLE

Genetically engineered, commonly referred to as genetically modified (GM), crops have historically been developed to minimize the extent of insect damage and to simplify herbicide use for weed management (Benbrook, 2004). From 1996 to 2019, the global area growing GM crops increased from 1.7 to 190.4 million hectares. The United States plants approximately 71.5 million hectares of GM crops, followed by Brazil (52.8 million hectares), Argentina (24.0 million hectares), Canada (12.5 million hectares), and India (11.9 million hectares). Of the total area planted worldwide, GM soybean, corn, cotton, and canola represent 48, 32, 14, and 5 percent, respectively (ISAAA, 2019). Globally, livestock probably consume 70 to 90 percent of the GM crops produced, while in the United States, 95 percent of food-producing animals may consume diets containing GM ingredients (Van Eenennaam and Young, 2014). Existing evidence for the potential negative consequences and positive benefits of the commercialization of GM crops has been evaluated and reviewed (NASEM, 2016). That committee suggested that producers of soybeans, corn, and cotton have experienced positive economic outcomes through improvements in productive efficiencies. The committee also reviewed several peer-reviewed publications (Phipps et al., 2003; Nemeth et al., 2004; Calsamiglia et al., 2007; Guertler et al., 2009; Rizzi et al., 2012; Einspanier, 2013; Furgał-Dierżuk et al., 2015) that examined milk from dairy cows consuming GM crops. None reported the detection of whole transgenes or GM proteins in the milk these animals produced; however, fragments of chloroplast DNA have been detected. These conclusions are supported by a more recent review further supporting the notion that recombinant DNA cannot be reliably or consistently detected in milk from dairy cows consuming GM feedstuffs (Van Eenennaam and Young, 2017).

Presently, GM corn traits designed to specifically improve the nutritional quality or feed value of corn silage are not available, and those commercially available are designed to facilitate agronomic practices (e.g., herbicide and insect resistance) or, as in one case, industrial ethanol production. More recently, a corn containing an α-amylase enzyme that is activated during the dry milling ethanol process has also been introduced. Improvements in feed efficiency in beef cattle consuming this corn have been reported (Jolly-Breithaupt et al., 2019). In dairy studies containing corn silage incorporating the α-amylase enzyme, improved milk and protein yields have been reported (Rebelo et al., 2020; Welchez et al., 2020). There are also GM corn hybrids that contain nutritional enhancements, but these traits have been introduced through conventional breeding practices and not through genetic engineering. One example is the brown midrib trait for reduced lignin and improved fiber digestibility. In general, there is little difference in the chemical composition of corn silage GM hybrids and genetically similar non-GM counterparts. Thus, it is not surprising that a meta-analysis comparing GM hybrids and isoline controls did not find any differences in milk production and composition (Ferraretto and Shaver, 2015). Genetic engineering may serve as a tool to manipulate the chemical composition of feedstuffs, and this, in turn, may be beneficial in improving efficiency and altering milk composition. Two examples of this are GM reduced-lignin alfalfa and high–oleic acid soybeans. Lignin negatively affects fiber digestion (Palmonari et al., 2014); thus, reducing the lignin content of alfalfa may be advantageous. A GM reduced-lignin alfalfa is now commercially available (McCaslin et al., 2014). To date, no feeding studies evaluating fiber digestibility and milk production in dairy cattle fed these commercialized reduced-lignin alfalfa varieties have been published. In a study using growing Angus heifers, feeding reduced-lignin alfalfa did not affect DMI, BW, or average daily gain (Staudenmeyer et al., 2017). In addition, in vitro fiber digestibility was similar, but in vivo fiber digestibility was not tested. Other studies have demonstrated that the GM reduced-lignin alfalfa forage had less lignin and

higher in vitro NDF digestibility compared with reference alfalfa varieties when harvested at the same time (Grev et al., 2017; Sulc et al., 2017). Linolenic and linoleic acids (18:2 and 18:3) play a role in milk fat depression; thus, reducing their concentration in soybeans may prove beneficial when they are fed to cows. In addition, increased intake of 18:1 by cows results in more monounsaturated FAs in milk, and this may improve milk quality, especially as it relates to consumer perceptions and expectations. A GM high–oleic acid soybean has been developed and results in greater 18:1 and less 18:2 and 18:3 FAs (Szabala et al., 2014; Lopes et al., 2017). Cows fed extruded soybean meal from high–oleic acid soybeans had increased milk fat concentration and reduced *trans* fatty acids in milk compared with cows fed extruded meal from conventional soybeans (Lopes et al., 2017).

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14

Dairy Cattle Nutrition and the Environment

INTRODUCTION

Agriculture has seen a tremendous increase in productivity over the past century in the United States through the use of various technologies. Improved housing facilities, waste handling, breeding, and feeding balanced rations allowed livestock to be raised in increasingly larger and more concentrated animal feeding operations. The intensification in the U.S. dairy industry is such that farms with more than 500 milking cows accounted for 63 percent of the milk supply in 2012 (USDA, NASS, 2013), up from 45 percent a decade before (USDA, 2005). Consolidation of large numbers of dairy cattle into small land areas to improve the efficiency of milk production may contribute to environmental problems unless animals are fed and managed properly, including consideration of number of animals per unit of land available for manure application. From an environmental standpoint, the primary concerns are the nutrients nitrogen (N) and phosphorus (P); the greenhouse gases carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O); and other odorous compounds such as ammonia (NH_3), volatile organic compounds (VOCs), and hydrogen sulfide (NRC, 2003). Excretion of excess minerals in manure and their application in soil raises salinity and toxicity concerns, particularly in irrigated fields.

Dairy cattle play a key role in human food production by converting forages and poor-quality feeds into human edible products. However, this conversion is associated with an environmental cost, which can be unavoidable, that is, as a by-product of a necessary fermentation process or avoidable, for example, nutrients consumed in excess of requirement (Dijkstra et al., 2013a). Dairy cattle must be fed to meet their requirements with minimal excesses of nutrients in the diet if the efficiency of nutrient use and milk production by dairy cows are to be maximized and nutrient losses to the environment reduced. In 2008, the contribution of the entire dairy sector to the U.S. greenhouse gas (GHG) emissions was estimated to be 134 Tg CO_2 equivalents (CO_2e) or 1.9 percent of the U.S. total, with CH_4, N_2O, and CO_2 contributing 44, 13,

and 41 percent, respectively (Thoma et al., 2013). Although the dairy industry's environmental footprint is small compared to other industries (e.g., oil and gas), there has been continued pressure to reduce its footprint through national and state regulations. Reducing dairy production's impact on the environment such as air, soil, and water quality will contribute to the industry's long-term environmental sustainability (Kebreab, 2013; von Keyserlingk et al., 2013).

METHANE

CH_4, which has a global warming potential 28 times that of CO_2 over a 100-year horizon (IPCC, 2013), emitted from dairy operations is a significant contributor to GHG emissions. In 2014, dairy cattle contributed 25 percent of total enteric CH_4 emissions from livestock (USEPA, 2014). CH_4 emissions represent a loss of about 3.8 to 7.4 percent (5.6 percent on average) of gross energy intake (GEI) in U.S. dairy cattle (Kebreab et al., 2008a). Most CH_4 production occurs in the reticulorumen, with only 13 percent produced in the lower tract (Murray et al., 1976), and with rectal emissions accounting for about 2 to 3 percent of the total CH_4 emissions from the animal (Murray et al., 1976; Munoz et al., 2012).

Factors Affecting Methane Emissions

CH_4 production is positively and linearly related to the amount of feed consumed. Feed intake accounts for 60 to 80 percent of the variation in CH_4 production (Mills et al., 2003; Ellis et al., 2007; Moraes et al., 2014). The rest of the variation could be accounted for by differences in nutrient composition, uptake and utilization, and other factors. Although absolute CH_4 production (g/d) varies by breed, several studies have shown that breed has little impact on CH_4 produced per unit of intake (CH_4 yield) or per unit of product (CH_4 intensity) in ruminants (Fraser et al., 2014; Moraes et al., 2014). Ellis et al. (2010) evaluated enteric CH_4 production equations used in whole-farm models and concluded that

the simple, more generalized equations performed worse than those that attempted to represent important aspects of diet composition. The type of dietary carbohydrates (fiber versus nonfiber) fermented in the rumen plays a major role in determining the profile of rumen volatile fatty acid (FA) production (Murphy et al., 1982; Mills et al., 2001) and consequently CH_4 production (Moe and Tyrrell, 1979). Fermentation of fiber compared to starch results in greater acetate production, which increases hydrogen production and, consequently, enteric CH_4 production (Bannink et al., 2008). The same authors reported a shift toward production of propionate as pH in the rumen decreased, which uses hydrogen in the rumen and reduces enteric CH_4 production. Thermal stress may influence CH_4 production. Under prolonged cold conditions, Bernier et al. (2012) reported CH_4 yield of 5.2 percent of GEI compared to 7.1 percent of GEI under thermoneutral conditions. This is related to increased rumen rate of passage, which ultimately reduces the extent and rate of rumen fermentation (see Chapter 5). Heat stress, on the other hand, may increase CH_4 yield due to longer retention of feed in the rumen or may decrease CH_4 yield because of lower feed intake and reduced rumen pH levels in response to reduced cation availability, as well as a consequent rise in the acetate to propionate ratio. Further research is required to quantify the effect of heat stress on enteric CH_4 emissions.

Mitigation Options to Reduce Enteric Methane Emissions

Hristov et al. (2013a) extensively reviewed enteric CH_4 mitigation options and identified several opportunities. The mitigation strategies can be classified into (1) feed manipulation, (2) rumen modifiers, and (3) increasing animal production through genetics and management (Knapp et al., 2014). Feed manipulation through nitrate (NO_3) inclusion was considered to have the highest potential mitigation effect because up to 50 percent reduction in enteric CH_4 production in sheep and cattle has been observed due to NO_3 provision as an alternative electron acceptor (van Zijderveld et al., 2010, 2011; Hristov et al., 2013a). Several studies have shown that lipids have a suppressive effect on rumen microbes and CH_4 production (Martin et al., 2010; Grainger and Beauchemin, 2011). Eugene et al. (2008) reported a 9 percent reduction in CH_4 production in dairy cows due to lipid supplementation, but dry matter intake (DMI) was reduced, so there was no difference in CH_4 yield (i.e., CH_4 production/kg DMI). Moraes et al. (2014) quantified the response to dietary fat and, with all dietary factors being equal, observed an average decrease of 0.045 to 0.09 Mcal (0.19 to 0.38 MJ) of CH_4 for every percentage increase in dietary fat. A meta-analysis by Knapp et al. (2014) on the effect of lipid source showed that each percentage unit of diet crude fat (CF) from rumen-inert, seed, oil, and endogenous lipid sources decreased CH_4 intensity (i.e., CH_4 per unit of energy-corrected milk) by 0.78 ± 0.20, 0.71 ± 0.20, 1.12 ± 0.20, and 1.01 ± 0.38 g/kg, respectively. Greater DMI, feeding nonstructural carbohydrates, and improving forage

quality have low to medium potential impacts (Hristov et al., 2013a) and were expected to reduce enteric fermentation by 5 to 15 percent (Knapp et al., 2014). In view of competition with human edible feed resources, Hristov et al. (2013a) recommended increasing forage digestibility and digestible forage intake among the major CH_4 mitigation practices.

Rumen modifiers considered to have low potential effects include ionophores and tannins (Hristov et al., 2013a). Appuhamy et al. (2013) quantified the effect of an ionophore (monensin) in dairy cows to be -12 ± 6 g CH_4/d (mean CH_4 production = 338 g/d) when adjusted for dose, DMI, and lipid intake. Care should be taken in extending CH_4 reduction results from in vitro studies to the commercial farm. For example, supplementing tea saponin (0.52 percent dry matter [DM]) reduced methanogenesis in vitro but increased CH_4 yield in vivo (Guyader et al., 2017). Until recently, the use of rumen modifiers or additives to reduce CH_4 production has been less successful compared to diet manipulation. However, several experiments have shown the potential for an inhibitor, 3-nitrooxypropanol, to reduce enteric CH_4 production in beef and dairy cattle (e.g., Hristov et al., 2015). Dijkstra et al. (2018) conducted a meta-analysis using 11 published studies and reported that at an average dose of 81 mg/kg of DM, 3-nitrooxypropanol reduced CH_4 production 39 percent in dairy cattle. Bromoform and chloroform are halogens that have been found to interfere directly with the methanogenesis pathway (Goel et al., 2009). The red microalgae (*Asparagopsis* spp.) bromoform and other halogens, which have antimethanogenic properties, reduced enteric CH_4 emissions in vitro (Kinley et al., 2016) and in vivo (Roque et al., 2019). Up to 60 percent reduction in enteric CH_4 production has been observed at 1 percent of organic matter (OM) inclusion rate, but the authors caution that further work is needed to determine the long-term effects on productivity and animal health (Roque et al., 2019). Effects of these and other rumen modifiers on CH_4 production have been recently reviewed (Honan et al., 2021).

Management strategies to reduce CH_4 emissions were reviewed by Hristov et al. (2013b). Increased productivity is considered as having greatest potential because of dilution of maintenance. From 1990 to 2012, enteric CH_4 emissions from dairy cattle increased 6 percent, cow numbers decreased 2 percent, and milk production increased 36 percent, indicating that while emissions per head increased, there was a 22 percent decline per unit of milk produced (USEPA, 2014). Capper et al. (2009) estimated that the total carbon footprint for the entire dairy industry was reduced by 41 percent in 2007 compared to 1944 in the United States. In the Netherlands, from 1990 to 2008, yield of fat- and protein-corrected milk (FPCM) increased by 34 percent and CH_4 production increased by 16 percent per cow, but CH_4 intensity per unit FPCM decreased by 13 percent (Bannink et al., 2011). Based on modeling projections, precision diet formulation on a weekly and monthly basis may improve animal performance and consequently CH_4 emission intensity (White and Capper, 2014). Other management strategies with low to medium

potential to mitigate enteric CH_4 emissions include use of recombinant bovine somatotropin, growth promoters, genetic selection, improved animal health, reduced animal mortality, fibrolytic enzymes, and reduced forage maturity (Hristov et al., 2013b; Tewoldebrhan et al., 2017). Mitigation options that can be implemented in intensively managed dairy production systems currently (2020) may have a combined potential to reduce enteric CH_4 intensity by 15 to 30 percent (Knapp et al., 2014). However, using effective feed additives may reduce CH_4 intensity by more than 50 percent.

Enteric Methane Prediction Equations

Assessment of the efficiency of animal production and its subsequent environmental footprint requires quantification of each nutrient consumed, used, excreted, or lost to the environment. Mathematical models are widely used to predict the environmental impact of livestock operations and can be used to assess mitigation options and policy decisions. Appuhamy et al. (2016) evaluated 38 extant models developed to predict enteric CH_4 emissions from lactating dairy cows. The authors collected an extensive data set from around the world and evaluated each model with regional data. For North America, the highest-ranked model (which is also the recommended equation for dairy cows) was a modified version of a model developed by Nielsen et al. (2013) that uses DMI (kg/d), estimated digestible neutral detergent fiber (dNDF, percentage of DM), and FA (percentage of DM) contents:

$$\text{Methane (Mcal/d)} = 0.294 \, (+0.019) \times \text{DMI} - 0.347 \, (+0.093) \times \text{FA} + 0.0409 \, (+0.012) \times \text{dNDF}$$

(Equation 14-1a)

Appuhamy et al. (2016) also evaluated previous and updated versions of the models (tier 2) recommended by the Intergovernmental Panel on Climate Change (IPCC, 1997, 2006). The earlier version of the IPCC model uses CH_4 conversion factor (Ym, percent of GEI) of 6.0 percent, which is more in agreement with literature data from North America (5.7 ± 0.9; Appuhamy et al., 2016; Jayasundara et al., 2016) and also in agreement with Kebreab et al. (2008a), who reported 5.6 percent compared to the updated IPCC Ym of 6.5 percent. Similarly, in their evaluation of CH_4 prediction equations used in whole-farm models, Ellis et al. (2010) reported better predictive capacity of the tier 2 method using Ym of 6.0 percent compared with Ym of 6.5 percent.

Moraes et al. (2014) developed equations to estimate CH_4 production for heifers and nonlactating dairy cattle based on indirect calorimetry measurements containing 414 and 591 records, respectively. The recommended equation for heifers is as follows:

$$\text{Methane (Mcal/d)} = -0.038 \, (0.071) + 0.051 \, (0.001) \, \text{GEI} + 0.0091 \, (0.0014) \, \text{NDF}$$

(Equation 14-1b)

For nonlactating dairy cows, the recommended equation is the following:

$$\text{Methane (Mcal/d)} = 0.69 \, (0.048) + 0.053 \, (0.001) \, \text{GEI} - 0.045 \, (0.012) \, \text{CF}$$

(Equation 14-1c)

In both equations, GEI is in Mcal/d, and NDF and CF are in percentages of DM.

Manure Methane and Volatile Solids Prediction Equations

According to IPCC (2006) tier 2 guidelines, CH_4 emissions from manure are determined based on estimated volatile solids and emission factors for various manure management systems. OM in livestock manure consisting of biodegradable and nonbiodegradable fractions are known as volatile solids. Using an extensive data set, Appuhamy et al. (2014) and Appuhamy et al. (2018) developed prediction equations to calculate volatile solids:

$$\text{Volatile solids (kg/d)} = 0.364 \, (\pm 0.007) \, \text{DMI} + 0.026 \, (\pm 0.004) \, \text{NDF} - 0.078 \, (\pm 0.008) \, \text{CP}$$

(Equation 14-2)

where DMI is in kg/d, and crude protein (CP) and NDF contents are in percentages of DM.

However, only digestible OM generates CH_4; therefore, CH_4 emissions should be based on digestible volatile solids (i.e., volatile solids—lignin). Appuhamy et al. (2018) developed a mathematical model for estimating digestible volatile solids outputs by lactating dairy cows:

$$\text{Digestible volatile solids (kg/d)} = 0.334 \, (\pm 0.007) \, \text{DMI} + 0.029 \, (\pm 0.006) \, \text{HC} - 0.058 \, (\pm 0.008) \, \text{CP}$$

(Equation 14-3)

where DMI is in kg/d, and CP and hemicellulose (HC = neutral detergent fiber—acid detergent fiber contents) are in percentages of DM.

It is recommended to use the volatile solids prediction equations given above when predicting CH_4 from manure management using the IPCC (2006) methodology.

NITROGEN

N is of primary environmental concern because of losses of organic N and ammonium via wind and water erosion, NH_3 through volatilization, NO_3 through leaching and denitrification, and oxides of N as a result of nitrification–denitrification processes (Eckard et al., 2010; Cavigelli et al., 2012). The main causes of N loss from the animal are inefficient utilization of feed N in the rumen, undigested feed and microbial true protein, microbial nucleic acids synthesized in the rumen, N use inefficiency for maintenance and milk protein synthesis,

and amino acids (AAs) absorbed in excess of requirement (Dijkstra et al., 2013c; Hristov et al., 2013b). The impact of these N losses from agricultural systems on surface- and groundwater quality, soil pH, biodiversity, and pathogens has been well documented (Tamminga, 1992).

Factors Affecting Nitrogen Excretion

The main driver of N losses from cattle is N consumed in feed. Dairy cows secrete in milk, on average, 21 to 33 percent of the N they consume (Calsamiglia et al., 2010), with almost all of the remaining N excreted in feces and urine. Using a large database, Reed et al. (2015) calculated an average total manure N excretion of 69 percent of N intake. In a meta-analysis, Huhtanen and Hristov (2009) concluded that dietary CP concentration is the most important dietary factor influencing milk N efficiency, with ruminal degradation of CP being of lesser importance. Similarly, of all single dietary and animal factors evaluated in a meta-analysis by Spek et al. (2013c) to predict N excretion in urine, dietary CP concentration and milk urea N level were by far the best predictors. Differences in amount and, to a smaller extent, digestibility of N in feed affect not only the total amount excreted but also the partitioning of N into milk, urine, and feces (Castillo et al., 2001b; Kebreab et al., 2002). Such a distinction of manure N excretion into fecal and urinary N excretion is of significance, because variation in dietary N supply will affect urinary N output, which is more susceptible to leaching and volatile losses than fecal N and of larger importance to reduce environmental impact (Dijkstra et al., 2013b). Dijkstra et al. (2013c) calculated the theoretical upper limit of N use efficiency to be 43 percent at maximal milk secretion for a cow weighing 650 kg and producing 40 kg/d of fat- and protein-corrected milk.

Mitigation of Nitrogen Losses from Cattle Operations

The wide variation in efficiency of conversion of feed N into products suggests that major improvements in reducing N excretion are possible (Dijkstra et al., 2013c). There is little opportunity to reduce N losses related to incomplete digestion of microbial protein, synthesis of microbial nucleic acids, and animal maintenance requirements (Dijkstra et al., 2013c). The most effective strategy to reduce N excretion and limit impact on the environment is to decrease the dietary CP content (Castillo et al., 2000; Kebreab et al., 2001; Spek et al., 2013c). Several studies have shown that total N excretion increases as dietary N intake increases, with urinary N excretion increasing at a greater rate than fecal excretion (Castillo et al., 2000; Huhtanen and Hristov, 2009; Kebreab et al., 2010). Feeding a diet that contains CP above the requirement will also increase energy expenditure slightly to cover the cost of urea synthesis. Because CP is usually a costly nutrient, feeding excess CP also inflates feed costs to producers. Reduction of N intake decreases the amount

of N excreted (Huhtanen et al., 2008) and may improve N use efficiency (Kebreab et al., 2010). Furthermore, as milk production per cow increases, because of dilution of maintenance N use, efficiency should increase with a concomitant reduction in N excretion per unit of product (Capper, 2011). However, reducing dietary N below requirement will impair productivity (Law et al., 2009). In addition to reduction of CP content, other dietary strategies such as optimizing rumen fermentation and microbial protein synthesis, passage of nutrients to the small intestine, and efficiency of absorbed amino acid (AA) utilization for milk protein synthesis can all be effective mitigation options (NRC, 2001). Efficiency of N utilization is affected by availability of energy, and generally milk production is increased with greater concentration of energy in the diet. In a multivariate analysis, Reed et al. (2014) showed that as the metabolizable energy content of the diet increases, efficiency of N use increased with diminishing returns. Modifying rumen microflora, particularly those involved in peptide degradation and AA deamination, may increase efficiency of N utilization (Calsamiglia et al., 2010). Further work is needed to better understand factors controlling urea transport across the rumen wall and take advantage of ruminants' ability to recycle urea (Calsamiglia et al., 2010). However, when N intake is low and rumen microorganisms might benefit from additional supply of N, close to 100 percent of urea synthesized in the liver is recycled to the gastrointestinal tract (Reynolds and Kristensen, 2008), and consequently, there is little potential for urea recycling to compensate low CP diets. Postrumen metabolism of AAs in the portal-drained viscera and the liver contributes to N excretion. Lapierre et al. (2005) estimated, on average, 35 percent of AAs are lost during absorption, and the liver removes 45 percent of absorbed AAs, giving rise to significant amounts of urea excreted in urine. Further opportunities to decrease N losses postrumen also arise from proper balancing of diets for individual AAs (Haque et al., 2012). Feeding diets that contain lower concentrations of CP supplemented with balanced quantities of rumen-protected AAs should reduce excretion of N in urine. Milk protein yield is particularly responsive to essential AA supply to the mammary gland, and improvements in the ability to model AA supply and use should improve N use efficiency.

Nitrogen Excretion Prediction Equations

Precise estimates of N excretion from livestock lead to better quantification of manure N, which is a basis for estimating N volatilization, leaching, runoff, and emission. Several models have been developed to predict N excretion from lactating dairy cattle and heifers to assess the efficiency of cattle production and calculate national inventories of N_2O emissions. Although the accompanying model will calculate N excretion based on the mass-balance approach, some equations are provided here to aid input–output type of analysis. For lactating cows, if DMI, dietary CP concentration, milk

yield, and milk protein concentrations are known, mass balance can be used to estimate manure:

$$\text{Manure N (g/d)} = (\text{DMI} \times \text{DietCP}) / 0.625$$
$$- (\text{Milk} \times \text{MilkCP}) / 0.638 - 5$$

$$\text{(Equation 14-4)}$$

where DMI and Milk are in kg/d, and DietCP and MilkCP are in percentages. The 5 is an estimate of body growth over three lactations (assumed to be 150 kg of growth over 915 days).

Johnson et al. (2016) evaluated 45 models to predict N excretion when milk protein yield is not known. The following equations were recommended for lactating dairy cows:

$$\text{Urine N (g/d)} = 12.0 \ (\pm 5.80)$$
$$+ 0.333 \ (\pm 0.011) \ \text{N intake (g/d)}$$

$$\text{(Equation 14-5)}$$

$$\text{Fecal N (g/d)} = -18.5 \ (\pm 3.59)$$
$$+ 10.1 \ (\pm 0.169) \ \text{DMI (kg/d)}$$

$$\text{(Equation 14-6)}$$

$$\text{Total Manure N (g/d)} = 20.3 \ (\pm 4.72)$$
$$+ 0.654 \ (\pm 0.009) \ \text{N intake (g/d)}$$

$$\text{(Equation 14-7)}$$

$$\text{Milk N (g/d)} = -19.0 \ (\pm 3.21)$$
$$+ 8.13 \ (\pm 0.245) \ \text{DMI (kg/d)}$$

$$\text{(Equation 14-8)}$$

For heifers and nonlactating cows, the following equations were recommended:

$$\text{Urine N (g/d)} = 14.3 \ (\pm 3.18)$$
$$+ 0.51 \ (\pm 0.12) \ \text{N Intake (g/d)}$$

$$\text{(Equation 14-9)}$$

$$\text{Feces N (g/d)} = 0.35 \ (\pm 1.73)$$
$$+ 0.32 \ (\pm 0.0064) \ \text{N Intake (g/d)}$$

$$\text{(Equation 14-10)}$$

$$\text{Total Manure N (g/d)} = 15.1 \ (\pm 2.50)$$
$$+ 0.83 \ (\pm 0.018) \ \text{N Intake (g/d)}$$

$$\text{(Equation 14-11)}$$

The root mean square prediction error (as a percentage of mean observed values), which measures the prediction performance, was 25, 16, 11, 14, 36, 17, and 13 percent for Equations 14-5 to 14-11, respectively.

Ammonia

Dairy production contributes to NH_3 emission, which can create human respiratory problems by forming fine particulate matter with other compounds and animal health hazards when concentrations reach critical levels in confined spaces. NH_3 emission can also cause regional degradation of terrestrial and aquatic ecosystems (NRC, 2003; Kampa and Castanas, 2008) and represents a net loss of manure fertilizer value. NH_3 emission is regulated by the U.S. Environmental Protection Agency (EPA) through the Clean Air Act Amendments of 1990 (Pub. L. 101-549). About 90 percent of NH_3-N originates from urine N and the rest from feces within a 10-day period after excretion (Lee et al., 2011). Large variation in NH_3 emission estimates is reported mainly due to several factors affecting measurement, including wind, temperature, time of day, and year. In a recent meta-analysis, Bougouin et al. (2016) identified housing system, season, diet composition, and milk production as major factors affecting NH_3 emissions from dairy cattle. Hristov et al. (2011) calculated a daily average of 59 g of NH_3 emission per cow based on a compilation of studies, with a large standard deviation of 65 g/d. However, when averaged over a year, NH_3 emissions measured in open-lot dairy housing systems in Idaho, Texas, and California were more consistent and ranged between 120 and 150 g/d per cow (Leytem et al., 2011). Emissions from freestall and open-freestall dairies were lower at 10 to 100 g/d per cow (Leytem et al., 2013). In general, stall NH_3 emissions are much higher than pasture NH_3 emissions (McQuilling and Adams, 2015). On average for grazing cows, 25 percent of the N excreted in manure (Hristov et al., 2011) and 3 to 15 percent of urine N in the field or 4 to 52 percent from urine patches (Oenema et al., 2008) may be lost as NH_3 depending on soil type, moisture, temperature, wind speed, and the concentration and forms of N in urine. NH_3 emission can be reduced by dietary manipulation, increasing milk yield, manure treatment, and capture and treatment of emitted gases (Hristov et al., 2011; Bougouin et al., 2016). Decreasing the dietary CP content is probably the most effective strategy to decrease NH_3 emission from dairy manure (Bougouin et al., 2016) due to reduced N substrate in the excreta, particularly urine N, and as a consequence reduces the environmental impact (Frank et al., 2002; Frank and Swensson, 2002; Agle et al., 2010). Urine N, because it is 52 to 94 percent urea, is more susceptible to leaching and volatile losses than fecal N (Reynal and Broderick, 2005; Dijkstra et al., 2013b). Thus, reducing urinary N excretion will greatly reduce environmental impact. However, a trade-off with other losses of N may occur. If a smaller fraction of manure N is emitted as NH_3, losses of NO_3 or N_2O may increase (van Groenigen et al., 2008), and simultaneously, lowering all of these losses is a challenge.

Milk urea N has been used as a proxy for urine urea N because the two are correlated (Burgos et al., 2007; Powell et al., 2011, 2014). A number of factors, including body weight, urine production, and time and frequency of feeding and milking, have been shown to affect the relationship between milk urea N and urinary N excretion (Spek et al., 2013b). Powell et al. (2014) calculated that each 1-mg/dL

decrease in milk urea N was associated with a reduction in NH_3 emissions from manure of 7 to 12 percent. However, the relationship between milk and urine urea N is affected by mineral concentration of the diet, which negatively affected milk urea N but not urine urea N (Spek et al., 2012, 2013a; Eriksson and Rustas, 2014). Variation in urine volume also affected the relationship between milk urea and urinary N excretion. Care should therefore be taken when using milk urea N as a proxy for urine urea N (Spek et al., 2013b).

Nitrous Oxide

Nitrogenous excretions from dairy cattle can directly contribute to overall N_2O emissions (Hristov et al., 2011), which have a global warming potential 265 times that of CO_2 over a 100-year horizon (IPCC, 2013). In addition, indirect emissions of N_2O occur after atmospheric deposition of NH_3 and nitrogen oxides (NOx) from animal housing and manure storage and various N sources from leaching and runoff (IPCC, 2006). In 2012, about 4 percent of N_2O emissions in the United States were attributed to the breakdown of N in livestock manure (USEPA, 2014). For N_2O to occur, manure must undergo several transformations mediated by microbes: hydrolysis and mineralization of organically bound N into ammonium (NH_4^+), nitrification to nitrite (NO_2), and NO_3 in the aerobic environment followed by anaerobic reduction to elemental N, with intermediate production of N_2O and nitric oxide (NO) through denitrification (Li et al., 2012). Factors affecting N_2O emissions include temperature, moisture content, availability of easily degradable organic carbon, and oxidation status of the environment (Montes et al., 2013). Because of environmental conditions, the fraction of manure N lost as N_2O is generally below 2 to 3 percent, with a few studies reporting up to 10 percent (Groenigen et al., 2005; Luo et al., 2010). In open-lot dairies, Leytem et al. (2011) measured N_2O emissions ranging from 19 to 33 g/d per cow over a year, which were greater than for open-freestall dairies measuring 5 to 37 g/d per cow (Leytem et al., 2013). Urine is the main source of volatile N emissions; therefore, manipulating the route of N excretion is an important N_2O mitigation tool. The portion of urine N released as N_2O depends on the urinary N composition, soil type, soil wetness, and soil temperature. Emissions are relatively low when the soil is dry or very wet and relatively high when the water-filled pore space in soil ranges from 60 to 80 percent (Dijkstra et al., 2013b). In soil, urinary hippuric acid, creatine, and creatinine decompose more slowly than urea, and hippuric acid may act as a natural inhibitor of N_2O emissions (Dijkstra et al., 2013b). About 92 percent of N_2O emissions occur in cropping systems, and mitigation options related to such systems are reviewed by Cavigelli et al. (2012). Successful mitigation options in cropping systems such as adding urease and artificial nitrification inhibitors (e.g., dicyandiamide) are generally effective in controlling N_2O emissions (Clough

et al., 2009). Manure storage and treatment-based mitigation options for reducing N_2O emissions have been extensively reviewed by Montes et al. (2013). Prediction of N_2O emissions is challenging because of the various factors involved that have an impact on N_2O formation. Most of the quantification methods of N_2O emissions from the dairy sector are based on static emission factors from IPCC (2006), which do not account for the wide range of variability inherent in the dairy industry. As a result, the methodology is associated with a high degree of uncertainty and will not be appropriate to use for assessment of mitigation options. Additional research on quantification and impact of dairy production on N_2O and other GHG emissions in the United States is warranted.

Water Quality

Dairy operations tend to be concentrated in certain regions of the country, which results in long-distance redistributions of substantial amounts of N and other nutrients (Kellogg et al., 2000). Nutrients accumulate near dairy operations, often in quantities that exceed the nutrient needs of the crops being grown within reasonable transportation distance (Rosenstock et al., 2014). These nutrients contribute to hypereutrophication of estuaries and N leaching to groundwater (Kellogg et al., 2000). Contamination of groundwater can have negative consequences on water supplies and their suitability for human consumption and use (Townsend et al., 2003). For example, excessive consumption of NO_3 in drinking water has been associated with methemoglobinemia or "blue baby syndrome" in humans, stomach cancer, and NO_3 poisoning in animals (Pasten-Zapata et al., 2014). It also contributes to surface-water contamination over longer time frames as groundwater reenters surface drainage networks (Meals et al., 2010; Wick et al., 2012). NO_3 export into adjacent surface water bodies may induce an increased level of nutrients (eutrophication) affecting biodiversity, mammals, birds, and fish adversely by producing toxins and reducing oxygen levels (Pasten-Zapata et al., 2014). Harter et al. (2002) assessed NO_3 and salt leaching to shallow groundwater near dairies in San Joaquin Valley, California. They estimated minimum average annual groundwater NO_3-N and salt loading from manure-treated forage fields to be 280 and 4,300 kg/ha, respectively. Leaching rates for ponds were estimated to be about 0.8 m/year, at least locally. Over six decades, there was over a 10-fold increase in NO_3 loading in two agricultural areas in California. Meeting safe drinking water standards would require leaching reductions of over 70 percent from current levels through reductions in excess manure application, which accounts for nearly half of all groundwater N loading, and through synthetic N management improvements (Rosenstock et al., 2014). In manure-treated fields, proper nutrient management will be a key to protecting groundwater quality, particularly in regions overlying alluvial aquifers (Harter et al., 2002).

VOLATILE ORGANIC COMPOUNDS

VOCs are defined as any carbonaceous compounds that participate in atmospheric photochemical reactions, excluding CO_2, carbon monoxide, carbonic acids, metallic carbides, or carbonates (USEPA, 2011). Some of the common VOCs include alcohols, aldehydes, ketones, esters, ethers, aromatic hydrocarbons, and halogenated hydrocarbons (Filipy et al., 2006). These gases are important air pollutants because they contribute to the formation of ozone, which is a constituent of photochemical smog in the presence of oxygen and sunlight (Carter, 1994). Some of the common VOCs emitted from dairy production include acetic acid from fermented feeds and manure (Shaw et al., 2007; Alanis et al., 2008); acetaldehyde from fermented feeds (Howard et al., 2010); ethanol from fermented feeds, manure lagoons, and housing (Filipy et al., 2006; Chung et al., 2010; Howard et al., 2010); methanol from enteric fermentation and manure (Shaw et al., 2007); and acetone from manure lagoons and housing (Filipy et al., 2006; Chung et al., 2010).

Good silage-making practices such as rapid filling, adequate packing, and the use of inoculants and preservatives can reduce VOC emissions from fermented feeds (Muck, 1988; Place and Mitloehner, 2013). Minimizing the exposed surface area of silos reduces emissions substantially because greater emissions occur within the first 12 hours of silage being exposed to air (Hafner et al., 2010). Water-soluble VOCs from animal housing could be reduced by flushing the barn floors with water (Chung et al., 2010); however, this may increase VOC concentrations in the lagoon (Place and Mitloehner, 2013). Biofiltration systems also have the potential to reduce VOC emissions by passing exhaust air from housing or manure storage systems through a filter containing microorganisms and "trapping" emissions (Martens et al., 2001; Pagans et al., 2007). More research is required to understand and quantify the sources and the factors influencing the VOC emissions in dairy operations.

INTEGRATED APPROACHES

The ultimate objective in reducing the environmental burden of dairy production should be focused on net reduction because mitigation options that have one environmental benefit may negatively impact another. In this respect, the level of analysis (at animal, farm, or food chain levels) is of large significance. For example, van Middelaar et al. (2013) evaluated the effect of replacing grass silage with corn silage on GHG emissions at all three levels using a linear programming model in combination with a mechanistic model of enteric fermentation and life cycle assessment. Although at animal, farm, and food chain levels, this strategy reduced annual GHG emissions by 12.8, 17.8, and 20.9 kg CO_2e per ton FPCM, converting grassland into corn land resulted in nonrecurrent emissions of more than 900 kg CO_2e per ton FPCM. Although enteric

CH_4 emissions contribute the greatest to whole-farm emissions (Thoma et al., 2013), manure management, particularly N_2O emissions, is also a significant contributor, mainly due to greater global warming potential. The processes involved in CH_4 and N_2O production are often antagonistic because the former is produced under anaerobic conditions, whereas the latter requires oxygen; therefore, some practices such as composting that result in the reduction of CH_4 production may increase subsequent N_2O emissions (Montes et al., 2013). Ellis et al. (2012) showed that mitigation options aimed at reducing urinary N excretion may result in elevated CH_4 emissions depending largely on the type of carbohydrate consumed. CH_4 production declines if starch or digestible nutrients escaping rumen fermentation replace protein in the diet but rises if dietary fiber levels increase. Reducing dietary CP by increasing fiber will likely increase CH_4 production while decreasing N_2O. Dijkstra et al. (2011) estimated an increase of on average 0.30 g CH_4 per gram urinary N decrease for various nutritional interventions with grass silage–based diets aimed to improve milk N efficiency. Using standard emission factors for direct and indirect N_2O emissions, the estimated N_2O emission reduction (in CO_2 equivalents) resulting from decreased manure N output was more than offset by a rise in enteric CH_4 production. Similarly, Sauvant et al. (2014) reported that CH_4 production per kilogram digested OM decreased in a linear fashion with increasing dietary CP concentration, which will likely increase N_2O emissions. Analysis of 1,111 records from calorimetry chambers at USDA Beltsville, Maryland, using Holstein and Jersey cows also showed a weak negative relationship, possibly because degradation of OM may be enhanced by greater dietary CP concentration (Colmenero and Broderick, 2006; Nousiainen et al., 2009). As expected, CH_4 production showed a positive linear relationship with neutral detergent fiber (NDF) content and negative linear relationship with metabolizable energy intake. Animals fed low CP diets produced manure with a slower mineralization rate of N, which reduced N_2O emissions when land applied (Powell and Broderick, 2011); however, CH_4 emissions may increase because of higher OM content. If the amount of N is not sufficient for crop growth, the need for higher rate of inorganic N fertilizer inclusion might also increase overall N_2O emissions. Moraes et al. (2012) developed a linear programming model to formulate minimum-cost diets when environmental policies are present. In their evaluations, imposing CH_4 restrictions increased N losses from the animal. Van Middelaar et al. (2014) reported extruded linseed or NO_3 supplementation to reduce CH_4 intensity by 42 and 33 kg/ton FPCM at the animal level, respectively, but total GHG emission at the food chain level was reduced by only 9 and 32 kg/ton FPCM, respectively. In view of these results, a major challenge in reducing N losses from dairy cattle is to find an optimal nutritional balance without increasing enteric CH_4 production.

The relationship between manure NH_3 volatilization and N_2O emission is also complex because if a mitigation technol-

ogy reduces NH_3 losses, the preserved ammonium may later increase soil N_2O emissions (Petersen and Sommer, 2011). On the other hand, losses of N as NH_3 reduce the availability of N for nitrification and denitrification processes and, consequently, N_2O formation. In their review, Montes et al. (2013) listed several opportunities for mitigation; however, only decreasing manure storage time was recommended for reducing both GHG and NH_3 emissions. In pasture-based production systems, improving forage quality is often accomplished by increasing N fertilizer application rates, which can have a negative impact on urinary N excretion and thus NH_3 and N_2O emissions.

MINERALS

Phosphorus

P is an essential mineral for ruminants (see Chapter 7). In dairy cattle, more than 55 percent of the P consumed may be excreted in manure depending on P availability, efficiency of feed conversion, and the amount of P consumed in excess of the animal's requirement (Kebreab et al., 2005b; Vitti and Kebreab, 2010; Klop et al., 2013). The rest is excreted mostly in feces with less than 1 g P/d in urine unless cows are fed 20 to 30 percent in excess of requirement (Wu et al., 2000). Apart from P being a finite resource, manure P has a potential to contribute to environmental degradation, particularly degradation of water quality. Manure phosphate applied to land is usually adsorbed onto soil particles, so it does not leach into water tables or waterways but builds up in the soil (Pierzynski et al., 1994). This becomes a problem when the P-laden soil particles are washed into surface water. In the United States, the median contribution of P from animal agricultural sources to the nation's watersheds is 26 percent, compared to 17 percent from commercial fertilizer and 3 percent for point sources (Smith and Alexander, 2000). Due to environmental impact, there have been substantial public interest and policies to reduce P excretion and manure P applied to agricultural soils. For example, the U.S. EPA is mandated to enforce P reductions of at least 0.6 million kg/year in the Chesapeake Bay watershed to maintain water quality (USEPA, 2009).

There are several ways to reduce P excretion from dairy cattle. Kebreab et al. (2012) broadly divided mitigation options for P excretion into two categories: (1) increasing efficiency of P utilization and (2) improving or optimizing P availability in feed. Several surveys revealed that dairy cattle in the United States (Dou et al., 2003; Castillo et al., 2013) and Canada (Kebreab et al., 2008b) were routinely fed diets that include 0.45 to 0.50 percent P, which is in excess of animal requirements (NRC, 2001; Wu et al., 2001; Valk et al., 2002). Castillo et al. (2013) reported that the median P concentration in a typical California dairy diet was 1.3 times the NRC (2001) requirements. Excess P is excreted mainly in feces, and there is a positive linear relationship between P intake and P in excreta

(Kebreab et al., 2005b; Klop et al., 2013). Using a mechanistic model, Hill et al. (2008) predicted that total P in the diet had a greater effect on P excretion than any other diet fraction. In a meta-analysis of dairy cattle data, Klop et al. (2013) found P intake to be the major predictor of P excreted in feces, and small but significant effects of dietary NDF content, which increased fecal P excretion, and of dietary CP content and milk production level, which decreased fecal P excretion, were also found. Therefore, reducing P intake by matching animal P requirement with available P in the diet is the most effective way of mitigating P excretion in dairy cattle. Recent advances in biotechnology, such as nutritional genomics, may offer genome-tailored individual animal requirements to closely match up with dietary supply (Kebreab et al., 2012).

Availability of dietary P can be increased when nonruminants are fed exogenous phytase; however, because rumen bacteria synthesize phytase, exogenous phytase does not greatly affect P availability to ruminants (see Chapter 7 for additional discussion). Continued development of phytase through improved understanding of its ability to break down organic P may produce more effective classes of phytases, which could increase P availability, which should reduce the need for dietary P and decrease fecal P (Kebreab et al., 2013).

The amount and type of carbohydrate in diet can affect P use efficiency. Kebreab et al. (2005a) reported that dairy cows excreted up to 15 percent less P when fed readily available carbohydrate sources such as starch compared to structural carbohydrate sources. Using a greater amount of starch rather than fiber may have led to greater incorporation of nutrients by microbes, including P. Hill et al. (2008) showed that the efficiency of using P increased when the energy content of the diet increased due to greater P incorporation in milk. In animals on pasture, rotational grazing has reduced total P load in runoff by 64 percent and improved soil infiltration compared to traditional grazing (Haan et al., 2006). A survey of the northeastern United States showed that 13 percent of dairy producers use this method and had better economic and environmental performance than producers using traditional grazing systems (Winsten et al., 2010).

Phosphorus Excretion Prediction Equations

Alvarez-Fuentes et al. (2016) evaluated 10 extant models and developed empirical models to predict P output in feces. The authors developed two sets of models, using all variables that are correlated with P output in one set and, in the second, selecting variables that are routinely available in commercial dairies. Among the extant models, those developed by Weiss and Wyatt (2004) and Klop et al. (2013) performed best when evaluated with data collected from published studies conducted after 2000 (Equations 14-12 and 14-13, respectively).

$$\text{Fecal P (g/d)} = -2.3 \ (\pm 4.2) + 0.63 \ (\pm 0.046)$$
$$\times \text{P intake (g/d)} \quad \text{(Equation 14-12)}$$

$$\text{Fecal P (g/d)} = 19.9\ (\pm 5.07) + 0.79\ (\pm 0.060)$$
$$\times \text{P intake (g/d)} - 1.04\ (\pm 0.127) \times \text{Milk yield (kg/d)}$$
$$\text{(Equation 14-13)}$$

Among the new models developed by Alvarez-Fuentes et al. (2016), the following model had the best performance:

$$\text{Fecal P (g/d)} = 0.73\ (\pm 0.03) \times \text{P intake (g/d)}$$
$$- 0.37\ (\pm 0.08) \times \text{Milk yield (kg/d)}$$
$$\text{(Equation 14-14)}$$

Comparing the three models (Equations 14-12 to 14-14), the more complex models that use P intake and milk yield had similar performances, which was slightly better than the simplest model requiring only P intake. Therefore, depending on availability of data, any of the three models are recommended for use.

Other Minerals

The limited land base of intensive dairy production necessitates importation of nutrients, including minerals in feed. If imported minerals do not leave the farm, mineral accumulation occurs, which has a negative impact on soil and water quality. Surveys have shown substantial mineral surpluses in dairy operations (Hristov et al., 2007; Castillo et al., 2013). In general, most dairy diets provide more mineral than NRC (2001) requirements. A survey of 39 commercial dairies in California showed that the median concentrations of minerals in diets above NRC (2001) requirements were 1.4× for calcium (Ca), 10.6× for magnesium (Mg), 10.5× for sodium (Na), 13.6× for chlorine (Cl), 1.6× for potassium (K), 1.4× for sulfur (S), 1.8× for copper (Cu), 24.7× for iron, 42.0× for manganese, 1.5× for selenium (Se), and 1.6× for zinc (Zn; Castillo et al., 2013). Depending on the source, drinking water can also add to total mineral consumption (Castillo et al., 2013), which, when excreted and applied to soil, can contribute to salinity problems, particularly in irrigated fields. The efficiency of utilization and excretion of minerals varies widely depending on the amount, requirement of the animal, the form of mineral (bioavailability), and interaction or coavailability between the minerals consumed. Excretion of minerals by cows fed various practical diets range 68 to 85 percent of intake for Ca, 86 to 93 percent for K, 95 to 98 percent for Mg, 45 to 95 percent for Na, and 58 to 87 percent for Cl (Meyer and Robinson, 2007). Taylor et al. (2018) developed models to predict Ca, Mg, and Se. The authors reported that DMI and mineral concentrations are strong predictors of mineral excretion.

Minerals such as Cu and Zn are less mobile in soil and may accumulate over time (Brock et al., 2006) and can be toxic to plants and foraging animals (Ferket et al., 2002). High Mg concentration in manure has been reported to inhibit crystallization of stable phosphate forms in sandy soils, enhancing the release of P to the environment (Josan et al., 2005). Manure S can be a significant source of S emissions and odor because it is a precursor of hydrogen sulfide (NRC, 2003). Dietary manipulation to reduce mineral consumption and more precise formulation of rations to meet animal mineral requirements are the most likely mitigation strategies to reduce mineral excretions from livestock operations (Hristov et al., 2007).

TOTAL MANURE

Several equations have been developed to estimate urine and total manure (urine plus wet feces) output by dairy cows (Wilkerson et al., 1997; Bannink et al., 1999; Wattiaux and Karg, 2004; Nennich et al., 2005; van der Stelt et al., 2008; Weiss et al., 2009). Manure is mostly water with DM concentrations usually less than 15 percent. The major factors influencing output of manure are DMI, diet digestibility, and intake of certain minerals, most important, Na and K. N (CP) intake also has some effect. Intake of indigestible DM, rather than DMI, should be a better predictor of manure output. However, equations are usually developed for practical application, and in the field, diet indigestibility cannot be measured. Na and K are major drivers of water flux (see Chapter 7), and increasing intake of those minerals usually increases intake of water, which increases urine output and the water concentration of feces. Most of the published equations were derived from lactating cow data; however, Nennich et al. (2005) includes equations for heifers and dry cows that were derived from limited data. The equations used in the software were chosen because they included independent variables that are major sources of variation in manure output and readily available in most situations. Because of numerous other variables influencing manure output, these equations are not adequate for regulatory purposes. The following equations are used in the software to estimate manure output in kg/d for various classes of animals:

$$\text{Growing heifers: Manure output} = 4.16 \times \text{DMI}$$
$$- \text{BW} \times 0.0246 \quad \text{(Equation 14-15a)}$$

(Nennich et al., 2005)

$$\text{Dry cows: } 0.00711 \times \text{BW} + 0.324 \times \text{CP} + 0.259$$
$$\times \text{NDF} + 8.05 \quad \text{(Equation 14-15b)}$$

(Wilkerson et al., 1997)

$$\text{Lactating cows: } 2.63 \times \text{DMI} + 9.4 \quad \text{(Equation 14-15c)}$$

(Nennich et al., 2005) where DMI is in kg/d, BW is in kg, and CP and NDF are percentages of diet DM.

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15

Feed By-Products

INTRODUCTION

By-product feeds are defined as "secondary products produced in addition to the principal product" (AAFCO, 2016). These secondary products originate from a wide range of industries, including the food, fiber, beverage, and bioenergy industries. By-products usually originate from production processes where at least a portion of the nutrients from the raw input is removed, the extent of which varies both within and by production process. By-products often represent cost-effective sources of protein and energy and may even improve palatability of many rations (Van Soest, 1994). By-products are often considered inedible by humans, but when fed to a dairy cow, they are converted to high-quality human food. The nutrient and chemical composition of by-products can vary depending on manufacturer, geographical region, or site of origin and often changes over time. Production processes such as excessive heating during drying can reduce the nutritional value and overall quality of by-products.

Beyond their nutritional value, feeding by-products to livestock is advantageous for several reasons. First, most cannot be consumed by humans, and consequently, the use of by-product feeds increases the overall efficiency of human-consumable inputs by the dairy industry. Second, by-products reduce the amount of grain used by livestock, resulting in increased grain available for human consumption (Karlsson et al., 2018). Third, the feeding of by-products to livestock eliminates the need for waste disposal from a variety of industries (Bampidis and Robinson, 2006). Fourth, the production of by-products may even represent a safer feed for cattle. Such is the case with sugar beets, which, if fed, are more likely to cause ruminal acidosis than the fibrous but highly digestible by-product, beet pulp (Crawshaw, 2004). Furthermore, many of these feeds originate from human food production, which follows higher standards than that of feed production. The inclusion of by-products into diets of dairy cattle may be limited by a number of nutritional, technical, and socioeconomic aspects.

The objective of this chapter is to identify major feed by-products used in the dairy industry and to provide clarity for the origin and nomenclature of feeds that are listed, with the chemical composition described in Chapter 19, while also noting any limitations or challenges associated with their use. Where applicable, published reviews are emphasized. Many published studies have been designed to include one or more by-products and replace forages or common commodities such as corn or soybean meal. In some cases, the inclusion rates are high, and often such experiments illustrate the adaptability of the modern dairy cow to produce a high-quality food product. This chapter will summarize key studies but will stop short of recommending so-called optimal inclusion rates as such justification is multifactorial in nature. Furthermore, safe and effective use of these products should follow general feeding recommendations outlined in this report. Inclusion of by-products may result in positive associative effects that are not properly accounted for in many nutrition models, such as the case when rumen pH is increased when starch is replaced with digestible fiber (Bradford and Mullins, 2012). This chapter groups each by-product feed into one of five categories and includes a brief description of feeds, including nutrient composition, availability, and impact on milk production.

POORLY DIGESTIBLE FIBER

Feeds in this class can be useful because they provide effective fiber that stimulates rumination and the formation of a rumen mat; they can be useful in diluting the diet concentrations of highly fermentable carbohydrates such as starch, which can reduce or modify the concentrations of organic acids produced in the rumen (Allen et al., 2009). Compared to forages, many of these feeds have a smaller particle size, and this may have a faster rumen passage rate and greater feed intake. When not used as feed, some products within this category are used in other applications on the dairy farm such as a source of bedding.

Corncobs and Residue

The structural makeup of corn residue is about 21 percent cobs, 54 percent stalks, 22 percent leaves, and 13 percent husks (NRC, 1983). Corncobs are high in fiber, low in protein, and poorly digested (Nangole et al., 1983), but compared to most by-products, the particle size of corncobs is fairly coarse (Mertens, 1997). Partial replacement of alfalfa silage with corncobs has been evaluated, and despite the fact that the concentration of energy in the diet was reduced, cows consumed more feed and milk yield was not negatively affected (Depies and Armentano, 1995). When used to replace corn, the addition of corncobs negatively affected milk yield likely because of reduced supply of digestible energy (Soper et al., 1977). In general, crop residues such as corn stover are poorly digested but may be improved through alkaline treatment (Klopfenstein and Owen, 1981). Example of these treatments includes sodium hydroxide (NaOH), ammonia (NH_3), calcium hydroxide ($Ca(OH)_2$), potassium hydroxide (KOH), and calcium oxide (CaO) (Watson et al., 2015). Although safety concerns surround alkaline treatment, replacing wheat hay with corn residue treated with NaOH in rations fed to dairy cattle increased fiber digestibility and energy-corrected milk (Jami et al., 2014). The replacement of Chinese wild rye, corn silage, or corn grain with distillers grains and corn stover treated with CaO has shown promise to maintain production and reduce feed costs, but more research is needed, especially in high-producing cows (Shi et al., 2015).

Cottonseed Hulls

Cottonseed hulls are the outer covering of cottonseed (AAFCO, 2016) and a product of the mechanical removal of oil and meal from the cottonseed. Despite the low nutrient content, cottonseed hulls are highly palatable to cattle (Rogers et al., 2002). Cottonseed hulls may be used to partially replace forage fiber, but to maintain milk production, increased inclusion of energy sources such as corn grain is likely required (Shin et al., 2012). When cottonseed hulls were included at 8 percent of the diet dry matter (DM), rumination activities were reduced while feed intake was increased (Kononoff and Heinrichs, 2003). Cottonseed hulls may be useful when included in the starter diet of calves (Hopkins, 1997), but it should be noted that young animals may be particularly sensitive to gossypol. The addition of cottonseed hulls in a low-fiber calf starter mix increased feed intake, average daily gain, and postweaning body weight (Hill et al., 2009a). Although generally low in protein, the concentration may increase if greater internal portions of the seed itself are present.

Cotton Gin Trash

This is the lowest-value residue produced from the ginning of cotton and formally referred to as "cotton plant by-product" and contains cotton burrs (husks), leaves, stems, lint, immature seeds, and/or dirt (AAFCO, 2016). Cotton gin trash is generally considered a poor-quality feed for dairy cattle, and the chemical composition is highly variable. Gin trash is high in lignin and, due to contamination of soil, is also high in ash. Cotton gin trash is also very coarse in texture and possess a low bulk density, making transportation difficult. Historically, there has been risk of cattle consuming this feed experiencing toxicity due to the insecticide known as disulfoton; however, use of this has been reduced (Rogers et al., 2002). When cotton gin trash replaced dehydrated alfalfa cubes, milk yield was reduced (Brown et al., 1979).

Oat Hulls

Oat hulls are a by-product of oat milling and are a high-fiber feed. This fiber is also highly lignified. Although the extent of fiber digested in the rumen is poor, the degree of cell wall lignification varies by genotype, and this affects the extent to which fiber is digested (Thompson et al., 2000). Methods of increasing the digestibility of fiber through chemical treatment such as alkaline hydrogen peroxide (Cameron et al., 1991a,b; Titgemeyer et al., 1991) have been evaluated, and when treated oat hulls were fed to cows in mid-lactation in place of alfalfa and corn silage, feed intake and production of fat-corrected milk increased (Cameron et al., 1991a). This method has not been widely adopted due to the caustic nature of the substance and associated safety risks (Shreck, 2013).

Peanut Hulls and Peanut Skins

Peanut by-products may contain mycotoxins, with aflatoxins being the most common. Producers should obtain an analysis of aflatoxin content to prevent possible aflatoxin poisoning of cattle and contamination of milk (Hill, 2002). Peanut hulls are not commonly fed to dairy cattle because the digestibility is extremely low (Huffman and Duncan, 1952). Ruminal DM digestibility of peanut hulls is 25 percent but may be increased to 40 percent through chemical treatment with NH_3 or NaOH (Barton et al., 1974). Peanut hulls are often ground and, as a result, low in effective fiber. Compared to hulls, peanut skins are higher in protein and fat while also lower in fiber. Peanut skins are also high in tannins, which may react with proteins and form protein–tannin complexes and reduce protein availability and may negatively affect palatability (West et al., 1993).

Pineapple Cannery Waste

In regions of the world where pineapples are grown, planting and harvesting of pineapple occur year round (Bartholomew et al., 2003). The nutrient content and in vitro digestibility of postharvest pineapple plant material, namely roots, stump, ratoon stems, green leaves, and dried leaves, have been evaluated (Kellems et al., 1979). Pineapple cannery waste is composed

of the outer peel (shell), crown and bud ends of the fruit, fruit trimmings, the inner core, and the pomace. The exact proportions of these parts and their associated chemical composition vary by variety and processing methods (Devendra, 1985). This feed may be fed either fresh or ensiled (Suksathit et al., 2011; Gowda et al., 2015), but the DM content is low, and if not stored correctly, it may spoil quickly (Nhan et al., 2009).

Rice Hulls

Rice hulls or husks consist of the outer covering of the rice grain and along with rice bran is a by-product of rice grain milling (Vadiveloo et al., 2009; AAFCO, 2016). This feed is low in protein and high in fiber and ash. It may be used as a low-quality animal feed but also as a fertilizer, an industrial energy source, or even as a filler for lignocellulosic fiber-thermoplastic composites (Vadiveloo et al., 2009). The digestibility of rice hulls is poor, and this is rarely fed to dairy cattle as a source of energy (Daniels and Hashim, 1977).

Sugarcane Bagasse, Silage, or Hay

This is a poor-quality roughage and is the pulp remaining from the removal of leaves and tops and the extraction of sugar from sugar cane (Fadel, 1999; AAFCO, 2016). This fibrous feed contains approximately 80 percent neutral detergent fiber (NDF), but the digestibility is poor because it is high in silica and lignin (Walford, 2008). Chemical treatment of bagasse increases its digestibility and milk production when fed (Randel et al., 1972).

Tomato Pomace

This by-product is produced during the production of tomato paste, juice, sauce, or ketchup and is usually composed of water, skins, seeds, and other hard tissues of the fruit (El Boushy and Poel, 1994). When produced, the moisture content is high, and as a result, it is often dried to aid in transportation and storage (Weiss et al., 1997). Tomato pomace is high in fiber but also contains approximately 5 percent pectin (Del Valle et al., 2007). A recent advancement in processing allows the separation of seeds from pulp and skin/peel with the seeds being produced as a feed by-product. Compared to tomato pomace, this feed is high in fat and protein and can replace whole cottonseed in rations fed to lactating cows without affecting milk production (Cassinerio et al., 2015).

DIGESTIBLE ENERGY

This class of by-product feeds is fed to dairy cattle because they have moderate to high digestibility and can be used to replace either forages or grains. These by-products are low in starch, but they often contain sugars, digestible fiber, and soluble fiber that contribute energy to rumen microbes, which, in turn, produce volatile fatty acids (FAs) and microbial protein (Dann et al., 2014). These products can replace higher-starch feeds. For example, concentration of starch was reduced from 27 to 18 percent with the addition of nonforage fiber sources, namely, beet pulp and brewers grains, without affecting the flow of microbial protein out of the rumen (Hristov and Ropp, 2003). The inclusion rate of these by-products can be exceptionally high without any negative effects on milk production if diets are formulated properly.

Almond Hulls

Almond hulls are a by-product from harvesting procedures for almond nuts (Fadel, 1999). Almond hulls are composed of primarily the mesocarp surrounding the fruit (Grasser et al., 1995). High in digestible fiber, this by-product is also high in fermentable sugars, which varies by variety (Offeman et al., 2014). Although a common feedstuff, few studies have evaluated its effect on milk production. As a partial replacement for forage, almond hulls have maintained milk production in one study (Aguilar et al., 1984) but not in another (Williams et al., 2018). The chemical composition of almond hulls has been reported to be affected not only by the variety of almond but also by the amount of debris present (DePeters et al., 2020). Like many by-products, proper dry storage is important to maintain the nutritional quality of this feedstuff as moisture may lead to mold growth and washout loss of sugars.

Beet Pulp

The production, chemical composition, and nutritional value of beet pulp have been reviewed (Kelly, 1983; Münnich et al., 2017). Beet pulp is the by-product when sugar is extracted from sugar beets (Fadel, 1999) and may be fed in wet, dry, pelleted, or ensiled forms and may also contain varying amounts of added molasses (Asadi, 2007). Beet pulp is high in NDF but also contains appreciable and variable concentrations of soluble fiber and sugars (DePeters et al., 2000). Beet pulp can be used as a replacement of high-starch grains in rations fed to dairy cattle. When beet pulp replaced high moisture corn and was included at up to 24 percent of the diet DM, milk production was maintained and rumen microbial nitrogen efficiency was not affected (Voelker and Allen, 2003a,b,c). The ruminal digestibility of fiber in beet pulp is rapid (DePeters et al., 1997) and is thought to at least be in part due to the higher arabinose content of hemicellulose. Adding molasses increases the sugar content and dilutes the concentration of fiber (Fadel et al., 2000). Sugar beet pulp silages in France were assayed for common mycotoxins, and although low concentrations were detected in 20 percent of the samples, the concentrations were not high enough to present a health risk for either animals or consumers (Boudra et al., 2015). Like many by-products, the chemical composition of beet pulp is known to vary among sources, and thus

chemical composition based on source may be useful for diet formulation procedures (Arosemena et al., 1995).

Citrus Pulp

Resulting from the extraction of juice, citrus pulp is made up of the ground peel, pulp, and seed residues of citrus fruit. Although this feed may contain any citrus crop, based on worldwide citrus production, oranges probably are the source of more than two-thirds of the citrus pulp produced (Crawshaw, 2004; Bampidis and Robinson, 2006). Citrus pulp is low in protein but supplies energy in the form of fermentable fiber, pectin, and sugars. The production and physical characteristics as well as the nutrient composition and value of citrus pulp have been reviewed (Bampidis and Robinson, 2006). Cattle consume this feedstuff in the wet form, but it is often dried to improve shelf-life and to enhance delivery logistics. When fresh citrus pulp is used, it should not be stored for long periods of time as remaining sugars will support secondary fermentation and mold growth and may attract insects (Bampidis and Robinson, 2006). Dehydrated citrus pulp containing mold can be the cause of citrinin toxicosis. Citrinin is produced by *Aspergillus* and *Penicillium* spp. (Gupta, 2007). During the dehydration process, CaO or $Ca(OH)_2$ may be added to the residue to release bound water (Arthington et al., 2002). Therefore, dried citrus pulp is usually high in Ca but low in phosphorus (P) and must be considered when formulating diets (Bath et al., 1980). A summary of studies in which citrus pulp was fed to lactating dairy cattle replacing corn grain or other high-starch ingredients concluded milk production and composition are usually maintained (Bampidis and Robinson, 2006). Although citrus pulp is commonly fed to dairy cattle with no ill effects, a delayed or type IV hypersensitivity reaction has occurred in a small number of cows consuming citrus pulp and is lethal (Saunders et al., 2000; Iizuka et al., 2005). Citrus pulp naturally contains phytochemicals, such as essential oils, which are antimicrobial, and feeding citrus pulp to ruminants has reduced both cecal and rectal populations of *Escherichia coli* O157:H7 (Callaway et al., 2011). Citrus pulp may contain pesticide residues, but given the concentrations commonly detected and typical inclusion rates, milk safety concerns are not likely (Fink-Gremmels, 2012).

Crude Glycerol

A by-product of biodiesel production, as the name implies, this by-product is high in glycerin but also contains low concentrations of water, ash, trace minerals, free FAs, and methanol (Ma and Hanna, 1999). A portion of glycerol may escape rumen fermentation and be available as a glucogenic substrate (Werner Omazic et al., 2015). The use of glycerol in dairy rations has been reviewed (Donkin, 2008; Meral et al., 2015). This feed has been used to replace energy sources in the diets of lactating cows and has maintained (Donkin et al., 2009; Carvalho et al., 2011) or increased (Shin et al., 2012; Gaillard et al., 2018) milk yield.

Fruit and Vegetable By-Product

The chemical composition and nutritional characterization of fruit and vegetable waste have been reviewed (Angulo et al., 2012). This feed is usually used where the fruits and vegetables are grown, but occasionally it is transported further distances (Froetschel et al., 2014). Because this feed may contain almost any fruit or vegetable in either a whole or processed state, the chemical composition is highly variable. As a result, adequate sampling and analysis of this feed are important. Given the high moisture content, this feed is highly perishable but in some cases may be ensiled and then fed to dairy cattle (Yang et al., 2010; Kotsampasi et al., 2017). Several studies have attempted to characterize microbes, which may be present in this by-product, and although present, these studies did not detect levels that would be considered dangerous for livestock (Sancho et al., 2004; Angulo et al., 2012). Nonetheless, care should be taken to store it in conditions that will not encourage spoilage and contamination.

Potato Waste

Potato waste by-products can contain filter cake, steam peel, potato screenings, cull potatoes, and cooked or dried potato products (Crawshaw, 2004; Nelson, 2010). The different potato by-products, their associated chemical composition, and their use as a feed for cattle have been reviewed (Nelson, 2010). In addition, the use of the potato for feed has been reviewed (Whittemore, 1977). The chemical composition of these by-products varies widely. Consequently, it is important to adequately sample and analyze the product that is fed. The DM content of potato waste is usually low, and consequently, cost of transportation and storage may be a challenge. However, the high moisture and starch content often enable producers to ensile this feedstuff effectively. Compared to potato waste from processing, the DM and fat contents of dried potato products are higher (Rooke et al., 1997). The starch from potatoes is approximately 25 percent amylose and 75 percent amylopectin (French, 1973) and is generally less fermentable in the rumen than the starch found in most grains (Monteils et al., 2002; Mosavi et al., 2012). Potato waste may contain a number of antinutritional constituents or toxic substances. Sprouted and sunburned or green potatoes may contain toxic glycoalkaloids most commonly α-chaconine and α-solanine (see Chapter 17). Although pesticides are used in potato production, if used properly, the risk to animal health is minimal (Nelson, 2010). Although animals may learn how to safely chew whole or coarsely chopped potatoes, feeding these to cattle may result in choking and death (Bradshaw et al., 2002).

Soyhulls

Early in the oil recovery process, the hull or seed coat is removed from the soybean. The hull accounts for about 8 percent of bean DM. Historically, soyhulls were finely

ground and blended with the meal to obtain a crude protein (CP) content of 44 percent. This practice was a means of disposing of the hulls, which were of low value. Today, much of the soybean meal marketed does not contain soyhulls making them available for cattle fed. Soyhulls are often ground because it increases bulk density, aiding in pelleting and transportation (Anderson et al., 1988). Soyhulls are often heated to inactivate antinutritional factors (Johnson et al., 2008). The characteristics and nutritional value of soyhulls have been reviewed (Ipharraguerre and Clark, 2003). Although the fiber may be extensively digested by rumen microbes, in vivo digestion is lower than that in vitro or in situ digestibility (Ipharraguerre and Clark, 2003). This discrepancy may be in part due to the fine particle size of soyhulls, making them pass out of the rumen rapidly and as a consequence limit the extent of rumen digestion (Drackley, 2000). The concentration of CP is about 10 percent and can contribute appreciable amounts of rumen-degradable protein. Soybean hulls are generally included in the rations of lactating dairy cattle as a source of digestible energy and may replace either forages or concentrates (Firkins and Eastridge, 1992; Ipharraguerre and Clark, 2003; Ranathunga et al., 2010). Replacing corn grain with soyhulls can have positive effects on rumen fermentation because they contain little if any starch, which helps maintain rumen pH. When corn grain in a diet was reduced from 40 to 1 percent by replacing it with soyhulls, yield of milk and milk components was maintained (Ipharraguerre et al., 2002a,b). As much as 30 percent of the diet DM may be soyhulls without negatively affecting ruminal fermentation, diet digestibility, or production of dairy cows.

Wheat Middlings

A by-product of the wheat milling process, wheat middlings are composed of wheat bran, shorts, germ, flour, and other assorted portions from the tail of the mill such as red dog (AAFCO, 2016). Shorts are mostly made up of fine bran particles while red dog consists mostly of the aleurone layer with small particles of bran, germ, and flour (Blasi et al., 1998). The chemical composition of wheat middlings has been reviewed (Boros et al., 2004; Slominski et al., 2004; Rosenfelder et al., 2013). This by-product is considered an energy feed because it is higher in starch and fiber but moderate in protein. It is also a good source of P (Erickson et al., 1985). The protein contained in this feed is highly degradable in the rumen (Batajoo and Shaver, 1998). Wheat middlings are commonly used to replace high-starch grains such as corn (Acedo et al., 1987; Bernard, 1997; Dann et al., 2014) but may also be used to replace some forage fiber (Wagner et al., 1993), but the small particle size reduces the effectiveness of fiber (Depies and Armentano, 1995). The chemical composition of wheat middlings may vary and is influenced by wheat type and variety as well as growing conditions, grade of flour produced, and the proportion of bran included (Blasi et al., 1998; Rosenfelder et al., 2013). Although preparatory

processes carried out before milling reduce the mycotoxin content of the grain, mycotoxins in these by-products may be up to 8-fold higher than the flour (Cheli et al., 2013).

Wheat Bran

Wheat bran originates from the wheat milling process and is composed of the pericarp and outer seed tissues, including the aleurone layer, while also containing varying amounts of endosperm (Rosenfelder et al., 2013; AAFCO, 2016). Compared to middlings, the feeding of wheat bran to dairy cattle is less common (Ertl et al., 2016), but it has greater nutritional value than rice bran (Tahir et al., 2002).

PROTEIN FEEDS

This class of by-product feeds is fed to dairy cattle because they contain high concentrations of protein. The extent to which protein is digested in the rumen and in the small intestine varies by feedstuff (Paz et al., 2014).

Animal Products

The use and safety of animal feed ingredients has been reviewed (Clark et al., 1987; Sapkota et al., 2007; Jayathilakan et al., 2012). About 30 to 50 percent of each animal used in the production of food is not consumed by humans. Instead, it goes through the rendering process in which it is exposed to heat while moisture is extracted and fat is separated. The resulting feed by-products include meat and bone meal, meat meal, poultry meal, hydrolyzed feather meal, blood meal, and animal fats (Meeker, 2006). Compared to oilseed meals, animal by-products supply more essential amino acids (AAs), especially lysine (Lys; Ravindran and Blair, 1993). The 2003 discovery of the first U.S. case of bovine spongiform encephalopathy (BSE) and concerns for bacterial contamination of animal feed on human bacterial illnesses have brought about establishment of new adjustments to existing restrictions on the use of many of these products (Garcia et al., 2006; Sapkota et al., 2007). In an attempt to prevent the spread of transmissible spongiform encephalopathy in the United States, the U.S. Food and Drug Administration (FDA, 2008) prohibits this use of specific cattle origin materials from the feed of all animals (see Chapter 17 for details). Regulations can change with time, and users of animal products for feed must be aware of and follow all current regulations regarding their use.

Blood Meal

Blood meal is high in CP, rumen-undegradable protein (RUP), and Lys (Boucher et al., 2009b). Subsequent to the slaughter of cattle, pigs, or poultry, blood is collected while avoiding contamination of hair, ingesta, or urine and processed to produce blood meal. The aim of blood processing is to obtain a dry, stable, nutrient-rich product that can be

ground to an even particle size. Blood meal is hydroscopic and must be processed to less than 12 percent DM and stored in dry facilities (Leoci, 2014). Blood is dried using several methods; the process begins with a coagulation process followed by batch drying, flash drying, or spray drying (Almeida et al., 2013). Very little blood meal is produced using *batch-drying* techniques, which essentially involve removal of water by cooking, steaming, or hydrolyzation. In *flash drying,* moisture in blood is first removed using a mechanical dewatering process, and it may be further condensed by cooking. The remaining semisolid material then undergoes rapid drying using a drum or ring drying process (AAFCO, 2016). In drum drying, blood is applied as a thin layer onto the outer surface of revolving horizontal drums that are internally heated by steam. After the product is dried, it is removed from the drum with a scraper and the dried blood is ground into flakes or powder (Tang et al., 2003). In ring drying, blood is simultaneously ground and dispersed into a high-velocity air stream (Pearson and Dutson, 1992). Whole blood or separated plasma and red albumin may be dried through the spray-dried process (Almeida et al., 2013). In *spray drying*, moisture is removed using low temperatures and evaporators under vacuum until the material is approximately 30 percent solids, after which it is further dried by spraying with a draft of warm dry air (AAFCO, 2016). The method of drying affects the availability of protein and AAs in this by-product (Batterham et al., 1986; Messman and Weiss, 1994).

Fish Meal

Fish meal is high in undegradable protein and methionine (Met; Chalupa and Sniffen, 1996; Santos et al., 1998); however, the consistency of the rumen-undegradable portion of protein can vary greatly across sources (Boucher et al., 2009a). The intestinal digestibility of RUP of this feed is high and the Na content may vary (Taghizadeh et al., 2005). Fish meal is also a good source of essential FAs (Ravindran and Blair, 1993). Fish meal can be made of clean, dried, ground tissue of whole fish and/or cuttings (AAFCO, 2016), which are then cooked, pressed, dried, and ground. Resulting fish meal may originate from a variety of different types of fish, including anchovy, herring, menhaden, pilchards, sardines, sharks, grayfish, catfish, and pollock (Ockerman and Hansen, 2000). The production process of fish meal has been reviewed (Ghaly et al., 2013), as has the use of fish meal in ruminant diets (Hussein and Jordan, 1991). The type of fish and drying temperatures can affect its chemical composition, the ruminal disappearance, and the intestinal digestibility (Opstvedt et al., 1984; González et al., 1998; Boucher et al., 2009b). There are essentially two different methods used in the production of fish meal. In the wet process, which is most common, oil is removed while it is not removed in dry processing (Pearson and Dutson, 1992). Replacing blood meal with fish meal can have positive effects on milk production (Moussavi et al., 2007) but not always (Mattos et al., 2002). Replacing soybean meal with

fish meal often increases the yield of milk protein, a response likely due to increasing the supply of limiting AAs such as Lys or Met (Polan et al., 1997). Feeding of fish meal can increase the intake of eicosapentaenoic acid and docosahexaenoic acid, which may have positive effects on cow fertility (Burke et al., 1997; Mattos et al., 2002; Staples et al., 2005). These FAs will also be found in the milk, but the observed differences are not directly proportional to intake (Wright et al., 2003) because of extensive rumen biohydrogenation and preferential deposition into body tissue (AbuGhazaleh et al., 2004). No effects of feeding fish meal were observed on the physical, chemical, sensory, and processing properties of milk. However, fat globule size was smaller, churning time of cream was longer, and butter possessed a soft texture—effects all likely a result of changes in the FA composition of milk fat (Avramis et al., 2003). The inclusion of fish meal may negatively affect palatability of a total mixed ration (Shaver, 2005). This by-product is used in pet foods and in aquaculture, and as a result, less is available for livestock feed.

Feather Meal

Approximately 5 to 7 percent of the total body weight of domestic fowl is composed of feathers (Onifade et al., 1998). The production processes of feather meal have been reviewed (Papadopoulos, 1985; El Boushy et al., 1990). Using steam and pressure, feather meal is usually hydrolyzed to disrupt the structure of keratin to increase digestibility and to produce a sterilized product (Blasi et al., 1991; El Boushy and van der Poel, 1994). During this process, sulfur (S) is volatilized, and as a result, the S content of feather meal may be used to evaluate the extent of hydrolysis (Moritz and Latshaw, 2001). In some cases, both acidic and enzymatic additives may be used to reduce odor and improve nutritive value (El Boushy et al., 1990). Although high in CP and sulfur AAs, feather meal is low in histidine (His), Lys, and Met (Goedeken et al., 1990; Klemesrud et al., 2000; Stahel et al., 2014). Combining both feather meal and blood meal is an effective way to increase RUP and essential AA content (Grant and Haddad, 1998; Bargo et al., 2001). The combination of adding feather meal to bone meal to increase the supply of cysteine (Cys) to spare Met did not meet the Met needs of lactating cows (Pruekvimolphan and Grummer, 2001).

Meat and Bone Meal, Porcine

A rendered product made up of porcine tissues, meat and bone meal does not contain added blood, hair, hoof, hide, manure, or digesta (AAFCO, 2016). This by-product is high in RUP and essential AAs. Intestinal digestibility of RUP is also high (Howie et al., 1996; Klemesrud et al., 1997). When compared to all animal protein sources, this by-product usually has the lowest concentration of protein but is high in Ca and P because of the varying presence of bone (Ravindran and Blair, 1993; Traylor et al., 2005). The nutritional quality

and sources of variation of meat and bone meal have been reviewed (Hendriks et al., 2002, 2004). When replacing soybean meal, meat and bone meal can increase efficiency of protein utilization, likely due to the high concentration of RUP, high intestinal digestibility of this feed, and increasing the supply of essential AAs (Akayezu et al., 1997).

Poultry By-Product Meal

Poultry by-product meal consists of ground, rendered carcass parts and, except what may be unavoidable, does not contain feathers (AAFCO, 2016). This by-product is extensively used by the pet food industry, and as a result, less is available for livestock feed (Dozier and Dale, 2005). Its chemical composition has been reviewed (Dale et al., 1993; Dozier and Dale, 2005). In addition to a high protein content, this feed is high in fat, P, Ca, and essential AAs. It can be fed to cattle and used as a source of RUP (Bohnert et al., 1998, 1999) and essential AAs (Mäntysaari et al., 1989; Polan et al., 1997).

Whey

The composition of whey and several whey components as well as responses to animals consuming these products has been reviewed (Schingoethe, 1976; Kosikowski, 1979). Whey is high in moisture, lactose, and Na. Despite the fact that casein is removed during the cheese-making process, it contains moderate concentrations of protein. Like all dairy products, whey is also a good source of Ca and P. Whey is the principal by-product of the dairy processing industry. *Sweet whey* is a by-product of cheese production while *acid whey* is a by-product of the production of acidified products, namely, cottage cheese, yogurt, or casein. Given the diversity of milk processing techniques and products produced, variation in chemical composition of by-products designated as whey or whey products is high. The increased use of microfiltration and production of Greek yogurt has increased the production of acid whey. Acid whey is lower in protein and higher in minerals, notably Ca, than sweet whey (Smith et al., 2016). This difference in composition has made the development of methods to produce dry whey ingredients from Greek yogurt problematic. Therefore, the liquid products are available for animal feed (Lagrange et al., 2015). Animals consuming whey may exhibit increased urination, likely a response to the high concentration of Na. Animals may also scour while the erosion of teeth has also been observed. Whey may also be deproteinized, resulting in the by-product called whey permeate, which is much lower in protein but is still high in lactose, minerals, and moisture.

Canola Meal

Canola is a trademarked name for rapeseed, which contains <2 percent erucic acid in the oil and <30 μmol alkenyl glucosinolates per gram of oil-free DM. This is reduced from 25 to 45 percent erucic acid and 50 to 100 μmol glucosinolates in conventional rapeseed meal (Bell, 1993). Glucosinolates are bitter, they impair palatability, and interfere with the synthesis of thyroid hormones by impairing the uptake of iodine (Woyengo et al., 2016). Canola meal is the meal remaining after the extraction of oil from *Brassica campestris*, *Brassica napus*, or *Brassica juncea* seeds by either mechanical or solvent extraction methods (AAFCO, 2016). The chemical composition, ruminal degradability, and lactational performances of dairy cows consuming canola meal have been reviewed (Mustafa et al., 2000; Huhtanen et al., 2011; Martineau et al., 2013). Compared to soybean meal, canola meal contains less protein and energy, but the protein is less degradable in the rumen. In a meta-analysis, Huhtanen et al. (2011) found that canola meal was at least as good as soybean meal and that some improved responses are due to increases in feed intake. A more recent meta-analysis conducted by Martineau et al. (2013) evaluated the replacement of other sources of protein with canola meal. In general, milk and milk protein yields increased with canola meal. Canola meal contains goitrogenic compounds that can reduce the transfer of iodine into milk (Laarveld et al., 1981; Weiss et al., 2015). See Chapter 7 for more details. Bell (1993) outlined other constituents of canola meal that may exert antinutritional effects. Canola meal contains sinapine, which may negatively affect palatability, but this has not been reported in cattle. Sinapine affects the flavor of eggs from chickens, but no research has been conducted on potential effects on milk flavor. Canola meal contains approximately 1.5 to 3.0 percent tannins, which may interfere with protein digestion.

Cottonseed Meal

This by-product is produced when the oil from cottonseed has been removed. Oil may be removed either through mechanical or solvent extraction, and the resulting meal must not contain less than 36 percent CP (AAFCO, 2016). The protein content and quality of cottonseed meal are high, and when replacing either soybean meal or canola meal, little difference in milk production is usually observed (Brito and Broderick, 2007). In a number of studies, milk protein decreased when cows consume cottonseed meal (Maesoomi et al., 2006; Brito and Broderick, 2007). This may be because of the lower concentration of Lys. In addition, the bioavailability of Lys in cottonseed meal may be low because under heat, gossypol binds to proteins, notably the epsilon amino group of Lys (Reiser and Fu, 1962; Blauwiekel et al., 1997).

Soybean Meal

Soybean meal products are discussed in Chapter 19.

Sunflower Meal

A by-product of the process of extracting oil from sunflower seed, sunflower meal has moderate concentrations of

TABLE 15-1   Major Coproducts Resulting from the Dry Grain Corn-Milling Process

Name	Brief Description
Condensed distillers solubles	Resulting from the removal of ethyl alcohol by the distillation from the yeast fermentation of grain by condensing this stillage to a semisolid state.
Corn bran	Produced when the corn ethanol facility dehulls and degerms the grain subsequent to fermentation. This feed has not gone through the fermentation process.
Corn germ	Produced when the corn ethanol facility dehulls and degerms the grain subsequent to fermentation. This feed has not gone through the fermentation process. Because the germ contains much of the fat found in the corn kernel, in addition to fiber, the product is also high in fat.
Deoiled distillers dried grains with solubles	Resulting product from solvent extraction of oil from corn distillers dried grains with solubles (DDGS), and fat content may be as low as 3 percent on an as-fed basis. Term solvent extracted is not required by AAFCO (2016).
Distillers dried grains with solubles	Resulting from the removal of ethyl alcohol by distillation from the yeast fermentation of grain or a grain mixture by condensing and drying at least 75 percent of the solids of the whole stillage.
Distillers dried solubles	Resulting from the removal of ethyl alcohol by distillation from the yeast fermentation of grain or a grain mixture by separating the coarse grain fraction of the whole stillage and drying it.
High-protein distillers grains	Remains after the corn ethanol facility dehulls and degerms the grain and then is fermented. In most cases, solubles are not added back as traditionally done in the dry milling process, but when this does occur, this product is called high-protein distillers with solubles. The protein content of this feed is similar to soybean meal and contains less phosphorus than traditional DDGS.
Modified wet distillers grains with solubles	Partially dried and contain approximately 50 to 54 percent moisture (Mello et al., 2012).
Reduced-fat distillers dried grains with solubles	Produced when the solubles are centrifuged before they are added to the distillers grains.
Wet distillers grains	Resulting from the removal of ethyl alcohol by the distillation from yeast fermentation of grains.
Wet distillers grains with solubles	Resulting from the removal of ethyl alcohol by distillation from the yeast fermentation of grain or a grain mixture by condensing and drying at least 75 percent of the solids of the whole stillage.

protein and fiber (Arieli et al., 1999). In general, it is a good source of digestible protein, but the fiber is highly resistant to rumen digestion (Van Soest, 1994). The chemical composition of sunflower meal has been reviewed (Lardy and Anderson, 2002; Lomascolo et al., 2012; Ítavo et al., 2015). Sunflower meal is high in copper and has been documented to contribute to copper toxicity in sheep, which are particularly sensitive to this mineral (García-Fernández et al., 1999). This feed is also high in S containing AAs, such as Met, but compared to soybean meal is low in Lys. The chemical composition of sunflower meal varies depending on the method of processing. Solvent extraction methods are most efficient at removing oil, resulting in sunflower seed with a low fat content, but mechanical expeller or extrusion methods also exist (Drackley and Schingoethe, 1986; Lardy and Anderson, 2002). The extent of hull removal affects the chemical composition. Sunflower meal probably contains more degradable protein than soybean meal or canola meal (Lardy and Anderson, 2002). Sunflower meal has been used effectively as a replacement for soybean meal without affecting the yield or composition of milk (Schingoethe et al., 1977). Sunflower meal is also used in calf starter diets (Drackley and Schingoethe, 1986) and was more palatable than canola meal (Miller-Cushon et al., 2014).

Safflower Meal

Compared to soybean or canola, safflower is a minor oilseed crop (Ekin, 2005). A by-product of the process in which oil is extracted out of the safflower seed, safflower meal is good source of both protein and fiber. The crude fat content varies depending on the extraction process but is lowest when solvent extraction is used. The protein appears to be relatively resistant to degradation by rumen microbes (Dixon et al., 2003a,b). The feed contains two phenolic glucosides, namely, matairesinol-β-glucoside and the purgative 2-hydroxyarctiin-β-glucoside that may make the feed bitter and unpalatable to cattle (Jin et al., 2010). Glucosinolates have been reviewed (Tripathi and Mishra, 2007).

ENERGY/PROTEIN BY-PRODUCTS

Corn Ethanol Production, the Dry Grind Process

Over 30 percent of the corn crop in the United States is probably used to produce fuel ethanol. Since approximately 2004, the fuel ethanol industry has experienced dramatic growth. A number of reviews have been published outlining the chemical composition and use of corn milling coproducts in rations for dairy cattle (Schingoethe et al., 2009; Hollmann et al., 2011a,b; Bradford and Mullins, 2012; Paz et al., 2014; Böttger and Südekum, 2018). The growth of the dry milling industry has brought about increased availability of corn milling coproducts (see Table 15-1). The dry milling process (see Figure 15-1) is used at approximately 80 percent of the corn ethanol facilities in the United States, and it begins when the entire corn kernel is ground through a hammer mill
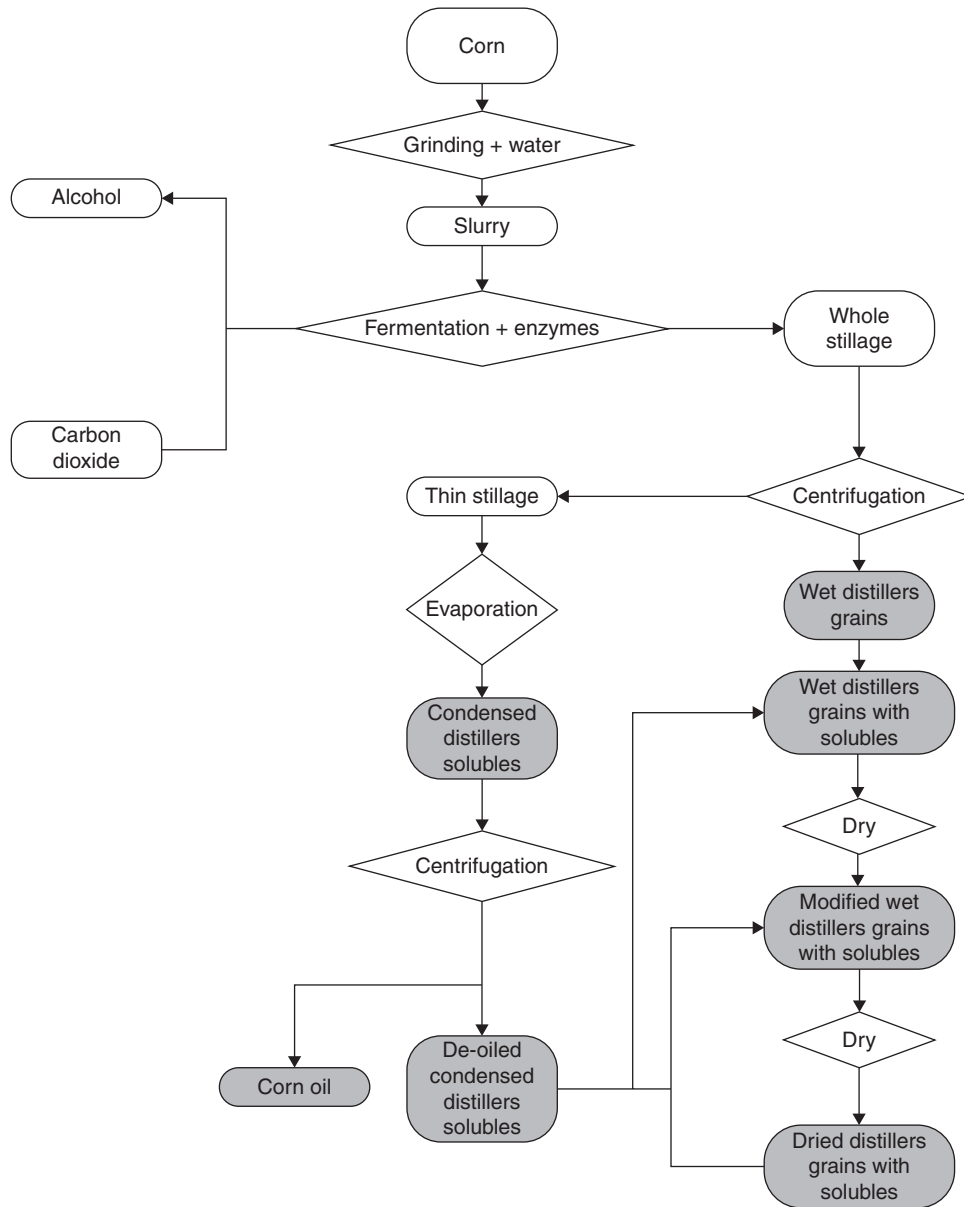
FIGURE 15-1   Schematic representation of the dry milling ethanol process for corn ethanol production. Diamonds denote a processing step and ovals denote a product. Feed coproducts are shaded gray.

and hot water is added (Rosentrater, 2015). After about 50 to 60 hours of fermentation, the resulting mash contains about 15 percent ethanol (Bothast and Schlicher, 2005). The ethanol is distilled off, and the remaining residue is called whole stillage and contains yeast cells, nonfermented solids, and water. The stillage can be centrifuged, resulting in "thin stillage," which contains 5 to 10 percent solids, and wet distillers grains (WDG). The thin stillage can be evaporated, producing syrup that can be blended with WDG to produce WDG with solubles that are often dried to produce dried distillers grains with solubles (DDGS). Compared to corn grain, protein, fat, and most minerals increase about 3-fold, but concentrations of Na, S, and Ca may be greater because exogenous sources

of these minerals may be added during the production process (NRC, 2012).

Corn milling coproducts may contain mycotoxin that were present in the incoming grain. If they withstand the fermentation process, the concentrations of these toxins can increase 3-fold in the final feedstock because a large proportion of starch is removed during the process (Fink-Gremmels, 2012). The toxins can include aflatoxins, deoxynivalenol, fumonisins, ergot alkaloids, and zearalenone. A 2-year (2006 to 2008) study evaluating 235 DDGS samples collected from 20 U.S. corn ethanol plants and 23 export shipping containers found that none of the samples contained aflatoxins or deoxynivalenol concentrations greater than FDA guidelines, and no more
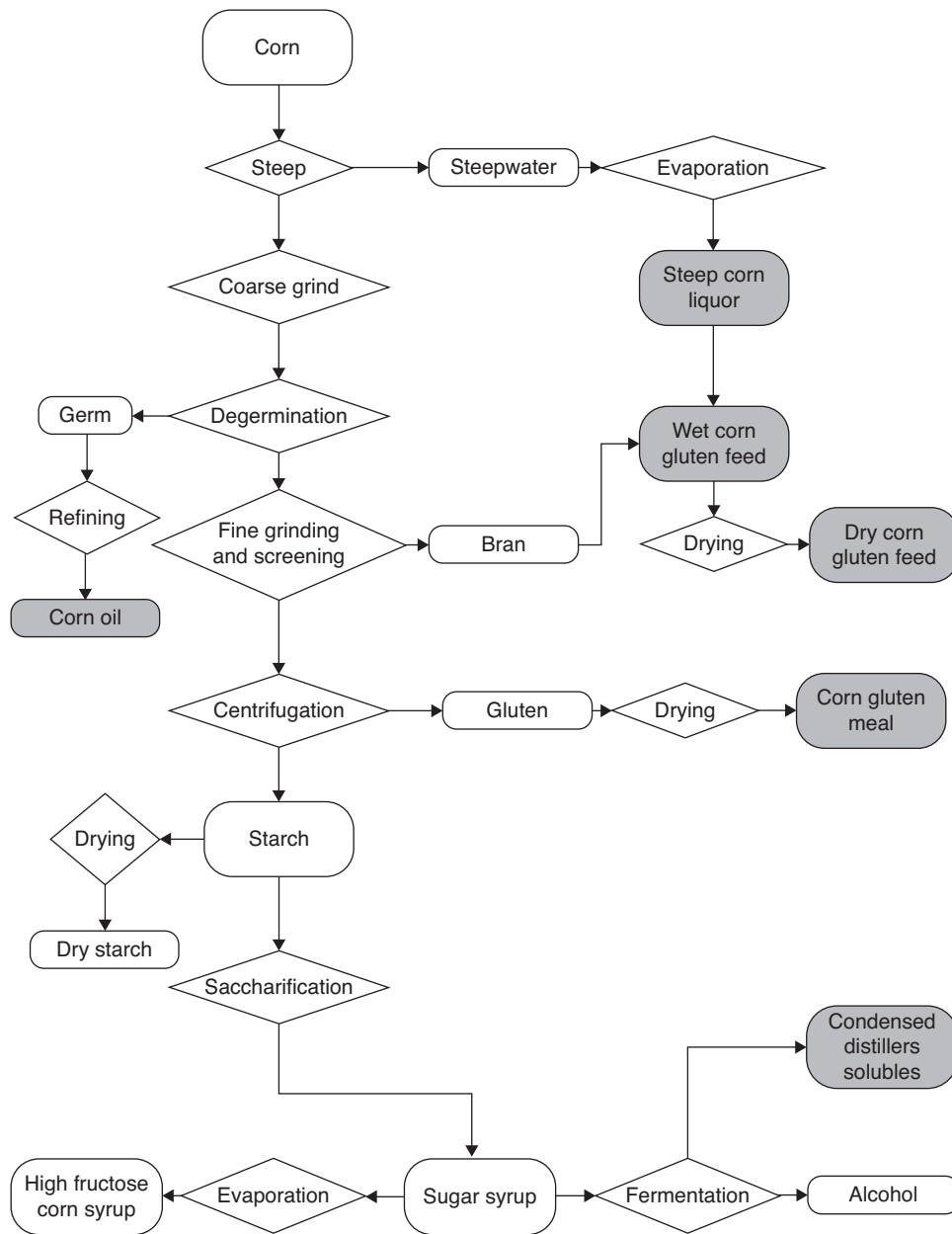
FIGURE 15-2   Schematic representation of the wet milling process for corn ethanol and high-fructose corn syrup. Diamonds denote a processing step while the ovals are products; feed coproducts are shaded gray.

than 10 percent of the samples contained fumonisin at concentrations higher than the recommendation for feeding equids and rabbits (Zhang et al., 2009). Fumonisins were detected at concentrations lower than FDA guidelines for use in animal feed. In addition, none of the samples contained T-2 toxins higher than the detection limit, while zearalenone concentrations were lower than the detection limit in most samples.

The Wet Milling Process

Compared to the dry grind process, the wet milling process (see Figure 15-2) is more energy and capital intensive (Bothast

and Schlicher, 2005). After cleaning, the corn is steeped in a dilute solution of sulfurous dioxide for approximately 40 hours to start breaking down the protein in the grain. The resulting steepwater can be condensed, resulting in a product that may be sold as *steep corn liquor* (referred to by AAFCO [2016] as condensed fermented corn extractives) or used as a source of nutrients for subsequent fermentation. This liquor is composed of a mixture of soluble components of corn grain containing both protein and complex sugars (Crawshaw, 2004). At this point, the corn kernel goes through a series of grinds, differential separations, and centrifuges. The germ is isolated, from which the oil is extracted. The remaining coproduct at this stage is *corn germ*

meal (wet milled) (Crawshaw, 2004; AAFCO, 2016). Once the germ is removed, the residue is milled to release the starch and aid in removal of the hull or bran. The bran is then pressed, and usually the corn steep liquor is added back to produce *corn gluten feed*. Corn gluten feed may be sold in either wet or dry forms. With much of the fiber removed, the remaining material is centrifuged to separate the gluten and starch fractions. The remaining *corn gluten meal* is high in zein protein and usually is used in the pet food and poultry industries. The starch from the wet milling process can be further converted to dextrose and then fermented to produce fuel ethanol or used in other industrial fermentation processes. If used to produce ethanol, a resulting coproduct is distillers solubles. The solubles produced by the wet milling industry contain yeast cells and unfermented sugars and protein, but because the germ has been removed, it does not contain high concentrations of fat. The starch may be further processed into a number of food-grade products such as corn syrup and high-fructose corn sweetener (Stock et al., 2000). Corn germ from the wet milling process has been fed to dairy cattle (Miller et al., 2009).

Brewers Grains

Beer ranks among the top five most consumed beverages in the world (Fillaudeau et al., 2006), and its by-product is brewers grains, or brewers spent grains. Brewers grains are mostly made up of the husk-pericarp-seed coat layers of the barley grain while hop residue will also be present (Mussatto et al., 2006; McCarthy et al., 2013). Brewers grains may also include corn, wheat, rice, and other common sources of carbohydrates. Chemical variations of this by-product exist both across and within a brewery (Westendorf et al., 2014) and are largely a function of barley variety, harvest time, malting, and mashing conditions as well as the quality and type of added adjuncts (Mussatto et al., 2006). Brewers grains contain cellulose, hemicellulose, lignin, protein, vitamins, and minerals (Priest and Stewart, 2006). The phenolic component is believed to have bioactive antioxidant potential but has not been studied in ruminants (McCarthy et al., 2013). As with other by-products from the food industry, fresh brewers grains are almost always a safe and nutrient-dense feed for dairy cows. The high moisture content of wet brewers grains makes it an unstable feedstuff, but it can be preserved by reducing the moisture content by pressing and then drying at the production facility (Aliyu and Bala, 2013). Brewers yeast is a by-product of the brewing industry and can be fed to cattle (Grieve, 1979). Brewers yeast is distinguished from yeast culture and yeast extract and other yeast-containing feeds (AOAC, 2016). Yeast used by brewers was selected or developed based on their effects on beer flavor or to optimize beer-making fermentation. Yeast used as direct feed additives was selected or developed for effects in the rumen (see Chapter 16). Usually available in a wet form, brewers yeast may be dried (Steckley et al., 1979a,b). When wet brewers grains were added to the rations of lactating dairy

cattle at 9 and 17 percent of the diet DM in replacement of forage, no effects on milk production were observed (Firkins et al., 2002). The RUP content of wet brewers grains is usually lower than that of WDG because RUP is lower in barley (brewers grains) than it is for corn (distillers grains). In addition, brewers grains are not exposed to heat during a distillation process. Drying brewers grain increases the RUP content because of heat exposure.

Bakery Waste

The chemical composition of bakery waste can be highly variable (Waldroup et al., 1982; Slominski et al., 2004) and originates from bread, cereal, or cookie production. The feed composition database of this publication distinguishes these sources. All types are high in starch while cookie waste is usually high in crude fat and sucrose or other simple sugars. In general, bakery waste contains a high concentration of energy and may be used as a partial replacement for cereal grains (Humer et al., 2018).

Cottonseed, Whole

The nutrient composition and impact on animal performance of cottonseed and cottonseed meal has been reviewed (Coppock et al., 1987; Arieli, 1998). There are two types of cottonseed available in the United States: (1) upland cotton (*Gossypium hirsutum*) or high lint and (2) Pima cotton (*Gossypium barbadense*), which is delinted. Pima cottonseed makes up only about 5.5 percent of the U.S. cotton production and is higher in fat, protein, and gossypol (Broderick et al., 2013). This by-product is usually fed after it has been cracked. Upland cottonseed is usually fed whole and is higher in fiber and should be stored in a facility where it is protected from moisture and well ventilated to prevent the formation of condensation and mold. Cottonseed should be tested for gossypol and mycotoxins. Details on both those toxins are in Chapter 17. Whole cottonseed is an excellent source of effective fiber for dairy cattle and often increases milk fat when fed (Clark and Armentano, 1993). The seed coat at least partially protects the oil from rumen microbes and biohydrogenation (Palmquist and Jenkins, 1980). Partial replacement (15 percent of diet DM) of both corn silage and alfalfa silage with whole cottonseed did not affect milk production and composition (Firkins et al., 2002). Cottonseed is an excellent source of protein and can effectively replace soybean meal in the ration of lactating dairy cows (Broderick et al., 2013).

High-Fat By-Products

Animal Fats

Animal fats are a by-product of the meat industry, and in the United States, these most commonly originate from beef or pork processing. In the rendering process, heat and pres-

sure are used to separate lipid material from meat tissues. The American Fats and Oils Association (Columbia, SC) outlines marketing grades for animal fats that are based on characteristics such as melting point, color, density, moisture, and impurities (USDA, 2013). These grades include tallow, choice white grease, and yellow grease. These specifications are not based on the species of origin but rather outline the technical specifications above (Pearson and Dutson, 1992). Commercially rendered fat from cattle is often referred to as tallow, but it is technically defined as fat possessing titer temperature (temperature at which FAs of a given fat solidify) greater than 40°C. Rendered fat from swine is usually commercially referred to as lard or grease, but technically, lard and grease have a titer equal to or less than 40°C (Ockerman and Hansen, 2000). The nutritional quality of different animal fats is dependent on the FA composition, which is a function of the animal species from which they originate. The FA profile of the diet fed to swine has a major impact on the FA profile of rendered pork fat. Because of rumen biohydrogenation by rumen microbes, diet has less of an impact on the FA profile of rendered beef fat. Compared to beef fat, pork fat is softer, and this is a due to the higher concentration of linoleic acid. Pork fat is also lower in myristic acid (unless pigs are fed beef fat) and is also essentially void of *trans* unsaturated FAs (Berger, 1997). Yellow grease originates from restaurant cooking practices but may also originate from rendering plants producing lower-quality greases. The impact of animal fats on milk production is related to the associated effects on intake, rumen fermentation, and digestibility of the FAs (see Chapter 4). Animal fats are also added to milk replacers (Jenkins et al., 1986; Hill et al., 2009b) to increase energy density. Animal fats may be added to calf starters (Hill et al., 2015) to increase energy density but are more frequently used to control dust. Because animal fat sources are susceptible to oxidation (Shurson et al., 2015; Joseph, 2016), antioxidants are often added (Buck, 1991).

Rice Bran

Rice bran is largely made up of the pericarp and germ of the rice grain but may also contain other constituents of the rice plant. In some cases, a portion of the oil is removed through the use of solvents (AAFCO, 2016). Both conventional (Nörnberg et al., 2004; Wang et al., 2015; Criscioni and Fernández, 2016) and defatted (Chaudhary et al., 2001) rice bran can be fed successfully to cattle.

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16

Feed Additives

INTRODUCTION

Feed additives are optional diet ingredients that are not nutrients but can affect digestion, metabolism, and production. They are not required to maintain good health and high production, but production and health can be improved by some additives. Their modes of action may or may not be understood. Only additives that are approved for use in the United States (in 2019) and are the subject of peer-reviewed research involving dairy cattle are included in this review. Feed additives that also act as nutrients are discussed in the appropriate chapter. For example, buffers and chromium are discussed in Chapter 7, and niacin, biotin, and β-carotene are discussed in Chapter 8.

IONOPHORES

Ionophores are polyether antibiotics (not used in human or veterinary medicine) produced by a variety of actinomycetes that alter the flux of ions across biological membranes. Gram-negative bacteria contain a complex outer membrane and are usually unaffected by ionophores, but Gram-positive bacteria lack the outer membrane and are more sensitive to ionophores. Ionophores generally decrease the proportion of Gram-positive bacteria and increase the proportion of Gram-negative bacteria; however, changes in microbial populations are much broader than that. The ionophore monensin also causes substantial shifts in bacterial communities within those broad classes (Kim et al., 2014a,b). Furthermore, dietary ingredients and nutrients interact with monensin on altering ruminal microbial populations.

In the United States, monensin and lasalocid are approved to be fed to dairy calves and growing heifers and are used as a coccidiostat for young calves (see Chapter 10) and to improve feed efficiency in growing heifers; however, in the United States, only monensin is approved for dry and lactating cows with the label claim of improving feed efficiency (solids-corrected milk/dry matter intake [DMI]). A substantial database exists regarding metabolic (both ruminal and cow), production, and health responses to monensin, and several qualitative (McGuffey et al., 2001; Ipharraguerre and Clark, 2003) and quantitative reviews (Duffield et al., 2008a,b,c; Appuhamy et al., 2013) are available. Ionophores alter production and concentrations of ruminal fermentation end products by altering ruminal bacterial populations and by altering metabolism of certain bacteria (McGuffey et al., 2001). When fed to dairy cows, methane (CH_4) production can be reduced, and the molar proportion of acetate is decreased while the molar proportion of propionate is increased. When monensin is fed to beef animals, changes in rumen volatile fatty acids (FAs) and CH_4 production are generally consistent (reviewed by McGuffey et al., 2001). However, with dairy cows fed typical diets, rumen volatile FA profiles often are not affected by monensin (Martineau et al., 2007; Oelker et al., 2009; Mathew et al., 2011). Although CH_4 production can be reduced when monensin is fed to dairy cows, the response is less and more variable than when fed to beef cattle (Appuhamy et al., 2013). Higher-forage diets and substantially greater DMI are likely reasons for the difference in ruminal responses to ionophores between beef and dairy cattle.

The increased production of propionate is one reason why cows fed monensin usually have significantly greater plasma concentrations of glucose (Duffield et al., 2008a). Production of glucose from propionate increased and tissue oxidation of glucose decreased when monensin was fed to dairy cows (Markantonatos and Varga, 2017). Monensin feeding significantly lowers concentrations of β-hydroxybutyrate and nonesterified FAs in plasma during the immediate postpartum period (Duffield et al., 2008a). In agreement with those results, cows fed monensin during the peripartum period had significantly lower risk of ketosis, mastitis, and displaced abomasum (Duffield et al., 2008c). The effects on mastitis and displaced abomasum may be indirect via a reduction in ketosis because ketosis is a risk factor for both mastitis and displaced abomasum.

Monensin often increases plasma urea concentrations in lactating dairy cows (Duffield et al., 2008a), but ionophore feeding is generally associated with decreased concentrations of ruminal ammonia (Ruiz et al., 2001). The reason for the apparently paradoxical results is unclear.

Including ionophores in diets fed to dairy cows has not markedly affected nutrient digestibility, with most responses being from 0 to about a 3 percent increase in dry matter (DM), organic matter, or energy digestibility (Haïmoud et al., 1995; Knowlton et al., 1996; Plaizier et al., 2000; Morris et al., 2018b; Tebbe et al., 2018). The mode of action is not clear but may be related to slightly lower DMI and altered ruminal microbial populations. Monensin also has very modest effects on feeding behavior (more frequent and more meals per day) in peripartum cows (Mullins et al., 2012), which may help stabilize the rumen. Effects of ionophore on digestibility of carbohydrates (e.g., neutral detergent fiber [NDF] and starch) in dairy-type diets are small and inconsistent; however, apparent protein digestibility is often increased with ionophores (Plaizier et al., 2000; Ruiz et al., 2001; Benchaar et al., 2006; Martineau et al., 2007; Morris et al., 2018b). Ionophore feeding has increased flow of feed protein out of the rumen (Haïmoud et al., 1995), and if that protein is more digestible than microbial protein, that could increase protein digestibility. Ionophores also increase absorption of certain minerals (see Chapter 7).

A meta-analysis using data from 36 papers and more than 9,600 cows concluded that monensin reduced DMI by 2.3 percent, increased milk yield by 2.3 percent, and reduced both milk fat and milk protein percentage but increased protein yield (did not affect fat yield). Energetic efficiency (milk energy plus energy change in body divided by energy intake) was significantly increased by 2.5 percent (Duffield et al., 2008b). The DMI equations do not include a monensin term because the database used for those equations was not adequate to separate a monensin effect. However, if monensin is included in the diet within the range of approximately 200 to 450 mg/d (approximate range in Duffield et al., 2008b), the model increases the digestible energy concentration of the diet by 2 percent (Fairfield et al., 2007) and reduces CH_4 energy by 5 percent (Odongo et al., 2007), which results in an approximate increase in metabolizable energy of 2.7 percent, which equals a 2.5 percent increase in net energy for lactation (NEL). Improvement in efficiency is likely caused by a combination of factors described above, including slightly improved digestibility, increased propionate production, and reduced CH_4.

Based on a meta-analysis, monensin often reduces milk fat percentage; however, significant heterogeneity among studies was found, and numerous individual studies report no effect of monensin on milk fat percentage. Generally, monensin is more likely to reduce milk fat percentage when fed in diets with greater concentrations of C18:3 (Duffield et al., 2008b). Increasing dietary concentrations of both C18:1 and C18:2 linearly reduced milk fat percentage, but no interactions

between monensin feeding and type or amount of dietary unsaturated FA were observed (He et al., 2012). In addition, Mathew et al. (2011) reported that feeding monensin reduced milk fat concentration, but the effect occurred whether supplemental fat (mix of 18-carbon unsaturated FAs) was fed or not. Including almost 30 percent of diet DM as distillers grains caused significant milk fat depression, and that was exacerbated by addition of monensin (Morris et al., 2018b). Diets were high in C18:2 but also high in sulfur and had a low dietary cation anion difference, which can also reduce milk fat (see Chapter 7). The causes of the variability in milk fat response to monensin have yet to be explained fully. Monensin supplementation often increases the concentrations of *trans* FAs including *trans*-10, *cis*-12 conjugated linoleic in milk with or without an effect on milk fat concentrations (da Silva et al., 2007; Oelker et al., 2009; Mathew et al., 2011; He et al., 2012; Morris et al., 2018a).

Growing Heifers

When fed to beef animals consuming high-starch diets, ionophores usually decrease feed intake but have little effect on daily gain, thereby improving feed conversion efficiency (reviewed by NASEM, 2016). In studies specific to dairy heifers, feed intake is not significantly reduced by ionophore supplementation (Baile et al., 1982; Meinert et al., 1992; Steen et al., 1992), and average daily gain or efficiency of feed utilization is often increased, but the differences have not always been significant (Baile et al., 1982; Meinert et al., 1992). Similar results are observed when beef animals are fed ionophores in high-forage diets (Bretschneider et al., 2008). Type and dose of ionophore, forage quality, and interactions between those factors can affect growth responses to ionophores. A reduction in days to conception (Baile et al., 1982) or age at first breeding and age at first calving (Meinert et al., 1992) has been reported. When ionophores were fed to recently weaned calves (approximately 12 weeks old), no or negative effects on growth rate have been reported (Cabral et al., 2013; Chapman et al., 2016).

YEAST AND DIRECT-FED MICROBIALS

A direct-fed microbial (DFM) as defined by the U.S. Food and Drug Administration is a feed additive that contains viable microorganisms. Based on descriptions by the Association of American Feed Control Officials (AAFCO), yeast, if viable, is considered a DFM. Yeast culture products and certain other yeast products (e.g., brewer's yeast) do not contain appreciable, if any, viable cells and are not DFMs. *Saccharomyces cerevisiae* is the most commonly fed yeast DFM; bacterial DFMs include various *Propionibacterium* and *Lactobacillus* species or strains including *Enterococcus faecium*, *Prevotella bryantii*, and *Megasphaera elsdenii*. Observed responses to DFMs and yeast products include greater milk yields, altered milk composition, greater feed

intake, greater feed efficiency, altered ruminal organic acid profiles, and higher ruminal pH. Proposed modes of action of yeast products and DFMs include altering ruminal bacterial population, including increased number of lactic acid–using bacteria, synthesis of growth factors or vitamins, reduction of oxygen concentrations within the rumen, and increased overall microbial activity and mass of microbes (Yoon and Stern, 1995; Seo et al., 2010). In nonruminants, DFMs have effects within the intestines, and this may also occur in ruminants.

Two meta-analyses have been conducted to quantify production responses by dairy cows fed yeast products (Desnoyers et al., 2009; Poppy et al., 2012). Both meta-analyses included studies that fed *S. cerevisiae* culture products. One analysis included only studies evaluating production responses by dairy cows fed a yeast product from a single company (Poppy et al., 2012). The other analysis included yeast from multiple companies fed to ruminants (Desnoyers et al., 2009). Several of the same studies were included in both analyses. Both analyses concluded that yeast culture increases milk yield and yield or concentration of milk fat. Yeast increased milk protein yield in one meta-analysis (Poppy et al., 2012) but not in the other. In one meta-analysis (Desnoyers et al., 2009), yeast culture increased DMI, but in the other analysis (Poppy et al., 2012), yeast increased DMI in early lactation (<70 days in milk) but reduced it in later lactation. Milk yield and, to a lesser extent, DMI responses were positively related to dose of yeast (Desnoyers et al., 2009). Cows fed yeast had slightly but significantly higher rumen pH and volatile FA concentrations and lower lactic acid concentrations. Total tract organic matter was increased significantly by about 1.1 percent.

Several species or classes of bacteria have been evaluated as potential DFM for cattle, but the number of studies for each type of bacteria is limited and not adequate for a meta-analysis. In addition, bacterial DFMs are often fed in combination with yeast products, which makes attributing responses to a specific DFM impossible. An extensive qualitative review on responses to bacterial DFMs is available (McAllister et al., 2011). Although bacterial DFMs may have multiple modes of action, they are often classified as lactic acid producers or lactic acid utilizers (Seo et al., 2010). Lactic acid–producing bacteria that have been fed to dairy cows include *Enterococcus faecium* and various *Lactobacillus* spp. One proposed mode of action is that these bacteria will produce low amounts of lactic acid constantly over time, which will stimulate growth of lactic acid–using bacteria. When a large influx of fermentable carbohydrate into the rumen occurs, the greater population of lactic acid–using bacteria will help attenuate rumen lactic acid concentrations and ruminal pH. Some data are available showing that at least regarding ruminal pH, this may occur (Nocek et al., 2002). When *E. faecium* was fed in combination with yeast products, feed intake and milk yield and milk component yields were increased (Nocek et al., 2003); however, this effect may be from the yeast, the bacteria, or their combination. Other

suggested modes of action for lactic acid–producing bacteria include antibacterial activity against specific bacteria and alteration of intestinal microbiome resulting in improved immune response (McAllister et al., 2011). Lactic acid utilizers that have been evaluated as DFM include *Megasphaera elsdenii* and various *Propionibacterium* spp. Wild-type *M. elsdenii* is a major lactic utilizer in the rumen, and various strains have been fed. Dairy cows dosed with *M. elsdenii* have or tended to have higher concentrations of ruminal propionate and lower acetate to propionate ratios, but milk production, milk composition, feed intake, and ruminal pH have not been affected (Hagg et al., 2010; Aikman et al., 2011). Propionibacteria can ferment lactate into propionate, but they can also produce propionate from alternative pathways. Increased ruminal propionate production could increase glucose synthesis, which could increase milk yield or metabolic efficiency. When propionibacteria were fed to dairy cows, efficiency or milk yield was increased in three of four studies (Francisco et al., 2002; Stein et al., 2006; Raeth-Knight et al., 2007; Weiss et al., 2008). In nonruminants and calves, DFM can modify microbial populations within the intestine, which can reduce certain diseases and enhance efficiency. This area has not been researched extensively in ruminants, but such research could lead to a better understanding of how DFM works.

SILAGE INOCULANTS

The effects of silage inoculants on the fermentation of silage are beyond the scope of this book (see Muck et al., 2018, for a discussion on this topic); however, since the inoculants and its end products are ultimately consumed by cows, they can be considered a feed additive. Silage inoculants can be broadly classified as homofermentative lactic acid bacteria (LAB) and obligate heterofermentative LAB. Based on a meta-analysis (Oliveira et al., 2017), cows fed silage inoculated with homofermentative LAB (this classification also includes facultative heterofermentative LAB, but they produce essentially only lactic acid) produced more milk than cows fed uninoculated silage likely because of greater DMI. Digestibility and feed efficiency were generally unaffected. The mode of action is unclear, but lower concentrations of some potentially hypophagic compounds (e.g., butyrate or ammonia) may be involved. Based on rumen in vitro studies, silage inoculation may also alter ruminal fermentation. For example, Jalč et al. (2009) reported less CH_4 production when inoculated silage was incubated in an in vitro system compared to control silage. Muck et al. (2018) reviewed studies that evaluated cow responses to silage inoculated with obligate heterofermentative LAB (*Lactobacillus buchneri* was the only species evaluated) and concluded the DMI was not affected by inoculation with *L. buchneri*. A field study on 39 farms reported no effect on DMI or milk yields when cows were fed silage inoculated with *L. buchneri* (Kristensen et al., 2010).

ENZYMES

The exogenous enzymes used as feed additives are produced by fungi or bacteria and can have fibrolytic, proteolytic, or amylolytic activities (or any combination of those). A substantial number of studies have been conducted evaluating the value of feeding exogenous enzymes to dairy cattle, and a comprehensive list of individual papers is cited in reviews (Ortiz-Rodea et al., 2013; Adesogan et al., 2014; Meale et al., 2014; Arriola et al., 2017); newer papers not included in those reviews are also available (Daniel et al., 2016; Romero et al., 2016; Tewoldebrhan et al., 2017). The most common type of enzyme additive has fibrolytic activity, and although responses are quite modest, they usually tend to increase DM and fiber digestibility (Arriola et al., 2017). Small but significant increases in feed intake and milk and milk component yields are also expected with those enzymes (Arriola et al., 2017). Although a meta-analysis indicates modest responses in digestibility and production are likely, substantial variation in responses among studies is evident. Variation in response is caused by experimental conditions (e.g., responses are less likely in Latin square–type experiments than longer-term experiments), type of animal (e.g., early lactation cows are more likely to respond than later lactation cows), enzyme type, and probably several dietary interactions (Adesogan et al., 2014). Various types of amylases have also been evaluated (DeFrain et al., 2005; Rojo et al., 2005; Tricarico et al., 2008; Klingerman et al., 2009; Gencoglu et al., 2010; Ferraretto et al., 2011; Weiss et al., 2011), and in most studies, modest improvements in digestibility, feed efficiency, or milk yield were reported. Increases in in vivo DM or NDF digestibility, but not starch digestibility, have been reported even though the additives did not have appreciable fibrolytic activity.

The obvious assumed mode of action is that the hydrolytic activity of the enzyme digests nutrients either while in the feed bunk or within the rumen. However, compared to the total enzymatic activity within the rumen and intestine, the amount of enzymatic activity added is minor. Beauchemin et al. (2004) and Adesogan et al. (2014) discussed potential modes of action for enzymes, and they include (1) preingestion hydrolysis, (2) continued enzymatic activity within the rumen, (3) synergistic effects with microbial enzymes, (4) enhanced bacterial attachment to feed particles, and (5) stimulation of microbial growth within the rumen. In nonruminants, some enzymes reduce viscosity of intestinal contents, thereby enhancing digestibility, but whether exogenous enzymes maintain activity postruminally is unknown.

ESSENTIAL OILS AND OTHER PHYTONUTRIENTS

Phytonutrients are plant-derived compounds that can have antimicrobial activity and direct effects on mammalian cells (Oh et al., 2017). Essential oils, a type of phytonutrient, are secondary plant metabolites that can be extracted via steam distillation. From a nutritional standpoint, they are neither essential nor oil. The compounds often have an aroma or essence, and they are liquid and hydrophobic—hence the name essential oils. Because many of these compounds have antimicrobial activity, they have been evaluated as rumen modifiers. Extracts of plants used as seasonings in human diets such as garlic, cinnamon, oregano, rosemary, turmeric, capsaicin, cloves, and others have been studied in vitro and in vivo, and several reviews are available (Calsamiglia et al., 2007; Benchaar et al., 2008; Benchaar and Greathead, 2011; Cobellis et al., 2016). Cobellis et al. (2016) provide an extensive listing of experiments evaluating effects of numerous essential oils on in vitro rumen fermentation. In most studies, in vitro DM disappearance and production of CH_4, ammonia, and volatile FAs were reduced when essential oils were added (supplementation rates were often much higher than would be used in vivo). The reduction in rumen CH_4 production has potential benefits with regards to environmental impact and energetic efficiency; however, in most studies, the decrease in CH_4 production was associated with a decrease in DM disappearance that likely would mitigate any potential benefits. Some essential oils reduce ruminal populations of Archaea and protozoa, which could reduce CH_4 production. Unfortunately, those reductions frequently occur in concert with reductions in fiber-digesting bacteria (Cobellis et al., 2016).

With some exceptions, in vitro responses to various essential oils are reasonably consistent (e.g., reduced CH_4 production). When fed to dairy heifers (Chapman et al., 2016) or cows, responses have been inconsistent, but most studies report no effects on intake, milk production, or milk composition (Benchaar et al., 2006; Yang et al., 2007; Tassoul and Shaver, 2009; Tager and Krause, 2011; Tekippe et al., 2011, 2013; Flores et al., 2013; Hristov et al., 2013; Vendramini et al., 2016). In a few studies, milk yield (Kung et al., 2008; Ferreira de Jesus et al., 2016), milk per unit of DMI (Tassoul and Shaver, 2009; Tekippe et al., 2011; Hristov et al., 2013), and in vivo fiber digestibility (Benchaar et al., 2006; Tekippe et al., 2013) have been increased with essential oil supplementation. Various measures of immune function, inflammation, hepatic function, and other physiological function have not been affected to any great extent by essential oil supplementation (Drong et al., 2017a,b).

Source of essential oil, dose, and diet may affect response, but the available data are inadequate to quantify sources of variation. Duration of supplementation can affect response, but results differ among studies. Blanch et al. (2016) supplemented a mix of essential oils to cows, and it took 15 days before an increase in milk yield was observed. In another study (Klop et al., 2017), in vivo CH_4 production was reduced by feeding essential oils during the first 2 weeks of the experiment, but no effects were found during the next 8 weeks. Based on in vitro data, essential oils hold promise, but additional research is needed to identify important sources of variation affecting in vivo responses to essential oils.

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17

Agents That Are Toxic to Dairy Cattle

INTRODUCTION

A variety of naturally occurring toxic agents can be present in harvested, grazed, or purchased feedstuffs consumed by dairy cattle. These include compounds produced by plants, fungi, and microbes naturally occurring in feeds and by microbes that may contaminate feed or water. Through ingestion of an adequate dose, these agents can cause dysfunction, sickness, or death to the animal and do not have a recognized nutritional value at other dosage levels. Some ingested toxic agents may pass into milk or animal tissues, which then may enter the human food supply. With the intent of preserving human health, this contamination of ruminant-derived foodstuffs is the basis for regulatory limits on levels in animal feeds imposed by the U.S. Food and Drug Administration (FDA). The major, naturally occurring toxic agents likely to be consumed by dairy cattle and are of interest for human health are addressed in this chapter. The effects of excess minerals are briefly discussed in Chapter 7 of this book, and an extended discussion can be found in *Mineral Tolerance of Animals* (NRC, 2005). Manufactured toxins, such as pesticides and herbicides, and toxic rangeland plants are not addressed.

This chapter contains a general overview of individual toxic agents, their potential to be transmitted to milk, and general management practices for prevention of toxicoses.[1]

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[1]For regulatory issues related to toxins, the action levels and specification as to whether they are expressed on an as fed or dry matter basis should be verified with current U.S. Food and Drug Administration (FDA) regulatory guidance. Further information on toxic agents may be found at the FDA poisonous plant database at www.accessdata.fda.gov /scripts/plantox/index.cfm; the FDA Mycotoxin Regulatory Guidance at www.ngfa.org/wp-content/uploads/NGFAComplianceGuide-FDARegulatoryGuidanceforMycotoxins8-2011.pdf; the *Merck Veterinary Manual*, 10th ed., 2010, C. M. Kahn, ed. Merck & Co, Inc., Whitehouse Station, NJ, at www.merckvetmanual.com/mvm/index.html; and FDA. 2012. *Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins*, 2nd ed., Lampel, K. A., ed. www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf.

Readers should seek the advice of veterinarians for treatment of specific toxicosis issues.

PLANT SECONDARY COMPOUNDS AND TOXIC AGENTS PRODUCED IN PLANTS

Plants and the fungi that live within them (endophytes) can produce and accumulate compounds that may be harmful to animals that consume them. Many of these compounds are part of the natural defense mechanisms of the plants. Their production can vary with environmental conditions, season, plant part, and cultivar.

Alkaloids in Feeds

Alkaloids are produced in cool-season forage grasses, including *Phalaris* spp. (e.g., reed canarygrass = *Phalaris arundinacea*) and *Lolium* spp. (e.g., perennial ryegrass = *Lolium perenne*; tall fescue = *Lolium arundinaceum*) (Cheeke, 1995). Sprouted and sunburned green potatoes may also contain toxic alkaloids. The alkaloids produced in each grass are diverse, although within a grass, a specific alkaloid may be responsible for much of the negative effect. The alkaloids may be intrinsic to the grass or produced by endophytes associated with the grass (Cheeke, 1995).

Phalaris spp. may contain at least eight different tryptamine and β-carboline alkaloids, some of which have structural similarity to the neurotransmitter serotonin (Cheeke, 1995). *Phalaris* staggers is a neurologic condition of cattle and sheep related to the consumption of *Phalaris* spp. It may be acute and reversible or a chronic, irreversible, and typically lethal form (Binder et al., 2010). Animals may suffer from a progression of symptoms of staggering, ataxia, recumbency, and death; symptoms may be delayed by 1 month or more from the time of *Phalaris* ingestion (Binder et al., 2010). Even in the absence of such symptoms, the performance of animals consuming *Phalaris* spp. is less than what would be expected from the composition of the grass (Cheeke, 1995).

Lambs fed low-alkaloid cultivars of reed canarygrass had similar dry matter intakes (DMIs) and dry matter (DM) and protein digestibilities as those fed timothy (Tosi and Wittenberg, 1993). Ensiling reed canarygrass did not alter alkaloid content of the forage (Tosi and Wittenberg, 1993).

Production of alkaloids in tall fescue and perennial ryegrass is associated with the presence of endophytes (*Neotyphodium coenophialum* and *Neotyphodium lolii*, respectively) (Canty et al., 2014). The major alkaloids in endophyte-infested tall fescue are lolines and ergovaline, with the latter, an ergot alkaloid with vasoconstrictive effects, being most detrimental to animal health (Porter, 1995). However, ergovaline toxicosis is likely augmented by other alkaloids in the infected grass (Porter, 1995). Symptoms of tall fescue toxicosis in cattle include lameness, body weight (BW) loss, dull appearance, rough haircoat, dry gangrene of the extremities, elevated body temperatures, elevated respiration rates, altered hoof growth (Jacobson et al., 1963), fat necrosis (Stuedemann et al., 1985), and reduction in serum prolactin concentrations (Hurley et al., 1981). Responses to the toxins are affected by environmental temperature (e.g., elevated body temperatures and respiration rates in warmer weather, increased lameness, and dry gangrene in cold weather) (Jacobson et al., 1963), but toxins are present throughout the growing season (Hemken et al., 1981). Animal toxicosis has been reported to occur at 50 ng ergovaline/g of tall fescue grass (Porter, 1995). Replacement of endophyte-infected forage with low-endophyte or endophyte-free tall fescue or other grasses, interseeding legume forages, or other approaches to diluting the potential dose of alkaloids usually reduce the risk of tall fescue toxicosis.

Perennial ryegrass contains at least two classes of toxins: lolitrem alkaloids and ergopeptide alkaloids (Hovermale and Craig, 2001). Tremorgens, including lolitrem B, are potent neurotoxins that cause ryegrass staggers. This toxicosis is characterized by incoordination, staggering, head shaking, and collapse, although death is not a direct consequence of the intoxication (Cheeke, 1995). Lolitrem B concentrations are greatest in the basal halves of leaf sheaths, lowest in the leaf blades, and greater in the spring season (di Menna et al., 1992). Because the site of greatest lolitrem B concentrations in the plant is in the basal portion of the leaf sheath, the staggers syndrome is most often seen in closely grazed pastures (Cheeke, 1995). Ryegrass staggers can occur at 5 µg of lolitrem B/g of grass (Porter, 1995). Recovery is spontaneous in 1 to 2 weeks if animals are fed nontoxic pastures or feeds (Merck, 2010c).

Cows fed endophyte-infected perennial ryegrass containing a maximum daily dose of approximately 35 mg lolitrem B produced milk with a maximum of 5 ng lolitrem B/mL milk (Finch et al., 2013). Lolitrem B concentrations in milk returned to almost zero 8 days after withdrawal of lolitrem B–containing forage. References were not found for dairy cattle describing the transfer to milk of alkaloids associated with *Phalaris* spp. or other *Lolium* spp. A study evaluating

effects of endophyte-infected tall fescue on ewes was unable to detect ergovaline in the milk at a limit of detection of 0.15 ng/mL when animals were fed diets averaging 497 µg ergovaline/kg of feed DM (Zbib et al., 2014). Further work is needed to determine carryover into milk of plant alkaloids and factors that may affect it.

Bracken Fern

Bracken fern (*Pteridium aquilinum*) is broadly distributed in the United States and contains a variety of toxins, including ptaquiloside (Potter and Baird, 2000). Poisoning may occur through consumption of bracken fern in pasture or hay. Consumption of the fern by cattle can result in the presence of blood in the urine, extensive hemorrhages throughout the body, aplastic anemia, and development of tumors (papillomas and carcinomas) in the bladder (Langham, 1957; Pamukcu et al., 1976). Extracts of the plant have mutagenic and carcinogenic properties and can pass through into milk to affect suckling calves (Evans et al., 1972) and cause cancers in milk-fed mice (Pamukcu et al., 1978). One report indicated that ptaquiloside is excreted in milk in a linear, dose-dependent fashion. The concentration in milk was approximately 8.6 ± 1.2 percent of the amount ingested by the cow (Alonso-Amelot et al., 1996). Ingestion of bracken fern toxins has been associated with various cancers and health disorders in humans (Shahin et al., 1999). Bracken fern also contains thiaminases, but these are less likely to affect ruminants than nonruminants due to the production of thiamin in the rumen (Merck, 2010c). Preventing cattle from consuming bracken fern through removal of the fern from pastures and hay fields or providing ample nonfern forage in pastures is strongly recommended.

Coumarin

Coumarin is a polyphenolic compound naturally occurring in many plant materials, including sweet clover forage (*Melilotus alba*, *Melilotus officinalis*). When coumarin is oxidized to dicumarol (3,3′-methylene-bis[4-hydroxycoumarin] or bishydroxycoumarin), it becomes a powerful anticoagulant (Stahmann et al., 1941). Molds convert coumarin to dicumarol, which interferes with synthesis of vitamin K coagulation factors and prothrombin (Radostits et al., 1980). "Sweet clover disease" is a hemorrhagic disease of cattle and sheep that have consumed moldy or spoiled sweet clover forage in which the blood ceases to clot (Roderick, 1931). The hemorrhaging may be visible, such as substantial bleeding that does not stop after injury or surgery, or may not be visible if the bleeding is internal. Consumption of hay with as-fed dicumarol concentrations of 20 to 30 mg/kg over several weeks may cause poisoning and death in cattle (Merck, 2010c). Calves born from cows consuming forage containing dicumarol also suffer from the hemorrhagic disorder (Roderick, 1931) due to transfer of the toxic agent across the

placenta during pregnancy (Merck, 2010c). In time, animals can recover if feed free of dicumarol is provided. Information regarding passage of dicumarol to milk was not found.

Gallotannin

Gallotannin is a hydrolysable tannin found in blossoms, buds, young leaves, and acorns of oaks (*Quercus* spp*.*) that can be poisonous to cattle. Gallotannins hydrolyze to lower molecular weight compounds that denature cell proteins, resulting in cell death and then in necrosis and lesions in the gastrointestinal tract, liver, and kidneys. Clinical signs of "oak poisoning" of cattle occur in 3 to 7 days from the time of ingestion and include anorexia, depression, brisket edema, rumen atony, and constipation changing to bloody diarrhea (Merck, 2010c). Blood urea N and creatinine may be elevated, consistent with renal failure, and ulceration or necrosis of the ruminal, omasal, and abomasal linings is present (Pérez et al., 2011). If animals consume other feeds after ceasing consumption of oak materials, they generally recover completely. Prevention of the consumption of gallotannin-containing material is recommended. Management strategies to accomplish this include maintaining adequate forage access when cattle graze, keeping cattle off pastures with oak trees until trees are mature, and closely observing animals for acorn consumption so they may be removed from the pasture if this occurs. Gallotannins have also been found in the leaves of red maple (*Acer rubrum*) (Agrawal et al., 2013), but no reports were found associating these with toxicoses in cattle. Information was not found on passage of the toxic principles into milk.

Glucosinolate Goitrogens

These are a class of naturally occurring, sulfur-containing compounds produced by plants of the *Brassica* spp. such as rape (*Brassica campestris*) and mustard (*Brassica* spp.) that negatively affect iodine metabolism. See the Iodine section in Chapter 7 for details.

Gossypol

This naturally occurring, toxic, yellow pigment is found in cottonseed (*Gossypium* spp.) and cottonseed products, including cottonseed meal. Free rather than bound gossypol is the biologically active form (Randel et al., 1992). The content of free gossypol in cottonseed kernels (*Gossypium hirsutum*) varies from 0.59 to 2.35 percent of DM, with an average of 1.32 percent and standard deviation of 0.35 percent (Pandey and Thejappa, 1975). This is approximately equivalent to about 0.9 percent of the weight of whole cottonseed on an as-received basis. Efforts have been devoted to genetically selecting cotton varieties with lower levels of gossypol.

Although the rumen microbes can detoxify gossypol, feeding excessively high levels of free gossypol to cows

(Lindsey et al., 1980) or to animals without functionally developed rumens (Risco et al., 1992) can result in sickness or death of the animal. For lactating dairy cows, no detrimental effects on DMI or on lactation performance were observed with diets containing cottonseed or cottonseed meal providing 0 to 1,050 mg free gossypol/kg diet DM (Mena et al., 2001). However, cows in that study showed increased erythrocyte fragility with free gossypol at the 1,050 mg/kg level. Diets with up to 200 mg/kg of DM of free gossypol had no deleterious effects on calves through 90 days of feeding, but provision of 400 mg/kg or more free gossypol was toxic (Risco et al., 1992). Accordingly, feeding cottonseed products to young calves is not recommended. Gossypol can also affect reproductive function of bulls. Compared to control animals, yearling bulls consuming cottonseed meal (7 percent) and hulls (18 percent) or whole cottonseed (15 percent) and cottonseed hulls (17 percent, values as percentage of diet DM) for 2 months showed histological changes indicative of damage to the spermatogenic tissues and associated cells (Arshami and Ruttle, 1988). The damage was partially reversed after animals were fed a gossypol-free diet for 2 months. Reproductive function in cows is relatively insensitive to gossypol (Randel et al., 1992).

Gossypol was not detected in the milk of cows consuming diets containing 1,510 mg free gossypol/kg DMI, even when the animals showed negative responses related to gossypol toxicity (Lindsey et al., 1980). The sensitivity of the gossypol assay used in that study was 0.5 µg gossypol/mL of milk. In a later study, lactating dairy cows consuming 10 and 15 percent of diet DM as whole cottonseed providing 817 and 1,295 free gossypol mg/kg diet DM, respectively, had milk gossypol concentrations of 0.13 and 0.22 mg gossypol/kg milk after consuming the diets for 60 days (Wang et al., 2012). No gossypol was detected in the milk of cows consuming the control diet that contained no cottonseed product or the diets containing cottonseed meal and that provided no more than 117 mg free gossypol/kg diet DM. The allowable amount of free gossypol in cottonseed products added to food intended for human consumption is 450 mg/kg (FDA, 2015d).

Nitrate

Nitrate (NO_3^-) is a normal component of the vegetative portions of plants and may also be present in ground water. Concentrations can increase to toxic levels in plants grown under conditions of drought, reduced light intensity (Reid and Jung, 1980), and increasing nitrogen (N) fertilization (Murphy and Smith, 1967). Different plant species differ in the degree to which they accumulate NO_3^-, with sudangrass (*Sorghum sudanense*), orchardgrass (*Dactylis glomerata*), and tall fescue (*Festuca arundinacea*) accumulating NO_3^- to the greatest extent and alfalfa (*Medicago sativa*) and wheat (*Triticum aestivum*) the least (Murphy and Smith, 1967). Corn (*Zea mays*) was not evaluated in that study, but it can

accumulate potentially toxic levels of NO_3^-. NO_3^- toxicity occurs when excessive amounts of NO_3^- are converted to nitrite (NO_2^-) in the rumen and then not completely converted to ammonia (Carlson and Breeze, 1984). The NO_2^- is absorbed into the blood and converts hemoglobin to methemoglobin, which cannot transport oxygen (Reid and Jung, 1980), and turns the blood chocolate brown in color. In addition, when high NO_3^- forage is ensiled, NO_3^- can be converted to nitrogen dioxide (NO_2), the toxic component in silo gas (Wang and Burris, 1960), which can cause lung damage or death to people or animals that inhale the gas. The amount of NO_3^- that can be toxic is variable, depending on the concentration in the plant, the rate at which the plants are consumed, the adaptation of the animal to the higher NO_3^- feed, and what other feeds are provided (Merck, 2010c). Early signs of toxicosis include subnormal body temperature, muscular tremor, weakness, and ataxia; brown-tinted mucous membranes develop as the amount of methemoglobin in the blood increases (Merck, 2010c). Consult Cooperative Extension bulletins (e.g., Strickland et al., 2011) for specific information on recommended forage and animal management practices for avoiding NO_3^- toxicity in livestock. Feeding NO_3^- can reduce enteric methane emissions; risk of toxicity due to increased NO_3^- consumption can be reduced through gradual acclimation of animals to dietary NO_3^- (Lee and Beauchemin, 2014).

Data were not found on NO_3^- or NO_2^- concentrations in milk from animals consuming naturally occurring elevated levels of NO_3^-. However, in a study in which up to 150 g of potassium nitrate (KNO_3; 92 g NO_3^-) was provided to lactating cows as a single, liquid oral dose, milk NO_3^- concentration increased to a maximum of 35.6 mg NO_3^-/L (standard deviation, 10.1 mg/L) and then returned to predose levels by 50 hours (Baranová et al., 1993). Level of milk production, diets, and feed intakes were not specified for the study. NO_3^- and NO_2^- concentrations in commercially available milk (2 percent milk fat) were 0.20 and 0.0002 mg/100 mL of milk, respectively (Hord et al., 2011). For comparison, human milk in that study contained 0.31 and 0.001 mg/100 mL for NO_3^- and NO_2^-, respectively. The FDA limits NO_3^- measured as N to 10 mg/L bottled water (equivalent to 44 mg NO_3^-/L) (FDA, 2015b) or NO_3^- as sodium nitrate ($NaNO_3$) used as a preservative in cured or preserved fish or meat products to not more than 500 mg/kg (364 mg NO_3^-/kg product) (FDA, 2015c).

Prussic Acid

Prussic acid is another name for hydrocyanic acid (HCN). Cyanide poisoning of livestock occurs when the cyanogenic glycosides held in vacuoles within plant cells come in contact with plant or microbial enzymes capable of cleaving off and releasing HCN (Merck, 2010c). The HCN blocks the action of cytochrome oxidase in cellular respiration (Kingsbury, 1958). The total amount of glycoside and free HCN in the plant as well as the rate of ingestion of the toxic plants and

the size of the animal are important to determining whether poisoning will occur (Kingsbury, 1958). One study reported a minimum lethal dose of about 4.4 mg of HCN/kg of BW per hour, with death following ingestion of a lethal dose within 15 minutes to a few hours (Kingsbury, 1958). Alternately, approximately 2 mg of HCN/kg of BW may be a toxic dose for most animals (Merck, 2010c).

Prussic acid is found in vegetative matter from *Sorghum* spp. (Johnsongrass, sorghums, sudangrass), oats, wheat, rye, ryegrass, millet, chokecherry, and wild cherry, among many other sources. The *Sorghum* spp. are of greatest concern, particularly when grazed, because intakes can be high. A generalized ranking for potential HCN accumulation in sorghum types is Johnsongrass > sorghums > sorghum–sudan hybrids > sudangrass (Strickland et al., 2014). The potential HCN content of sorghum forage varies by variety and decreases with increasing maturity past 45 days (Gorashi et al., 1980) but increases with N fertilization (McBee and Miller, 1980). Amounts of HCN and risks of poisoning are likely to increase under stress conditions, such as drought or freeze damage, and are generally highest in young growing plants (i.e., short plants), the youngest material in older plants, and leaves as compared to stems (Strickland et al., 2014). Consult Cooperative Extension bulletins (e.g., Strickland et al., 2014) for specific information on recommended management practices for avoiding prussic acid poisoning of cattle grazing *Sorghum* spp.

HCN was shown to pass into milk in goats by virtue of increased blood HCN values in kids that suckled dams dosed with 1, 2, or 3 mg of potassium cyanide/kg of BW per day (Soto-Blanco and Górniak, 2003). Information regarding the degree of transfer of HCN into milk was not found.

Trypsin Inhibitor

This globulin protein found in raw soybeans irreversibly binds with trypsin, inhibiting the action of the small intestinal protease (Kunitz, 1947). The inhibition of trypsin reduces protein digestibility in the small intestine. Heat treatment of soybeans or soybean meal denatures the inhibitor (Rackis, 1974), and most commercially available soybean meal is heat treated. Although typically posing no major issues to mature ruminants because the inhibitor is degraded and inactivated in the rumen (Hoffmann et al., 2003), feeding raw soybeans to young calves reduces protein digestion and impairs performance.

Another naturally occurring trypsin inhibitor is found in bovine colostrum; trypsin inhibition activity in colostrum appears to decline with day postcalving (Laskowski and Laskowski, 1951). In contrast to the deleterious effects of raw soybean meal fed to young calves, addition of soybean trypsin inhibitor to colostrum in the first two feedings increased serum concentrations of immunoglobulin G (IgG) and immunoglobulin M (IgM) in neonatal Jersey calves,

suggesting that the treatment improved transfer of passive immunity (Quigley et al., 1995). It seems likely that the natural presence of trypsin inhibitor in colostrum effectively reduces the degradation of immunoglobulins.

MYCOTOXINS

Mycotoxins are naturally occurring toxins produced by molds. Their prevalence in plant-derived feeds depends on growing conditions, damage to the plant, moisture/humidity and availability of oxygen during storage, concentration or dilution during processing, and so on. Mycotoxins may be present in the variety of feeds provided to cattle, including silages, grains, pasture, hays, and by-product feeds, and can impair animal performance. In addition to direct effects on the animal, some mycotoxins may have antibiotic properties that can affect rumen microbiota (Gallo et al., 2015) and so may have an indirect impact on performance. Because the molds and their toxins may not be evenly mixed throughout feed sources, appropriate sampling to determine the presence or absence of toxins can be a challenge. For mycotoxins that are regulated in feeds, the FDA mandates the analysis of feeds be performed by methods found in "(1) the most recent edition of the Official Methods of Analysis of the AOAC, (2) the FDA Laboratory Information Bulletins, or (3) peer reviewed literature" (FDA, 2005a). Advanced detection technologies such as liquid chromatography coupled to mass spectroscopy are allowing detection of more of these fungal metabolites, laying the basis for future investigations on their impact (Gruber-Dorninger et al., 2017).

Aflatoxin

Aflatoxins are a group of toxins (B_1, B_2, G_1, G_2) produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus* (FDA, 2012a). They can be found in corn, sorghum, rice, cottonseed, peanuts, and a variety of other food crops (FDA, 2012a). Climatologically, the production of alfatoxins is associated with above-average temperatures and below-average rainfall during the growing season. Toxins may be produced while crops are in the field or in storage if moisture content and temperatures support mold growth (Merck, 2010c). Aflatoxins can cause health disorders in animals, with calves being very susceptible to their effects and adult ruminants being relatively resistant (Merck, 2010c). Exposure to aflatoxins can cause impaired liver function and reduced feed intake (Fink-Gremmels, 2008). Aflatoxins are potential carcinogens.

The greatest concern with aflatoxins is their potential impact on human health. Unlike some other mycotoxins, aflatoxin B_1 is not modified after incubation with rumen fluid (Kiessling et al., 1984), but it is converted to aflatoxin M_1 in the liver, and M_1 can pass into the milk. An estimated rate of aflatoxin B_1 as aflatoxin M_1 carryover into milk is 2.0 to 6.2 percent of intake (Finks-Gremmel, 2008). Although aflatoxin M_1 is "by far, not as hazardous as the parent compound,"

the FDA limits the allowable levels in milk to 0.5 µg/kg, primarily because milk tends to provide a substantial part of the diets of infants and children (FDA, 2012a). To reduce human exposure to aflatoxins in animal products, limits are placed on the concentrations of aflatoxins allowable in the diets of different classes of cattle. Current FDA regulations for dairy animals limit aflatoxin to no more than 20 µg/kg for corn, peanut products, cottonseed meal, and other animal feeds and feed ingredients (FDA, 1994). For dairy animals raised as beef cattle, corn or peanut products intended for finishing animals may contain 300 µg/kg aflatoxin; cottonseed meal intended for beef cattle may contain 300 µg/kg aflatoxin regardless of age (FDA, 1994). The allowable aflatoxin values are likely on an approximately 88 percent DM basis to make them equivalent to as fed dry corn grain, cottonseed, and peanut products.

Deoxynivalenol

Deoxynivalenol (DON; vomitoxin) is a trichothecene mycotoxin produced by *Fusarium* spp. and other mold species. Feeds that contain DON also typically contain zearalenone, another *Fusarium*-produced mycotoxin (Mirocha et al., 1976). Among potential sources, wheat can be a major source of DON, but contamination has been reported for all major grain commodities (Price et al., 1993; Bianchini et al., 2015). Forages may also contain DON (Keller et al., 2013). Deoxynivalenol can reduce the synthesis of proteins in affected animals, but the main apparent effect is usually reduced feed intake (Pestka, 2007). Cattle, however, appear to be largely resistant to the negative effects of DON on feed intake and milk production (Côté et al., 1986), possibly because ruminal and intestinal microbes convert DON to de-epoxy DON (DOM-1) (King et al., 1984; Pestka, 2007). In a bioassay for toxicity, DOM-1 did not inhibit growth of yeast cells as compared to DON, which inhibited growth at 23 mg/L, the concentration that was midway between the baseline and the concentration that gave maximal effect (Binder et al., 1997). However, DON can depress microbial protein production (Dänicke et al., 2005). As shown in swine, other co-occurring toxins such as fusaric acid may increase the negative effects of DON (Smith et al., 1997).

No DON was detected in the milk of cows provided with diets containing 66 mg/kg DON for 5 days (detection limit 1 µg/kg; Côté et al., 1986). The metabolite DOM-1 in the milk ranged from undetectable levels to 26 µg/kg, varying greatly by cow, but was no longer detectable 16 hours after the last DON feeding. At DON ingestion levels of 18.8 to 60.8 mg/d, milk concentrations ranged from 0.11 to 0.22 µg/kg for DON and 1.5 to 2.9 µg/kg for DOM-1. For DON, ≤0.02 percent of intake was found in milk (Seeling et al., 2006). The FDA advisory level for DON that apparently applies to dairy cattle is 5 mg/kg in grains and grain by-products with the added recommendation that these ingredients not exceed 40 percent of the diet (FDA, 2005a); DON values are on an 88 percent DM basis (National Grain and Feed Association, 2011).

Fumonisins

Fumonisins are mycotoxins produced in the field or in storage by a variety of *Fusarium* spp., including *Fusarium verticillioides* and *Fusarium proliferatum* (Voss et al., 2007). The toxins are found predominantly in corn grain and associated products (FDA, 2001). However, they have also been found in small grain products (Batatinha et al., 2007) and can be found in sorghum and millet (Nelson et al., 1991). High levels of contamination have been associated with hot and dry weather followed by periods of high humidity during the growing season. Many different fumonisins are produced, with FB_1 and FB_2 having the greatest concentrations under natural conditions and the greatest toxicological significance (Thiel et al., 1992). Fumonisin FB_1 can cause pulmonary edema in swine (Harrison et al., 1990) and leukoencephalomalacia in horses, and it can be hepatotoxic and hepatocarcinogenic in mice (Thiel et al., 1992). It has been statistically associated with an incidence of esophageal cancer in humans (Thiel et al., 1992), but it has not yet been proven to cause disease in humans (Voss et al., 2007). Ruminants are largely tolerant of fumonisins apparently because they are minimally absorbed. In beef steers, more than 80 percent of ingested FB_1 and FB_2 was excreted in the feces (Smith and Thakur, 1996). The estimated carryover rate into milk of fumonisin B_1 is 0 to 0.05 percent of intake (Finks-Gremmel, 2008). The FDA (2005a) guidance on fumonisins in animal feeds recommends maximum levels of fumonisins $FB_1 + FB_2 + FB_3$ of 15 mg/kg in the total rations for bulls, lactating dairy cows, and breeding stock and 30 mg/kg for cattle that are ≥3 months old and fed for slaughter; fumonisin values are on a dry weight basis (National Grain and Feed Association, 2011).

Ochratoxin

Ochratoxin A is produced by *Aspergillus* and *Penicillium* spp. (Merck, 2010c). It may be found in small grains, corn, and other feeds that have molded (Pohland et al., 1992). It is extensively degraded to nontoxic ochratoxin α and phenylalanine in ruminal, reticular, and omasal digesta samples (Hult et al., 1976), leaving functioning ruminants relatively resistant to all but very high (12 mg ochratoxin A/kg BW) pulse doses (Ribelin et al., 1978). Calves with functioning rumens may suffer no ill effects of ochratoxin A, but milk-fed calves that received a pulse dose of ≥1 mg/kg of BW died (Sreemannarayana et al., 1988). However, milk-fed calves would probably not consume that much because they consume limited amounts of grain. Studies in Europe have detected ochratoxin A in milk (range of 5 to 40 μg/kg) in 1 to 15 percent of the milk samples tested, depending on the study (Battacone et al., 2010). No tolerances or guidance have been established for levels of ochratoxin in animal feeds (FDA, 2005a).

Patulin

Patulin has received limited study for its effects on dairy cattle. It is produced by several of the *Aspergillus*, *Penicillium*, and *Byssochlamys* species of molds (Puel et al., 2010). Although there is no definitive information on its health effects in ruminants, it alters rumen fermentation in vitro, depressing microbial protein production, substrate digestibility, and volatile fatty acid production (Tapia et al., 2005). There are no tolerances or guidance established for its content in feeds, or information on its carryover into milk. As a food contaminant, it is more commonly associated with apples and their products (FDA, 2005b).

Zearalenone

Zearalenone is a mycotoxin with estrogenic effects produced by *Fusarium* spp. Feeds that contain zearalenone also typically contain deoxyvalenol, which is another *Fusarium* spp.–produced mycotoxin (Mirocha et al., 1976). Zearalenone has been detected in moldy grains, in silages (Kalač, 2011), and in grass and legume pastures at levels that could affect animal performance (Reed et al., 2004). In pastures, the concentrations were independent of mean annual rainfall, date of sampling, pasture height, and pasture age. Production of zearalenone is favored by high humidity and low temperatures during the growing season. More than 90 percent of zearalenone is converted to zearalenol by rumen microbes, with approximately twice as much α-zearalenol produced compared to β-zearalenol; protozoa were more active in this conversion than were bacteria (Kiessling et al., 1984). In rat uterus bioassays, α-zearalenol is three times more estrogenic than zearalenone, and β-zearalenol is equal in activity to the parent compound (Hagler et al., 1979). The mycotoxin and its degradation products can bind to estradiol-17β receptors with clinical effects indistinguishable from excessive estrogen administration (Merck, 2010c). Dietary concentrations of more than 10 and 20 mg/kg may cause reproductive dysfunction in dairy heifers and mature cows, respectively; young male cattle may become infertile (Merck, 2010c). The concentrations of zearalenone and α- and β-zearalenol in milk from cows consuming 238 to 1,125 μg zearalenone/d were below detection limits (1, 3, and 1 μg/kg, respectively) (Seeling et al., 2005). Mirocha et al. (1981) reported a 0.7 percent carryover of zearalenone and its metabolites into milk. No tolerances or guidance have been established by the FDA for zearalenone in animal feeds (FDA, 2005a).

MICROBES AS TOXIC AGENTS IN FEED OR WATER

Botulism, *Clostridium botulinum*

The neurotoxin producing *Clostridium botulinum* is a widely distributed anaerobic spore-forming rod found in soils, sediments in streams and bodies of water, and the intestinal

tracts of animals (FDA, 2012b). The neurotoxin is produced during the growth of the organism. Of the seven types of botulinum toxin, types A, B, E, and F cause human botulism; types C and D cause botulism in animals (FDA, 2012b), but botulism in cattle may also be caused by types A and B (Lindström et al., 2010). The signs of botulism include muscle paralysis and weakness, including progressive motor paralysis, problems in chewing and swallowing, inability to rise, and death (Merck, 2010b). Signs of poisoning may occur within 24 hours to several weeks after ingestion (Myllykoski et al., 2009). Immunization of cattle against type C and D botulism has been used as a preventive measure (Merck, 2010b).

Intoxication of humans or animals most commonly occurs when they consume foods or feeds contaminated with the toxin but may also occur if *C. botulinum* grows and produces toxin in the intestinal tract. With cattle, contaminated feeds are the most common cause. Forages contaminated with decayed carcasses of animals accidentally incorporated during forage harvest are a known source of toxin (Myllykoski et al., 2009). Because *Clostridia* and their spores are ubiquitous, they do contaminate forages, but production of the botulinum toxin is dependent on storage conditions. The higher the water content of the forage and the higher the pH, the greater potential for toxin production (Notermans et al., 1979). Grass or small grain silages that are not well wilted and contain insufficient sugars to support the acid production needed for preservation and acidification are at higher risk to contain the toxin. Spoiled or toxin-contaminated feeds must not be fed to prevent botulism poisoning.

It appears unlikely that botulinum toxin is transmitted via milk from cattle suffering from botulism (Lindström et al., 2010). A greater concern is the contamination of milk products with *C. botulinum* spores, which then produce botulinum toxin under storage conditions. The small number of reported outbreaks associated with milk products suggests a low incidence of spores in milk or the presence of competing bacteria that reduce the potential for clostridial growth (Lindström et al., 2010). However, attention to appropriate thermal processing, fermentation, maintenance of appropriate storage temperature, and avoiding contamination during and after processing of dairy products are critical to food safety, as it is for any processed food products. Standard pasteurization conditions of 72°C for 15 seconds inactivate at least 99.99 percent of type A and B botulinum toxins added to milk (Weingart et al., 2010).

Cryptosporidiosis, *Cryptosporidium parvum*

Cryptosporidium parvum, the cause of cryptosporidiosis, is an obligate, intracellular protozoan parasite that is transmitted by ingestion of oocysts shed in feces of infected animals (FDA, 2012c). Contamination of water or feed is a common route of infection, although transmission of oocysts from animal to animal, as well as indirectly by human transmission, is possible (Merck, 2010a). Cryptosporidiosis

is mostly a disease of young calves and can be found in 48 percent (Garber et al., 1994) to 70 percent (Merck, 2010a) of calves 1 to 3 weeks of age. The clinical symptoms of cryptosporidiosis in calves include transient, mild to severe diarrhea, usually with complete recovery. Treatments for cryptosporidiosis in calves are not currently available in the United States (Merck, 2010a). Reducing the incidence of infection through avoidance of transmitting oocytes between calves and through contaminated materials is recommended. Some but not all disinfectants or disinfecting methods are effective in reducing oocyst infectivity. *Cryptosporidium parvum* oocysts are resistant to disinfection with chlorine (Shields et al., 2008). Effective disinfectants include 5 percent ammonia, formalin, freeze-drying, ammonium hydroxide, hydrogen peroxide, chlorine dioxide, and 10 percent formol saline (Merck, 2010a); caution is urged in determining which of these are appropriate and approved for use under farm and feeding conditions. Temperatures less than 0°C and greater than 65°C destroy infectivity of oocytes; allowing calf feces to dry reduces infectivity, and allowing cleaned calf rearing houses to dry for several weeks before reuse is recommended (Merck, 2010a).

People coming in direct contact with feces from infected calves or ingesting oocyst-contaminated soil, water, or food may become infected. For immunocompetent individuals, cryptosporidiosis may present as diarrhea and abdominal cramps that last for 1 to 10 days (Current et al., 1983). After the diarrhea resolves, individuals can excrete oocysts for the next several months (FDA, 2012c). Immunodeficient individuals are at a greater risk of severe health impact and may suffer from prolonged and severe diarrhea (Current et al., 1983). Cryptosporidiosis can be a waterborne disease because of outbreaks associated with drinking water and recreational water (Painter et al., 2016), but theoretically, any food touched by an infected food handler or contaminated with an environmental source of oocysts (contaminated fecal material, contaminated water supplies) can infect people consuming those products (FDA, 2012c). Produce (Painter et al., 2013), apple cider (Blackburn et al., 2006), and unpasteurized milk (Harper et al., 2002; Rosenthal et al., 2015) have been implicated in cryptosporidiosis cases. Pasteurization appears to destroy infectivity of *Cryptosporidium* oocysts. *C. parvum* oocysts suspended in water or whole milk and pasteurized at 71.7°C for 5, 10, or 15 seconds were found not to be infective (0 of 177 mice), whereas all mice (80 of 80) became infected when dosed with nonpasteurized oocytes in water or whole milk; dose was 10,000 oocysts (Harp et al., 1996).

Cyanobacteria

Cyanobacteria are a diverse group of photosynthetic bacteria, including the toxigenic genera *Microcystis*, *Anabaena*, and *Planktothrix* (Wiegand and Pflugmacher, 2005). Their growth can be accelerated with increased inputs of nutrients

such as phosphorus and N such that they can form blooms in surface water (Bláha et al., 2009). Rather than being infectious, these microbes can produce an array of toxins that include hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, and irritant toxins (Wiegand and Pflugmacher, 2005). Ingestion of water from a source supporting a cyanobacterial bloom can result in acute poisoning. Death may occur in a few hours to a few days. Signs of poisoning may include coma, muscle tremors, paddling, and labored breathing; hemorrhage and necrosis of the liver occur (Merck, 2010c). Surviving animals may recover but may show signs of photosensitization and should be housed out of direct sunlight and offered ample uncontaminated water and good-quality feed (Merck, 2010c). Tests with dairy cattle in which lethal cell concentrations of the cyanobacterium *Microcystis aeruginosa* were provided in the water (Orr et al., 2001) or in which the cyanobacterial toxin microcystin-LR, a cyclic heptapeptide, was dosed daily (Feitz et al., 2002) showed less than 0.2 ng microcystin-LR/L of milk. Interference by milk proteins made it difficult to measure the toxin at lower concentrations (Orr et al., 2001). The World Health Organization (WHO) suggests a tolerable daily limit of 0.04 µg microcystin-LR/kg of BW for humans (WHO, 2003).

Enterohemorrhagic *Escherichia coli* O157:H7

Certain serotypes of *Escherichia coli* such as *E. coli* O157:H7 produce Shiga toxins and cause severe illness in humans (Riley et al., 1983). In humans, the symptoms of infection include bloody diarrhea and, in some cases, hemolytic uremic syndrome, which can result in acute renal failure (Pennington, 2010). *E. coli* O157:H7 is not pathogenic in cattle, but dairy cattle and calves can carry the organism with a prevalence ranging from 0.4 to 48.8 percent (Pennington, 2010). The contamination of foods with feces from infected animals and failure to destroy the organisms through cooking or pasteurization are the basis for outbreaks of this foodborne illness; pasteurization of milk is effective against transmission (Pennington, 2010). Feed and feed ingredients in which *E. coli* O157:H7 is detected are considered adulterated and not allowable as animal feed (FDA, 2005a).

Listeria monocytogenes

Listeria monocytogenes is a Gram-positive, aerobic or facultative anaerobic bacteria found in soil, moist environments, and decaying vegetation (FDA, 2012d). Silage, particularly when affected by aerobic spoilage, is a potential source of *Listeria* spp. contamination, as is bovine feces (Kalač, 2011). Silage pH <4.9 (Pauly and Tham, 2003) is associated with a low presence of *Listeria* spp., but some may survive at that pH for 30 days but typically not for more than 90 days. The incidence of *Listeria* spp. increases with increasing pH (Perry and Donnelly, 1990). Infection causes abortion, perinatal mortality, encephalitis, or meningitis in

ruminants (Merck, 2010b). The encephalitis may present as "circling disease," and animals may be anorectic, depressed, and disoriented (Merck, 2010b). With removal of offending feedstuffs and sanitation to prevent fecal contamination of feed and water to reduce the risk of reinfection, and with treatment of affected animals, the recovery rate in cattle approaches 50 percent (Merck, 2010b).

In addition to animal health concerns, *L. monocytogenes* contamination of foods is a human health concern. Most seriously affecting immunocompromised individuals, pregnant women, and the elderly, the fatality rate has been reported as 20 to 25 percent, and infection may cause abortion or stillbirth (Hitchins and Whiting, 2001). Otherwise healthy individuals may have no or mild symptoms (FDA, 2012d). Among the many foods associated with *L. monocytogenes* infection are raw milk, inadequately pasteurized milk, and soft cheeses, in addition to raw vegetables, meat and meat products, and raw or smoked fish (FDA, 2012d). Control of *L. monocytogenes* in foods can be difficult because of its ability to grow slowly at refrigeration temperatures and survival of freezing and use of salt as a food preservative (Hitchins and Whiting, 2001). Foods that are pasteurized "are not reasonably likely to contain *L. monocytogenes*" (FDA, 2008).

Salmonella *spp.*

Salmonella spp., particularly subspecies of *S. enterica*, are non-spore-forming Gram-negative bacteria that can cause enteric disease in cattle (Merck, 2010a). The serotypes *Typhimurium*, *Dublin*, and *Newport* are those likely to affect cattle (Merck, 2010a). *Salmonella* infection may be endemic to a herd (Merck, 2010a) or transmitted into herds by animals or humans bringing contaminated materials, introduction of infected animals into herds, or transmission by birds (McDonough et al., 1999) and rodents (Merck, 2010a). Contamination of feed and water by feces from infected animals is a primary route of transmission. Affected adult animals or those more than 1 week of age may show acute enteritis, with fever and diarrhea, whereas newborn calves may suffer depression, fever, pneumonia, and death (Merck, 2010a).

S. enterica is also the salmonella of greatest public health concern (FDA, 2012e) and has caused severe disease in people who drank infected raw milk (McDonough et al., 1999). Commercially sold feed and feed ingredients in which *Salmonella* are detected are considered adulterated and are not allowable as animal feed (FDA, 2005a). Recommended pasteurization procedures for milk kill *S. enterica* (Marth, 1969; FDA, 2015a).

PRIONS

Prions are small proteinaceous infectious particles that can cause transmissible spongiform encephalopathies (TSEs), which are degenerative disorders of the central

nervous system (Prusiner, 1982). The TSEs include kuru and Creutzfeldt–Jakob disease (CJD) in humans, scrapie in sheep and goats, chronic wasting disease in deer and elk, transmissible encephalopathy in mink, and bovine spongiform encephalopathy (BSE, "mad cow disease") in cattle. Unlike other infectious agents, prions are small segments of protein and do not contain genetic material made of nucleic acids.

Despite the name itself indicating an infectious agent, noninfectious forms of prions have been identified in animals (Collinge et al., 1996), although the normal function of prions is not completely understood. Those found in fungi can alter cellular processes and are "epigenetic determinants," in that they may be modified by molecular events other than changes in an organism's genetic code (Tuite and Serio, 2010). Prions, like most proteins, have a specific three-dimensional structure that is essential to them performing their function. The disease-causing prions are misfolded and can induce normally folded prions to become misfolded, as well. The accumulation of misfolded prions leads to TSE (FDA, 2012f).

The variant CJD (vCJD) in humans is caused by the same prion strain that causes BSE in cattle (Collinge et al., 1996; Hill et al., 1997) and is acquired by consumption of meat contaminated with the abnormal prions (FDA, 2012f). Bovine tissues with the highest risks of carrying the disease-causing prions are skull, brain, the nerves attached to the brain, eyes, tonsils, spinal cord, nerves attached to the spinal cord, and the distal small intestinal ileum (FDA, 2012f). Milk and bovine meat free of central nervous system tissue have, to date, shown no infectivity (FDA, 2012f). Other TSEs exist in animals, but these are not known to be transmitted to humans (FDA, 2012f). The most effective means of preventing infection of humans with vCJD is prevention of infection of cattle with BSE—hence, the ban since 1997 on feeding most ruminant protein products to ruminants with the exceptions of blood products, gelatin, and tallow as described in the Code of Federal Regulations (FDA, 2015e). Subsequently, in 2008, all cattle tissues at highest risk of carrying infective prions were banned for use in all animal feed (FDA, 2015f). As of 2018, six cases of BSE in cattle have been detected in the United States since 2003.

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18

Feed Analysis

INTRODUCTION

The following section provides guidance on analytical methods that may be used to determine values needed for the equations and models in this publication. If alternate methods are used, particularly for the empirical assays (those assays such as neutral detergent fiber [NDF] that define the analyte measured), the user is advised to verify that the alternate gives results comparable to the cited method.

Reference is made to cutting or abrasion mills that may be used to process feed samples for analysis. Cutting mills (e.g., a Wiley mill) are those in which the samples are cut by the action of rotating and stationary steel knives. In an abrasion mill (e.g., Udy or Cyclone mills), samples are carried on air currents in a circular chamber and strike the abrasive interior of the chamber. A 2-mm screen in an abrasion mill gives a grind equivalent to a 1-mm screen in a cutting mill because of the angle at which the particle hits the screen.

Often the concentrations of assayed components in a feed do not sum to 100 percent. In the past, a fraction that was calculated by difference (e.g., nitrogen [N]-free extract or nonfiber carbohydrates) was included so the sum would equal 100 percent. However, when a by-difference fraction is not included, deviations from 100 percent can occur even with accurate execution of analytical methods and often are not a problem. One source of error is the conversion of N measurements to crude protein (CP). By default, N times 6.25 equals CP, although actual factors for plant and animal products range from 5.18 to 6.38 (Jones, 1931); nonprotein N sources such as urea can have much smaller factors. Some components can be found in multiple fractions and are counted more than once when summing. NDF can contain N and ash, and those portions will also be counted in the CP and ash fractions. There are also many analytes that are not detected by routine assays that will reduce recovery from 100 percent (e.g., pectins, tannins). Where possible, information on analytical error related to specific analyses is included but is not available for all methods.

Obtaining a representative sample of a feedstuff is crucial, and proper sampling procedures must be followed wherever subsampling is taking place, from the farm to the laboratory. The variability in composition data related to sampling can be large. Sampling variance for corn and haycrop silages accounted for 30 to 81 percent of within-farm variance with daily sampling and 9 to 37 percent with monthly sampling, with the variation varying by the analyte evaluated (St-Pierre and Weiss, 2015). A high level of sampling variation implies a low level of confidence that the analysis of a single sample reflects the composition of the feed. Taking multiple samples of silages over time and using rolling averages in ration formulation is recommended (Weiss et al., 2014). A challenge with feeds that are fed out rapidly but that may vary substantially in composition within or between lots is that analyses may only give a retrospective on feeds already consumed. A useful manual on factors that affect subsampling in the field and laboratory, as well as recommended techniques and tools, was published by the Association of American Feed Control Officials (AAFCO, 2015). Practical recommendations for sampling silages and baled hay, with discussion on the issue of sampling variability, are found in Weiss et al. (2014).

CHEMICAL AND PHYSICAL ANALYSES

Dry Matter

Dry matter (DM) is usually determined by drying materials in a forced-air oven or microwave. Oven methods include 100 to 105°C overnight or to a constant weight, 135°C for 2 hours (AOAC Method 930.15). Drying samples at a lower temperature (55 to 60°C) in a forced-air oven for 24 hours before drying at 105°C for 48 hours can reduce the degree of browning and sample destruction that occurs. The portion of the feed that is lost in the drying process is referred to as "loss on drying."

Special Considerations

Samples intended for further analysis should not be dried at >60°C because of temperature-induced changes in protein solubility, lignin, and fiber concentrations (e.g., Van Soest, 1965). When large quantities of samples are dried, mixing the sample partway through the drying is important to fully dry the sample. Drying of samples at <60°C will result in lower DM values than those determined at higher temperatures. Use of a specific assay for water, such as a Karl Fischer titration, does not allow determination of DM by difference. The generally used measures of DM or loss on drying remove water and other volatile substances and are different from specific measurement for water content.

Ash/Organic Matter

Ash and organic matter (OM) are commonly determined by combusting feeds in a muffle furnace. A variety of approaches are used, including 600°C for 2 hours (AOAC Method 942.05), 500 to 600°C for 8 to 24 hours, and so on. The residue after ashing should be light gray to white in color. Other colors, including brown or black, are possible because of the presence of minerals or formation of carbonates. If the residue is black, verify that the sample has been completely ashed by reashing, making sure that there is adequate oxygen admission to allow full combustion and possibly verifying the muffle furnace temperature.

Crude Protein/Nitrogen

N content of feeds is commonly analyzed by Kjeldahl analysis or Dumas combustion analysis.

Special Considerations

Assuming 16 percent of N in CP, 6.25 is the factor usually used to calculate CP from N. Although other factors may be more appropriate, depending on the N fractions and amino acid (AA) composition of the protein matrix (see Jones [1931] for additional factors), the 6.25 factor became almost universal, except for milk protein, for which 6.38 is commonly used. However, based on the milk protein composition, a factor of 6.34 would be more appropriate (Karman and van Boekel, 1986). Measured components in blood meal often sum to >100 percent likely because of an inappropriate N to CP conversion factor.

Soluble Protein

Soluble protein content of feeds is the difference between total CP and CP remaining after feed is extracted with a borate–phosphate buffer (Licitra et al., 1996). No differentiation is made between amino and nonprotein nitrogenous materials. Soluble protein is not used to determine nutrient supply in the present NRC.

Neutral Detergent-Insoluble Protein

Neutral detergent residue is prepared by processing a sample as described for NDF analysis, with inclusion of sodium sulfite and heat-stable α-amylase in the extraction. The sample is then filtered under vacuum through filter paper, with subsequent soaks and rinses with boiling distilled water and acetone as described in the NDF procedure. The residue and filter paper may be dried at 55 to 60°C prior to analysis for N by Kjeldahl or Dumas combustion analysis. The neutral detergent insoluble protein (NDIP) is expressed on a CP basis as N×6.25.

Acid Detergent-Insoluble Protein

Acid detergent residue is prepared by processing a sample as described for acid detergent fiber (ADF) analysis. The sample is then filtered under vacuum through filter paper, with subsequent soaks and rinses as described in the ADF procedure. The residue and filter paper may be dried at 55 to 60°C prior to analysis for N by Kjeldahl or Dumas combustion analysis. The acid detergent insoluble protein (ADIP) is expressed on a CP basis as N×6.25.

Amino Acids

Determination of the AA content of proteins requires their hydrolysis. Protocols may be found in the Methods of Analysis (AOAC, 2006, or later), the research literature, and other resources (e.g., Rutherfurd and Gilani, 2009). Briefly, concentrations of all AAs in proteins are determined after hydrolysis in hydrochloric acid (HCl), except tryptophan (Trp), which requires an alkaline hydrolysis, with the duration of hydrolysis usually varying between 21 and 24 hours. Sulfur AAs, cysteine (Cys) and methionine (Met), must be protected prior to hydrolysis: the recommended procedure is a performic acid oxidation. Because the committee incorporated AA requirements into the model, special attention has been devoted to obtaining the true AA composition of each protein fraction to be used in the model.

Correction of Amino Acid Composition of Protein

Two general points can be extended to all proteins when assessing the AA composition obtained from protein hydrolysis. First, during hydrolysis, one molecule of water is added for each peptide bond that is cleaved. Therefore, 1 kg of pure protein perfectly hydrolyzed should yield approximately 1.15 kg of free AAs, the ratio varying slightly depending of the AA composition of the protein. However, the hydrolysis of 1 kg of protein usually yields less than 1 kg of free AA, and that difference is often incorrectly assumed to equal nonamino (or nonprotein) N compounds. Indeed, some AAs are not stable in acid conditions, whereas other AAs are not completely released from the protein structure because peptide bonds involving hydrophobic residues are

not all hydrolyzed by 24 hours. In practice, the 21- to 24-hour period has been chosen for practicality and laboratory cost to optimize the recovery of AAs that are labile in acid and of those AAs more resistant to hydrolysis (Robel and Crane, 1972; Rowan et al., 1992; Rutherfurd et al., 2008). Although this practice is usually adequate to rank feed ingredients based on AA composition, it limits the development of a factorial approach for AA supply and demand. On the supply side, the AA composition is estimated using results from hydrolyses with the abovementioned constraints. On the other hand, the requirement side includes proteins for nonproductive functions with a composition determined by hydrolysis and milk proteins, the major protein secretion, with an AA composition based on the residues obtained from DNA sequences of the different milk proteins.

In an attempt to improve the determination of AA composition of protein, multiple time point hydrolyses had been performed on pure proteins (Robel and Crane, 1972; Darragh et al., 1996), diets and digesta (Rowan et al., 1992), and goat milk (Rutherfurd et al., 2008). The number of hydrolysis times varied from 4 to 19, and periods of hydrolysis varied from 2 to 168 hours. The best estimates of AA composition were either the maximal value of each AA obtained or estimated using an equation that included hydrolysis rate and the loss rate of the labile AAs once they were released into the HCl. Based on a single set of analyses, Rowan et al. (1992) proposed different corrections factors for each type of sample. However, based on a literature search and on recent work, there is no clear indication that different correction factors should be used for each type of matrix (Lapierre et al., 2019), although this is worthy of further investigation. Therefore, a single set of correction factors, one for each AA, is proposed and has been adopted in this edition (see Table 18-1) to correct the AA composition of all types of proteins reported following 24-hour hydrolysis. The AA compositions reported in the feed tables in this current publication are as analyzed and reported without application of the correction factors. The implications of this are discussed in Chapter 6. In the text, if the AA composition is corrected for the incomplete recovery after a 24-hour hydrolysis, the concentration will be indicated as AA_{corr}. Note that once corrected with the proposed correction factors for incomplete recovery, the sum of the AA concentrations of a pure protein will yield approximately 1.15 times the weight of the hydrolyzed protein due to the addition of water. Some models (e.g., NorFor, 2011) correct the concentrations of each AA by the ratio of the molecular weight (MW) of the anhydrous AA (MW of the AA—18, the MW of water) relative to the MW of the free AA. That way, 1 kg of pure protein yields 1 kg of anhydrous AA. Using such a scenario, one would have to back-calculate from anhydrous AA to "hydrated" AA for various derivations (e.g., to determine the amount of an AA to be supplied as a rumen-protected AA). Therefore, to avoid confusion, the fluxes of AA reported by the model will always refer to the free "hydrated" AA.

TABLE 18-1   Correction Factors Proposed for Individual AAs to Estimate the True AA Concentration from Concentrations Obtained After a 24-Hour Hydrolysis[a]

Amino Acid	24-Hour Hydrolysis Correction Factor
Alanine	1.04
Arginine	1.06
Asparagine + aspartic acid	1.02
Cysteine	1.15
Glutamine + glutamic acid	1.05
Glycine	1.10
Histidine	1.07
Isoleucine	1.12
Leucine	1.06
Lysine	1.07
Methionine	1.05
Phenylalanine	1.06
Proline	1.04
Serine	1.12
Threonine	1.07
Tryptophan	1.06
Tyrosine	1.05
Valine	1.10

[a]Adapted from Lapierre et al., 2019.

Neutral Detergent Fiber

NDF is determined by gravimetric analysis of ground samples boiled under reflux for 1 hour in neutral detergent with heat-stable α-amylase and sodium sulfite, as well as filtered through coarse porosity crucibles (e.g., AOAC Method 2002.04; Mertens, 2002). Neutral detergent residues are dried and weighed, with NDF equal to the dry residue weight divided by the dry weight of the original sample. In this assay, α-amylase removes starch contamination and sodium sulfite solubilizes protein, thus reducing protein content of the residue. Selection of whether samples are expressed on a "with ash" or "ash-free" basis is dictated by the requirements of the application in which the values are used. Biogenic silica is solubilized by neutral detergent, but silica in soil is more resistant to solubilization and will contaminate the fiber residue. The analytical error (standard deviation) for replicate samples run in the same analytical run was approximately 0.4 percent for forages, 0.6 percent for concentrates <10 percent fat, 0.4 percent for concentrates >10 percent fat, and 0.5 percent overall, on an as-received basis (Mertens, 2002).

Special Considerations

Ash present in NDF is erroneously counted as OM if NDF is not reported on an ash-free basis. This is typically a small error, unless samples are contaminated with soil. Soil-contaminated samples should be evaluated on an ash-free basis or the original materials resampled. In most applica-

tions of the current model, NDF is not on an ash-free basis because the base literature from which requirements were derived did not report NDF on an ash-free basis.

Samples with >10 percent fat should be preextracted with acetone (Mertens, 2002) or NDF values may be inflated as the detergent solubilizes in a lipid layer during extraction.

The original method calls for grinding samples through the 1-mm screen of a cutting mill (e.g., a Wiley mill; Goering and Van Soest, 1970). Using a finer grind size (e.g., abrasion mill with 1-mm screen) may reduce NDF values if finer material passes through the filter used. The AOAC Method 2002.04 recommends use of sand or glass fiber filters as filtration aids. This can help greatly with difficult-to-filter samples.

Acid Detergent Fiber

ADF is determined by gravimetric analysis of ground samples boiled under reflux for 1 hour in acid detergent and filtered through coarse porosity crucibles (AOAC Method 973.18). Acid detergent residues are dried and weighed, with ADF equal to the dry residue weight divided by the dry weight of the original sample.

Special Considerations

Ash in the ADF residue is erroneously counted as OM if ADF is not reported on an ash-free basis, but as with NDF, the error is usually small unless samples are contaminated with soil or contain substantial amounts of biogenic silica. Soil-contaminated samples should be evaluated on an ash-free basis. Biogenic silica, the silica naturally incorporated into the structure of plants, is insoluble in acid detergent and will contaminate the acid detergent residue. Biogenic silica is found in both C3 and C4 grasses, including sugar cane. Forages suspected to contain higher levels of silica should be evaluated on an ash-free basis to avoid treating ash as carbohydrate.

Samples with >10 percent fat should be preextracted with acetone (Mertens, 2002) or ADF values may be inflated as the detergent solubilizes in a lipid layer during extraction.

When analyzing for ADF, care should be taken to boil samples for no more than 1 hour and to fully soak and wash all residual reagent from the residue. The acid nature of the reagent will remove more of the sample with longer boiling or further degrade the sample during drying. The original method calls for grinding samples through the 1-mm screen of a cutting mill (e.g., a Wiley mill; Goering and Van Soest, 1970), and finer grinding may reduce ADF concentrations.

Lignin

Lignin is determined as sulfuric acid lignin analyzed on acid detergent fiber residues (AOAC Method 973.18; AOAC, 2006).

Special Considerations

The 72 percent sulfuric acid used in this assay should be chilled to 15°C before use and crucibles maintained at 20 to 23°C. Failure to do so will result in increased solubilization of the sample and reduced measured lignin values.

Starch

Starch is commonly analyzed using enzymatic-colorimetric methods (e.g., Bach Knudsen, 1997, AOAC Method 2014.10; Hall, 2015). In these methods, the starch is gelatinized using heat and moisture, or alkali, followed by hydrolysis with enzymes specific to the α-1,4 and α-1,6 linkages in starch. A combination of heat-stable α-amylase (E.C. 3.2.1.1) and amyloglucosidase (glucoamylase; E.C. 3.2.1.3) or amylo-glucosidase alone is used. If heat and moisture are used for gelatinization, use of acidified buffers is recommended to avoid the conversion of a portion of the starch to maltulose (Dias and Panchal, 1987), which reduces starch recovery. Specific measurement of glucose with methods such as the glucose oxidase–peroxidase assay (e.g., Karkalas, 1985; McCleary et al., 1997), high-performance liquid chromatography (HPLC), or gas chromatography (GC) is recommended to avoid interference from other carbohydrates. The total glucose measured in enzymatically treated samples minus the free glucose in the original sample equals glucose from starch, and that glucose $\times$ 0.9 equals the amount of starch. The 0.9 factor reflects the ratio (162/180) of anhydroglucose (162 g/mol) to glucose (180 g/mol) after removal of one water molecule (18 g/mol) added during hydrolysis per glucose molecule bound into the polysaccharide chain. Glucose carried through the assay should give a value of 88 to 92 percent starch; pure sucrose should give a value less than 1 percent. The analytical error (standard deviation) for replicate samples of livestock feeds in the same analytical run was approximately 0.1 percent for low-starch feeds and 0.3 percent for high-starch feeds on an as-received basis (Hall, 2015).

Special Considerations

Samples should be ground to pass the 1-mm screen of an abrasion mill, the 0.5-mm screen of a cutting mill, or a 40-mesh screen. Use of alkali in the analysis may include starch that is resistant to digestion by mammalian enzymes in the total starch value, depending on the run conditions (McCleary et al., 2002). Use of enzyme preparations that release glucose from nonstarch carbohydrates inflates starch values. If analysis of sucrose and cellulose samples yields values greater than 1 percent glucose, the enzymes or assay conditions are releasing glucose from these nonstarch substrates. Do not use recovery values of starch control samples to adjust the starch values of other samples. This approach assumes that all samples behave similarly to the control samples, which may be incorrect.

In feeds including but not limited to cooked starchy feeds, bakery or candy by-products, and some corn hybrids, a portion of the starch may be hydrolyzed to material that is water soluble but analyzes as starch. Accordingly, it may be counted in both the starch fraction and water-soluble carbohydrates (WSC). A potential solution to this issue is to (1) use a starch assay to measure the starch in the water extract used to determine WSC and subtract it from the starch value or (2) determine the recovery factor for solubilized starch and maltooligosaccharides in the phenol–sulfuric acid assay to allow correction of the WSC for the solubilized starch. The recovery factor is determined by analyzing several different concentrations of maltooligosaccharides or solubilized starch using the usual standard carbohydrate (usually sucrose) for the WSC assay and determine the recovery of starch or maltooligosaccharides as recovery = detected g/actual g. Multiply the measured starch in the water extract by 1/recovery to estimate the portion of the WSC attributable to water-soluble starch. Correct the total starch or WSC for the solubilized starch. Determinations of total starch digestibility will include the water-soluble starch.

Water-Soluble Carbohydrates

WSC are determined by extraction of samples with water and analysis of the extract for carbohydrates (e.g., Faithfull, 2002; Udén, 2006; Hall, 2015). The water will solubilize monosaccharides (glucose, fructose, etc.), disaccharides (sucrose, lactose, maltose), oligosaccharides (stachyose, raffinose, maltooligosaccharides), and fructans. Use of a broad-spectrum carbohydrate detection method such as the phenol–sulfuric acid assay (Dubois et al., 1956) is preferable to a reducing sugar assay, particularly when samples high in fructans or lactose are analyzed (Hall, 2013, 2014) as fructans will be overestimated and lactose underestimated. The carbohydrate chosen as the standard for the detection method affects the WSC values. Different carbohydrates give different responses in both the phenol–sulfuric acid assay (Dubois et al., 1956) and reducing sugar assays (Weinbach and Calvin, 1935). Sucrose is commonly used as a standard in the detection method as it reflects the primary carbohydrate present in WSC in many feeds. However, if the analyst knows that a carbohydrate other than sucrose predominates, such as lactose in milk products or fructans in cool-season grasses, the carbohydrate that predominates should be used as the standard to obtain more accurate values.

Special Considerations

Samples should be ground to pass the 1-mm screen of an abrasion mill, the 0.5-mm screen of a cutting mill, or a 40-mesh screen. The phenol–sulfuric acid assay (Dubois et al., 1956), which uses 0.5 mL each of sample solution and 5 percent phenol solutions and 2.5 mL of acid, gives more

consistent results in workable volumes than the 80 percent phenol solution used by Dubois et al. (1956). The sample solution should be analyzed in duplicate with vortexing after each liquid addition.

Bakery products may give higher WSC values than expected because some of the starch hydrolyzes to maltooligosaccharides during baking. Maltooligosaccharides or soluble starch are water soluble and will analyze with the WSC. Analysis of the water extract using a starch assay to detect enzyme-released glucose minus free glucose will show what portion of the WSC is solubilized starch and maltooligosaccharides. See section on starch for details.

The values for ethanol-soluble carbohydrates (80 percent ethanol extraction) are similar to water-soluble carbohydrate values except for feeds containing substantial amounts of carbohydrate that are extracted by water but not ethanol, such as cool-season grasses with fructans and products containing lactose. Molasses products are typically analyzed for "total sugars as invert," and this value is given on the feed tag. Given that other WSC such as lactose have not been blended with the molasses, total sugars as invert may be used for the WSC value in molasses products.

Residual Organic Matter

Residual organic matter (ROM) is the fraction not accounted for by the major nutrients and is calculated (see Equation 3-1) by subtracting crude protein (CP), starch, NDF, and fatty acids (FAs) from OM. It contains water-soluble carbohydrates, ingested fermentation, and other short-chain FAs (such as acetic, lactic butyric acids, plant organic acids), glycerol (both free and the glycerol moiety of triglycerides), soluble fiber (e.g., pectins, gums), any other components not accounted for in the main feed fractions (e.g., tannins, waxes, pigments), and analytical error associated with determination of the main feed fractions. It is used for the calculation of energy values.

Fats/Fatty Acids

FA content of feeds is determined by GC of sample extracts in which all FAs present have been converted to methyl esters (Sukhija and Palmquist, 1988). Simultaneous methylation and extraction in this procedure more completely extracts FAs from feeds than does preextraction and subsequent methylation. The diverse lipid profiles in samples may recommend method variants—benzene, chloroform, or elevated temperature—to achieve more complete extraction and precision of analyses (Sukhija and Palmquist, 1988). Use of crude fat is not recommended as a measure of nutritionally useful lipid. In addition to FAs, crude fat (CF) contains plant waxes, cutin, pigments, and indigestible materials of similar solubilities. The use of CF as a percentage of DM minus 1 to estimate FA content in feeds (NRC, 2001) is no

longer recommended because it can overestimate FAs by up to 2 percent of DM in forages and mixed diets (Palmquist and Jenkins, 2003). Because of feed labeling, some feeds must be analyzed for CF and should be done via the AOAC Official Method 2003.05 (AOAC, 2006).

Special Considerations

Quantitative extraction of lipids prior to FA analysis is critical. Internal standards of FAs that do not occur in feeds should be added to adjust for loss of FAs during the analytical process (Palmquist and Jenkins, 2003; Jenkins, 2010). When extraction of lipids is followed by FA analysis, there is no concern about using solvent systems that also extract non-FA components, provided that these compounds do not subsequently manifest as unidentified FAs in the chromatogram and erroneously contribute to total FAs (Alves and Bessa, 2007).

Mid-infrared (MIR) analysis has been used to analyze FAs in milk (Soyeurt et al., 2011), and it is more accurate for major than for minor FAs, and accuracy was lower for MIR predictions of polyunsaturated FAs, n-3 FAs, and branched-chain FAs. The MIR predictions of FA content in milk (g/L milk) may be more accurate than those developed for FA determinations in milk fat (Soyeurt et al., 2011). See Jenkins (2010) for additional insights on factors that can affect FA analysis.

Minerals

Mineral analyses are commonly carried out with inductively coupled plasma mass spectrometry or atomic absorption, although there are also chemical gravimetric or colorimetric assays available for some minerals. Mineral values reported from near-infrared reflectance spectroscopy (NIRS) analyses are based on correlations of minerals with certain organic components of feeds. Because NIRS does not directly measure minerals, the results cannot be considered definitive.

Special Considerations

Chromium (Cr)—Samples processed through harvesting equipment and grinders with steel or chromed components will be contaminated with Cr from those sources; soil contamination may also elevate sample Cr (Spears et al., 2017). It appears that the degree of contamination is variable. Samples should be ground with a ceramic device.

Phosphorus (P)—For the current model, feed P is analytically partitioned into organic P and inorganic P. Organic P is calculated as total P − inorganic P. Total P can be determined spectrophotometrically by a molybdovanadate method (AOAC Method 965.17; AOAC, 2006) in addition to other spectroscopic methods. Inorganic P can be determined by extraction of samples with 0.5 M HCl and detection of P in the extract (Ray et al., 2012).

Particle Size

Use of the Penn State Particle Separator is recommended for use with the physically adjusted NDF (paNDF) system (Kononoff et al., 2003; Heinrichs, 2013). Four separator boxes with 19 mm (0.75 in.), 8 mm (0.31 in.), 4 mm (0.16 in.), and the solid pan are used, stacked from the largest sieve opening through the smallest with the pan on the bottom. Approximately 1.4 L of total mixed ration or silage is placed on the top screen. Boxes are shaken vigorously on a flat surface five times in one direction, then turned one-quarter turn, and shaken again. The 5 shaking reps are repeated a total of eight times to give a total of 40 shakes. The shakes should be done at a rate of 1.1 shakes per second over a distance of 17 cm (7 in.). Once completed, the material on each sieve is weighed. The DM on each sieve is determined using microwave, forced-air oven, or other suitable method. The percentage of the total sample DM on each sieve is determined.

DIGESTION ANALYSES

In Vitro Neutral Detergent Fiber Digestibility

Samples are anaerobically incubated in vitro with ruminal inoculum per Goering and Van Soest (1970). Samples for NDF are ground to pass a 1-mm screen of a cutting mill and are incubated for 30 or 48 hours for NDF. For neutral detergent fiber digestibility (IVNDFD), the entire contents of the flask are analyzed according to the method for NDF and the residue recovered through filtration under vacuum. Digestibility of NDF at a given time point is calculated on a DM basis as follows: (NDF as percentage of original feed − residual NDF as percentage of original feed) / NDF as percentage of original feed.

In Situ Measurement of A, B, and C Protein Fractions

Samples are ground to pass the 2-mm screen of a cutting mill. Samples are weighed into 40- to 60-μm pore size dacron/polyester bags that are then sealed. The ratio of sample weight to surface area of the bag should be 10 mg/cm^2 (Vanzant et al., 1998). Bags are presoaked in water and then incubated in the ventral rumens of at least two ruminally cannulated animals fed characterized diets, ideally including the feedstuff being incubated. Incubations are for at least five time points of incubation that include at least 0 and 48 hours (and another time of 72 hours for forages) such that a nonlinear mathematical model can fit the washout and extent fractions and the resultant k_d of the B pool. Endpoints <48 hours are problematic because for many rumen-undegradable protein (RUP) sources, the C fraction could not be resolved (Liebe et al., 2018). The committee recommends that a standard substrate be included. After removal, samples are subject to a consistent and thorough rinsing in a washing machine for

five 1-minute rinses in cold water. Correction for microbial contamination is highly recommended, particularly for forages (Krawielitzki et al., 2006; Kamoun et al., 2014) and fibrous by-products (Paz et al., 2014). Failure to correct for microbial contamination in incubated residues can reduce estimates of ruminally degradable protein (Alexandrov, 1998). Multiple models have been evaluated for in situ disappearance (López et al., 1999), but the committee recommends a first-order model for consistency of protein degradation and to use blank bags to assess infiltration of particulate matter.

Special Considerations

Loss of nonsoluble matter in the form of small particles from the in situ bags can inflate the value of the A fraction and reduce that of the B or C fraction (Maxin et al., 2013). Methods have been applied to address this issue (Maxin et al., 2013), but more work is needed to assess the approaches and the applicability of B fraction rates of digestion and C fraction indigestibility to the small particles.

Standard substrates have been included in in situ incubations to allow covariate adjustment of the results across incubation runs. However, this approach assumes that the other substrates in the incubation behave like the standard, that there is a body of data from multiple runs using the same standard for comparison, and that A, B, and C fractions can be adjusted to give accurately comparable values among runs. These assumptions may not be correct. The use of standards to flag incubations that have abnormally high or low digestibilities relative to other runs can be used to determine whether results from a total run should be discarded. The standard must be subsampled accurately and stored appropriately to avoid changes in composition and digestibility over time.

As stated previously (NRC, 2001), standardized approaches are strongly recommended, but often they are not stringently followed (Liebe et al., 2018). Hence, certain inherent assumptions or limitations should be reiterated. Decreasing particle size of ground forages reduces subsampling error, but the fractional k_d increases with decreasing particle size (Michalet-Doreau and Cerneau, 1991). Grinding through a 2-mm sieve appears to improve homogeneity of samples while minimizing particle losses of NDF (Krizsan et al., 2015) and presumably N. In the committee's database, forage processing ranged from being cut by scissors (especially for fresh forage simulating that grazed) to being ground through a 1- or 2-mm screen while frozen or after oven drying at <60°C. Bacterial contamination in residues from ruminal incubation is well known (NRC, 2001; Krawielitzki et al., 2006). This issue led to standardization for forages (Klopfenstein et al., 2001), but similar problems exist and deserve correction for fibrous by-products (Paz et al., 2014). When NDF exceeds 20 percent in a feedstuff, the committee recommends that studies correct for bacterial contamination. Infusion of ^{15}N followed by recovery of ^{15}N enrichment in particulate-phase bacteria and residual feed in the bags is one potential approach (Kamoun et al., 2014), but that is costly and

^{15}N enrichment might be stratified in the rumen. Assaying the residue and bacteria using quantitative polymerase chain reaction (qPCR) offers promise (Paz et al., 2014).

Intestinal Digestibility of Rumen-Undegradable Protein

Both in vitro and mobile bag techniques are used for determining intestinal digestibility of RUP of feedstuffs. Pre-incubation with ruminal inoculum is needed for some feeds prior to mobile bags being introduced into the duodenum (Hvelplund, 1985) or prior to the intestinal digestibility step in the original three-step procedure (Calsamiglia and Stern, 1995). Many laboratories have adopted the modified three-step procedure (Gargallo et al., 2006) for in vitro assessment, which has been modified to use small pore-size filter paper to recover undegraded but soluble proteins (Hristov et al., 2019). Although early intestinal digestibility values were derived from mobile bags retrieved from an ileal cannula to reduce error associated with bacterial contamination as bags passed through the colon, virtually all of the subsequent values since NRC (2001) were obtained with bags retrieved from the feces. An alternative in vitro method for measuring small intestinal digestibility of AA in RUP is that of Ross (2013). As of this writing, peer-reviewed publications on the method or comparison of its results to in vivo small intestinal digestibilities were not available. The results of this method were not used in the development of the nutritional model in this publication and have not been evaluated or tested for generating inputs for the National Academies of Sciences, Engineering, and Medicine model. Accordingly, the committee cannot provide recommendations for their use at this time.

Special Considerations

Procedures for measuring digestibility of RUP protein (dRUP) need to be standardized and further assessed for interactions with feed type. For example, pore size of the bag affects the digestibility values and likely pore size and substrate interact (Jarosz et al., 1994; Liebe et al., 2018). Insufficient comparisons have been made with in vivo approaches, particularly because of the limitation of using ileal cannulas in dairy cows (Hristov et al., 2019). As with ruminal incubations, dRUP assays also should have correction for bacterial N contamination, particularly if the feedstuff has residual NDF (Jarosz et al., 1994).

Bioavailability of Rumen-Protected Amino Acids

Rumen-protected AA (RP-AA) supplementation decisions require knowledge of the bioavailability of the AA from all ingredients in the diet, including the RP-AA. Commentary on these methods is provided in Chapter 6. There is no clear consensus about which method should be used; different methods can give different values. Different methods to estimate the bioavailability of commercial RP-AA have been extensively

detailed in Whitehouse (2016). These methods can be categorized as in vitro, in situ, or in vivo methods (Whitehouse et al., 2017). In vitro methodology relies on incubation of the RP-AA in rumen fluid collected from cows or in a buffer mimicking the rumen environment followed by in vitro exposure to conditions simulating the intestinal digestion (Calsamiglia and Stern, 1995). The in vitro method obviously lacks the effect of animal and does not include any interaction of the RP-AA with the other feed ingredients of the diet. The in situ methods rely on estimation of rumen and intestinal disappearance of RP-AA in polyester or dacron bags. Ruminal incubation is for a fixed time period thought to reflect mean exposure when fed. Bags containing the ruminal incubation residue are subsequently introduced into a duodenal cannula and recovered either at the ileum or in the feces (e.g., Jarosz et al., 1994; Overton et al., 1996; Berthiaume et al., 2000). The in situ method also does not expose the RP-AA to the feed ingredients or handling, lacks the effect of chewing and ruminating, and assumes that all particles leaving the bag are degraded. Sometimes, these methodologies are mixed—for example, an in situ rumen incubation followed by an in vitro estimation of intestinal digestibility (e.g., Bach and Stern, 2000). These two methodologies do not consider factors affecting the residence time of the RP-AA in the rumen, which affects degradation of certain types of RP-AA. For example, rumen degradation ranged from 9 to 37 percent for residence times varying from about 2 to 12 hours for an ethyl-cellulose protected product (Berthiaume et al., 2000).

In vivo methodologies are based on blood concentrations, labeled AA dilution, or production responses when the RP-AA is fed or introduced into the rumen. For blood concentration and production responses, the results need to be compared to either a base diet or the response to a known quantity of absorbed AA. Ideally, the known quantity is a postruminal infusion of the AA (e.g., Graulet et al., 2005; Koenig and Rode, 2001), but in some studies, the comparison was to an RP-AA with known bioavailability. Plasma concentration of lysine (Lys) and Met increased linearly with increasing levels of postrumen infusion of each AA (Whitehouse, 2016) up to at least 85 g/d of Lys (Borucki Castro et al., 2008) or 30 g/d of DL-Met (Rulquin and Kowalczyk, 2003). However, King et al. (1991) reported that at 180 g/d Lys, the relationship was strongly quadratic, whereas Lapierre et al. (2012) reported that at 15 g/d of DL-Met infused postruminally, the relationship was quadratic but with a significant linear component. Recommended methodologies are available (Whitehouse, 2016; Whitehouse et al., 2017). Key points include the use of both dietary and postrumen infusion of the test AA at rates similar to expected supplementation. The basal diet should be just adequate in MP and the AA of interest. To improve the accuracy of the method, Whitehouse (2016) proposed to express the concentration of the Lys relative to the total AA (excluding Lys) or the concentration of Met+Cys relative to the total AA (excluding the sulfur AA), rather than in absolute values of concentration, for RP-Lys and RP-Met, respectively.

The blood sampling schedule should at least cover one feeding interval, and sampling on more than one day should decrease the variability. The main advantage of this in vivo methodology is that the response obtained may include all of the factors that affect the bioavailability of the RP-AA. For example, mechanical mixing of RP-Lys as well as exposure to a total mixed ration (the lower the DM, the higher the effect) increased the rumen in situ release of Lys; these effects were different depending on the type of RP-Lys (Ji et al., 2016). The main disadvantage is that the trials are expensive, and the resulting bioavailability can have high variance (20 percent or greater) for single-point assessments (Rulquin and Kowalczyk, 2003; Borucki Castro et al., 2008; Whitehouse, 2016). Whether this technique will work for multiple AAs delivered by a protein source is not known. In addition, this methodology does not determine whether the loss of the RP-AA occurs before the ingestion of the RP-AA, in the rumen or across the intestine.

Another response that has been used is milk protein yield (MPY), which is based on regression just as for the blood concentration method above and is subject to the same considerations regarding linearity and range. Because MPY response is an indirect measurement, the confidence interval may be large, and that could compromise the accuracy of the estimation of the bioavailability (Whitehouse, 2016). In addition, one needs to ensure that the animals are deficient in the AA so that MPY response can be observed. This has been problematic in the past as the models have lacked precision, but the updated equations provided in this edition will hopefully reduce this problem. This method also might be useful when evaluating Met analogues, which might not increase blood Met concentrations but might affect MPY.

Another method uses the blood concentration response when a large dose of the residue from a ruminally incubated RP-AA is infused into the abomasum to estimate intestinal availability. The area under the blood concentration curve resulting from the infused RP-AA is compared to an abomasally dosed, unprotected AA to derive a bioavailability value. Ruminal incubation is generally for a set time as for the in situ methods. This method has been shown to produce results equivalent to the regression method discussed previously (Graulet et al., 2005). However, it likely becomes less accurate and more biased as the rate of release from the RP-form decreases. Adequate samples are needed to accurately calculate area under the curve and to better detect the start and end of the elevated concentrations. A second concern with this method is that the large dose of AA could stimulate catabolism. If the unprotected and protected doses are similar and absorption occurs over a similar time frame, the concern is likely not valid. However, the bioavailability and the rate of release of a naive RP-AA are unknown so that the dose of the unprotected AA may not be appropriate, which may necessitate a second trial. The method also relies on in situ incubations, which may not replicate the true ruminal effects. Graulet et al. (2005) observed consistent responses among the blood concentration regression methods and a pulse-dose

response; however, bioavailabilities determined from milk protein responses were not consistent with those from the pulse-dose method (Fleming et al., 2019). This suggests that the method may rank RP-forms correctly, but the results may be biased in terms of the absolute bioavailability estimates.

Isotopic dilution methods have been used, and they provide greater precision than other methods and are applicable to all feed ingredients (Borucki Castro et al., 2008; Estes et al., 2018; Huang et al., 2019). This approach relies on the plasma dilution of AA labeled with stable isotope (either ^{15}N or ^{13}C) administered into the bloodstream either continuously (Borucki Castro et al., 2008; Estes et al., 2018; Huang et al., 2019) or as a single dose (Borucki Castro et al., 2008; Maxin et al., 2013). Availability is derived by difference from a base diet or in comparison to provision of a known quantity of the AA. An extension of this method uses seleno-Met as a label with measurements of selenium concentrations in milk protein to deduce the dilution of seleno-Met and thus Met absorption (Weiss and St-Pierre, 2009). Results from these methods are promising as they are less invasive, are generally less expensive to conduct, are more precise (10 to 12 percent errors of determination for the ^{13}C method), and, for the ^{13}C-method, are broadly applicable across the essential AAs. The drawback is that they do not distinguish between ruminal and intestinal losses.

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19

Nutrient Composition of Feeds

INTRODUCTION

The historical background for systems of naming feeds and describing their chemical composition and nutritive value has been reviewed (Tyler, 1975; Harris et al., 1980). In the 1950s, the National Research Council (NRC) began publishing tables of nutrient composition of feeds (NRC, 1945, 1956) and cereals and forages (NRC, 1945, 1958). Additional publications followed that attempted to standardize nomenclature and update nutrient composition data (NRC, 1971, 1982; Fontenot et al., 1995). Data for this report were prepared in collaboration with the Feed Composition Committee of the National Animal Nutrition Program,[1] a National Research Support Project (NRSP-9).[2]

SOURCE OF DATA

Numerous feeds are incorporated into diets for dairy cattle. For example, in 1978, the International Network of Information Centres listed 17,000 registered feedstuffs (Tran and Lapierre, 1997). The committee attempted to construct tables of reliable data on the composition of common feeds fed to dairy cattle in North America. Where applicable, variations in moisture, processing, grade, and harvest practices are reflected in the name. Feedstuffs are also further assigned a classification (animal protein, calf feed, by-product/other, energy source, grain crop forage, grass/legume forage, calcium soaps, oil, nonprotein nitrogen, plant protein, pasture, and vitamin/mineral) and type (dry forage, wet forage, and concentrate). This classification scheme is used in various equations within the model. All names and classifications are unique to this report and do not follow the International Feed Name and Number system (Harris et al., 1980); however, the committee attempted to use feed names that are consistent with national feed control officials (AAFCO, 2016).

Data summarized in Table 19-1 later in this chapter were derived from data graciously donated by the following commercial laboratories: Cumberland Valley Analytical Services (Waynesboro, PA, but formerly Hagerstown, MD), Rock River Laboratory (Watertown, WI), Dairyland Laboratories (Arcadia, WI), and Dairy One (Ithaca, NY). Five years of feed composition data were requested from each lab in the spring of 2015 and received soon after this request. The individual fatty acid (FA; not total fatty acids [TFAs]) and amino acid (AA) data presented in Table 19-2 later in this chapter were provided by Cornell University (Higgs et al., 2015). AA data were not corrected for microbial contamination that may exist in residues isolated to estimate rumen escape of feed protein (Paz et al., 2014). Tables 19-1 and 19-2 contain a small number of values that originated from other sources, including literature data, the eighth revised edition of the *Nutrient Requirements of Beef Cattle* (NASEM, 2016), and unpublished data provided by university researchers. These values can be identified in the tables when a mean is reported but the corresponding number of observations (N) and standard deviation (SD) are not reported. Feed composition listed in this report reflects data available from the listed sources, and methods to generate them may not necessarily follow all analytical methods described in Chapter 18. Table 19-1 includes the mean, N, and SD, and when no data were available, these estimates are blank, but a value of zero indicates that the analyte was measured but the concentration was zero (or below detection limits). Based on SD, some nutrients do not follow a normal distribution, and the mean may not be the best indicator of central tendency, but to be consistent, means were used for all nutrients and feeds. Aside from dry matter (DM), all data are presented on a moisture-free or DM basis. Data that were generated from wet chemistry could not be differentiated from

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[1] See https://animalnutrition.org.

[2] The National Research Support Project (NRSP-9) was started in 2010 and is supported by the Experiment Station Committee on Organization and Policy, the State Agricultural Experiment Stations, and Hatch Funds provided by the National Institute of Food and Agriculture, U.S. Department of Agriculture.

data derived from near-infrared reflectance spectroscopy, and as a consequence, both sources of data were used. Mineral data, however, were only from wet chemistry analyses.

STATISTICAL ANALYSIS AND GENERATION OF DATA

The number of observations used for each nutrient and each feed can vary; consequently, variations in reliability or confidence also exist. Data in tables mostly reflect broad populations, and users should use data from more specific populations (e.g., specific production process or geographic area) when available. Mean values are estimates of central tendency of a population; a specific sample may differ greatly from the mean. Ranges and SD should be used to evaluate the reliability of the mean value to reflect the value of a specific sample. Means with a large N will better reflect the total population (but not necessarily a specific sample). A mean with a large SD may represent the total population but may be a poor estimate for a specific sample. New to this report is the greater detail provided regarding statistical variation, namely, minimum, maximum, 10th percentile, and 90th percentile. When the minimum or maximum differs greatly from the 10th percentile and 90th percentile values, respectively, the data likely do not follow a normal distribution, and the mean and SD may not be the appropriate statistics for central tendency and dispersion. For example, in Table 19-1, alfalfa meal has a mean iron (Fe) concentration of 951 mg/kg with an SD of 680. The minimum value is 1.4 SD units from the mean, but the maximum concentration is 4.1 SD units from the mean. These data are clearly skewed, with a few samples having very high concentrations of Fe; the median is 726 mg/kg or almost 24 percent less than the mean. Users are encouraged to evaluate all of the statistics (mean, SD, minimum, maximum, and 10th and 90th percentiles) before deciding whether the use of the mean is appropriate. Within feeds, the number of observations can differ across analytes, and this may represent a source of inconsistency when working with mean values because of covariance (Sauvant and Ponter, 2004). Sources of variation include analytical methodology and variation, subsampling practices, and factors such as crop variety, climate, soil type, length of storage, or method of processing (St-Pierre and Cobanov, 2007; St-Pierre and Weiss, 2015). Data from the tables are not intended to replace proper analytical testing and optimal sampling frequency but to serve as a reference on the nutrient composition for populations of feedstuffs.

Data generated from commercial testing laboratories generally classify feeds based on the feed name provided by the user, and because of ambiguities in feed names and other issues, feeds are often misclassified. Because of this problem, a statistical method developed to screen and classify feed data (Yoder et al., 2014) was used. The method was modified to operate on Python (Python Programming Language, v. 2.7; Tran et al., 2020). A total of 2.761×10^6 records received from the four labs were used to develop the procedures and to construct the table summarizing feed composition. Feed names and variables across labs were standardized, and obvious erroneous data points and duplicated samples were removed. Histograms and univariate analysis were used to identify and remove outliers having key nutrients outside of mean $\pm (3.5 \times SD)$. Key nutrients were the analytes used for within-laboratory clustering analysis to identify groups of feeds within a named feedstuff. Typically, the key nutrients were DM, crude protein (CP), neutral detergent fiber (NDF), ash, and sometimes lignin, starch, crude fat (CF), and hemicellulose. In addition, although water-soluble carbohydrates (WSC) are listed in Table 19-1, ethanol soluble carbohydrates (80 percent ethanol extraction) were used in the cluster analysis for some feeds. Two multivariate analyses (principal component analysis and clustering) were used to eliminate additional outliers and to identify potential subpopulations. Samples with a principal component score greater than $3.5 \times SD$ from the mean score were removed. Clustering analysis was conducted to identify the existence of subpopulations of feeds within a feed name. In a few cases, expertise of several committee members was relied on to identify subpopulations. Analytes used in the clustering steps are described in Tran et al. (2020). Aside from the clustering step that was programmed in Python to automatically run SAS (SAS Institute, Cary, NC) underneath, all steps were programmed automatically, followed by a manual evaluation step of the resulting Pearson correlation matrix and clusters. The input data of each of the four labs contained 42, 94, 162, and 270 feeds and were made up of 28 to 37 nutrients. The resulting database contains 173 feeds (1.489×10^6 records), and 111 feeds had more than one cluster or subpopulation. The sum of analytes will not necessarily total 100 percent because of analytical (e.g., ash contamination of NDF) and statistical (e.g., different sample size for different nutrients) issues. Nutrients listed in animal protein sources, which are high in CP, often sum to more than 100 percent. For these animal protein sources, the use of a nitrogen-to-CP conversion factor of 6.25 usually leads to an overestimation of the actual protein content (Mariotti et al., 2008). Residual organic matter (ROM), which is used in energy calculations of this report, is determined as 100 minus the sum of ash, FAs, CP, NDF, and starch. In rare situations, ROM was negative, but that was left in to partially compensate for the energy provided by the calculated excess nutrients. For convenience, the electronic feed library associated with the model contains some commonly commercially available supplements such as rumen-protected AAs and organic trace minerals, but fields are blank, and the user will have to use their own data, expertise, and judgment to populate these.

Energy

Table 19-1 lists the base digestible energy (DE), which was calculated from the mean nutrient data for each entry (see

below and Chapter 3 for more detail). The computer model will generate DE, metabolizable energy (ME), and net energy for lactation (NEL) values for the diet based on user inputs of specific nutrients and equations outlined in Chapter 3. Energy values depend on diet (not ingredient) composition and feed intake; therefore, the DE values in Table 19-1 are standardized to a base value. These values were calculated assuming a DM intake (DMI) of 3.5 percent of body weight, dietary starch concentration of 26 percent, and a diet NDF content of 30 percent so that endogenous fecal CP was equal to 15.6 g/kg DM or 0.088 Mcal/kg DM. Undigested bacterial CP was assumed to be 16.5 g/kg DM or 0.093 Mcal/kg based on the average quantity of microbial protein synthesized in the data set used to derive the microbial protein synthesis equation (1,875 g/d and average DMI was 22.7 kg/d), and endogenous ROM was assumed to be 34.3 g/kg DM or 0.137 Mcal/kg DMI. Total endogenous energy was calculated as $0.088 + 0.093 + 0.137 = 0.318$ Mcal/kg DMI.

For fat supplements, the digestion coefficients are listed in Chapter 4. For fat supplements that contain essentially only FAs (not triglycerides [TGs]), base DE is calculated using the following equation:

$$\text{Total FA (TFA)} \times \text{(Fat digestibility coefficient / 100)} \times 0.094 - 0.318 \qquad \text{(Equation 19-1)}$$

For fat supplements that are made up of mostly TGs, the base DE is calculated using the following equation:

$$(\text{TFA} \times \text{(Fat digestibility coefficient / 100)} \times 0.094) + [(100 - \text{Ash} - (\text{TFA}/1.06)) \times 0.96 \times 0.043] - 0.318 \qquad \text{(Equation 19-2)}$$

For feedstuffs made up of animal proteins, base DE is calculated using the base energy equations (see Chapter 3) except that the starch and NDF terms were deleted because animal products do not contain those compounds:

$$(0.73 \times \text{TFA} \times 0.094) + ((\text{CP} \times (\text{RDP / 100}) + (\text{CP} \times (\text{RUP / 100}) \times \text{dRUP})) \times 0.056) + (0.96 \times (100 - \text{TFA}/1.06 - \text{CP} - \text{Ash}) \times 0.04) - 0.318 \qquad \text{(Equation 19-3)}$$

Note: TFA and CP are percent of DM while rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) are percent of CP and dRUP (percentage of RUP).

For sugars, crude glycerol and other sugar alcohols, base DE is calculated as follows:

$$(100 - \text{Ash}) \times 0.040 \times 0.96 - 0.318 \qquad \text{(Equation 19-4)}$$

Note: The enthalpy of sugars and glycerol differs slightly (e.g., pure glycerol = 4.3 Mcal/kg and sucrose = 4.0 Mcal/kg), but for simplicity, the same enthalpy was used (4 Mcal/kg).

Total Fatty Acids

CF values represent the total ether-soluble content of a feed but are a poor index of the true FA content of many feeds. CF values are reported but not used in this edition because the concentration of FAs in a feed is the better measure of the true fat content of a feedstuff (Sukhija and Palmquist, 1988) and results in more accurate energy values. When available, the TFA value in the tables was from data collected from commercial laboratories; however, when actual TFA data were unavailable, FA was usually estimated as $CF - 1$, and neither N nor SD were reported. For forages, this estimate is not accurate, and a regression equation was used: $TFA = CF \times 0.5678$ (Daley et al., 2018) to generate estimated TFA concentrations. When this equation was used to estimate TFA, a mean TFA was reported, but N or SD was not reported.

Carbohydrates and Lignin

In some cases, data that were derived with different analytical techniques were used. Lignin and ash concentrations are only used to estimate energy values, and most lignin values were determined using sulfuric acid detergent lignin (ADL). Crude fiber concentrations are not presented because the values have little meaning nutritionally. WSC are not used in any calculations contained in this report and are listed in Table 19-1 for reference purposes only but do comprise a portion of the ROM fraction. In vitro rumen NDF digestibility (IVNDFD48) is reported, and 48-hour incubations were used. Values of various carbohydrate fractions (acid detergent fiber [ADF], NDF, WSC, and starch) and lignin for animal proteins were set to zero as any detection of these analytes represents artifacts of the assay and not the nutritive entities intended to be described by the assays (see Chapter 18). Values may differ when the chemical composition of a feed mixture containing animal proteins is determined through direct chemical analysis and when chemical composition is determined through computation using values from Table 19-1.

Minerals

Mean concentrations of minerals are in Table 19-1; however, before using these values, the reader should examine the SD. Soil concentrations of minerals are highly variable, and geographic differences exist for the mineral concentrations of many feeds. For most trace minerals, the SD is high and data generally fit a nonnormal distribution. Variable contamination with soil likely contributes to the high variation. A substantial source of variation is sampling error (St-Pierre and Weiss, 2015). When evaluating single sample results for copper (Cu), Fe, manganese (Mn), and zinc (Zn), caution should be exercised, especially for forages. Reliable data were not available for cobalt, chromium, and iodine in feedstuffs; thus, they are not included in Table 19-1. Concentrations of molybdenum

(Mo) are provided only in reference to Cu availability. Composition of common mineral supplements is listed in Table 19-3 later in this chapter.

Protein

Soluble protein is listed in Table 19-1 for reference purposes only, and values were not used in any model calculations. Oil from soybeans may be obtained through mechanical processes (extrusion/expelling) or through solvent extraction. These processes result in meals of different chemical composition and nutrient availability (Karr-Lilienthal et al., 2006). Extrusion is a process in which material is placed under pressure and is pressed, pushed, or protruded through a small orifice (Jiang et al., 2011; AAFCO, 2016). The resulting meal has a much higher fat content than solvent-extracted soybean meal (Giallongo et al., 2015). When oil is removed by grinding the soybean and then pressing the oil out under high pressure, the product is called soybean meal, expellers. In the current feed library, feedstuffs resulting from soybean processing are referred to as (1) soybean meal, solvent extracted, 48 percent CP; (2) soybean meal, extruded; and (3) soybean meal, expellers. A number of different branded products are considered soybean meal, expellers, 48 percent CP, but they are not differentiated in this report.

The RUP content of diets was determined by calculations outlined in Chapter 6. This method was also used to calculate the RUP content of individual feeds listed in Table 19-1, assuming a standard cow weighing 650 kg and consuming 23 kg of feed (i.e., 3.5 percent of body weight). This method uses different calculations based on feed classification. Parameters for rumen disappearance (A, B, and C) and coefficients for the digestibility of RUP (dRUP) were determined from values compiled from literature data and other sources. When data on protein fractions were not available, fractions from comparable feeds were assigned. Although differences in maturity of forages may affect these fractions, data were not always available, so the same values were assigned across maturity classes.

Feed Names and Maturity Classes

Common names were used to designate feeds. As in the previous edition, data for different species of cool-season grasses (i.e., C3 grasses) were combined into a single classification (cool-season grasses). The classification is appropriate because macronutrient composition does not vary greatly among different perennial cool-season grass species (Cherney et al., 1993). Similarly, common legumes such as alfalfa, clover, and trefoil were combined into a single classification (legume hay or legume silage). Where possible, maturity classifications as determined through cluster analysis were added. Within forages, entries were classified as immature, mid-maturity, and mature. Typically, less mature forages contain a lower concentration of NDF, but growing conditions may alter that relationship. Mean NDF concentrations included in each entry are in Table 19-1; however, unlike in the last edition, distinct NDF cutoff values were not used. With clustering analysis, some overlap of NDF (and other analytes) concentrations between different forage maturity classes occurs. Because of the widespread use of mixed legume and grass forages, entries were included for this type of forage. The difference in hemicellulose concentrations, estimated as the NDF minus ADF (Van Soest et al., 1991), between legumes and grasses was used to partition some forages into mostly (>70 percent) grass mixtures (>17 to 22 percent hemicellulose), mixtures with approximately equal grass and legume (>13 to 17 percent hemicellulose), and mostly (>70 percent) legume (10 to 13 percent hemicellulose). Maturity classification for mixed forages was also based on NDF concentrations. Maturity of corn silage was estimated from DM content. Generally, as corn plants mature, DM increases (Wiersma et al., 1993).

TABLE 19-1　Nutrient Composition, Digestibility, and Variability of Some Feedstuffs Commonly Fed to Dairy Cattle[a]

Name	Alfalfa Meal			Almond Hulls			Apple Pomace/ By-Product, Wet			Bakery By-Product, Bread Waste		
Feed ID Code	NRC16F1			NRC16F2			NRC16F3			NRC16F5		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	90.7	1.88	707	88.2	4.05	878	18.2	4.64	129	67.4	4.85	230
Ash, % DM	11.7	2.06	707	7.6	1.54	672	2.9	1.21	38	3.6	1.16	183
CP, % DM	19.5	1.99	705	5.3	1.06	869	6.4	1.64	129	14.9	1.91	230
A fraction, % of CP[b]	28			30			42			48		
B fraction, % of CP[b]	66			35			53			44		
C fraction, % of CP[b]	6			35			5			8		
Kd of B, %/h[b]	6.7			5.3			7.4			16.2		
RUP, % CP[c]	36			54			30			22		
dRUP, % of RUP[d]	75			50			80			90		
Soluble protein, % CP	37.0	5.72	602	37.0	11.24	543	19.7	8.80	81	20.6	8.10	140
ADIP, % DM[e]	1.62	0.161	69	1.45	0.525	213	1.98			0.62	0.274	73
NDIP, % DM[f]	3.97	0.406	67	1.94	0.586	173	3.38			1.04	0.488	72
ADF, % DM	33.9	3.29	688	27.6	7.16	874	38.6	8.10	113	3.2	2.39	208
NDF, % DM	42.9	4.66	707	33.0	7.90	869	45.7	8.47	129	6.3	4.47	230
IVNDFD48, % of NDF[g]	49.1	3.69	12	43.9	15.09	5	66.3	8.39	3	52.0		
Lignin, % DM	7.55	1.104	689	9.53	2.955	335	15.95	5.586	27	1.29	0.896	130
Starch, % DM	1.8	0.97	660	0.9	0.72	202	3.5	4.37	42	53.5	8.23	144
WSC, % DM[h]	8.3	1.84	561	36.4	6.76	37	25.5	17.85	12	11.4	4.74	24
TFAs, % DM	1.61	0.276	82	1.26			1.88			4.76		
Crude fat, % DM	2.35	0.460	699	2.52	1.034	459	5.97	2.928	38	5.76	2.730	229
DE base, Mcal/kg[i]	2.50			2.44			2.29			3.66		
Ca, % DM	1.50	0.295	619	0.27	0.055	743	0.15	0.057	96	0.22	0.119	190
P, % DM	0.27	0.054	618	0.12	0.031	743	0.14	0.035	97	0.26	0.125	191
Mg, % DM	0.30	0.059	621	0.12	0.026	741	0.08	0.023	97	0.09	0.062	190
K, % DM	2.33	0.450	622	2.82	0.425	745	0.84	0.313	97	0.33	0.235	191
Na, % DM	0.12	0.069	283	0.02	0.011	723	0.02	0.030	94	0.65	0.175	173
Cl, % DM	0.67	0.240	513	0.08	0.069	163	0.04	0.028	15	0.93	0.229	71
S, % DM	0.26	0.042	560	0.04	0.013	420	0.08	0.019	78	0.21	0.096	109
Cu, mg/kg DM	8.83	2.803	289	5.80	2.684	721	8.91	2.382	98	4.19	3.379	165
Fe, mg/kg DM	951	680.4	288	291	169.4	721	172	241.7	97	125	102.9	165
Mn, mg/kg DM	50	19.6	283	17	6.3	723	12	6.0	96	21	17.6	167
Zn, mg/kg DM	23	5.7	283	16	8.7	716	8	4.0	97	25	13.9	165
Mo, mg/kg DM	1.71	0.886	226	1.06	0.307	20	1.00	0.000	15	1.05	0.229	19

Name	Bakery By-Product, Cereal			Bakery By-Product, Cookies			Bakery By-Product Meal			Barley Grain, Dry, Ground		
Feed ID Code	NRC16F6			NRC16F7			NRC16F4			NRC16F8		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	91.1	2.61	236	89.3	2.59	1,241	90.1	2.42	149	88.7	2.43	2,404
Ash, % DM	3.5	1.62	145	4.6	1.52	797	4.4	1.54	150	2.8	0.58	2,075
CP, % DM	9.2	2.01	238	12.9	2.26	1,250	12.8	1.90	149	11.8	1.74	2,421
A fraction, % of CP[b]	34			48			48			30		
B fraction, % of CP[b]	63			44			44			61		
C fraction, % of CP[b]	4			8			8			9		
Kd of B, %/h[b]	20.0			16.2			16.2			22.7		
RUP, % CP[c]	19			22			22			22		
dRUP, % of RUP[d]	75			90			90			85		
Soluble protein, % CP	18.2	8.72	139	22.3	8.35	764	18.0	13.65	18	25.3	7.48	1,979
ADIP, % DM[e]	1.07	0.375	105	1.13	0.485	259	0.46	0.425	92	0.70	0.264	539
NDIP, % DM[f]	1.53	0.518	104	2.11	1.033	262	1.06	0.756	82	1.38	0.289	524
ADF, % DM	3.1	1.74	227	7.6	4.68	1,123	5.6	2.88	126	7.3	1.83	2,351
NDF, % DM	7.2	3.67	238	14.4	7.23	1,257	12.7	4.84	149	18.6	3.42	2,400
IVNDFD48, % of NDF[g]	52.0			52.0			52.0			51.5	13.35	22
Lignin, % DM	1.49	0.756	116	2.17	1.252	428	1.69	1.168	102	1.72	0.528	1,745

TABLE 19-1   Continued

Name	Bakery By-Product, Cereal			Bakery By-Product, Cookies			Bakery By-Product Meal			Barley Grain, Dry, Ground		
Feed ID Code	NRC16F6			NRC16F7			NRC16F4			NRC16F8		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Starch, % DM	47.6	9.94	182	36.2	10.33	817	51.7	7.81	107	56.7	4.32	2,417
WSC, % DM[h]	28.1	9.71	82	18.2	6.55	388				4.9	1.67	100
TFAs, % DM	1.95			9.04			7.68			1.31		
Crude fat, % DM	2.95	1.306	236	10.04	2.837	1,244	8.68	2.278	149	2.31	0.334	1,948
DE base, Mcal/kg[i]	3.44			3.59			3.60			3.36		
Ca, % DM	0.22	0.206	158	0.24	0.224	889	0.37	0.303	125	0.12	0.188	1,780
P, % DM	0.28	0.102	157	0.42	0.174	895	0.35	0.111	126	0.38	0.064	1,862
Mg, % DM	0.08	0.039	158	0.17	0.089	889	0.15	0.082	125	0.14	0.027	1,795
K, % DM	0.31	0.123	159	0.57	0.269	889	0.47	0.152	125	0.59	0.314	1,786
Na, % DM	0.59	0.271	224	0.59	0.270	943	0.60	0.224	100	0.02	0.017	559
Cl, % DM	0.79	0.282	114	0.80	0.323	491	0.62	0.154	14	0.15	0.049	257
S, % DM	0.12	0.027	128	0.17	0.039	674	0.17	0.040	111	0.14	0.030	1,377
Cu, mg/kg DM	3.33	1.645	154	6.57	4.129	903	6.17	2.558	89	5.53	2.625	716
Fe, mg/kg DM	189	201.6	156	172	121.2	895	237	133.2	90	89	54.2	716
Mn, mg/kg DM	22	12.1	157	40	22.8	905	30	12.4	90	20	5.9	714
Zn, mg/kg DM	55	39.8	146	41	20.8	900	31	14.7	90	34	9.4	714
Mo, mg/kg DM	1.00	0.000	19	1.08	0.269	407				1.05	0.211	173

Name	Barley Grain, Steam Rolled			Barley Hay			Barley Malt Sprouts			Barley Silage, Headed		
Feed ID Code	NRC16F1074			NRC16F9			NRC16F10			NRC16F11		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	88.7	2.43	2,404	89.4	2.55	1,096	81.2	27.84	145	37.0	5.40	1,266
Ash, % DM	2.8	0.58	2,075	8.6	2.33	849	5.9	1.41	145	5.9	1.33	1,276
CP, % DM	11.8	1.74	2,421	10.8	2.95	1,105	23.9	5.08	145	10.9	1.72	1,277
A fraction, % of CP[b]	30			57			47			57		
B fraction, % of CP[b]	61			33			45			33		
C fraction, % of CP[b]	9			10			8			10		
Kd of B, %/h[b]	22.7			7.0			13.3			7.0		
RUP, % CP[c]	22			27			24			27		
dRUP, % of RUP[d]	85			83			64			83		
Soluble protein, % CP	25.3	7.48	1,979	44.8	12.91	1,089	39.9	13.54	61	61.4	12.35	1,277
ADIP, % DM[e]	0.70	0.264	539	0.65			0.86			0.95	0.197	1,274
NDIP, % DM[f]	1.38	0.289	524	2.24			5.13			1.30	0.412	1,272
ADF, % DM	7.3	1.83	2,351	34.1	4.34	1,105	18.3	4.16	107	26.3	2.65	1,274
NDF, % DM	18.6	3.42	2,400	54.5	5.75	1,106	40.5	7.35	145	44.8	3.67	1,276
IVNDFD48, % of NDF[g]	51.5	13.35	22	61.3	9.32	32						
Lignin, % DM	1.72	0.528	1,745	4.07	1.293	846	2.61	1.590	70	3.81	0.488	1,275
Starch, % DM	56.7	4.32	2,417	7.3	6.64	791	8.6	6.38	73	23.9	6.05	1,277
WSC, % DM[h]	4.9	1.67	100	14.8	7.46	760	15.9	4.81	36			
TFAs, % DM	1.31			1.40	0.449	63	1.46			2.06	0.288	887
Crude fat, % DM	2.31	0.334	1,948	2.74	0.870	848	2.73	2.050	119	3.08	0.534	1,271
DE base, Mcal/kg[i]	3.44			2.62			3.13			2.86		
Ca, % DM	0.12	0.188	1,780	0.38	0.192	1,088	0.19	0.079	94	0.32	0.070	273
P, % DM	0.38	0.064	1,862	0.25	0.070	1,093	0.61	0.153	94	0.29	0.044	272
Mg, % DM	0.14	0.027	1,795	0.17	0.058	1,093	0.18	0.037	94	0.18	0.030	272
K, % DM	0.59	0.314	1,786	1.96	0.622	1,096	1.11	0.522	94	1.57	0.249	272
Na, % DM	0.02	0.017	559	0.38	0.338	237	0.05	0.037	78	0.11	0.073	272
Cl, % DM	0.15	0.049	257	0.95	0.533	766	0.36	0.125	62	0.47	0.269	271
S, % DM	0.14	0.030	1,377	0.17	0.052	1,091	0.33	0.091	80	0.18	0.034	268
Cu, mg/kg DM	5.53	2.625	716	6.78	2.953	228	9.93	3.270	68	5.96	1.669	583
Fe, mg/kg DM	89	54.2	716	422	524.9	227	180	78.8	68	172	111.3	573
Mn, mg/kg DM	20	5.9	714	36	22.4	225	46	12.4	69	31	10.6	579
Zn, mg/kg DM	34	9.4	714	30	19.1	192	65	16.3	62	26	6.7	580
Mo, mg/kg DM	1.05	0.211	173	1.32	0.642	145	1.36	0.515	64	0.77	0.148	2

TABLE 19-1 Continued

Name	Barley Silage, Mid-Maturity			Barley Silage, Vegetative			Beet Pulp, Dry			Beet Pulp, Dry, Molasses Added		
Feed ID Code	NRC16F12			NRC16F13			NRC16F14			NRC16F15		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	36.1	7.99	4,993	33.0	9.03	1,277	92.3	1.24	53	90.6	1.84	549
Ash, % DM	7.9	2.01	5,016	10.7	2.58	1,282	5.2	1.50	54	7.5	1.96	542
CP, % DM	11.4	2.36	5,030	14.2	2.84	1,281	9.9	1.36	54	8.9	1.00	547
A fraction, % of CP[b]	57			57			5			5		
B fraction, % of CP[b]	33			33			90			90		
C fraction, % of CP[b]	10			10			5			5		
Kd of B, %/h[b]	7.0			7.0			2.0			2.0		
RUP, % CP[c]	*27*			*27*			*71*			*71*		
dRUP, % of RUP[d]	83			83			80			80		
Soluble protein, % CP	59.9	11.57	5,030	65.0	8.38	1,278	20.6	8.10	50	19.3	9.14	440
ADIP, % DM[e]	1.11	0.225	3,666	1.39			1.30			1.79	0.772	112
NDIP, % DM[f]	1.60	0.456	3,655	2.00			5.46			4.23	0.985	112
ADF, % DM	34.1	4.13	5,034	37.7	3.79	1,282	28.2	2.37	54	26.1	3.06	516
NDF, % DM	52.9	5.19	5,033	56.6	5.42	1,280	46.9	4.27	54	39.7	3.47	547
IVNDFD48, % of NDF[g]	52.1	4.81	253	57.5	6.19	193	79.0			74.0		
Lignin, % DM	4.96	0.858	5,032	5.19	1.209	1,281	3.88	2.128	49	3.53	1.840	317
Starch, % DM	12.3	6.91	5,033	3.0	2.39	1,282	0.6	0.44	48	1.1	1.12	289
WSC, % DM[h]	5.9	2.44	482	6.9	3.59	756	5.7	1.55	3	13.8	4.42	23
TFAs, % DM	1.70	0.379	2,808	2.07			0.63			0.63		
Crude fat, % DM	3.13	0.575	5,023	3.65	0.612	1,278	1.06	0.308	49	1.19	0.374	400
DE base, Mcal/kg[i]	*2.62*			*2.52*			*2.75*			*2.74*		
Ca, % DM	0.46	0.251	1,661	0.53	0.228	1,275	0.77	0.252	51	1.18	0.332	504
P, % DM	0.31	0.053	1,666	0.35	0.061	1,280	0.08	0.014	50	0.09	0.035	501
Mg, % DM	0.19	0.052	1,660	0.19	0.050	1,277	0.26	0.039	51	0.25	0.046	506
K, % DM	2.00	0.575	1,667	2.72	0.642	1,279	0.49	0.229	50	0.59	0.386	503
Na, % DM	0.18	0.231	549	0.16	0.184	203	0.11	0.057	50	0.14	0.142	427
Cl, % DM	0.65	0.524	961	0.87	0.388	739	0.08	0.059	47	0.09	0.114	294
S, % DM	0.18	0.034	1,663	0.20	0.038	1,278	0.20	0.097	50	0.30	0.092	385
Cu, mg/kg DM	7.88	2.815	1,616	9.24	3.546	168	8.10	2.492	51	8.56	2.959	450
Fe, mg/kg DM	272	227.6	1,611	663	675.9	167	588	262.2	50	614	308.1	451
Mn, mg/kg DM	40	17.5	1,616	48	22.8	169	78	19.3	51	63	17.3	449
Zn, mg/kg DM	30	8.1	1,616	32	10.6	166	21	6.0	49	24	7.0	451
Mo, mg/kg DM	1.43	0.753	62	1.40	0.752	124	1.00	0.000	4	1.03	0.169	35

Name	Beet Pulp, Wet			Bermudagrass Hay			Bermudagrass Silage, Mature			Bermudagrass Silage, Mid-Maturity		
Feed ID Code	NRC16F16			NRC16F17			NRC16F18			NRC16F19		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	22.7	3.36	629	93.4	1.15	10,059	45.3	10.51	128	38.4	9.66	693
Ash, % DM	7.3	2.06	630	8.0	1.31	10,053	6.2	1.65	130	9.1	2.13	695
CP, % DM	9.1	1.08	631	11.0	2.56	10,071	10.3	1.64	130	14.6	2.56	696
A fraction, % of CP[b]	5			36			37			37		
B fraction, % of CP[b]	90			52			51			51		
C fraction, % of CP[b]	5			12			12			12		
Kd of B, %/h[b]	2.0			8.1			8.1			8.1		
RUP, % CP[c]	*71*			*34*			*34*			*34*		
dRUP, % of RUP[d]	80			65			65			65		
Soluble protein, % CP	16.3	7.73	249	33.0	4.99	8,917	47.0	10.09	130	54.5	9.75	697
ADIP, % DM[e]	0.88	0.201	50	0.84			1.15			1.64	0.484	32
NDIP, % DM[f]	2.22	0.706	50	4.16			2.44			3.46	1.141	32
ADF, % DM	27.2	2.92	329	34.9	3.42	10,056	42.8	2.64	130	39.0	3.10	695
NDF, % DM	44.1	6.49	632	65.4	3.79	10,064	70.8	3.28	130	64.1	4.57	697
IVNDFD48, % of NDF[g]	78.3	3.58	10	54.2	7.89	383	52.3	2.29	7	63.5	4.84	110
Lignin, % DM	3.18	2.334	161	5.41	1.199	10,073	7.69	1.091	130	5.90	1.261	696

TABLE 19-1 Continued

Name	Beet Pulp, Wet			Bermudagrass Hay			Bermudagrass Silage, Mature			Bermudagrass Silage, Mid-Maturity		
Feed ID Code	NRC16F16			NRC16F17			NRC16F18			NRC16F19		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Starch, % DM	0.9	0.64	174	4.9	3.05	9,869	3.4	1.87	127	2.4	1.50	620
WSC, % DM[h]	3.0	1.90	37	7.7	1.69	9,776	4.0	2.17	128	5.0	2.04	573
TFAs, % DM	0.64			1.25	0.441	169	1.35			1.44	0.568	92
Crude fat, % DM	0.99	0.373	492	1.93	0.335	10,026	2.38	0.662	130	3.37	0.703	685
DE base, Mcal/kg[i]	2.73			2.40			2.26			2.39		
Ca, % DM	1.12	0.349	314	0.49	0.091	10,038	0.47	0.101	129	0.51	0.120	663
P, % DM	0.13	0.120	318	0.19	0.047	10,023	0.24	0.053	130	0.31	0.057	666
Mg, % DM	0.26	0.048	321	0.21	0.045	10,032	0.20	0.047	129	0.24	0.051	660
K, % DM	0.57	0.366	320	1.71	0.378	10,043	1.52	0.428	130	2.41	0.575	665
Na, % DM	0.10	0.071	243	0.15	0.112	3,814	0.03	0.017	17	0.06	0.046	134
Cl, % DM	0.11	0.199	134	0.74	0.239	8,238	0.55	0.217	120	0.75	0.285	566
S, % DM	0.24	0.108	199	0.40	0.111	9,098	0.21	0.042	130	0.25	0.063	650
Cu, mg/kg DM	9.40	4.876	224	9.76	2.947	3,823	9.00	4.256	18	12.71	6.212	143
Fe, mg/kg DM	699	354.7	226	221	93.5	3,813	206	118.1	18	386	328.5	137
Mn, mg/kg DM	60	14.2	224	59	27.2	3,788	86	59.3	18	75	43.6	149
Zn, mg/kg DM	26	7.8	226	34	8.8	3,800	32	12.7	18	44	15.0	143
Mo, mg/kg DM	1.00	0.000	4	1.12	0.324	2,406	1.13	0.354	8	1.27	0.466	98

Name	Blood Meal, High dRUP			Blood Meal, Low dRUP			Brewers Grains, Dry			Brewers Grains, Wet		
Feed ID Code	NRC16F1000			NRC16F20			NRC16F21			NRC16F22		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	90.9	3.10	870	90.9	3.10	870	93.1	2.34	139	22.5	3.56	2,427
Ash, % DM	3.3	1.74	183	3.3	1.74	183	4.6	1.06	139	4.6	0.63	2,423
CP, % DM	95.1	3.15	845	95.1	3.15	845	25.3	4.56	139	28.1	4.00	2,441
A fraction, % of CP[b]	10			10			18			47		
B fraction, % of CP[b]	61			61			67			44		
C fraction, % of CP[b]	29			29			15			9		
Kd of B, %/h[b]	1.9			1.9			4.5			3.9		
RUP, % CP[c]	85			75			52			37		
dRUP, % of RUP[d]	85			65			74			83		
Soluble protein, % CP	19.5	20.96	135	19.5	20.96	135	17.7	13.47	136	11.2	4.85	1,842
ADIP, % DM[e]	4.25	4.211	31	4.25	4.211	31	3.12			2.94	1.249	317
NDIP, % DM[f]	5.67	4.497	30	5.67	4.497	30	8.60			3.95	1.334	299
ADF, % DM	0.0			0.0			24.7	5.61	139	23.8	2.66	2,292
NDF, % DM	0.0			0.0			51.8	10.59	139	49.3	4.94	2,439
IVNDFD48, % of NDF[g]							51.5	4.43	4	47.3	8.58	8
Lignin, % DM	0.00			0.00			6.66	2.133	136	6.64	1.112	2,179
Starch, % DM	0.0			0.0			6.5	2.76	126	5.2	3.88	1,966
WSC, % DM[h]	0.0			0.0			3.8	3.87	13	2.0	1.20	23
TFAs, % DM	1.31			1.31			8.31			7.61		
Crude fat, % DM	1.69	1.458	171	1.69	1.458	171	9.02	2.270	139	9.52	1.248	2,436
DE base, Mcal/kg[i]	4.56			3.82			2.98			3.11		
Ca, % DM	0.13	0.158	166	0.13	0.158	166	0.30	0.131	138	0.36	0.111	2,035
P, % DM	0.28	0.159	172	0.28	0.159	172	0.64	0.136	135	0.69	0.095	2,045
Mg, % DM	0.05	0.036	140	0.05	0.036	140	0.23	0.059	137	0.23	0.033	2,038
K, % DM	0.43	0.309	143	0.43	0.309	143	0.23	0.306	136	0.12	0.080	2,016
Na, % DM	0.42	0.198	133	0.42	0.198	133	0.02	0.012	77	0.02	0.017	699
Cl, % DM	0.35	0.127	67	0.35	0.127	67	0.09	0.075	46	0.06	0.043	306
S, % DM	0.74	0.225	137	0.74	0.225	137	0.30	0.059	134	0.32	0.056	1,853
Cu, mg/kg DM	6.05	4.309	134	6.05	4.309	134	15.97	7.000	77	10.38	5.401	766
Fe, mg/kg DM	2267	476.5	133	2267	476.5	133	350	315.0	78	223	82.0	764
Mn, mg/kg DM	4	3.5	125	4	3.5	125	54	12.5	78	53	11.8	769
Zn, mg/kg DM	33	14.0	131	33	14.0	131	86	13.3	49	94	17.9	662
Mo, mg/kg DM							1.92	0.885	77	2.34	0.861	300

TABLE 19-1 Continued

Name	Brewers Yeast, Dry			Brewers Yeast, Wet			Calcium Soaps			Candy (Not Chocolate) By-Product		
Feed ID Code	NRC16F23			NRC16F24			NRC16F25			NRC16F26		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	92.8	1.66	43	13.4	3.99	233	95.3			90.1	5.17	17
Ash, % DM	8.6	1.61	43	6.3	1.43	231	15.5			1.0	1.28	17
CP, % DM	50.7	6.86	43	43.3	8.36	234				2.4	1.53	17
A fraction, % of CP[b]	9			9						74		
B fraction, % of CP[b]	91			91						26		
C fraction, % of CP[b]	0			0						0		
Kd of B, %/h[b]	2.4			2.4						3.2		
RUP, % CP[c]	63			63						21		
dRUP, % of RUP[d]	93			93						90		
Soluble protein, % CP	45.3	16.44	38	59.4	27.34	12				26.2	26.48	16
ADIP, % DM[e]	0.56			0.48						0.46	0.410	16
NDIP, % DM[f]	2.73			2.33						0.84	0.783	16
ADF, % DM	3.6	3.68	11	5.6	4.51	12				1.5	1.81	16
NDF, % DM	1.6	2.14	38	11.5	9.33	12				2.3	1.40	16
IVNDFD48, % of NDF[g]												
Lignin, % DM	1.82	1.650	9	1.25	0.630	12				0.57	0.571	16
Starch, % DM	4.1	6.78	11	4.4	1.00	4				23.5	11.41	17
WSC, % DM[h]	4.1	1.69	33	14.0	7.78	2						
TFAs, % DM	0.11			2.34			84.50			0.25		
Crude fat, % DM	1.11	0.774	42	3.34	2.143	231	84.50			1.25	1.308	17
DE base, Mcal/kg[i]	3.94			3.85			5.42			3.48		
Ca, % DM	0.12	0.099	14	0.37	0.147	20				0.06	0.076	16
P, % DM	1.19	0.288	14	1.49	0.464	20				0.03	0.015	16
Mg, % DM	0.21	0.078	14	0.21	0.064	20				0.04	0.048	14
K, % DM	1.38	0.469	14	1.76	0.549	20				0.09	0.095	17
Na, % DM	0.08	0.072	14	0.08	0.065	20				0.15	0.150	17
Cl, % DM	0.20	0.090	10	0.79	0.456	3				0.15	0.145	15
S, % DM	0.86	1.644	13	0.47	0.072	18				0.04	0.021	16
Cu, mg/kg DM	101.14	254.822	14	19.25	17.550	20				1.46	0.660	13
Fe, mg/kg DM	135	131.6	14	100	56.2	20				37	45.4	17
Mn, mg/kg DM	26	39.0	14	7	2.9	20				4	4.5	16
Zn, mg/kg DM	60	34.4	9	60	17.6	16				8	12.8	16
Mo, mg/kg DM	1.25	0.622	12	3.32	1.701	19						

Name	Candy By-Product, High Protein			Canola Meal, Solvent Extracted			Canola Seed, Ground			Chocolate By-Product		
Feed ID Code	NRC16F27			NRC16F28			NRC16F29			NRC16F30		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	88.9	2.25	162	89.1	1.15	3,293	95.0	1.35	141	94.3	1.97	37
Ash, % DM	5.6	1.07	157	7.9	0.53	2,967	4.5	1.45	19	2.6	0.85	11
CP, % DM	14.6	1.67	160	41.5	1.55	3,437	23.4	2.70	143	10.0	1.84	37
A fraction, % of CP[b]	74			22			35			74		
B fraction, % of CP[b]	26			71			60			26		
C fraction, % of CP[b]	0			7			5			0		
Kd of B, %/h[b]	3.2			10.5			20.1			3.2		
RUP, % CP[c]	21			32			20			21		
dRUP, % of RUP[d]	90			74			50			90		
Soluble protein, % CP	26.9	7.32	32	25.0	5.84	895	49.3	19.83	25	27.1	12.99	29
ADIP, % DM[e]	2.76	1.231	17	2.50	0.789	478	2.79			1.01	0.497	9
NDIP, % DM[f]	3.97	1.725	17	4.75	1.614	452	4.53			1.92	1.060	9
ADF, % DM	19.5	4.48	93	20.3	2.17	1,367	20.2	7.32	31	8.3	3.04	37
NDF, % DM	29.7	5.37	162	29.0	2.78	1,503	28.7	9.07	31	13.2	4.20	37
IVNDFD48, % of NDF[g]				49.4	7.48	14						
Lignin, % DM	5.16	2.784	23	8.51	1.968	686	6.01	2.325	11	1.43	0.418	10

TABLE 19-1   Continued

Name	Candy By-Product, High Protein			Canola Meal, Solvent Extracted			Canola Seed, Ground			Chocolate By-Product		
Feed ID Code	NRC16F27			NRC16F28			NRC16F29			NRC16F30		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Starch, % DM	16.1	6.47	104	1.6	0.85	554	2.4	3.40	16	11.2	4.45	32
WSC, % DM[h]				11.0	1.41	51				39.6	5.46	8
TFAs, % DM	11.11			2.51			39.46			20.68		
Crude fat, % DM	12.11	2.697	163	3.51	0.856	3,389	40.46	3.990	142	21.68	3.457	36
DE base, Mcal/kg[i]	3.31			3.14			4.31			4.05		
Ca, % DM	0.32	0.306	35	0.79	0.116	1,211	0.44	0.098	22	0.15	0.079	32
P, % DM	0.49	0.177	35	1.15	0.107	1,212	0.69	0.098	22	0.28	0.094	32
Mg, % DM	0.30	0.102	35	0.62	0.058	1,185	0.33	0.055	23	0.12	0.035	31
K, % DM	1.22	0.423	35	1.36	0.136	1,185	0.85	0.151	23	0.51	0.162	32
Na, % DM	0.23	0.548	34	0.08	0.074	923	0.01	0.010	15	0.16	0.088	31
Cl, % DM	0.21	0.115	16	0.10	0.050	532	0.08	0.042	9	0.21	0.112	10
S, % DM	0.23	0.212	18	0.77	0.086	879	0.43	0.077	21	0.10	0.026	29
Cu, mg/kg DM	16.03	6.323	35	5.78	1.986	884	3.94	1.697	18	7.65	3.071	31
Fe, mg/kg DM	269	142.2	34	253	96.3	894	298	373.2	18	90	77.1	32
Mn, mg/kg DM	57	22.8	35	73	8.0	895	47	11.8	18	20	10.8	31
Zn, mg/kg DM	53	19.0	35	64	8.1	891	40	6.0	18	23	7.7	32
Mo, mg/kg DM	1.00	0.000	4	1.12	0.328	237	1.40	0.966	10	1.00	0.000	14

Name	Citrus Pulp, Dry			Citrus Pulp, Wet			Cool-Season Grass Hay, Mature			Cool-Season Grass Hay, Mid-Maturity		
Feed ID Code	NRC16F31			NRC16F32			NRC16F33			NRC16F34		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	88.2	1.35	354	18.3	5.49	158	89.8	2.86	27,990	88.3	4.24	6,033
Ash, % DM	7.1	1.40	219	6.8	1.90	73	6.7	1.79	28,264	8.6	1.76	6,032
CP, % DM	7.2	1.11	508	8.7	1.56	159	9.2	2.49	28,463	13.3	2.22	6,035
A fraction, % of CP[b]	42			42			30			30		
B fraction, % of CP[b]	53			53			56			56		
C fraction, % of CP[b]	5			5			14			14		
Kd of B, %/h[b]	7.4			7.4			5.5			5.5		
RUP, % CP[c]	30			30			42			42		
dRUP, % of RUP[d]	80			80			60			60		
Soluble protein, % CP	40.3	10.95	236	56.7	8.68	91	29.3	6.30	24,383	30.5	8.20	4,974
ADIP, % DM[e]	1.03	0.386	53	0.68			1.48	0.250	12,111	1.43	0.256	3,380
NDIP, % DM[f]	1.78	0.692	51	1.20			3.79	0.974	12,118	4.85	1.292	3,389
ADF, % DM	20.1	3.31	464	23.2	3.86	158	41.4	3.51	28,385	35.5	2.68	6,050
NDF, % DM	24.1	2.90	506	25.9	4.16	159	66.7	4.29	28,429	58.0	4.08	6,049
IVNDFD48, % of NDF[g]	86.5						55.8	9.04	1,031	67.8	8.16	82
Lignin, % DM	2.49	1.369	107	3.61	3.055	38	5.97	1.238	28,462	4.17	0.786	6,052
Starch, % DM	1.3	1.28	175	0.9	0.95	65	2.0	0.90	27,342	2.2	0.98	5,956
WSC, % DM[h]	23.0	7.44	147	11.5	9.23	46	10.8	4.39	15,366	15.2	3.99	2,618
TFAs, % DM	1.72			1.72			0.95	0.359	11,891	1.58	0.372	3,213
Crude fat, % DM	2.55	0.710	159	3.42	2.129	79	2.35	0.534	27,946	3.23	0.646	5,987
DE base, Mcal/kg[i]	3.02			2.97			2.34			2.53		
Ca, % DM	1.86	0.553	330	1.27	0.669	141	0.44	0.151	16,249	0.48	0.155	2,709
P, % DM	0.12	0.029	329	0.16	0.044	118	0.21	0.070	16,242	0.28	0.078	2,709
Mg, % DM	0.14	0.023	323	0.12	0.026	117	0.20	0.074	16,219	0.23	0.064	2,693
K, % DM	1.06	0.193	324	1.25	0.304	118	1.63	0.574	16,425	2.26	0.625	2,707
Na, % DM	0.06	0.039	317	0.05	0.074	115	0.06	0.095	6,824	0.10	0.132	1,560
Cl, % DM	0.11	0.048	92	0.12	0.060	34	0.58	0.412	12,555	0.78	0.483	1,705
S, % DM	0.10	0.036	169	0.11	0.026	98	0.15	0.057	12,997	0.20	0.049	1,717
Cu, mg/kg DM	6.85	2.504	314	5.48	2.132	116	8.33	3.473	11,150	9.21	3.115	2,601
Fe, mg/kg DM	108	106.7	313	153	183.9	115	196	163.5	11,060	217	167.3	2,577
Mn, mg/kg DM	10	4.8	314	12	8.2	116	93	63.0	11,070	86	47.8	2,583
Zn, mg/kg DM	13	5.8	308	11	4.2	118	26	9.3	11,036	27	8.2	2,580
Mo, mg/kg DM	1.37	1.469	23	1.07	0.267	14	1.53	0.982	4,464	1.69	1.100	1,211

continued

TABLE 19-1 Continued

Name	Cool-Season Grass Silage			Corn, Ear with Husk and Some Stalk, Ensiled, High Fiber			Corn, Ear with Husk and Some Stalk, Ensiled, Low Fiber			Corn and Cob Meal, Dry		
Feed ID Code	NRC16F35			NRC16F53			NRC16F54			NRC16F36		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	38.8	13.09	19,740	58.0	7.11	8,618	64.8	6.61	6,211	86.3	3.36	448
Ash, % DM	8.1	1.85	19,391	2.1	0.35	8,593	1.7	0.22	6,203	1.9	0.45	421
CP, % DM	13.4	2.72	19,702	7.9	0.82	8,607	7.8	0.69	6,209	8.4	1.12	444
A fraction, % of CP[b]	52			30			30			30		
B fraction, % of CP[b]	34			68			68			68		
C fraction, % of CP[b]	13			2			2			2		
Kd of B, %/h[b]	5.7			5.0			5.0			5.0		
RUP, % CP[c]	32			39			39			39		
dRUP, % of RUP[d]	60			61			61			61		
Soluble protein, % CP	48.0	10.53	19,674	43.8	15.85	8,584	37.9	14.55	6,200	21.1	6.24	423
ADIP, % DM[e]	1.60	0.333	6,946	0.61	0.173	4,863	0.60	0.149	3,169	0.67	0.120	173
NDIP, % DM[f]	3.69	0.961	6,961	0.92	0.313	4,843	0.86	0.320	3,157	1.24	0.496	173
ADF, % DM	39.0	3.63	19,734	12.3	2.22	8,595	8.4	1.66	6,201	8.8	2.34	449
NDF, % DM	62.1	4.90	19,702	24.9	4.07	8,584	18.1	3.08	6,205	19.0	4.28	449
IVNDFD48, % of NDF[g]	63.6	5.71	790	46.8	11.98	893	50.6	15.62	182	30.5	4.95	2
Lignin, % DM	5.82	1.263	19,730	2.01	0.446	8,238	1.75	0.386	6,122	1.86	0.425	401
Starch, % DM	1.9	1.07	18,982	56.7	4.17	8,582	64.6	3.21	6,202	62.1	5.23	425
WSC, % DM[h]	7.3	3.75	12,179	1.8	0.80	18	1.9	1.16	8	4.5	0.21	2
TFAs, % DM	1.84	0.406	6,511	2.89	0.488	3,712	3.11	0.398	2,713	3.26	0.536	166
Crude fat, % DM	3.63	0.742	19,140	3.37	0.456	8,283	3.56	0.425	6,131	3.69	0.726	405
DE base, Mcal/kg[i]	2.44			3.26			3.36			3.35		
Ca, % DM	0.55	0.174	12,844	0.07	0.029	4,751	0.04	0.017	3,404	0.05	0.031	267
P, % DM	0.31	0.061	12,850	0.26	0.032	4,780	0.28	0.032	3,407	0.29	0.062	269
Mg, % DM	0.21	0.052	12,809	0.12	0.019	4,774	0.11	0.013	3,405	0.12	0.031	272
K, % DM	2.29	0.615	12,860	0.52	0.104	4,767	0.45	0.047	3,409	0.46	0.071	271
Na, % DM	0.08	0.084	2,233	0.01	0.009	797	0.01	0.013	232	0.01	0.012	61
Cl, % DM	0.67	0.286	12,119	0.14	0.034	274	0.13	0.033	69	0.10	0.033	11
S, % DM	0.20	0.041	12,550	0.10	0.012	4,434	0.10	0.010	3,359	0.10	0.014	237
Cu, mg/kg DM	9.46	3.266	3,474	3.34	1.424	1,191	2.46	1.101	575	3.88	4.434	86
Fe, mg/kg DM	450	465.3	3,462	85	57.9	1,192	56	35.8	581	114	153.3	85
Mn, mg/kg DM	95	52.8	3,479	12	4.7	1,198	8	3.0	588	11	11.5	87
Zn, mg/kg DM	33	11.1	3,472	25	5.2	1,198	23	4.8	587	27	17.2	87
Mo, mg/kg DM	1.87	1.243	1,712	1.00	0.079	50	1.00	0.000	22	1.00	0.000	8

Name	Corn Cobs			Corn Germ			Corn Germ Meal			Corn Gluten Feed, Dry		
Feed ID Code	NRC16F37			NRC16F38			NRC16F39			NRC16F40		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	89.8	8.06	143	90.2	2.04	107	90.2	1.17	191	89.2	1.77	1,615
Ash, % DM	2.8	1.00	97	5.9	1.55	106	3.7	0.78	128	7.5	1.75	1,353
CP, % DM	3.0	0.77	144	15.4	1.57	107	26.1	1.76	192	23.2	2.48	1,621
A fraction, % of CP[b]	45			41			14			51		
B fraction, % of CP[b]	49			45			50			39		
C fraction, % of CP[b]	6			14			36			10		
Kd of B, %/h[b]	2.8			10.0			12.0			7.0		
RUP, % CP[c]	41			32			52			29		
dRUP, % of RUP[d]	60			73			73			79		
Soluble protein, % CP	36.0	10.60	91	35.4	14.25	18	24.5	4.76	46	45.7	10.77	920
ADIP, % DM[e]	0.35			0.78	0.612	27	4.15	1.051	3	4.44	3.256	407
NDIP, % DM[f]	0.85			2.67	1.767	26	9.88	1.153	3	9.88	6.279	342
ADF, % DM	46.3	6.32	144	10.1	4.82	108	15.2	2.06	186	11.5	2.11	1,581
NDF, % DM	83.8	5.28	142	27.0	6.62	109	44.8	3.39	192	35.7	4.62	1,619
IVNDFD48, % of NDF[g]	65.0						74.0			74.1	12.12	93
Lignin, % DM	4.20	1.793	123	2.69	2.014	102	2.92	1.536	25	2.31	1.218	578
Starch, % DM	1.1	0.83	70	27.6	10.46	45	19.4	2.69	40	15.5	4.63	485
WSC, % DM[h]	1.1	0.25	3				3.9	0.87	15	5.8	2.23	26

TABLE 19-1 Continued

Name	Corn Cobs			Corn Germ			Corn Germ Meal			Corn Gluten Feed, Dry		
Feed ID Code	NRC16F37			NRC16F38			NRC16F39			NRC16F40		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
TFAs, % DM	0.35			16.89			2.11			3.38		
Crude fat, % DM	0.62	0.292	73	17.89	3.417	107	3.11	1.516	190	3.91	1.112	1,305
DE base, Mcal/kg[i]	*2.41*			*3.63*			*3.12*			*3.21*		
Ca, % DM	0.04	0.032	92	0.03	0.029	37	0.04	0.027	135	0.07	0.045	1,338
P, % DM	0.06	0.019	93	1.17	0.381	38	0.83	0.147	137	1.07	0.203	1,431
Mg, % DM	0.04	0.010	92	0.43	0.159	38	0.25	0.073	137	0.43	0.080	1,230
K, % DM	0.96	0.355	93	1.22	0.453	38	0.46	0.124	134	1.47	0.312	1,232
Na, % DM	0.01	0.011	91	0.01	0.007	33	0.04	0.033	123	0.33	0.232	1,050
Cl, % DM	0.28	0.073	68	0.12	0.023	7	0.06	0.034	18	0.29	0.108	269
S, % DM	0.04	0.011	88	0.17	0.049	36	0.33	0.038	89	0.50	0.144	870
Cu, mg/kg DM	5.60	2.556	90	5.63	1.811	36	6.46	2.471	116	5.57	2.109	1,032
Fe, mg/kg DM	188	238.5	91	99	51.5	38	135	33.6	122	146	62.2	1,038
Mn, mg/kg DM	8	5.2	91	16	5.2	38	16	5.4	122	21	5.2	1,040
Zn, mg/kg DM	22	11.7	91	72	20.4	36	79	25.5	54	74	17.1	1,029
Mo, mg/kg DM	1.00	0.000	12	1.00	0.000	14	1.06	0.242	81	1.24	0.475	332

Name	Corn Gluten Feed, Wet			Corn Gluten Meal			Corn Grain Dry, Coarse Grind			Corn Grain Dry, Fine Grind		
Feed ID Code	NRC16F41			NRC16F42			NRC16F1071			NRC16F1070		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	45.6	5.51	1,001	90.5	1.64	221	86.9	2.04	11,264	86.9	2.04	11,264
Ash, % DM	7.2	1.42	683	2.8	0.95	130	1.5	0.23	9,972	1.5	0.23	9,972
CP, % DM	23.1	2.64	1,001	68.5	3.52	220	8.5	0.83	11,326	8.5	0.83	11,326
A fraction, % of CP[b]	51			8			23			23		
B fraction, % of CP[b]	39			72			70			70		
C fraction, % of CP[b]	10			20			7			7		
Kd of B, %/h[b]	7.0			2.5			5.4			5.4		
RUP, % CP[c]	*25*			*69*			*43*			*43*		
dRUP, % of RUP[d]	79			92			73			73		
Soluble protein, % CP	57.0	8.92	473	7.5	3.03	124	22.1	5.79	11,087	22.1	5.79	11,087
ADIP, % DM[e]	0.89	0.630	309	1.11	0.773	35	0.52	0.129	3,572	0.52	0.129	3,572
NDIP, % DM[f]	2.04	0.800	284	1.96	1.449	51	0.91	0.292	3,583	0.91	0.292	3,583
ADF, % DM	12.1	1.91	918	3.7	2.02	205	3.6	0.72	11,282	3.6	0.72	11,282
NDF, % DM	36.9	4.80	1,006	6.8	3.58	221	9.8	1.50	11,326	9.8	1.50	11,326
IVNDFD48, % of NDF[g]	76.7	10.44	23	73.0			62.3	17.61	27	62.3	17.61	27
Lignin, % DM	1.89	1.178	391	1.79	1.241	65	1.37	1.972	7,195	1.37	1.972	7,195
Starch, % DM	15.3	4.70	530	16.4	4.19	56	70.4	2.59	11,331	70.4	2.59	11,331
WSC, % DM[h]	4.0	0.86	91	1.6	0.77	18	2.9	0.84	201	2.9	0.84	201
TFAs, % DM	3.09	0.000	1	1.44			3.84	0.536	1,847	3.84	0.536	1,847
Crude fat, % DM	3.81	1.128	686	2.44	1.058	182	3.84	0.454	2,501	3.84	0.454	2,501
DE base, Mcal/kg[i]	*3.23*			*4.33*			*3.10*			*3.55*		
Ca, % DM	0.10	0.123	839	0.04	0.071	125	0.04	0.045	8,532	0.04	0.045	8,532
P, % DM	1.07	0.240	851	0.49	0.106	126	0.31	0.041	9,185	0.31	0.041	9,185
Mg, % DM	0.45	0.097	848	0.07	0.039	123	0.13	0.058	9,151	0.13	0.058	9,151
K, % DM	1.57	0.381	849	0.22	0.130	123	0.56	0.524	9,150	0.56	0.524	9,150
Na, % DM	0.20	0.142	742	0.05	0.039	110	0.02	0.027	1,321	0.02	0.027	1,321
Cl, % DM	0.25	0.102	212	0.08	0.030	80	0.10	0.043	828	0.10	0.043	828
S, % DM	0.50	0.088	685	0.97	0.113	100	0.10	0.015	5,343	0.10	0.015	5,343
Cu, mg/kg DM	6.54	2.592	694	5.23	3.534	100	2.07	0.951	1,264	2.07	0.951	1,264
Fe, mg/kg DM	179	91.0	697	122	66.8	112	39	18.3	1,274	39	18.3	1,274
Mn, mg/kg DM	23	9.4	700	6	3.5	112	7	3.5	1,347	7	3.5	1,347
Zn, mg/kg DM	77	16.8	681	29	10.9	112	23	6.6	1,357	23	6.6	1,357
Mo, mg/kg DM	1.30	0.474	173	1.13	0.341	31	0.91	0.040	122	0.91	0.040	122

TABLE 19-1   Continued

Name	Corn Grain Dry, Medium Grind			Corn Grain High Moisture, Coarse Grind			Corn Grain High Moisture, Fine Grind			Corn Grain Screenings		
Feed ID Code	NRC16F44			NRC16F1072			NRC16F45			NRC16F43		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	86.9	2.04	11,264	72.3	5.28	71,104	72.3	5.28	71,104	86.9	2.42	622
Ash, % DM	1.5	0.23	9,972	1.6	0.24	61,515	1.6	0.24	61,515	1.8	0.51	316
CP, % DM	8.5	0.83	11,326	8.5	0.81	71,052	8.5	0.81	71,052	8.6	0.94	623
A fraction, % of CP[b]	23			28			28			23		
B fraction, % of CP[b]	70			71			71			70		
C fraction, % of CP[b]	7			1			1			7		
Kd of B, %/h[b]	5.4			5.1			5.1			5.4		
RUP, % CP[c]	*43*			*39*			*39*			*43*		
dRUP, % of RUP[d]	73			90			90			73		
Soluble protein, % CP	22.1	5.79	11,087	33.1	11.78	70,804	33.1	11.78	70,804	25.0	6.20	603
ADIP, % DM[e]	0.52	0.129	3,572	0.44	0.135	27,705	0.44	0.135	27,705	0.63	0.198	47
NDIP, % DM[f]	0.91	0.292	3,583	0.77	0.304	27,925	0.77	0.304	27,925	1.02	0.331	47
ADF, % DM	3.6	0.72	11,282	3.5	0.75	70,657	3.5	0.75	70,657	4.4	1.66	610
NDF, % DM	9.8	1.50	11,326	9.6	1.65	70,894	9.6	1.65	70,894	11.7	2.59	620
IVNDFD48, % of NDF[g]	62.3	17.61	27	50.9	23.35	212	50.9	23.35	212			
Lignin, % DM	1.37	1.972	7,195	1.21	0.684	42,520	1.21	0.684	42,520	1.46	0.582	52
Starch, % DM	70.4	2.59	11,331	70.9	2.40	71,054	70.9	2.40	71,054	65.6	3.93	601
WSC, % DM[h]	2.9	0.84	201	3.0	0.71	540	3.0	0.71	540	2.8	0.59	14
TFAs, % DM	3.84	0.536	1,847	3.57	0.475	14,298	3.57	0.475	14,298	3.18	0.316	15
Crude fat, % DM	3.84	0.454	2,501	3.58	0.466	61,070	3.58	0.466	61,070	3.19	0.576	280
DE base, Mcal/kg[i]	*3.46*			*3.52*			*3.70*			*3.46*		
Ca, % DM	0.04	0.045	8,532	0.04	0.020	53,960	0.04	0.020	53,960	0.18	0.396	599
P, % DM	0.31	0.041	9,185	0.31	0.032	55,837	0.31	0.032	55,837	0.32	0.043	597
Mg, % DM	0.13	0.058	9,151	0.13	0.023	55,212	0.13	0.023	55,212	0.15	0.049	597
K, % DM	0.56	0.524	9,150	0.44	0.227	55,006	0.44	0.227	55,006	0.67	0.602	596
Na, % DM	0.02	0.027	1,321	0.02	0.019	2,354	0.02	0.019	2,354	0.02	0.032	68
Cl, % DM	0.10	0.043	828	0.12	0.050	1,228	0.12	0.050	1,228	0.09	0.056	20
S, % DM	0.10	0.015	5,343	0.11	0.011	28,478	0.11	0.011	28,478	0.11	0.020	36
Cu, mg/kg DM	2.07	0.951	1,264	1.60	0.784	3,131	1.60	0.784	3,131	3.21	1.675	66
Fe, mg/kg DM	39	18.3	1,274	38	16.1	3,687	38	16.1	3,687	165	145.9	67
Mn, mg/kg DM	7	3.5	1,347	6	1.8	3,843	6	1.8	3,843	12	7.1	66
Zn, mg/kg DM	23	6.6	1,357	23	4.6	3,864	23	4.6	3,864	30	10.5	66
Mo, mg/kg DM	0.91	0.040	122	1.00	0.000	104	1.00	0.000	104			

Name	Corn Grain, Steam-Flaked			Corn Hominy			Corn Silage, Immature			Corn Silage, Mature		
Feed ID Code	NRC16F46			NRC16F47			NRC16F49			NRC16F50		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	85.7	2.36	1,045	89.1	1.85	801	31.3	2.70	267,615	39.6	3.86	247,483
Ash, % DM	1.3	0.29	1,010	2.6	0.76	557	4.0	0.95	267,962	3.7	0.80	248,173
CP, % DM	8.0	0.65	1,050	10.1	1.62	803	7.9	0.97	267,961	7.5	0.85	248,152
A fraction, % of CP[b]	2			45			58			49		
B fraction, % of CP[b]	83			49			24			28		
C fraction, % of CP[b]	16			6			16			24		
Kd of B, %/h[b]	3.0			7.0			4.0			3.0		
RUP, % CP[c]	*69*			*30*			*33*			*44*		
dRUP, % of RUP[d]	90			90			70			70		
Soluble protein, % CP	13.9	5.30	564	28.3	8.00	423	52.7	11.63	267,910	51.3	12.31	248,144
ADIP, % DM[e]	0.63			0.51	0.289	135	0.84	0.143	137,979	0.80	0.129	134,544
NDIP, % DM[f]	1.28			1.07	0.530	132	1.26	0.301	138,093	1.19	0.265	134,562
ADF, % DM	3.4	0.72	588	5.7	1.97	735	25.5	3.05	267,953	23.2	2.83	248,237
NDF, % DM	8.6	1.34	591	16.9	5.22	802	42.6	4.45	267,954	39.3	4.14	248,167
IVNDFD48, % of NDF[g]	55.7	11.72	3	75.0	0.00	1	53.4	6.07	58,478	50.8	6.09	69,227
Lignin, % DM	1.26	0.330	454	1.59	0.824	249	3.15	0.569	268,063	2.97	0.528	248,304

TABLE 19-1   Continued

Name	Corn Grain, Steam-Flaked			Corn Hominy			Corn Silage, Immature			Corn Silage, Mature		
Feed ID Code	NRC16F46			NRC16F47			NRC16F49			NRC16F50		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Starch, % DM	71.7	2.81	578	55.6	9.30	488	30.2	6.09	267,777	35.5	5.18	248,239
WSC, % DM[h]	1.8	0.58	79	4.8	1.42	51	2.9	1.16	43,910	3.1	1.19	24,875
TFAs, % DM	3.14			5.38	1.204	5	2.32	0.401	180,768	2.36	0.369	173,952
Crude fat, % DM	3.14	0.808	1,013	7.21	2.347	803	2.96	0.410	266,941	2.86	0.362	247,726
DE base, Mcal/kg[i]	*3.63*			*3.50*			*2.93*			*2.88*		
Ca, % DM	0.06	0.209	540	0.06	0.159	656	0.24	0.050	167,224	0.23	0.043	160,966
P, % DM	0.24	0.055	578	0.52	0.174	657	0.24	0.029	167,750	0.23	0.027	161,246
Mg, % DM	0.10	0.034	575	0.21	0.069	653	0.17	0.037	167,298	0.16	0.033	160,873
K, % DM	0.40	0.342	574	0.66	0.272	652	1.05	0.223	167,677	0.92	0.199	161,324
Na, % DM	0.00	0.005	161	0.02	0.046	503	0.02	0.019	15,933	0.03	0.018	12,118
Cl, % DM	0.08	0.021	102	0.10	0.031	112	0.27	0.121	52,977	0.24	0.103	31,767
S, % DM	0.09	0.011	449	0.12	0.032	414	0.11	0.014	167,040	0.10	0.013	160,877
Cu, mg/kg DM	2.08	0.758	168	3.79	1.956	518	6.34	1.872	31,379	5.91	1.504	25,261
Fe, mg/kg DM	32	17.6	169	77	56.7	516	166	95.6	31,311	146	85.2	25,086
Mn, mg/kg DM	5	1.7	170	12	5.5	516	30	11.9	31,295	28	10.5	25,230
Zn, mg/kg DM	17	3.9	169	40	12.9	514	28	7.7	31,343	26	6.8	25,243
Mo, mg/kg DM	1.00	0.000	32	1.00	0.000	205	1.11	0.317	2,881	1.11	0.313	1,186

Name	Corn Silage, Typical			Corn Stalks, Ensiled, High DM			Corn Stalks, Ensiled, Low DM			Cotton Gin Trash		
Feed ID Code	NRC16F48			NRC16F52			NRC16F51			NRC16F55		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	35.4	5.38	535,422	85.0	6.53	1,877	41.2	13.01	1,695	90.3	3.08	528
Ash, % DM	3.8	0.91	535,923	9.0	4.32	1,108	10.5	5.03	1,310	14.5	6.95	237
CP, % DM	7.7	0.94	536,303	5.6	2.34	1,873	7.0	2.27	1,689	12.0	3.75	532
A fraction, % of CP[b]	60			60			60			30		
B fraction, % of CP[b]	24			24			24			35		
C fraction, % of CP[b]	16			16			16			35		
Kd of B, %/h[b]	4.1			4.1			4.1			5.3		
RUP, % CP[c]	*33*			*33*			*33*			*54*		
dRUP, % of RUP[d]	70			70			70			50		
Soluble protein, % CP	51.8	12.03	537,150	52.6	16.31	1,492	49.6	14.87	1,478	28.1	11.52	326
ADIP, % DM[e]	0.82	0.141	288,591	1.33	0.359	121	1.56	0.385	197	3.19	1.734	66
NDIP, % DM[f]	1.23	0.293	288,614	1.95	0.724	120	2.20	0.715	199	4.37	2.205	67
ADF, % DM	24.3	3.27	537,131	46.9	6.63	1,807	44.8	7.11	1,570	51.0	12.34	530
NDF, % DM	40.9	4.75	536,939	72.0	8.49	1,888	66.2	8.44	1,696	59.4	12.51	533
IVNDFD48, % of NDF[g]	52.0	6.25	130,789	56.2	8.74	296	52.8	11.02	117	21.3	14.50	3
Lignin, % DM	3.05	0.564	537,082	6.37	1.618	1,136	6.16	1.758	1,089	15.03	5.367	147
Starch, % DM	32.9	6.42	536,519	3.7	3.04	1,640	3.5	3.01	1,408	1.1	0.85	82
WSC, % DM[h]	3.0	1.22	70,737	5.9	3.97	345	5.2	3.67	458	2.2	0.72	6
TFAs, % DM	2.35	0.394	370,294	0.48	0.308	441	0.72	0.419	530	3.14		
Crude fat, % DM	2.92	0.390	535,609	1.21	0.580	1,019	1.84	0.714	1,182	4.01	2.270	182
DE base, Mcal/kg[i]	*2.93*			*2.16*			*2.19*			*1.67*		
Ca, % DM	0.24	0.048	336,426	0.39	0.215	1,729	0.48	0.555	1,371	1.69	0.975	433
P, % DM	0.23	0.029	337,092	0.14	0.073	1,736	0.19	0.069	1,412	0.24	0.104	430
Mg, % DM	0.17	0.036	335,977	0.22	0.081	1,730	0.23	0.072	1,392	0.32	0.113	427
K, % DM	0.99	0.222	337,045	1.10	0.429	1,721	1.35	0.510	1,393	1.96	0.634	431
Na, % DM	0.03	0.020	29,854	0.13	0.506	245	0.03	0.026	263	0.07	0.067	421
Cl, % DM	0.26	0.120	87,922	0.43	0.246	406	0.47	0.302	527	0.55	0.377	88
S, % DM	0.10	0.014	336,115	0.09	0.029	1,428	0.11	0.029	1,237	0.40	0.242	256
Cu, mg/kg DM	6.22	1.909	60,914	10.00	6.828	265	9.70	4.591	312	9.01	6.903	426
Fe, mg/kg DM	165	108.4	60,879	1182	1224.3	265	1303	1190.6	312	930	887.7	425
Mn, mg/kg DM	30	12.3	60,745	71	44.5	264	72	42.3	315	64	33.8	428
Zn, mg/kg DM	27	7.9	60,836	29	12.7	267	33	12.4	317	26	12.6	422
Mo, mg/kg DM	1.11	0.317	4,238	1.15	0.404	61	1.36	0.777	42	1.18	0.463	158

TABLE 19-1   Continued

Name	Cottonseed Hulls			Cottonseed Meal			Cottonseed Whole, Linted			Distillers Grains and Solubles, Dried, High Fat		
Feed ID Code	NRC16F57			NRC16F58			NRC16F56			NRC16F59		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	91.0	1.75	224	89.9	2.03	331	91.4	1.83	1148	89.1	1.74	5043
Ash, % DM	3.5	0.79	99	7.6	0.96	267	4.2	0.58	955	5.4	0.60	5,042
CP, % DM	7.0	2.18	231	46.7	3.84	340	23.3	2.65	1,271	30.2	1.68	5,038
A fraction, % of CP[b]	30			25			45			26		
B fraction, % of CP[b]	35			56			48			62		
C fraction, % of CP[b]	35			19			7			12		
Kd of B, %/h[b]	5.3			7.2			14.8			5.0		
RUP, % CP[c]	54			44			23			47		
dRUP, % of RUP[d]	50			83			74			75		
Soluble protein, % CP	20.7	12.08	115	14.5	4.00	259	26.6	9.59	650	15.7	3.69	4,689
ADIP, % DM[e]	2.77	0.536	41	1.72	0.442	75	2.73	1.656	422	2.85	0.733	1,564
NDIP, % DM[f]	3.60	0.893	40	2.22	0.800	74	2.84	3.250	369	3.83	1.111	1447
ADF, % DM	66.0	4.81	228	19.2	4.01	330	38.6	4.32	1,124	14.6	1.62	4,989
NDF, % DM	80.9	6.13	222	28.1	5.89	341	50.6	4.24	1,269	32.1	2.73	5,047
IVNDFD48, % of NDF[g]	22.4	7.92	5				12.8	8.34	7	71.5	8.24	19
Lignin, % DM	19.93	2.710	78	7.01	2.078	211	11.21	2.577	670	4.17	0.936	3,915
Starch, % DM	1.2	1.16	64	1.1	0.76	104	0.8	0.87	348	4.5	1.60	3,897
WSC, % DM[h]	2.1	1.35	15	8.5	1.75	33				4.6	2.01	227
TFAs, % DM	3.14			3.06			18.26			11.39	1.480	1,078
Crude fat, % DM	3.20	1.601	114	3.60	2.215	338	18.62	2.425	1,273	12.54	1.659	5,046
DE base, Mcal/kg[i]	1.58			3.32			3.15			3.49		
Ca, % DM	0.23	0.260	190	0.25	0.058	302	0.17	0.108	926	0.12	0.234	4,328
P, % DM	0.15	0.063	193	1.31	0.176	304	0.62	0.165	932	0.88	0.151	4,366
Mg, % DM	0.22	0.044	193	0.70	0.068	304	0.38	0.107	927	0.34	0.045	4,318
K, % DM	1.21	0.174	193	1.74	0.168	305	1.18	0.185	925	1.26	0.364	4,315
Na, % DM	0.02	0.023	190	0.17	0.099	283	0.02	0.065	858	0.21	0.111	2,376
Cl, % DM	0.07	0.046	47	0.08	0.023	127	0.08	0.071	326	0.19	0.047	1,585
S, % DM	0.10	0.046	118	0.48	0.066	236	0.24	0.081	634	0.67	0.156	4,070
Cu, mg/kg DM	5.18	2.600	191	12.48	4.190	282	7.56	3.385	854	4.15	2.417	2,397
Fe, mg/kg DM	83	119.2	192	208	172.2	276	72	42.1	853	94	29.1	2,414
Mn, mg/kg DM	26	11.4	189	23	3.6	279	17	5.1	859	18	9.0	2,428
Zn, mg/kg DM	19	11.1	190	66	10.5	271	36	7.5	854	64	10.0	2,395
Mo, mg/kg DM	1.00	0.000	23	1.76	0.862	126	1.13	0.331	201	1.11	0.308	1,103

Name	Distillers Grains and Solubles, Dried, High Protein			Distillers Grains and Solubles, Dried, Low Fat			Distillers Grains and Solubles, Modified Wet			Distillers Grains and Solubles, Wet		
Feed ID Code	NRC16F60			NRC16F61			NRC16F62			NRC16F63		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	91.1	1.40	665	89.9	1.54	5,083	49.2	5.59	2,553	33.2	2.84	3,070
Ash, % DM	4.0	1.51	647	5.3	0.62	5,081	5.6	0.80	2,580	4.5	1.02	3,091
CP, % DM	39.0	2.84	671	31.0	1.74	5,075	30.3	2.27	2,579	31.5	2.76	3,084
A fraction, % of CP[b]	26			26			26			26		
B fraction, % of CP[b]	62			62			62			62		
C fraction, % of CP[b]	12			12			12			12		
Kd of B, %/h[b]	5.0			5.0			5.0			5.0		
RUP, % CP[c]	47			47			44			42		
dRUP, % of RUP[d]	75			75			75			75		
Soluble protein, % CP	16.6	5.16	205	20.1	3.60	2,521	21.9	4.33	1,998	16.4	4.93	1,673
ADIP, % DM[e]	3.97	0.805	477	3.15	0.937	2,409	4.09	1.058	436	3.29	1.150	1,763
NDIP, % DM[f]	4.45	0.815	464	3.87	0.854	2,369	4.69	0.979	430	4.13	1.047	1,707
ADF, % DM	17.7	3.20	657	14.8	2.32	4,950	14.4	2.41	2,463	16.1	2.65	3,025
NDF, % DM	37.6	4.80	656	30.8	2.75	5,092	27.1	3.80	2,582	31.7	4.90	3,083
IVNDFD48, % of NDF[g]	62.7	8.33	3	47.2	19.97	11	52.8	10.57	3	25.5	5.46	2

TABLE 19-1   Continued

Name	Distillers Grains and Solubles, Dried, High Protein			Distillers Grains and Solubles, Dried, Low Fat			Distillers Grains and Solubles, Modified Wet			Distillers Grains and Solubles, Wet		
Feed ID Code	NRC16F60			NRC16F61			NRC16F62			NRC16F63		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Lignin, % DM	5.83	1.960	585	3.53	1.049	3,210	3.09	1.197	883	3.22	1.019	2,004
Starch, % DM	6.2	2.87	607	6.1	2.46	3,689	4.7	2.01	1,389	6.3	2.41	2,270
WSC, % DM[h]	5.4	2.94	10	8.0	2.90	57	9.7	3.01	46	7.3	2.41	26
TFAs, % DM	6.56			7.90			8.35			8.31		
Crude fat, % DM	7.56	2.001	664	8.90	1.555	5,107	9.35	1.716	2,586	9.31	2.001	3,095
DE base, Mcal/kg[i]	*3.34*			*3.44*			*3.50*			*3.50*		
Ca, % DM	0.08	0.052	631	0.11	0.229	4,837	0.21	0.365	2,466	0.13	0.219	2,800
P, % DM	0.64	0.278	622	0.89	0.156	4,885	0.86	0.236	2,471	0.76	0.193	2,826
Mg, % DM	0.23	0.105	612	0.34	0.052	4,840	0.35	0.074	2,461	0.28	0.077	2,818
K, % DM	0.75	0.320	617	1.21	0.291	4,829	1.45	0.592	2,469	1.10	0.487	2,819
Na, % DM	0.21	0.132	433	0.24	0.107	1,496	0.27	0.122	418	0.15	0.102	934
Cl, % DM	0.20	0.066	96	0.22	0.054	551	0.23	0.087	67	0.13	0.084	340
S, % DM	0.64	0.177	609	0.71	0.144	4,726	0.63	0.141	2,468	0.67	0.156	2,630
Cu, mg/kg DM	6.65	2.805	438	5.63	2.684	1,389	6.71	2.266	445	4.70	2.324	928
Fe, mg/kg DM	99	48.8	437	102	42.1	1,405	121	37.1	449	110	70.2	958
Mn, mg/kg DM	21	20.9	420	19	9.5	1,410	19	10.6	451	15	6.3	954
Zn, mg/kg DM	53	21.1	439	70	15.6	1,401	72	14.7	450	60	30.0	960
Mo, mg/kg DM	1.38	0.490	78	1.40	0.556	150						

Name	Distillers Solubles			Fat, Canola Oil			Fat, Corn Oil			Fat, Cottonseed Oil		
Feed ID Code	NRC16F64			NRC16F65			NRC16F66			NRC16F67		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	31.2	6.73	645	99.0			99.0			99.0		
Ash, % DM	11.1	2.67	818									
CP, % DM	22.6	4.25	1,248									
A fraction, % of CP[b]	26											
B fraction, % of CP[b]	62											
C fraction, % of CP[b]	12											
Kd of B, %/h[b]	5.0											
RUP, % CP[c]	*25*											
dRUP, % of RUP[d]	75											
Soluble protein, % CP	69.9	11.77	235									
ADIP, % DM[e]	0.78	0.297	351									
NDIP, % DM[f]	1.21	0.465	349									
ADF, % DM	3.2	1.79	471									
NDF, % DM	4.8	2.25	666									
IVNDFD48, % of NDF[g]												
Lignin, % DM	0.57	0.441	373									
Starch, % DM	4.0	1.78	560									
WSC, % DM[h]	28.7	0.01	2									
TFAs, % DM	9.99			88.00			88.00			88.00		
Crude fat, % DM	10.99	5.620	1,253	100.00			100.00			100.0		
DE base, Mcal/kg[i]	*3.62*			*6.22*			*6.22*			*6.22*		
Ca, % DM	0.13	0.082	938									
P, % DM	1.82	0.552	944									
Mg, % DM	0.77	0.215	942									
K, % DM	2.78	0.706	940									
Na, % DM	0.65	0.310	637									
Cl, % DM	0.50	0.136	300									
S, % DM	1.15	0.454	1,045									
Cu, mg/kg DM	9.26	17.620	638									
Fe, mg/kg DM	148	64.4	651									
Mn, mg/kg DM	32	7.9	651									
Zn, mg/kg DM	108	32.0	653									
Mo, mg/kg DM												

continued

TABLE 19-1   Continued

Name	Fat, Flaxseed Oil			Fat, Lard			Fat, Safflower Oil			Fat, Soybean Oil		
Feed ID Code	NRC16F69			NRC16F68			NRC16F70			NRC16F71		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	99.0			99.0			99.0			99.0		
Ash, % DM												
CP, % DM												
A fraction, % of CP[b]												
B fraction, % of CP[b]												
C fraction, % of CP[b]												
Kd of B, %/h[b]												
RUP, % CP[c]												
dRUP, % of RUP[d]												
Soluble protein, % CP												
ADIP, % DM[e]												
NDIP, % DM[f]												
ADF, % DM												
NDF, % DM												
IVNDFD48, % of NDF[g]												
Lignin, % DM												
Starch, % DM												
WSC, % DM[h]												
TFAs, % DM	88.00			88.00			88.00			88.00		
Crude fat, % DM	100.00			100.00			100.00			100.0		
DE base, Mcal/kg[i]	6.22			5.80			6.22			6.22		
Ca, % DM												
P, % DM												
Mg, % DM												
K, % DM												
Na, % DM												
Cl, % DM												
S, % DM												
Cu, mg/kg DM												
Fe, mg/kg DM												
Mn, mg/kg DM												
Zn, mg/kg DM												
Mo, mg/kg DM												

Name	Fat, Sunflower Oil			Fat, Tallow			Feather Meal			Fish Meal		
Feed ID Code	NRC16F72			NRC16F73			NRC16F74			NRC16F75		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	99.0			99.8			92.9	2.20	1,435	92.0	1.88	192
Ash, % DM							2.3	0.69	362	21.1	3.28	173
CP, % DM							90.6	3.75	1,433	69.2	4.25	189
A fraction, % of CP[b]							24			36		
B fraction, % of CP[b]							30			38		
C fraction, % of CP[b]							45			26		
Kd of B, %/h[b]							0.8			1.9		
RUP, % CP[c]							72			76		
dRUP, % of RUP[d]							68			76		
Soluble protein, % CP							9.9	7.01	106	22.4	8.17	72
ADIP, % DM[e]							9.28	3.246	4	1.04	0.537	2
NDIP, % DM[f]							12.70	2.133	4	4.05	2.192	2
ADF, % DM							0.0					
NDF, % DM							0.0					
IVNDFD48, % of NDF[g]												
Lignin, % DM							0.00					
Starch, % DM							0.0					
WSC, % DM[h]							0.0					

TABLE 19-1 Continued

Name	Fat, Sunflower Oil			Fat, Tallow			Feather Meal			Fish Meal		
Feed ID Code	NRC16F72			NRC16F73			NRC16F74			NRC16F75		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
TFAs, % DM	88.00			88.00			7.85			6.44		
Crude fat, % DM	100.00			99.80			8.92	2.742	506	10.48	1.869	191
DE base, Mcal/kg[i]	*6.22*			*5.80*			*4.12*			*3.63*		
Ca, % DM							0.50	0.219	114	5.56	1.665	98
P, % DM							0.32	0.119	115	3.16	0.795	99
Mg, % DM							0.04	0.018	92	0.24	0.078	67
K, % DM							0.18	0.129	93	0.93	0.286	69
Na, % DM							0.16	0.087	95	0.82	0.483	72
Cl, % DM							0.23	0.104	87	1.06	0.536	32
S, % DM							1.78	0.426	99	0.88	0.152	72
Cu, mg/kg DM							9.12	4.831	92	6.02	6.167	64
Fe, mg/kg DM							347	224.1	93	804	612.8	66
Mn, mg/kg DM							12	25.7	93	44	31.7	65
Zn, mg/kg DM							87	15.5	82	92	16.3	34
Mo, mg/kg DM							1.00	0.000	28	1.48	0.802	27

Name	Flaxseed			Flaxseed Meal			Fruit and Vegetable By-Product, Wet			Glycerol		
Feed ID Code	NRC16F96			NRC16F97			NRC16F77			NRC16F1075		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	92.7	1.68	175	89.6	1.37	150	19.8	11.77	1,142	80.0		
Ash, % DM	4.0	1.02	58	6.8	0.93	112	8.4	3.86	1,093	6.7		
CP, % DM	22.8	2.14	182	38.5	3.03	162	13.6	5.20	1140	0.8		
A fraction, % of CP[b]	18			18			42			100		
B fraction, % of CP[b]	67			67			53			0		
C fraction, % of CP[b]	15			15			5			0		
Kd of B, %/h[b]	5.4			5.4			7.4			0.0		
RUP, % CP[c]	*49*			*49*			*30*			*0*		
dRUP, % of RUP[d]	84			84			80			100		
Soluble protein, % CP	43.1	10.30	46	39.0	10.22	70	41.0	19.51	948	100.0		
ADIP, % DM[e]	0.98	0.388	7	1.71	0.469	37	1.18	0.882	442	0.00		
NDIP, % DM[f]	3.55	0.889	6	4.35	1.512	33	1.71	1.225	440	0.00		
ADF, % DM	19.1	5.94	126	16.9	2.85	100	22.4	13.34	1096	0.0		
NDF, % DM	30.4	7.54	131	30.4	6.11	112	28.7	15.01	1143	0.0		
IVNDFD48, % of NDF[g]	57.0	12.73	2	49.0	0.00	1	89.0			0.0		
Lignin, % DM	6.42	2.735	22	6.23	1.723	69	4.65	5.484	545	0.00		
Starch, % DM	2.4	2.12	46	2.1	1.69	45	10.1	10.15	540	0.0		
WSC, % DM[h]	3.9	0.73	15	5.9	0.84	14	31.2	21.96	22	1.4		
TFAs, % DM	33.41			3.08			6.13			5.24		
Crude fat, % DM	34.41	5.569	183	3.20	1.797	160	7.13	4.349	711	6.24		
DE base, Mcal/kg[i]	*4.10*			*3.24*			*3.04*			*3.29*		
Ca, % DM	0.24	0.049	95	0.44	0.070	95	0.72	0.556	1071	0.08		
P, % DM	0.59	0.088	97	0.95	0.085	96	0.34	0.130	1078	0.19		
Mg, % DM	0.37	0.043	96	0.65	0.054	90	0.19	0.099	1082	0.07		
K, % DM	0.79	0.101	95	1.30	0.103	90	2.01	1.039	1082	0.53		
Na, % DM	0.04	0.018	93	0.13	0.056	81	0.23	0.224	1069	2.48		
Cl, % DM	0.08	0.043	22	0.07	0.026	42	0.47	0.285	443	4.49		
S, % DM	0.24	0.027	44	0.40	0.035	80	0.21	0.090	483	1.18		
Cu, mg/kg DM	12.64	2.896	92	21.01	4.127	78	10.98	7.453	1065	5.08		
Fe, mg/kg DM	98	64.8	91	312	224.4	78	621	704.5	1070			
Mn, mg/kg DM	30	5.4	91	52	8.2	77	35	26.8	1069	22		
Zn, mg/kg DM	45	8.0	90	75	9.7	77	35	46.7	1072	7		
Mo, mg/kg DM	1.00	0.000	49	1.00	0.000	28	1.76	1.359	15			

TABLE 19-1   Continued

Name	Grain Screenings, Source Unknown			Grain Sorghum Hay			Grain Sorghum Silage, Mature			Grain Sorghum Silage, Mid-Maturity		
Feed ID Code	NRC16F79			NRC16F80			NRC16F81			NRC16F82		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	89.2	2.83	117	92.6	1.60	1,124	31.3	4.32	2,218	29.8	4.71	3,744
Ash, % DM	6.0	2.16	118	7.0	1.66	1,124	5.9	1.61	2,223	8.1	3.02	3,752
CP, % DM	16.2	4.19	118	8.8	1.75	1,122	8.2	1.56	2,226	8.9	1.57	3,767
A fraction, % of CP[b]	23			42			42			42		
B fraction, % of CP[b]	70			38			38			38		
C fraction, % of CP[b]	7			20			20			20		
Kd of B, %/h[b]	5.4			4.1			4.1			4.1		
RUP, % CP[c]	*43*			*43*			*43*			*43*		
dRUP, % of RUP[d]	73			70			70			70		
Soluble protein, % CP	40.4	11.75	68	35.3	12.36	1,110	35.6	9.83	2,222	45.1	10.07	3,758
ADIP, % DM[e]	0.92	0.346	48	0.82			9.30	1.574	543	9.22	1.837	2,585
NDIP, % DM[f]	2.40	1.040	49	2.75			12.84	3.268	541	13.64	3.670	2,586
ADF, % DM	17.7	7.53	84	30.6	4.52	1,124	28.9	3.09	2,227	34.1	3.16	3,759
NDF, % DM	34.1	9.99	118	48.1	6.69	1,122	45.7	4.46	2,227	53.1	3.94	3,756
IVNDFD48, % of NDF[g]	35.9	0.00	1	57.5	6.11	724	56.2	6.83	1,248	55.3	5.98	392
Lignin, % DM	3.65	1.267	55	4.54	0.865	1,123	4.25	0.779	2,223	5.03	0.858	3,767
Starch, % DM	21.8	6.82	47	22.2	7.35	1,125	23.2	6.27	2,228	14.3	4.90	3,766
WSC, % DM[h]				7.3	5.95	938	11.0	6.96	1,426	4.9	4.40	1,155
TFAs, % DM	2.91			1.36			1.93	0.312	404	1.56		
Crude fat, % DM	3.91	2.171	98	2.39	0.590	1,120	2.52	0.537	2,221	2.75	0.468	3,750
DE base, Mcal/kg[i]	*3.03*			*2.64*			*2.73*			*2.52*		
Ca, % DM	0.26	0.235	82	0.28	0.085	1,117	0.28	0.082	1,705	0.37	0.110	1,443
P, % DM	0.66	0.334	84	0.22	0.054	1,120	0.22	0.039	1,706	0.23	0.045	1,446
Mg, % DM	0.30	0.162	83	0.22	0.063	1,120	0.20	0.047	1,705	0.24	0.057	1,441
K, % DM	1.05	0.324	84	1.24	0.338	1,125	1.27	0.268	1,710	1.55	0.360	1,446
Na, % DM	0.03	0.019	70	0.02	0.014	105	0.02	0.017	119	0.02	0.021	373
Cl, % DM	0.23	0.134	32	0.43	0.207	976	0.40	0.165	1,275	0.59	0.244	1,355
S, % DM	0.20	0.035	36	0.11	0.027	1,111	0.12	0.024	1,715	0.13	0.025	1,453
Cu, mg/kg DM	10.56	4.873	70	8.17	3.304	110	7.88	2.224	218	8.39	2.407	1,232
Fe, mg/kg DM	251	189.5	71	224	157.5	106	276	200.5	223	528	400.0	1,244
Mn, mg/kg DM	105	72.5	71	39	19.3	108	46	18.7	221	57	22.3	1,241
Zn, mg/kg DM	66	28.9	70	45	24.1	47	33	10.4	221	36	11.8	1,259
Mo, mg/kg DM				1.10	0.342	72	3.00	2.260	66	1.14	0.348	65

Name	Grass–Legume Mixtures, Mix Hay			Grass–Legume Mixtures, Mix Silage			Grass–Legume Mixtures, Predominantly Grass, Hay, Mature			Grass–Legume Mixtures, Predominantly Grass, Hay, Mid-Maturity		
Feed ID Code	NRC16F89			NRC16F90			NRC16F85			NRC16F84		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	86.4	4.38	22,911	40.0	11.22	36,299	87.4	4.15	36,179	89.2	5.93	4,659
Ash, % DM	9.2	1.88	24,357	9.5	1.62	36,059	7.5	1.93	36,267	9.4	1.72	4,603
CP, % DM	12.1	3.18	24,462	17.7	2.60	36,189	10.9	2.94	36,440	15.6	2.89	4,650
A fraction, % of CP[b]	41			61			41			41		
B fraction, % of CP[b]	49			30			49			49		
C fraction, % of CP[b]	10			9			10			10		
Kd of B, %/h[b]	13.0			10.6			13.0			13.0		
RUP, % CP[c]	*26*			*22*			*26*			*26*		
dRUP, % of RUP[d]	70			70			70			70		
Soluble protein, % CP	27.0	8.68	24,076	50.7	9.92	36,287	29.7	8.65	34,815	34.2	6.80	4,108
ADIP, % DM[e]	1.65	0.423	7,858	1.91	0.486	18,574	1.31	0.299	27,083	0.99	0.291	4,162
NDIP, % DM[f]	4.12	1.261	7,807	3.70	0.915	18,611	3.57	1.120	27,147	1.33		
ADF, % DM	39.3	4.85	24,461	34.8	3.93	36,228	40.0	4.22	36,367	33.8	2.90	4,650
NDF, % DM	58.2	5.67	24,461	51.2	4.94	36,240	62.0	4.82	36,398	54.7	4.18	4,654
IVNDFD48, % of NDF[g]	54.5	7.25	1,069	61.1	7.19	574	47.5	9.18	7,379	67.5	9.54	196

TABLE 19-1 Continued

Name	Grass–Legume Mixtures, Mix Hay			Grass–Legume Mixtures, Mix Silage			Grass–Legume Mixtures, Predominantly Grass, Hay, Mature			Grass–Legume Mixtures, Predominantly Grass, Hay, Mid-Maturity		
Feed ID Code	NRC16F89			NRC16F90			NRC16F85			NRC16F84		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Lignin, % DM	6.70	1.521	24,478	5.90	1.423	36,252	6.08	1.511	36,460	4.28	0.969	4,657
Starch, % DM	2.7	2.26	9,892	2.1	1.03	35,763	2.5	1.08	29,258	1.5	1.13	4,557
WSC, % DM[h]	10.1	2.87	2,301	8.1	3.64	17,445	10.9	4.11	8,772	12.6	3.88	4,510
TFAs, % DM	1.37	0.483	20,816	2.03	0.455	17,547	1.29	0.519	18,868	1.93		
Crude fat, % DM	2.66	0.589	24,417	4.04	0.676	36,156	2.43	0.568	36,029	3.39	0.630	4,578
DE base, Mcal/kg[i]	2.38			2.60			2.43			2.64		
Ca, % DM	0.74	0.313	16,636	0.87	0.253	17,747	0.51	0.171	16,308	0.63	0.163	4,627
P, % DM	0.25	0.076	16,645	0.34	0.050	17,727	0.22	0.065	16,321	0.33	0.068	4,623
Mg, % DM	0.21	0.060	16,594	0.25	0.044	17,689	0.22	0.068	16,268	0.27	0.065	4,621
K, % DM	1.91	0.693	16,652	2.54	0.513	17,742	1.55	0.579	16,272	2.34	0.621	4,623
Na, % DM	0.04	0.052	2,646	0.06	0.064	2,036	0.07	0.089	4,947	0.08	0.100	1,179
Cl, % DM	0.43	0.285	4,368	0.59	0.281	17,526	0.48	0.324	10,263	0.65	0.378	4,189
S, % DM	0.02	0.016	16,305	0.23	0.035	17,673	0.14	0.051	15,134	0.22	0.046	4,205
Cu, mg/kg DM	9.98	4.645	3,782	10.73	2.820	4,949	8.75	3.274	8,762	0.52	0.406	34
Fe, mg/kg DM	309	423.9	3,755	451	396.4	4,936	261	240.6	8,724	10	4.6	1,215
Mn, mg/kg DM	65	43.6	3,770	72	35.2	4,928	89	57.8	8,733	295	342.7	1,205
Zn, mg/kg DM	28	13.7	3,767	32	7.7	4,926	27	9.2	8,728	83	49.2	1,195
Mo, mg/kg DM	1.91	1.217	587	1.79	1.006	1,755	1.54	0.915	2,226	29.65	10.458	1,182

Name	Grass–Legume Mixtures, Predominantly Grass, Silage			Grass–Legume Mixtures, Predominantly Legume, Hay, Immature			Grass–Legume Mixtures, Predominantly Legume, Hay, Mature			Grass–Legume Mixtures, Predominantly Legume, Silage		
Feed ID Code	NRC16F83			NRC16F87			NRC16F86			NRC16F88		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	39.6	10.99	49,300	88.6	4.36	4,767	85.3	4.85	2,636	41.0	10.33	40,430
Ash, % DM	8.1	1.75	49,257	10.0	1.46	4,830	9.0	1.67	2,626	10.3	1.70	40,235
CP, % DM	14.3	2.65	49,294	20.3	2.58	4,860	17.4	2.78	2,636	20.0	2.06	40,423
A fraction, % of CP[b]	41			44			34			52		
B fraction, % of CP[b]	49			49			51			39		
C fraction, % of CP[b]	10			7			15			9		
Kd of B, %/h[b]	13.0			15.1			9.5			8.2		
RUP, % CP[c]	26			22			34			27		
dRUP, % of RUP[d]	70			75			65			72		
Soluble protein, % CP	49.2	10.51	49,238	37.2	6.50	4,625	32.0	6.40	2,622	53.6	9.55	40,425
ADIP, % DM[e]	1.61	0.377	49,224	1.61	0.277	884	1.83	0.324	2,610	1.99	0.467	21,276
NDIP, % DM[f]	3.51	0.885	49,216	4.25	0.902	887	4.38	1.113	2,621	3.54	0.861	21,276
ADF, % DM	37.0	3.90	49,299	32.9	3.68	4,877	38.7	2.89	2,636	33.9	3.43	40,396
NDF, % DM	57.7	4.62	49,300	43.9	4.80	4,877	51.2	2.85	2,636	45.9	3.82	40,424
IVNDFD48, % of NDF[g]	55.2	8.36	2,810	49.9	6.46	420	20.1		1	56.2	5.94	1,247
Lignin, % DM	5.62	1.240	49,299	6.92	1.103	4,866	8.27	0.988	2,634	6.60	1.198	40,404
Starch, % DM	2.6	1.24	46,473	1.9	0.88	4,763	2.5	0.80	2,609	2.1	1.10	40,070
WSC, % DM[h]				9.0	2.48	3,884				6.6	2.52	18,858
TFAs, % DM	1.98	0.431	43,787	1.78	0.307	891	1.23	0.296	2,476	1.99	0.419	20,669
Crude fat, % DM	3.75	0.611	49,224	2.63	0.506	4,812	2.27	0.407	2,623	3.81	0.663	40,370
DE base, Mcal/kg[i]	2.55			2.64			2.40			2.61		
Ca, % DM	0.55	0.187	3,177	1.28	0.255	3,960	1.24	0.332	52	1.26	0.251	19,239
P, % DM	0.30	0.071	3,181	0.30	0.058	3,961	0.27	0.086	52	0.35	0.046	19,212
Mg, % DM	0.23	0.066	3,162	0.30	0.063	3,952	0.28	0.072	52	0.27	0.040	19,144
K, % DM	2.40	0.759	3,179	2.24	0.529	3,967	2.52	0.748	52	2.77	0.461	19,225
Na, % DM	0.13	0.133	856	0.07	0.090	961	0.06	0.077	52	0.05	0.046	3,573
Cl, % DM	0.82	0.473	851	0.56	0.333	3,687	0.43	0.356	44	0.57	0.267	19,051
S, % DM	0.18	0.046	3,117	0.24	0.047	3,742	0.21	0.075	44	0.24	0.034	19,168
Cu, mg/kg DM	10.66	5.566	6,644	10.24	2.483	1,135	10.29	2.492	744	10.94	3.401	7,416
Fe, mg/kg DM	395	322.2	6,565	285	245.4	1,126	421	409.9	738	473	499.3	7,399
Mn, mg/kg DM	87	44.3	6,580	44	21.3	1,141	56	26.3	742	57	28.4	7,394
Zn, mg/kg DM	32	8.0	6,594	26	5.8	1,131	26	7.1	749	30	7.8	7,389
Mo, mg/kg DM	2.77	2.006	10	1.86	1.138	823	1.81	0.634	6	1.69	0.899	3,235

TABLE 19-1   Continued

Name	Legume Hay, Immature			Legume Hay, Mature			Legume Hay, Mid-Maturity			Legume Silage, Immature		
Feed ID Code	NRC16F91			NRC16F92			NRC16F93			NRC16F94		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	89.3	3.22	85,479	87.7	3.57	17,360	88.1	2.95	100,858	41.6	9.98	103,397
Ash, % DM	11.0	1.46	85,949	10.0	1.49	17,325	10.8	1.44	101,438	11.1	1.84	102,570
CP, % DM	21.5	1.97	86,336	18.1	1.93	17,414	20.7	2.37	102,002	22.1	1.93	103,311
A fraction, % of CP[b]	43			39			45			62		
B fraction, % of CP[b]	51			49			45			29		
C fraction, % of CP[b]	7			12			9			9		
Kd of B, %/h[b]	17.8			14.0			17.8			13.1		
RUP, % CP[c]	*21*			*27*			*22*			*21*		
dRUP, % of RUP[d]	65			65			65			70		
Soluble protein, % CP	41.1	8.55	85,771	37.9	6.13	17,213	34.6	6.97	100,429	55.2	10.05	102,955
ADIP, % DM[e]	1.51	0.334	50,671	1.75	0.273	6,501	0.74	0.160	45,707	1.60	0.323	94,318
NDIP, % DM[f]	2.80	0.945	50,575	3.89	0.946	6,536	1.85	0.596	45,773	2.68	0.776	94,556
ADF, % DM	30.7	3.08	86,414	37.2	2.98	17,409	32.1	3.96	101,978	32.0	3.49	103,291
NDF, % DM	37.7	3.69	86,443	46.6	3.34	17,405	41.1	4.84	101,963	38.7	3.71	103,272
IVNDFD48, % of NDF[g]	51.4	8.48	6,645	43.4	5.07	677	52.4	9.10	49,252	53.3	7.94	26,119
Lignin, % DM	6.59	0.776	86,373	8.12	0.812	17,417	6.64	1.148	101,932	6.55	1.016	103,223
Starch, % DM	2.3	0.70	65,803	2.3	0.98	11,948	1.5	0.85	25,588	1.9	0.83	70,071
WSC, % DM[h]	9.8	1.87	20,423	9.8	1.87	5,609	9.0	1.84	26,447	7.3	2.59	1,475
TFAs, % DM	1.54	0.360	56,590	1.21	0.344	10,725	1.50	0.466	27,726	1.98	0.414	74,017
Crude fat, % DM	2.55	0.439	85,735	2.25	0.428	17,271	2.08	0.313	101,238	3.22	0.463	102,857
DE base, Mcal/kg[i]	*2.68*			*2.46*			*2.63*			*2.70*		
Ca, % DM	1.51	0.225	41,783	1.37	0.232	10,915	1.40	0.255	101,582	1.30	0.174	34,542
P, % DM	0.29	0.047	42,028	0.28	0.049	10,980	0.28	0.050	101,747	0.35	0.047	34,742
Mg, % DM	0.32	0.062	41,874	0.29	0.053	10,968	0.32	0.063	101,407	0.33	0.051	34,596
K, % DM	2.49	0.555	42,018	2.34	0.479	10,920	2.39	0.630	101,742	2.79	0.598	34,760
Na, % DM	0.20	0.137	6,313	0.11	0.109	1,163	0.21	0.141	13,990	0.16	0.129	4,890
Cl, % DM	0.76	0.325	23,579	0.66	0.295	5,707	0.76	0.324	35,929	0.80	0.364	6,058
S, % DM	0.19	0.041	41,508	0.12	0.029	10,787	0.18	0.050	101,242	0.22	0.033	34,406
Cu, mg/kg DM	10.23	4.756	19,685	9.71	2.972	3,028	9.69	2.615	6,646	11.44	2.761	20,771
Fe, mg/kg DM	430	333.9	19,685	380	340.9	2,998	421	316.4	6,612	685	583.9	20,686
Mn, mg/kg DM	43	14.4	19,591	44	18.2	3,007	38	13.3	6,636	62	27.1	20,650
Zn, mg/kg DM	26	8.5	19,672	24	6.1	3,011	26	12.7	6,645	30	6.9	20,768
Mo, mg/kg DM	2.75	1.839	2,045	2.23	1.744	637	2.53	1.710	2,948	3.51	2.376	216

Name	Legume Silage, Mid-Maturity			Meat and Bone Meal, Porcine			Millet Hay			Millet Silage		
Feed ID Code	NRC16F95			NRC16F98			NRC16F99			NRC16F100		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	42.9	11.35	99,804	96.1	1.05	376	87.5	3.26	862	29.3	11.07	518
Ash, % DM	10.6	1.73	98,949	26.2	3.24	314	11.2	2.19	429	11.7	3.07	448
CP, % DM	20.5	2.19	99,680	56.6	3.19	411	10.7	3.73	876	13.0	3.75	521
A fraction, % of CP[b]	52			32			28			38		
B fraction, % of CP[b]	39			42			53			29		
C fraction, % of CP[b]	9			26			19			33		
Kd of B, %/h[b]	8.2			8.8			5.0			3.7		
RUP, % CP[c]	*27*			*44*			*47*			*52*		
dRUP, % of RUP[d]	70			61			60			55		
Soluble protein, % CP	49.3	9.95	99,506	14.9	3.85	113	34.7	11.87	865	47.0	10.38	518
ADIP, % DM[e]	1.25	0.254	47,887	2.84	0.803	30	0.91			1.13		
NDIP, % DM[f]	2.24	0.647	47,958	11.83	2.344	10	3.49			3.36		
ADF, % DM	33.7	3.27	99,624				39.7	4.86	872	39.2	4.87	521
NDF, % DM	43.2	3.94	99,573				61.9	5.58	873	59.7	5.90	522
IVNDFD48, % of NDF[g]	49.4	7.18	48,021				64.0	7.31	138	61.2	6.98	61
Lignin, % DM	7.42	1.283	99,678				5.64	1.629	445	5.48	1.680	451
Starch, % DM	2.0	1.09	19,262				2.9	3.11	338	3.5	4.02	505
WSC, % DM[h]	6.3	2.47	11,060				8.4	3.94	332	6.2	4.02	331

TABLE 19-1 Continued

Name	Legume Silage, Mid-Maturity			Meat and Bone Meal, Porcine			Millet Hay			Millet Silage		
Feed ID Code	NRC16F95			NRC16F98			NRC16F99			NRC16F100		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
TFAs, % DM	2.32	0.577	46,724	7.45			1.09	0.375	122	1.47	0.225	5
Crude fat, % DM	2.87	0.486	99,133	11.90	1.621	315	1.95	0.673	445	2.79	0.789	452
DE base, Mcal/kg[i]	2.59			3.25			2.25			2.25		
Ca, % DM	1.25	0.175	90,914	9.29	2.297	373	0.54	0.265	864	0.55	0.224	519
P, % DM	0.35	0.044	91,354	4.59	0.991	372	0.28	0.070	868	0.33	0.083	520
Mg, % DM	0.30	0.043	90,871	0.28	0.111	220	0.31	0.097	865	0.34	0.110	518
K, % DM	2.82	0.510	91,218	0.91	0.563	223	2.71	0.769	868	2.88	0.928	521
Na, % DM	0.12	0.109	6,596	0.74	0.403	185	0.03	0.036	41	0.06	0.114	69
Cl, % DM	0.64	0.295	17,573	0.49	0.140	86	1.07	0.636	303	1.05	0.455	340
S, % DM	0.14	0.024	91,173	0.51	0.126	109	0.18	0.056	865	0.20	0.050	517
Cu, mg/kg DM	10.59	2.817	3,974	20.01	22.084	174	9.12	3.122	34	11.65	3.702	55
Fe, mg/kg DM	534	456.8	3,950	451	256.4	175	286	224.5	34	491	418.6	55
Mn, mg/kg DM	55	24.2	3,952	24	45.0	176	105	114.8	34	110	76.7	54
Zn, mg/kg DM	29	18.4	3,949	160	77.3	175	43	19.5	33	46	14.9	56
Mo, mg/kg DM	2.09	1.486	1,378				1.58	1.176	24	2.08	1.645	36

Name	Molasses			Oat Grain, Rolled			Oat Hay			Oat Hulls		
Feed ID Code	NRC16F101			NRC16F102			NRC16F103			NRC16F104		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	65.4	14.17	13	89.3	2.14	911	89.5	2.65	14,004	91.4	1.85	69
Ash, % DM	16.0	4.87	10	3.2	0.57	717	7.4	2.09	12,910	5.8	1.31	32
CP, % DM	9.3	4.36	32	12.2	1.75	910	8.5	2.44	13,998	5.0	1.58	68
A fraction, % of CP[b]	74			72			35			10		
B fraction, % of CP[b]	26			20			53			51		
C fraction, % of CP[b]	0			8			12			39		
Kd of B, %/h[b]	3.2			26.4			4.3			1.4		
RUP, % CP[c]	21			16			42			80		
dRUP, % of RUP[d]	100			72			70			62		
Soluble protein, % CP	95.9	0.00	1	27.1	6.44	669	39.5	7.75	13,769	36.7	16.09	16
ADIP, % DM[e]	0.00			0.77	0.377	107	1.03	0.284	1,444	0.67		
NDIP, % DM[f]	0.00			1.21	0.658	104	2.05	1.004	1,441	4.16		
ADF, % DM	0.2	0.19	3	14.5	3.70	910	37.5	4.47	14,112	39.6	6.33	59
NDF, % DM	0.6	0.41	4	28.6	6.45	910	59.0	6.04	14,124	73.6	8.19	67
IVNDFD48, % of NDF[g]				35.9	14.72	7	56.0	6.55	2,598			
Lignin, % DM	0.00			3.20	0.756	648	4.71	1.475	12,949	6.54	1.920	19
Starch, % DM	0.8	0.52	5	44.7	6.06	872	4.1	2.67	12,306	10.6	4.92	21
WSC, % DM[h]	60.0	8.86	551	2.9	0.94	99	17.8	7.55	10,657	1.0		
TFAs, % DM	0.00			4.80			1.45	0.575	1,625	1.82		
Crude fat, % DM	0.61	0.428	30	5.68	1.424	709	2.36	0.581	12,862	1.94	0.827	31
DE base, Mcal/kg[i]	3.07			3.27			2.51			2.23		
Ca, % DM	0.73	0.653	17	0.13	0.172	805	0.32	0.163	12,804	0.16	0.230	46
P, % DM	0.26	0.276	17	0.38	0.082	812	0.21	0.055	12,881	0.15	0.065	46
Mg, % DM	0.28	0.235	17	0.14	0.031	790	0.14	0.046	12,837	0.11	0.055	46
K, % DM	4.49	1.708	17	0.56	0.323	798	1.70	0.592	12,897	0.56	0.218	46
Na, % DM	1.51	0.547	11	0.01	0.009	238	0.42	0.282	3,081	0.02	0.008	25
Cl, % DM	2.02	0.952	8	0.14	0.077	117	0.94	0.504	10,681	0.13	0.042	8
S, % DM	0.64	0.259	20	0.16	0.041	606	0.13	0.049	12,708	0.09	0.025	21
Cu, mg/kg DM	36.71	110.239	14	6.58	3.116	265	6.77	3.118	3,561	7.63	2.386	24
Fe, mg/kg DM	216	326.0	14	129	83.0	269	257	243.4	3,519	198	97.5	24
Mn, mg/kg DM	74	219.0	14	53	17.0	270	66	31.1	3,541	49	15.3	24
Zn, mg/kg DM	112	295.5	14	33	10.1	265	20	8.0	3,530	22	6.5	24
Mo, mg/kg DM				1.36	0.603	124	1.37	0.708	1,119	1.00	0.000	8

TABLE 19-1 Continued

Name	Oat Silage, Immature			Oat Silage, Mid-Maturity			Pea Hay			Pea Silage		
Feed ID Code	NRC16F105			NRC16F106			NRC16F107			NRC16F108		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	33.7	9.47	1,712	35.8	10.08	14,245	89.5	2.22	79	31.7	9.86	96
Ash, % DM	13.4	2.90	1,495	10.4	2.58	12,534	9.1	2.04	80	11.4	3.31	98
CP, % DM	18.5	2.72	1,715	12.9	2.89	14,251	15.9	3.29	80	17.0	3.80	99
A fraction, % of CP[b]	46			45			45			52		
B fraction, % of CP[b]	31			31			46			39		
C fraction, % of CP[b]	24			24			9			9		
Kd of B, %/h[b]	5.4			5.4			17.8			8.2		
RUP, % CP[c]	*41*			*42*			*22*			*27*		
dRUP, % of RUP[d]	65			65			65			72		
Soluble protein, % CP	57.6	11.26	1,707	58.9	10.70	14,145	45.9	14.07	79	59.0	10.02	99
ADIP, % DM[e]	1.09	0.320	904	1.21	0.283	4,102	1.48			2.23	0.630	4
NDIP, % DM[f]	2.94	1.167	905	2.08	0.827	4,103	3.60			3.86	0.725	4
ADF, % DM	31.7	3.70	1,718	38.8	4.06	14,224	32.0	4.38	80	37.1	4.80	99
NDF, % DM	45.8	4.28	1,719	57.4	5.40	14,251	43.4	6.65	80	52.5	7.04	99
IVNDFD48, % of NDF[g]	59.3	9.27	136	54.1	6.34	878	58.5	0.71	2	57.7	8.66	6
Lignin, % DM	3.89	1.072	1,506	5.39	1.138	12,561	5.78	1.452	80	6.42	1.644	99
Starch, % DM	1.8	1.21	1,610	3.2	2.97	13,764	8.6	4.97	80	3.4	3.31	95
WSC, % DM[h]	4.3	2.53	13	6.7	3.72	5,412	9.0	6.14	80	4.5	2.49	89
TFAs, % DM	2.24	0.380	853	1.77	0.413	3,358	1.69			1.68	0.440	4
Crude fat, % DM	4.23	0.593	1,494	3.64	0.631	12,564	2.98	1.153	80	3.80	0.777	98
DE base, Mcal/kg[i]	*2.58*			*2.41*			*2.66*			*2.46*		
Ca, % DM	0.72	0.254	820	0.51	0.212	10,360	1.04	0.257	80	0.88	0.296	92
P, % DM	0.40	0.051	818	0.34	0.058	10,381	0.28	0.069	80	0.34	0.059	95
Mg, % DM	0.25	0.065	813	0.20	0.051	10,353	0.27	0.075	79	0.24	0.070	94
K, % DM	3.18	0.531	816	2.68	0.671	10,395	2.05	0.599	79	2.86	0.815	95
Na, % DM	0.30	0.350	51	0.23	0.210	1,280	0.11	0.081	11	0.04	0.025	17
Cl, % DM	1.19	0.604	53	0.90	0.430	5,524	0.55	0.254	75	0.72	0.342	84
S, % DM	0.26	0.034	817	0.19	0.037	10,389	0.20	0.045	79	0.21	0.052	94
Cu, mg/kg DM	10.03	8.149	274	8.42	3.428	2,505	9.27	5.159	11	11.06	5.446	16
Fe, mg/kg DM	1031	737.6	272	611	578.0	2,478	1169	1213.3	11	1205	1032.2	16
Mn, mg/kg DM	115	50.2	275	70	39.7	2,497	45	21.3	11	56	33.8	16
Zn, mg/kg DM	34	8.5	271	30	9.1	2,504	32	19.2	10	35	17.9	16
Mo, mg/kg DM				1.47	0.744	591	2.20	2.683	5	1.57	0.756	14

Name	Peanut Hay			Peanut Hulls			Peanut Meal, Expellers			Peanut Skins		
Feed ID Code	NRC16F109			NRC16F110			NRC16F111			NRC16F112		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	91.3	1.22	275	94.0	1.51	174	94.0	1.00	173	92.4	2.38	74
Ash, % DM	10.4	2.37	275	4.6	2.84	23	6.5	1.99	13	3.2	1.17	37
CP, % DM	12.0	3.39	275	8.9	1.16	175	42.6	5.52	172	16.2	4.75	74
A fraction, % of CP[b]	45			27			62			27		
B fraction, % of CP[b]	45			69			36			70		
C fraction, % of CP[b]	9			4			2			4		
Kd of B, %/h[b]	17.8			6.4			16.1			6.0		
RUP, % CP[c]	*22*			*37*			*15*			*39*		
dRUP, % of RUP[d]	65			38			94			38		
Soluble protein, % CP	35.7	8.77	249	21.3	8.10	122	37.7	12.49	10	20.9	14.15	50
ADIP, % DM[e]	1.74			2.63			1.95			2.87	0.654	8
NDIP, % DM[f]	3.74			5.42			5.63			4.01	1.417	8
ADF, % DM	37.9	5.72	274	59.8	4.74	173	15.9	8.78	9	36.5	17.09	73
NDF, % DM	45.8	6.34	274	69.6	3.93	174	22.9	8.99	9	47.4	16.49	74
IVNDFD48, % of NDF[g]	40.1	6.98	14									
Lignin, % DM	8.48	2.031	263	24.25	3.181	16	5.39	4.404	10	17.85	10.914	21
Starch, % DM	4.0	2.84	252	1.3	0.89	13	6.4	2.75	8	3.2	3.18	6
WSC, % DM[h]	10.3	2.84	244	3.5	2.44	6				11.9		

TABLE 19-1   Continued

Name	Peanut Hay			Peanut Hulls			Peanut Meal, Expellers			Peanut Skins		
Feed ID Code	NRC16F109			NRC16F110			NRC16F111			NRC16F112		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
TFAs, % DM	1.31			3.21			7.31			18.61		
Crude fat, % DM	2.30	1.070	258	4.21	1.468	135	8.46	1.744	173	19.61	9.706	51
DE base, Mcal/kg[i]	2.37			1.57			3.73			2.74		
Ca, % DM	1.34	0.309	272	0.26	0.107	152	0.35	0.419	14	0.30	0.080	67
P, % DM	0.17	0.060	270	0.09	0.025	153	0.57	0.214	14	0.16	0.069	67
Mg, % DM	0.48	0.210	270	0.12	0.062	151	0.41	0.232	14	0.18	0.059	67
K, % DM	1.68	0.479	271	0.64	0.159	153	1.19	0.151	14	0.58	0.129	67
Na, % DM	0.04	0.069	103	0.02	0.021	141	0.07	0.120	14	0.03	0.102	64
Cl, % DM	0.63	0.334	236	0.12	0.080	5	0.26	0.279	8	0.05	0.057	11
S, % DM	0.15	0.042	249	0.09	0.019	127	0.30	0.072	11	0.15	0.029	39
Cu, mg/kg DM	9.91	5.349	122	12.52	3.437	152	13.21	4.644	14	40.75	22.153	67
Fe, mg/kg DM	578	597.2	120	647	307.4	151	622	597.6	14	293	282.5	67
Mn, mg/kg DM	69	37.7	122	51	12.0	153	34	14.0	14	27	14.3	67
Zn, mg/kg DM	29	12.2	121	16	4.9	153	48	17.3	14	32	10.8	67
Mo, mg/kg DM	2.43	3.857	68	1.29	0.572	35	5.30	1.418	10	1.83	1.337	12

Name	Peanuts			Peas			Pineapple Cannery Waste			Potato By-Product Meal		
Feed ID Code	NRC16F113			NRC16F114			NRC16F115			NRC16F116		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	94.1	2.44	38	88.9	1.70	226	23.4	8.09	39	23.0	7.72	234
Ash, % DM	3.0	0.43	9	3.6	1.01	65	6.1	2.19	37	5.6	2.86	232
CP, % DM	25.6	3.95	38	24.3	2.31	232	7.0	1.68	58	10.0	3.25	238
A fraction, % of CP[b]	26			57			42			5		
B fraction, % of CP[b]	73			42			53			90		
C fraction, % of CP[b]	0			1			5			5		
Kd of B, %/h[b]	9.3			16.0			7.4			2.0		
RUP, % CP[c]	29			15			30			57		
dRUP, % of RUP[d]	90			89			80			80		
Soluble protein, % CP	35.1	24.14	16	75.1	8.03	76	45.5	12.78	51	39.1	20.62	104
ADIP, % DM[e]	1.17			1.02			0.93			0.94	0.551	79
NDIP, % DM[f]	3.38			3.65			1.66			1.63	1.080	79
ADF, % DM	17.3	12.49	37	7.9	2.57	209	36.4	5.47	58	10.7	6.90	196
NDF, % DM	24.0	13.92	38	12.2	3.68	231	62.4	7.37	58	14.4	9.88	232
IVNDFD48, % of NDF[g]				71.6	19.19	5	65.0	0.00	1			
Lignin, % DM	5.85	5.479	8	0.95	0.531	32	6.24	4.349	36	3.00	2.505	112
Starch, % DM	2.8	0.92	9	43.0	5.74	65	3.4	7.34	32	57.5	15.33	172
WSC, % DM[h]				8.3	1.19	12	8.2	7.41	19	7.1	0.00	1
TFAs, % DM	41.24			1.14			0.94			1.78		
Crude fat, % DM	42.24	8.079	23	2.08	1.426	97	1.94	0.845	37	2.78	2.818	237
DE base, Mcal/kg[i]	4.60			3.67			2.42			3.16		
Ca, % DM	0.09	0.059	24	0.11	0.059	143	0.43	0.286	55	0.17	0.139	175
P, % DM	0.38	0.071	24	0.43	0.091	147	0.15	0.046	55	0.25	0.065	194
Mg, % DM	0.20	0.035	24	0.14	0.028	148	0.14	0.101	55	0.11	0.048	197
K, % DM	0.68	0.082	24	1.10	0.203	148	1.56	0.648	53	1.39	0.787	205
Na, % DM	0.40	1.193	25	0.01	0.006	129	0.02	0.044	52	0.12	0.147	176
Cl, % DM	0.41	0.523	2	0.12	0.027	21	0.53	0.239	29	0.29	0.218	71
S, % DM	0.20	0.029	18	0.19	0.029	78	0.14	0.048	51	0.15	0.056	86
Cu, mg/kg DM	6.91	1.505	23	8.47	1.717	131	9.84	3.537	55	6.93	2.842	165
Fe, mg/kg DM	75	66.4	23	119	118.6	129	799	844.4	54	348	249.9	152
Mn, mg/kg DM	21	7.6	24	17	8.9	129	123	85.0	55	17	8.7	158
Zn, mg/kg DM	33	8.2	24	38	8.0	131	19	10.7	53	22	8.8	166
Mo, mg/kg DM	2.70	2.179	20	3.31	2.828	120	1.00	0.000	16			

TABLE 19-1   Continued

Name	Poultry By-Product Meal			Rice, Grain			Rice Bran			Rice Bran, Defatted		
Feed ID Code	NRC16F117			NRC16F123			NRC16F118			NRC16F119		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	95.5	2.63	266	87.9	1.43	77	92.4	1.70	338	89.5	1.75	44
Ash, % DM	14.2	3.21	266	1.8	1.62	66	10.0	2.76	194	12.3	4.57	31
CP, % DM	65.6	13.49	265	8.1	1.08	76	14.8	1.55	344	18.5	2.40	42
A fraction, % of CP[b]	5			31			31			31		
B fraction, % of CP[b]	91			54			45			45		
C fraction, % of CP[b]	5			15			24			24		
Kd of B, %/h[b]	2.0			19.1			3.4			3.4		
RUP, % CP[c]	*71*			*29*			*53*			*53*		
dRUP, % of RUP[d]	90			88			85			85		
Soluble protein, % CP	28.2	8.39	53	17.9	8.57	73	30.5	13.03	105	18.2	6.80	27
ADIP, % DM[e]	3.89		1	0.41			0.78	0.698	6	1.08		
NDIP, % DM[f]	30.34			1.12			2.18	1.139	3	3.54		
ADF, % DM				4.6	4.74	76	13.8	4.69	330	12.7	2.93	44
NDF, % DM	0.0	0.00	0.00	6.4	5.69	76	23.1	6.12	338	25.9	7.30	44
IVNDFD48, % of NDF[g]							23.3	14.01	3			
Lignin, % DM	0.00	0.00	0.00	1.55	1.403	70	4.94	1.785	111	4.46	1.757	17
Starch, % DM	0.0	0.00	0.00	75.8	7.65	75	22.2	9.13	155	21.5	12.60	19
WSC, % DM[h]	0.0	0.00	0.00	1.4	1.38	16	8.0	2.81	83	9.7	3.65	6
TFAs, % DM	11.78			1.24			12.00			2.16		
Crude fat, % DM	12.78	3.166	266	2.00	1.060	68	18.50	4.260	345	3.16	1.334	44
DE base, Mcal/kg[i]	*4.25*			*3.48*			*3.22*			*2.85*		
Ca, % DM	4.31	1.432	263	0.02	0.022	71	0.78	1.105	237	0.83	0.885	33
P, % DM	2.48	0.812	263	0.26	0.116	72	1.77	0.431	240	2.48	0.875	31
Mg, % DM	0.16	0.019	71	0.10	0.050	72	0.78	0.192	230	1.08	0.340	31
K, % DM	0.89	0.147	72	0.25	0.118	72	1.40	0.347	230	1.81	0.514	31
Na, % DM	0.38	0.054	72	0.01	0.005	71	0.02	0.041	219	0.02	0.007	33
Cl, % DM	0.55	0.131	5	0.07	0.039	59	0.13	0.130	44	0.08	0.032	12
S, % DM	0.74	0.058	52	0.10	0.015	71	0.17	0.022	125	0.20	0.043	27
Cu, mg/kg DM	14.14	9.257	72	3.96	3.505	67	8.64	5.783	221	5.67	3.585	33
Fe, mg/kg DM	233	84.3	71	83	181.2	70	216	194.9	223	178	91.5	33
Mn, mg/kg DM	45	22.3	71	37	28.6	73	183	62.5	226	232	90.8	33
Zn, mg/kg DM	122	36.4	46	21	5.7	72	61	13.5	202	76	20.7	32
Mo, mg/kg DM	1.20	0.401	46	1.00	0.000	40	1.14	0.347	158	1.28	0.455	29

Name	Rice Hulls			Rice Silage, Headed			Rice Silage, Vegetative			Rumen-Protected Lysine		
Feed ID Code	NRC16F120			NRC16F121			NRC16F122			NRC16F1002		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	91.0	2.59	17	41.1	8.61	59	42.9	17.30	78	98.0		
Ash, % DM	17.4	4.18	16	12.5	2.86	59	16.2	4.28	78			
CP, % DM	3.7	1.78	24	7.1	1.06	59	8.3	2.16	78	75.0		
A fraction, % of CP[b]	27			62			62			25		
B fraction, % of CP[b]	69			29			29			0		
C fraction, % of CP[b]	4			9			9			75		
Kd of B, %/h[b]	6.4			10.0			10.0					
RUP, % CP[c]	*37*			*22*			*22*			*78*		
dRUP, % of RUP[d]	38			72			72			98		
Soluble protein, % CP	16.0	6.36	9	45.4	13.74	59	41.8	12.11	78			
ADIP, % DM[e]	6.47	1.458	2	0.69			0.80					
NDIP, % DM[f]	8.13	0.530	2	1.85			2.15					
ADF, % DM	65.0	8.82	17	31.8	5.20	58	43.6	4.65	78			
NDF, % DM	75.1	6.50	15	41.9	6.73	59	61.5	5.18	78			
IVNDFD48, % of NDF[g]												
Lignin, % DM	16.62	2.730	3	4.67	1.312	59	4.66	1.288	78			
Starch, % DM	7.6	0.06	3	32.2	8.48	59	7.6	3.99	78			

TABLE 19-1 Continued

Name	Rice Hulls			Rice Silage, Headed			Rice Silage, Vegetative			Rumen-Protected Lysine		
Feed ID Code	NRC16F120			NRC16F121			NRC16F122			NRC16F1002		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
WSC, % DM[h]							1.8	1.62	3			
TFAs, % DM	0.27			1.62			1.47					
Crude fat, % DM	1.27	0.638	10	2.86	0.605	58	2.59	0.661	78			
DE base, Mcal/kg[i]	1.17			2.49			2.18					
Ca, % DM	0.24	0.466	25	0.21	0.063	59	0.27	0.078	77			
P, % DM	0.12	0.157	25	0.23	0.043	59	0.25	0.063	77			
Mg, % DM	0.09	0.126	25	0.14	0.023	58	0.17	0.039	77			
K, % DM	0.44	0.360	25	1.17	0.323	58	1.91	0.396	77			
Na, % DM	0.07	0.157	12	0.02	0.014	57	0.02	0.024	77			
Cl, % DM	0.10	0.022	4	0.34	0.103	32	0.53	0.143	19			
S, % DM	0.09	0.128	17	0.11	0.026	59	0.15	0.052	77			
Cu, mg/kg DM	57.36	179.958	14	11.77	5.268	57	11.92	5.721	77			
Fe, mg/kg DM	389	367.6	14	408	230.3	56	523	325.8	77			
Mn, mg/kg DM	281	360.6	14	508	242.0	57	556	276.1	77			
Zn, mg/kg DM	340	1160.7	14	36	8.0	58	39	10.7	78			
Mo, mg/kg DM				1.46	0.836	46	1.81	0.973	72			

Name	Rumen-Protected Methionine			Rye Annual Fresh, Immature			Rye Annual Fresh, Mid-Maturity			Rye Annual Hay, Immature		
Feed ID Code	NRC16F1001			NRC16F124			NRC16F125			NRC16F126		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	98.0			15.8	3.27	240	19.5	3.41	64	90.0	1.76	2,416
Ash, % DM				10.9	1.02	240	9.7	0.97	64	11.1	1.62	2,413
CP, % DM	75.0			27.5	2.87	239	20.5	2.63	64	22.9	6.22	2,417
A fraction, % of CP[b]	25			57			57			57		
B fraction, % of CP[b]	0			33			33			33		
C fraction, % of CP[b]	75			10			10			10		
Kd of B, %/h[b]				6.0			6.0			6.0		
RUP, % CP[c]	78			28			28			28		
dRUP, % of RUP[d]	98			65			65			65		
Soluble protein, % CP				25.4	5.38	237	28.6	5.70	62	39.2	12.57	2,373
ADIP, % DM[e]				1.42			1.06			1.69		
NDIP, % DM[f]				6.84			5.09			5.69		
ADF, % DM				24.8	2.60	240	28.2	2.20	64	27.2	5.07	2,417
NDF, % DM				42.9	4.04	240	49.1	3.63	64	47.0	6.35	2,415
IVNDFD48, % of NDF[g]				83.6	10.71	2				85.5	6.98	209
Lignin, % DM				3.14	0.810	239	3.70	0.628	64	3.44	1.190	2,409
Starch, % DM				4.9	1.37	4	4.9			2.0	1.47	2,398
WSC, % DM[h]				5.8	0.70	2	7.7	0.06	2	12.8	5.26	2,075
TFAs, % DM				3.07	0.563	233	2.44	0.379	64	2.53		
Crude fat, % DM				5.03	0.408	240	4.37	0.439	64	4.46	0.952	2,397
DE base, Mcal/kg[i]				2.94			2.78			2.80		
Ca, % DM				0.48	0.173	240	0.51	0.169	64	0.59	0.176	2,404
P, % DM				0.49	0.051	239	0.39	0.040	63	0.40	0.082	2,403
Mg, % DM				0.29	0.039	239	0.27	0.044	64	0.26	0.074	2,401
K, % DM				3.17	0.494	240	2.64	0.396	64	3.17	0.702	2,407
Na, % DM										0.51	0.425	855
Cl, % DM										1.46	0.612	2,316
S, % DM				0.40	0.048	240	0.30	0.037	64	0.29	0.068	2,367
Cu, mg/kg DM										9.43	3.255	840
Fe, mg/kg DM										406	347.7	844
Mn, mg/kg DM										113	63.5	852
Zn, mg/kg DM										37	15.0	751
Mo, mg/kg DM										1.49	1.361	498

TABLE 19-1   Continued

Name	Rye Annual Hay, Mature			Rye Annual Hay, Mid-Maturity			Rye Annual Silage, Immature			Rye Annual Silage, Mature		
Feed ID Code	NRC16F127			NRC16F131			NRC16F128			NRC16F129		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	92.7	1.36	460	90.3	3.45	2,532	34.7	9.46	4,819	67.2	7.34	232
Ash, % DM	6.3	1.28	459	9.3	2.00	2,359	11.0	2.69	4,838	8.7	1.87	232
CP, % DM	7.6	2.30	459	12.0	4.26	2,533	16.4	2.91	4,833	8.3	2.22	232
A fraction, % of CP[b]	57			57			57			57		
B fraction, % of CP[b]	33			33			33			33		
C fraction, % of CP[b]	10			10			10			10		
Kd of B, %/h[b]	6.0			5.9			5.9			5.9		
RUP, % CP[c]	*28*			*28*			*29*			*29*		
dRUP, % of RUP[d]	65			65			65			65		
Soluble protein, % CP	36.0	6.92	427	41.0	11.29	2,451	65.7	14.16	4,840	46.8	9.13	231
ADIP, % DM[e]	0.56			1.09	0.275	731	1.06	0.295	4,813	0.70		
NDIP, % DM[f]	1.89			2.50	1.104	731	2.11	1.062	4,803	1.17		
ADF, % DM	42.7	4.12	460	36.7	5.43	2,534	33.1	3.84	4837	42.9	3.39	232
NDF, % DM	66.8	5.33	460	57.3	7.59	2,532	50.5	4.80	4833	66.2	3.76	232
IVNDFD48, % of NDF[g]	45.9	8.72	33	59.8	13.10	64	34.0	0.00	1	66.3	4.44	29
Lignin, % DM	6.21	1.156	460	4.84	1.377	2,365	3.76	0.690	4828	5.71	0.933	232
Starch, % DM	2.0	1.58	449	2.2	1.56	2,189	1.7	1.06	4614	1.4	1.41	231
WSC, % DM[h]	12.7	5.21	430	14.0	6.39	1,396				12.3	5.11	219
TFAs, % DM	1.01			1.47	0.626	699	1.95	0.372	4499	1.25		
Crude fat, % DM	1.77	0.604	457	2.89	0.944	2,359	4.10	0.527	4,819	2.20	0.493	231
DE base, Mcal/kg[i]	*2.36*			*2.50*			*2.64*			*2.32*		
Ca, % DM	0.37	0.114	455	0.51	0.213	1,955	0.57	0.194	266	0.36	0.104	232
P, % DM	0.18	0.059	459	0.28	0.073	1,963	0.42	0.110	269	0.25	0.060	232
Mg, % DM	0.16	0.047	459	0.20	0.062	1,959	0.23	0.054	270	0.16	0.049	232
K, % DM	1.42	0.464	459	2.31	0.645	1,965	3.63	1.035	270	2.07	0.502	232
Na, % DM	0.13	0.151	151	0.26	0.237	557	0.16	0.161	266	0.07	0.091	123
Cl, % DM	0.69	0.380	403	1.09	0.521	1,526	1.14	0.706	255	0.85	0.299	222
S, % DM	0.13	0.040	429	0.17	0.058	1,899	0.25	0.062	258	0.15	0.037	231
Cu, mg/kg DM	5.67	2.427	153	8.14	3.309	951	10.39	3.841	1,610	6.68	2.766	110
Fe, mg/kg DM	175	141.8	150	365	343.3	946	709	723.1	1,614	396	454.6	110
Mn, mg/kg DM	115	65.0	151	90	52.6	950	72	34.4	1,611	89	60.8	110
Zn, mg/kg DM	25	12.6	150	30	11.5	943	36	10.3	1,611	27	9.4	110
Mo, mg/kg DM	1.24	0.468	59	1.42	0.729	238				1.59	1.276	79

Name	Rye Annual Silage, Mid-Maturity			Rye Grain			Safflower Meal			Sorghum Forage, Silage, Immature		
Feed ID Code	NRC16F130			NRC16F132			NRC16F133			NRC16F135		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	34.9	10.48	11,836	86.0	2.41	27	94.0	3.00	47	29.2	6.53	1,474
Ash, % DM	10.3	2.14	11,821	2.6	0.44	10	4.9	0.70	33	10.7	2.39	1,479
CP, % DM	14.4	3.53	11,850	11.8	1.62	28	26.2	4.18	47	11.7	1.83	1,479
A fraction, % of CP[b]	57			31			23			58		
B fraction, % of CP[b]	33			54			71			24		
C fraction, % of CP[b]	10			15			6			16		
Kd of B, %/h[b]	5.9			19.1			10.4			4.0		
RUP, % CP[c]	*29*			*29*			*31*			*33*		
dRUP, % of RUP[d]	65			88			75			70		
Soluble protein, % CP	61.7	11.91	11,817	33.0	5.31	15	31.2	11.79	20	51.1	9.00	1,473
ADIP, % DM[e]	1.23	0.317	2,956	0.55	0.118	6	1.52			8.26	1.611	525
NDIP, % DM[f]	2.05	0.827	2,968	1.50	0.243	6	2.10			20.15	5.668	520
ADF, % DM	38.3	4.91	11,835	5.4	1.43	24	40.1	3.98	35	36.4	2.92	1,480
NDF, % DM	58.0	6.67	11,848	16.0	3.53	26	55.4	4.36	35	56.7	3.40	1,478
IVNDFD48, % of NDF[g]	61.9	6.99	1,751				25.5	0.71	2	58.5	6.47	151
Lignin, % DM	4.93	1.249	11,845	1.55	0.740	6	13.79	1.774	13	4.92	0.918	1,477

TABLE 19-1   Continued

Name	Rye Annual Silage, Mid-Maturity			Rye Grain			Safflower Meal			Sorghum Forage, Silage, Immature		
Feed ID Code	NRC16F130			NRC16F132			NRC16F133			NRC16F135		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Starch, % DM	1.5	1.17	11,387	57.6	6.06	23	1.2	0.88	10	8.1	1.70	1,476
WSC, % DM[h]	8.8	4.60	5,327				5.1	1.07	5	4.3	3.42	880
TFAs, % DM	1.68	0.409	2,818	1.45			3.88			1.74	0.486	491
Crude fat, % DM	3.88	0.723	11,782	2.15	0.536	10	5.35	3.825	47	3.06	0.582	1,465
DE base, Mcal/kg[i]	2.48			3.41			2.51			2.45		
Ca, % DM	0.48	0.185	8,970	0.25	0.362	19	0.32	0.050	21	0.43	0.115	969
P, % DM	0.37	0.070	9,025	0.38	0.065	19	0.65	0.149	21	0.25	0.047	977
Mg, % DM	0.19	0.048	9,016	0.17	0.062	19	0.34	0.070	21	0.24	0.057	970
K, % DM	2.91	0.682	9,034	0.97	0.813	19	1.03	0.135	20	1.91	0.423	975
Na, % DM	0.15	0.187	1,562	0.01	0.005	2	0.03	0.034	20	0.03	0.029	135
Cl, % DM	0.92	0.417	5,460	0.05	0.000	1	0.23	0.043	4	0.70	0.270	912
S, % DM	0.21	0.048	9,009	0.14	0.000	1	0.25	0.044	9	0.15	0.027	981
Cu, mg/kg DM	9.53	3.743	2,366	5.00	1.732	3	21.67	4.004	21	11.83	3.687	265
Fe, mg/kg DM	493	411.6	2,363	52	8.5	3	240	100.3	20	891	612.7	265
Mn, mg/kg DM	75	42.0	2,371	40	14.6	3	29	6.9	20	67	28.8	267
Zn, mg/kg DM	34	11.3	2,385	38	5.3	3	66	21.2	18	44	14.8	266
Mo, mg/kg DM	1.57	0.964	922				1.00	0.000	14	1.70	0.864	87

Name	Sorghum Forage, Silage, Mature			Sorghum Grain, Ground			Sorghum Grain, Steam-Flaked			Sorghum Hay		
Feed ID Code	NRC16F136			NRC16F137			NRC16F1073			NRC16F138		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	28.7	7.33	3,894	88.9	1.14	1,000	88.9	1.14	1000	91.4	1.45	2,034
Ash, % DM	9.9	2.77	3,910	2.5	0.34	882	2.5	0.34	882	8.5	2.68	2,041
CP, % DM	11.3	2.65	3,913	12.5	1.13	999	12.5	1.13	999	10.2	3.71	2,042
A fraction, % of CP[b]	49			24			24			28		
B fraction, % of CP[b]	28			56			55			53		
C fraction, % of CP[b]	24			20			20			19		
Kd of B, %/h[b]	3.0			5.0			5.0			5.0		
RUP, % CP[c]	44			50			50			47		
dRUP, % of RUP[d]	70			69			69			60		
Soluble protein, % CP	51.0	8.99	3,904	20.2	4.91	908	20.2	4.91	908	41.4	10.28	2,007
ADIP, % DM[e]	8.31	1.710	1,428	0.83	0.406	19	0.83	0.406	19	0.95		
NDIP, % DM[f]	16.17	4.374	1,432	1.60	0.456	21	1.60	0.456	21	3.18		
ADF, % DM	39.2	3.50	3,905	3.9	1.06	956	3.9	1.06	956	38.8	5.00	2,041
NDF, % DM	61.6	4.27	3,911	6.1	1.90	956	6.1	1.90	956	63.0	5.93	2,036
IVNDFD48, % of NDF[g]	63.6	6.67	416							60.8	8.71	1,165
Lignin, % DM	5.15	1.125	3,895	1.09	0.364	868	1.09	0.364	868	5.00	1.465	2,034
Starch, % DM	2.7	1.94	3,899	72.6	2.35	994	72.6	2.35	994	3.2	2.54	2,033
WSC, % DM[h]	5.3	3.38	2,180	2.2	0.50	17	2.2	0.50	17	10.3	5.26	1,894
TFAs, % DM	1.44	0.382	1,265	2.92			2.92			1.24		
Crude fat, % DM	3.07	0.653	3,893	4.09	0.358	910	4.09	0.358	910	2.18	0.609	2,026
DE base, Mcal/kg[i]	2.38			3.29			3.62			2.39		
Ca, % DM	0.49	0.139	2,529	0.10	0.100	922	0.10	0.100	922	0.39	0.130	2,027
P, % DM	0.29	0.066	2,572	0.38	0.039	919	0.38	0.039	919	0.22	0.077	2,038
Mg, % DM	0.27	0.064	2,529	0.15	0.015	923	0.15	0.015	923	0.29	0.089	2,019
K, % DM	2.47	0.673	2,567	0.41	0.181	929	0.41	0.181	929	2.06	0.760	2,035
Na, % DM	0.02	0.031	452	0.03	0.093	101	0.03	0.093	101	0.04	0.061	210
Cl, % DM	0.83	0.353	2,257	0.17	0.264	57	0.17	0.264	57	0.94	0.382	1,898
S, % DM	0.17	0.039	2,569	0.12	0.009	861	0.12	0.009	861	0.14	0.056	2,007
Cu, mg/kg DM	9.88	3.399	857	8.74	16.495	100	8.74	16.495	100	8.63	4.360	222
Fe, mg/kg DM	516	434.4	857	71	39.3	100	71	39.3	100	381	414.1	221
Mn, mg/kg DM	60	28.3	860	27	28.0	100	27	28.0	100	44	26.4	216
Zn, mg/kg DM	38	12.1	869	23	5.2	88	23	5.2	88	37	19.0	205
Mo, mg/kg DM	1.44	0.721	236	1.09	0.282	47	1.09	0.282	47	1.18	0.425	119

continued

TABLE 19-1 Continued

Name	Sorghum Soybean Silage			Sorghum–Sudangrass Hay			Sorghum–Sudangrass Silage			Soybean Hay		
Feed ID Code	NRC16F139			NRC16F142			NRC16F140			NRC16F143		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	32.3	11.35	34	90.5	3.72	1,991	31.7	9.44	7,137	91.6	2.65	390
Ash, % DM	9.2	1.92	34	10.0	2.78	2,026	10.9	3.07	7,165	9.0	1.88	389
CP, % DM	11.7	3.59	34	9.8	3.22	2,028	12.3	3.25	7,197	20.1	5.38	389
A fraction, % of CP[b]	60			28			38			45		
B fraction, % of CP[b]	24			53			30			45		
C fraction, % of CP[b]	16			19			32			9		
Kd of B, %/h[b]	4.1			5.0			3.7			17.8		
RUP, % CP[c]	*33*			*47*			*51*			*22*		
dRUP, % of RUP[d]	70			60			55			65		
Soluble protein, % CP	50.9	10.85	34	38.6	9.61	2,028	47.3	10.00	7,170	36.9	9.11	389
ADIP, % DM[e]	1.14			1.05	0.327	41	1.15	0.226	2,940	1.97		
NDIP, % DM[f]	2.63			2.57	0.686	41	1.90	0.562	1,506	3.62		
ADF, % DM	35.2	5.04	34	39.3	5.38	2,032	38.9	3.88	7,194	31.3	5.04	388
NDF, % DM	54.4	8.41	34	61.0	6.68	2,033	59.5	5.16	7,187	40.3	7.37	389
IVNDFD48, % of NDF[g]	54.0	0.00	1	54.4	8.58	1,549	54.9	7.91	2,201	50.5	9.44	37
Lignin, % DM	5.80	1.410	34	4.94	1.407	2,033	5.25	1.254	7,187	7.23	1.305	390
Starch, % DM	9.9	8.05	34	3.7	3.02	1,919	2.8	2.83	7,077	5.7	2.94	388
WSC, % DM[h]	4.2	3.47	33	10.4	4.18	947	5.6	3.39	1,785	7.8	3.14	376
TFAs, % DM	1.81			1.10	0.462	87	1.63	0.432	1,440	1.91		
Crude fat, % DM	3.19	0.660	34	1.93	0.590	2,023	3.01	0.606	7,177	3.36	1.440	388
DE base, Mcal/kg[i]	*2.48*			*2.34*			*2.31*			*2.68*		
Ca, % DM	0.62	0.339	33	0.37	0.128	1,994	0.52	0.184	5,714	1.41	0.266	389
P, % DM	0.27	0.061	34	0.24	0.066	2,026	0.30	0.071	5,756	0.28	0.078	389
Mg, % DM	0.28	0.089	34	0.25	0.066	2,007	0.26	0.070	5,712	0.42	0.159	388
K, % DM	2.08	0.601	34	2.00	0.591	2,023	2.46	0.742	5,759	1.85	0.579	389
Na, % DM	0.03	0.035	9	0.03	0.027	87	0.06	0.063	759	0.01	0.011	182
Cl, % DM	0.78	0.357	31	0.98	0.410	724	0.81	0.378	2,031	0.40	0.225	385
S, % DM	0.16	0.053	34	0.14	0.047	2,025	0.16	0.043	5,757	0.24	0.056	388
Cu, mg/kg DM	12.00	3.937	9	9.39	2.621	60	11.42	4.194	769	8.76	1.694	181
Fe, mg/kg DM	584	608.0	9	359	282.1	60	899	886.6	771	419	744.2	178
Mn, mg/kg DM	40	13.8	9	62	31.6	60	77	43.1	773	71	29.0	178
Zn, mg/kg DM	34	10.6	9	39	13.7	59	39	12.6	779	36	17.9	165
Mo, mg/kg DM	1.00	0.000	7	1.45	0.856	51	1.60	0.881	218	1.67	1.282	87

Name	Soybean Hulls			Soybean Meal, Expellers			Soybean Meal, Extruded			Soybean Meal, Solvent Extracted, 48% CP		
Feed ID Code	NRC16F144			NRC16F145			NRC16F146			NRC16F134		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	90.4	1.90	1,169	91.2	2.13	811	93.4	2.06	114	89.3	1.12	1,169
Ash, % DM	5.2	0.69	690	6.7	0.64	483	5.7	0.42	68	7.2	0.55	1,139
CP, % DM	11.9	1.43	1226	47.6	2.15	872	40.4	2.16	114	52.6	1.69	1,400
A fraction, % of CP[b]	27			9			18			18		
B fraction, % of CP[b]	70			91			80			79		
C fraction, % of CP[b]	4			0			2			2		
Kd of B, %/h[b]	6.4			2.4			8.7			9.0		
RUP, % CP[c]	*37*			*63*			*45*			*33*		
dRUP, % of RUP[d]	68			93			91			91		
Soluble protein, % CP	26.3	6.38	478	14.7	8.61	457	17.4	9.70	69	23.1	8.16	788
ADIP, % DM[e]	1.15	0.245	94	0.91	0.520	144	1.40	1.185	16	0.63	0.216	146
NDIP, % DM[f]	3.65	0.569	90	3.61	2.348	146	2.12	2.086	16	1.00	0.580	144
ADF, % DM	47.9	3.07	996	10.1	2.09	744	10.6	2.67	90	7.2	1.58	1,119
NDF, % DM	66.7	3.63	1,046	19.6	4.20	793	18.4	3.47	88	11.1	3.07	1,220
IVNDFD48, % of NDF[g]	88.1	7.26	9							85.7	11.37	3
Lignin, % DM	2.57	0.752	222	2.06	1.090	261	2.15	0.835	49	1.08	0.520	560
Starch, % DM	1.0	0.70	197	1.8	0.93	318	1.5	1.07	42	1.9	1.05	403

TABLE 19-1 Continued

Name	Soybean Hulls			Soybean Meal, Expellers			Soybean Meal, Extruded			Soybean Meal, Solvent Extracted, 48% CP		
Feed ID Code	NRC16F144			NRC16F145			NRC16F146			NRC16F134		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
WSC, % DM[h]	2.7	0.91	53	11.8	1.24	99	9.3	1.79	17	13.0	2.07	118
TFAs, % DM	1.61			6.12			15.08			1.08	0.000	1
Crude fat, % DM	1.89	0.862	618	7.12	2.368	872	20.42	2.144	113	1.82	0.870	1,387
DE base, Mcal/kg[i]	*2.70*			*3.90*			*4.16*			*3.99*		
Ca, % DM	0.64	0.075	955	0.34	0.073	515	0.27	0.046	79	0.40	0.099	983
P, % DM	0.13	0.047	955	0.72	0.066	519	0.63	0.073	79	0.74	0.069	985
Mg, % DM	0.28	0.032	896	0.31	0.033	492	0.25	0.019	75	0.33	0.032	947
K, % DM	1.40	0.147	899	2.24	0.210	494	1.90	0.124	76	2.42	0.209	951
Na, % DM	0.01	0.010	755	0.01	0.014	363	0.01	0.008	63	0.02	0.018	626
Cl, % DM	0.04	0.029	169	0.06	0.042	223	0.07	0.036	35	0.06	0.038	364
S, % DM	0.12	0.025	446	0.40	0.040	407	0.33	0.025	60	0.41	0.033	829
Cu, mg/kg DM	7.58	1.770	775	15.12	2.620	377	13.20	3.292	70	16.06	2.103	657
Fe, mg/kg DM	464	92.4	770	196	100.8	374	119	37.3	69	187	112.8	654
Mn, mg/kg DM	21	6.6	773	39	9.6	379	29	6.1	70	41	7.9	659
Zn, mg/kg DM	47	8.1	767	53	7.7	372	44	15.8	70	53	7.6	657
Mo, mg/kg DM	1.05	0.221	118	2.72	1.268	189	2.85	1.318	47	4.32	2.086	460

Name	Soybean Silage			Soybeans, Whole Raw			Soybeans, Whole Roasted			Spelt Grain		
Feed ID Code	NRC16F147			NRC16F148			NRC16F149			NRC16F150		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	37.5	9.79	672	89.1	2.84	180	94.0	2.23	972	87.4	2.23	17
Ash, % DM	10.1	2.59	671	5.3	0.47	103	5.6	0.54	533	4.8	2.73	17
CP, % DM	18.0	3.27	673	40.0	2.12	216	40.0	2.07	1,001	12.9	3.32	17
A fraction, % of CP[b]	57			26			18			31		
B fraction, % of CP[b]	35			74			77			54		
C fraction, % of CP[b]	7			0			5			15		
Kd of B, %/h[b]	12.2			9.3			9.3			19.0		
RUP, % CP[c]	*21*			*25*			*29*			*29*		
dRUP, % of RUP[d]	65			90			87			88		
Soluble protein, % CP	49.0	10.26	673	42.1	29.94	35	15.6	7.71	457	28.8	7.15	15
ADIP, % DM[e]	1.92	0.507	207	0.66	0.189	22	0.97	0.789	111	0.87	0.518	12
NDIP, % DM[f]	2.88	1.082	207	1.66	1.015	22	2.79	1.975	98	1.50	1.063	12
ADF, % DM	35.6	4.76	673	7.0	1.65	43	10.1	2.80	714	21.3	13.02	17
NDF, % DM	45.3	6.59	673	11.9	3.36	62	18.4	4.44	733	39.3	16.90	17
IVNDFD48, % of NDF[g]	47.7	7.43	38				84.3	9.95	4			
Lignin, % DM	7.81	1.482	673	1.52	1.745	28	1.80	0.841	248	3.44	0.960	13
Starch, % DM	4.5	3.20	652	4.2	1.84	10	1.5	1.16	159	41.3	18.45	17
WSC, % DM[h]	4.3	2.29	314				9.8	1.61	26			
TFAs, % DM	2.86	1.421	153	16.99			15.35			1.66		1
Crude fat, % DM	4.26	1.718	668	20.73	1.664	212	21.26	1.907	1,004	2.44	0.877	15
DE base, Mcal/kg[i]	*2.56*			*4.33*			*4.16*			*3.00*		
Ca, % DM	1.33	0.325	475	0.27	0.078	87	0.28	0.118	623	0.09	0.033	5
P, % DM	0.32	0.087	476	0.65	0.070	87	0.63	0.100	628	0.38	0.139	5
Mg, % DM	0.37	0.099	474	0.27	0.027	85	0.26	0.030	586	0.26	0.258	5
K, % DM	1.99	0.597	476	2.03	0.153	86	1.90	0.201	585	0.51	0.095	5
Na, % DM	0.02	0.032	55	0.01	0.006	70	0.02	0.041	441	0.03	0.030	5
Cl, % DM	0.47	0.344	298	0.04	0.018	10	0.07	0.034	125	0.08		1
S, % DM	0.23	0.047	471	0.34	0.028	19	0.32	0.034	384	0.14		1
Cu, mg/kg DM	11.44	3.331	133	11.86	4.358	74	13.66	2.707	461	8.13	6.034	8
Fe, mg/kg DM	664	566.7	128	103	28.1	72	131	72.3	458	166	98.3	8
Mn, mg/kg DM	79	39.3	132	26	7.7	73	31	8.7	457	63	26.2	8
Zn, mg/kg DM	40	12.5	134	51	7.2	74	46	8.8	459	54	33.7	8
Mo, mg/kg DM	1.57	0.935	30				3.00	2.285	239			

continued

TABLE 19-1   Continued

Name	Sudangrass Hay, Mature			Sudangrass Hay, Mid-Maturity			Sudangrass Silage, Mature			Sudangrass Silage, Mid-Maturity		
Feed ID Code	NRC16F151			NRC16F152			NRC16F153			NRC16F154		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	93.1	1.61	3,621	83.1	4.16	10	31.5	9.44	280	31.5	10.68	1,605
Ash, % DM	9.6	1.44	3,573	15.0	0.01	2	10.6	2.29	281	12.3	3.57	1,610
CP, % DM	8.1	2.16	3,626	14.7	2.73	10	9.5	1.85	282	13.4	3.09	1,618
A fraction, % of CP[b]	28			28			38			38		
B fraction, % of CP[b]	53			53			30			30		
C fraction, % of CP[b]	19			19			32			32		
Kd of B, %/h[b]	5.0			5.0			3.7			3.7		
RUP, % CP[c]	*47*			*47*			*51*			*51*		
dRUP, % of RUP[d]	60			60			55			55		
Soluble protein, % CP	34.9	5.00	3,605	36.9	2.56	9	49.3	9.19	281	49.9	9.71	1,615
ADIP, % DM[e]	1.38	0.273	102	2.52			1.00			1.40	0.277	713
NDIP, % DM[f]	3.61	1.086	102	6.58			2.25			3.16	0.897	715
ADF, % DM	41.6	2.73	3,606	36.9	2.21	10	43.8	3.50	282	39.0	4.20	1,619
NDF, % DM	65.8	3.30	3,626	54.6	3.70	10	66.6	3.96	282	60.7	4.73	1,617
IVNDFD48, % of NDF[g]	55.8	6.36	110				61.0	5.31	18	64.6	5.44	47
Lignin, % DM	5.08	1.004	3,579	5.92	0.191	2	6.34	1.336	282	4.99	1.079	1,618
Starch, % DM	1.5	0.99	3,547	2.0	0.91	3	2.1	1.57	275	1.8	1.37	1,527
WSC, % DM[h]	9.9	2.39	3,365				4.0	2.79	269	5.2	3.32	868
TFAs, % DM	0.99	0.371	83	1.05	0.318	2	1.53			1.61	0.462	674
Crude fat, % DM	1.69	0.300	3,567	2.58	0.177	2	2.69	0.554	281	3.24	0.656	1,610
DE base, Mcal/kg[i]	*2.29*			*2.18*			*2.16*			*2.27*		
Ca, % DM	0.45	0.086	3,533	1.25	1.203	10	0.45	0.116	281	0.52	0.146	934
P, % DM	0.21	0.041	3,521	0.35	0.128	10	0.27	0.060	280	0.32	0.069	934
Mg, % DM	0.30	0.070	3,524	0.28	0.050	10	0.25	0.065	278	0.28	0.075	931
K, % DM	2.11	0.399	3,527	2.59	0.646	10	2.40	0.556	282	2.85	0.718	938
Na, % DM	0.03	0.032	664	0.03			0.03	0.029	66	0.04	0.062	175
Cl, % DM	1.19	0.335	3,197	1.19			0.85	0.327	265	0.97	0.396	906
S, % DM	0.13	0.030	3,517	0.20	0.072	10	0.16	0.037	279	0.20	0.043	935
Cu, mg/kg DM	8.20	3.111	712	8.20			10.29	3.242	68	12.06	4.158	418
Fe, mg/kg DM	264	288.9	707	264			564	471.5	66	1079	920.3	417
Mn, mg/kg DM	40	12.7	710	40			66	37.7	66	74	39.6	417
Zn, mg/kg DM	32	9.0	709	32			37	12.9	68	43	13.4	414
Mo, mg/kg DM	1.33	0.579	564	1.33			1.29	0.645	49	1.96	1.349	112

Name	Sugarcane Bagasse Hay			Sugarcane Bagasse Silage			Sunflower Meal			Sunflower Seed		
Feed ID Code	NRC16F155			NRC16F156			NRC16F157			NRC16F158		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	93.2	4.21	174	34.6	12.34	115	90.2	1.89	112	92.7	1.88	70
Ash, % DM	6.1	3.39	96	6.2	3.29	44	7.4	0.99	80	3.5	0.92	48
CP, % DM	3.9	2.09	177	5.0	2.30	114	37.0	4.34	112	20.1	3.00	70
A fraction, % of CP[b]	28			38			42			66		
B fraction, % of CP[b]	53			30			53			32		
C fraction, % of CP[b]	19			33			5			2		
Kd of B, %/h[b]	5.0			3.7			29.2			17.0		
RUP, % CP[c]	*47*			*52*			*16*			*14*		
dRUP, % of RUP[d]	60			55			90			80		
Soluble protein, % CP	41.9	13.54	133	52.6	14.76	74	27.3	8.52	69	50.5	14.97	10
ADIP, % DM[e]	1.99			1.78			1.64	0.539	5	0.84		
NDIP, % DM[f]	2.92			1.95			2.36	0.822	2	1.71		
ADF, % DM	62.6	12.49	179	55.0	12.22	114	29.0	5.42	90	24.4	11.43	28
NDF, % DM	76.9	9.12	173	72.0	11.43	115	40.2	5.95	91	35.2	11.96	49
IVNDFD48, % of NDF[g]	32.0	2.83	2	44.0	5.26	7	36.5	12.02	2			
Lignin, % DM	17.69	5.764	100	13.16	6.093	59	9.07	2.114	58	7.21	5.263	8
Starch, % DM	0.8	0.70	79	1.0	0.99	38	1.1	0.95	59	0.6	0.65	14

TABLE 19-1   Continued

Name	Sugarcane Bagasse Hay			Sugarcane Bagasse Silage			Sunflower Meal			Sunflower Seed		
Feed ID Code	NRC16F155			NRC16F156			NRC16F157			NRC16F158		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
WSC, % DM[h]	5.3	3.58	59	9.6	8.76	20	9.2	1.99	17			
TFAs, % DM	0.72			0.91			1.02			37.20		
Crude fat, % DM	1.26	1.031	111	1.60	0.832	47	2.20	1.349	111	39.00	9.621	70
DE base, Mcal/kg[i]	*1.55*			*1.83*			*2.99*			*4.16*		
Ca, % DM	0.34	0.236	139	0.25	0.182	81	0.46	0.143	79	0.20	0.081	19
P, % DM	0.05	0.055	139	0.07	0.069	79	1.13	0.223	81	0.73	0.246	19
Mg, % DM	0.10	0.084	143	0.10	0.063	79	0.60	0.097	80	0.39	0.096	19
K, % DM	0.34	0.308	146	0.80	0.791	82	1.62	0.220	80	0.98	0.330	19
Na, % DM	0.02	0.023	142	0.03	0.037	79	0.04	0.053	68	0.01	0.011	17
Cl, % DM	0.14	0.148	69	0.29	0.471	34	0.15	0.034	42	0.10	0.016	7
S, % DM	0.08	0.056	131	0.14	0.121	74	0.45	0.070	71	0.25	0.049	11
Cu, mg/kg DM	7.14	5.232	143	6.84	3.095	80	32.87	5.208	77	20.11	4.319	19
Fe, mg/kg DM	1535	1390.1	141	821	805.5	79	275	151.2	77	104	81.5	19
Mn, mg/kg DM	73	39.5	139	58	35.7	81	48	10.6	77	30	9.6	19
Zn, mg/kg DM	16	7.8	134	16	5.7	77	84	13.2	50	56	11.2	19
Mo, mg/kg DM	1.12	0.431	26	1.55	0.999	20	1.13	0.345	52	1.00	0.000	13

Name	Sunflower Silage			Sweet Corn Cannery Waste			Tapioca (Cassava)			Tomato Pomace		
Feed ID Code	NRC16F159			NRC16F160			NRC16F161			NRC16F162		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	21.6	6.30	25	22.5	5.18	1,446	87.7	2.00	104	24.7	20.10	4
Ash, % DM	12.6	2.36	35	5.1	1.67	1,264	3.2	1.82	104	5.5	1.90	3
CP, % DM	13.3	2.63	35	9.8	1.31	1,455	2.5	0.98	104	19.3	4.80	22
A fraction, % of CP[b]	42			30			23			42		
B fraction, % of CP[b]	53			68			70			53		
C fraction, % of CP[b]	5			2			7			5		
Kd of B, %/h[b]	29.2			5.0			5.4			7.4		
RUP, % CP[c]	*15*			*39*			*43*			*30*		
dRUP, % of RUP[d]	90			61			73			80		
Soluble protein, % CP	49.2	6.06	35	50.7	11.06	1,394	41.5	17.54	102			
ADIP, % DM[e]	2.44			6.78	2.146	125	0.59			3.80	0.100	2
NDIP, % DM[f]	4.24			10.70	4.217	125	1.10			8.00		1
ADF, % DM	36.6	5.67	35	32.0	4.16	1,451	6.0	2.90	104	47.6	2.80	4
NDF, % DM	45.3	6.39	35	56.3	7.36	1,456	8.1	3.48	105	60.0	5.80	4
IVNDFD48, % of NDF[g]				72.0	5.63	58	11.0		1			
Lignin, % DM	8.20	1.438	35	3.19	0.823	1,177	1.80	1.128	104	13.30	10.800	3
Starch, % DM	1.5	1.79	34	10.2	5.15	1,454	78.9	6.71	105	1.2		
WSC, % DM[h]				4.8	2.60	392	2.3	1.31	10			
TFAs, % DM	3.06			3.81	1.212	738	0.48			12.30		
Crude fat, % DM	5.39	2.658	35	5.08	1.539	1,189	0.70	0.296	99	13.30	4.900	4
DE base, Mcal/kg[i]	*2.42*			*2.82*			*3.27*			*2.63*		
Ca, % DM	1.12	0.225	35	0.24	0.094	1,365	0.18	0.110	104	0.22	0.110	10
P, % DM	0.38	0.066	35	0.27	0.047	1,368	0.09	0.030	104	0.47	0.200	10
Mg, % DM	0.71	0.149	35	0.20	0.046	1,367	0.08	0.027	104	0.28	0.070	9
K, % DM	3.91	0.988	35	1.11	0.332	1,364	0.61	0.328	101	0.98	0.260	9
Na, % DM	0.02	0.011	35	0.02	0.016	253	0.02	0.020	102	0.12	0.230	9
Cl, % DM	1.38	0.292	34	0.32	0.226	430	0.07	0.034	14			
S, % DM	0.24	0.040	35	0.13	0.021	1,289	0.04	0.013	100	0.15	0.060	6
Cu, mg/kg DM	11.15	1.395	34	8.85	3.374	234	3.98	3.083	102	11.00	3.000	9
Fe, mg/kg DM	399	541.5	34	470	394.5	234	521	539.4	103	541	574.0	9
Mn, mg/kg DM	25	11.2	34	33	16.5	233	34	30.1	103	11	3.0	9
Zn, mg/kg DM	34	12.3	25	40	12.3	234	13	6.4	101	54	10.0	9
Mo, mg/kg DM	1.86	2.268	7	1.51	1.755	35	1.00	0.000	17	1.80	0.300	9

TABLE 19-1   Continued

Name	Triticale Grain			Triticale Hay			Triticale Plus Pea Silage			Triticale Silage, Mature		
Feed ID Code	NRC16F163			NRC16F164			NRC16F165			NRC16F166		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	88.4	1.37	316	91.0	1.93	1,085	34.2	9.28	620	30.1	9.80	3,757
Ash, % DM	2.1	0.45	296	8.5	2.41	1,035	10.3	2.22	619	10.1	2.24	3,763
CP, % DM	12.1	2.07	317	10.3	3.79	1,088	16.0	2.58	620	14.2	2.82	3,763
A fraction, % of CP[b]	31			56			56			56		
B fraction, % of CP[b]	54			33			33			33		
C fraction, % of CP[b]	15			11			11			11		
Kd of B, %/h[b]	19.1			5.9			5.9			5.9		
RUP, % CP[c]	39			30			30			30		
dRUP, % of RUP[d]	88			65			65			65		
Soluble protein, % CP	31.3	9.05	302	46.8	10.48	1,081	62.6	9.79	619	62.7	12.60	3,758
ADIP, % DM[e]	0.38			0.51			2.17			0.81		
NDIP, % DM[f]	1.89			1.87			5.70			1.69		
ADF, % DM	4.4	1.00	311	38.3	6.17	1,089	37.1	3.15	620	37.2	3.95	3,763
NDF, % DM	14.1	2.55	315	60.0	7.46	1,086	55.7	4.27	620	58.6	4.42	3,766
IVNDFD48, % of NDF[g]	41.0		2	63.7	9.29	182	65.1	4.82	70	58.5	5.53	425
Lignin, % DM	1.79	0.493	296	4.84	1.345	1,032	5.46	1.125	620	4.33	1.274	3,748
Starch, % DM	61.2	4.25	313	3.1	2.67	969	2.8	1.86	610	1.7	1.72	3,717
WSC, % DM[h]	8.0	2.61	28	14.0	6.63	966	6.0	2.96	614	10.1	5.19	3,065
TFAs, % DM	1.55			1.46	0.448	53	2.13			2.48	0.332	2
Crude fat, % DM	1.73	0.349	296	2.29	0.705	1,028	3.76	0.633	619	3.47	0.693	3,750
DE base, Mcal/kg[i]	3.44			2.48			2.50			2.55		
Ca, % DM	0.09	0.105	312	0.33	0.133	1,051	0.68	0.175	618	0.38	0.130	3,722
P, % DM	0.35	0.062	313	0.24	0.076	1,084	0.35	0.061	620	0.34	0.068	3,756
Mg, % DM	0.13	0.021	312	0.15	0.056	1,077	0.21	0.042	615	0.17	0.042	3,745
K, % DM	0.51	0.162	311	1.99	0.691	1,081	2.76	0.644	619	2.82	0.666	3,749
Na, % DM	0.01	0.004	44	0.04	0.059	266	0.08	0.072	385	0.05	0.046	383
Cl, % DM	0.12	0.029	34	0.77	0.457	943	0.71	0.265	571	0.86	0.437	3,018
S, % DM	0.14	0.034	300	0.15	0.053	1,078	0.20	0.031	617	0.20	0.037	3,752
Cu, mg/kg DM	5.19	1.110	42	6.26	2.562	256	8.79	2.268	388	8.91	3.271	357
Fe, mg/kg DM	51	12.9	42	184	158.1	251	666	711.0	388	455	298.2	360
Mn, mg/kg DM	43	13.8	42	38	18.8	254	45	21.0	387	48	20.8	358
Zn, mg/kg DM	29	8.4	42	25	8.9	254	28	5.6	387	35	11.5	361
Mo, mg/kg DM	1.00	0.000	20	1.44	0.802	163	1.22	0.465	351	30.1	9.80	3,757

Name	Triticale Silage, Mid-Maturity			Urea			Wheat Bran			Wheat Grain, Ground		
Feed ID Code	NRC16F167			NRC16F168			NRC16F169			NRC16F170		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	33.3	7.67	1,111	99.0			90.1	1.16	186	85.7	16.69	2,011.07
Ash, % DM	12.4	2.02	1,111				5.5	0.81	94	2.1	0.64	1,856
CP, % DM	17.8	2.09	1,107	281.0			17.4	1.60	187	13.5	2.36	2,120
A fraction, % of CP[b]	56			100			43			31		
B fraction, % of CP[b]	33			0			51			54		
C fraction, % of CP[b]	11			0			6			15		
Kd of B, %/h[b]	5.9			0.0			24.2			19.1		
RUP, % CP[c]	30			0			18			28		
dRUP, % of RUP[d]	65						69			88		
Soluble protein, % CP	66.8	9.44	1,115	100.0			39.9	5.99	119	28.4	6.76	1,746
ADIP, % DM[e]	1.01						0.61			0.45	0.228	306
NDIP, % DM[f]	2.12						3.10			1.59	0.479	295
ADF, % DM	34.8	3.00	1,113				13.8	2.45	186	4.2	1.46	1,978
NDF, % DM	52.2	3.80	1,114	0.0			40.1	6.22	187	12.5	2.71	2,044
IVNDFD48, % of NDF[g]	57.5	5.44	221				43.3	9.61	3	55.7	17.36	7
Lignin, % DM	4.31	1.105	1,109	0.00			4.15	1.020	82	1.52	0.589	1,679
Starch, % DM	1.5	1.03	1,097	0.0			20.8	6.20	113	63.0	4.40	2,038

TABLE 19-1 Continued

Name	Triticale Silage, Mid-Maturity			Urea			Wheat Bran			Wheat Grain, Ground		
Feed ID Code	NRC16F167			NRC16F168			NRC16F169			NRC16F170		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
WSC, % DM[h]	7.5	3.22	504				8.0	1.20	42	5.9	1.58	72
TFAs, % DM	2.38	0.473	6				4.02			1.78		
Crude fat, % DM	4.08	0.598	1,109				4.39	0.743	111	1.98	0.361	1,808
DE base, Mcal/kg[i]	2.56			2.21			3.06			3.56		
Ca, % DM	0.52	0.191	1,107				0.13	0.091	167	0.10	0.158	1,694
P, % DM	0.41	0.062	1,111				1.05	0.249	167	0.36	0.076	1,727
Mg, % DM	0.19	0.046	1,112				0.43	0.095	157	0.13	0.032	1,710
K, % DM	3.42	0.586	1,112				1.22	0.237	158	0.47	0.229	1,711
Na, % DM	0.06	0.045	110				0.03	0.127	155	0.01	0.018	452
Cl, % DM	1.11	0.469	503				0.11	0.035	69	0.13	0.099	227
S, % DM	0.24	0.034	1,110				0.19	0.021	130	0.15	0.028	1,471
Cu, mg/kg DM	11.44	6.001	91				10.76	2.950	156	4.45	2.350	551
Fe, mg/kg DM	616	415.6	91				163	68.9	157	71	63.2	557
Mn, mg/kg DM	57	32.6	92				133	30.8	156	43	15.2	558
Zn, mg/kg DM	42	11.3	91				77	13.3	139	32	10.5	557
Mo, mg/kg DM	1.66	0.901	73				1.37	0.633	146	1.00	0.000	121

Name	Wheat Hay, Headed			Wheat Hay, Vegetative			Wheat Middlings			Wheat Silage, Headed		
Feed ID Code	NRC16F171			NRC16F172			NRC16F173			NRC16F174		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	90.6	3.46	1,065	90.5	2.98	2,404	88.3	1.38	2,723	34.8	4.94	6,375
Ash, % DM	8.1	2.12	1,070	8.2	2.51	2,404	5.9	0.46	2,239	10.5	2.19	6,393
CP, % DM	9.9	2.18	1,069	10.5	2.89	2,402	19.1	1.25	2,731	10.7	1.79	6,403
A fraction, % of CP[b]	35			35			48			62		
B fraction, % of CP[b]	53			53			44			29		
C fraction, % of CP[b]	12			12			8			9		
Kd of B, %/h[b]	4.3			4.3			16.2			10.0		
RUP, % CP[c]	42			42			22			23		
dRUP, % of RUP[d]	70			70			57			72		
Soluble protein, % CP	39.8	9.24	1,036	41.6	9.14	2,329	40.1	5.50	274	69.5	9.36	6,357
ADIP, % DM[e]	5.93	1.412	540	6.38	1.713	581	0.69	0.302	82	1.17	0.243	3,507
NDIP, % DM[f]	11.90	5.123	547	15.99	6.988	582	2.77	0.711	70	1.55	0.434	3,502
ADF, % DM	33.6	3.80	1,053	36.1	4.70	2,398	13.2	1.83	774	35.1	3.28	6,401
NDF, % DM	52.8	5.19	1,071	58.0	6.03	2,409	38.7	4.62	754	51.1	4.25	6,404
IVNDFD48, % of NDF[g]	57.2	8.36	18	59.3	8.28	58	48.6	8.84	5	61.4	3.81	41
Lignin, % DM	4.91	0.946	811	4.79	1.393	2,281	3.77	0.939	221	4.99	0.704	6,391
Starch, % DM	12.2	3.99	795	3.4	2.13	2,298	22.9	5.60	565	13.0	5.85	6,410
WSC, % DM[h]	9.6	4.19	257	17.3	7.45	1,679	7.9	1.33	126	6.5	2.85	2,802
TFAs, % DM	1.01	0.316	401	0.89	0.342	503	3.85			1.53	0.301	3,168
Crude fat, % DM	2.09	0.474	828	2.19	0.528	2,272	4.35	0.600	760	3.06	0.445	6,366
DE base, Mcal/kg[i]	2.52			2.48			3.07			2.51		
Ca, % DM	0.31	0.114	655	0.29	0.140	1,876	0.14	0.122	2,120	0.30	0.093	3,164
P, % DM	0.23	0.050	658	0.21	0.064	1,883	1.21	0.147	2,130	0.29	0.048	3,182
Mg, % DM	0.14	0.037	640	0.14	0.046	1,884	0.45	0.092	656	0.13	0.033	3,180
K, % DM	1.65	0.503	645	1.75	0.557	1,883	1.23	0.217	654	2.03	0.421	3,183
Na, % DM	0.05	0.079	438	0.07	0.099	663	0.02	0.022	502	0.06	0.100	870
Cl, % DM	0.62	0.314	418	0.69	0.364	1,676	0.10	0.023	143	0.74	0.257	2,984
S, % DM	0.16	0.042	421	0.16	0.054	1,737	0.20	0.025	339	0.17	0.031	3,186
Cu, mg/kg DM	7.52	3.484	570	7.87	2.824	824	11.51	3.822	506	7.68	3.422	2,615
Fe, mg/kg DM	303	222.3	571	403	365.5	826	153	44.1	499	543	347.4	2,604
Mn, mg/kg DM	51	24.9	575	59	27.0	826	133	27.6	505	46	17.3	2,625
Zn, mg/kg DM	25	8.4	574	27	10.9	817	90	16.3	452	28	8.3	2,616
Mo, mg/kg DM	1.65	0.965	49	1.50	0.868	292	1.64	0.811	273	1.58	0.834	493

TABLE 19-1   Continued

Name	Wheat Silage, Vegetative			Wheat Straw			Whey, Dry			Whey, Wet		
Feed ID Code	NRC16F175			NRC16F176			NRC16F177			NRC16F178		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
DM, % as fed	35.6	9.04	11,669	89.5	3.14	7,940	92.6	1.86	73	22.9	10.42	453
Ash, % DM	9.9	2.68	11,025	8.0	2.54	7,924	9.7	1.40	69	13.5	4.41	303
CP, % DM	13.4	3.19	11,685	4.5	1.54	7,936	17.8	5.28	72	7.4	2.98	437
A fraction, % of CP[b]	62			10			90			90		
B fraction, % of CP[b]	29			51			10			10		
C fraction, % of CP[b]	9			39			0			0		
Kd of B, %/h[b]	10.0			1.4			5.0			5.0		
RUP, % CP[c]	*22*			*80*			*11*			*11*		
dRUP, % of RUP[d]	72			62			95			95		
Soluble protein, % CP	65.0	10.78	11,653	36.0	10.85	7,838	91.1	4.65	48	53.7	22.20	185
ADIP, % DM[e]	1.14	0.286	4,461	1.26	0.170	3,098	0.22	0.110	34	0.34	0.262	94
NDIP, % DM[f]	1.90	0.725	4,453	1.51	0.409	3,097	0.53	0.658	35	0.67	0.444	94
ADF, % DM	37.0	4.26	11,673	53.1	4.06	7,903	0.3	0.19	45	0.9	0.81	211
NDF, % DM	56.6	5.50	11,677	76.9	4.72	7,928	0.6	0.52	62	2.2	1.64	229
IVNDFD48, % of NDF[g]	59.0	6.12	1,034	41.8	6.09	362						
Lignin, % DM	4.79	1.139	11,058	8.19	1.333	7,898	0.23	0.159	37	0.32	0.198	100
Starch, % DM	2.5	2.35	11,544	1.8	1.10	5,679	1.4			1.4	0.95	80
WSC, % DM[h]	9.2	4.89	5,028	4.2	1.69	2,477	56.1	2.16	108	50.6	16.27	568
TFAs, % DM	1.42	0.365	4,112	0.55	0.340	2,837	5.27			0.97		
Crude fat, % DM	3.50	0.647	11,038	1.49	0.454	7,924	6.27	0.449	49	1.97	2.052	169
DE base, Mcal/kg[i]	*2.53*			*1.96*			*3.62*			*3.16*		
Ca, % DM	0.43	0.201	7,414	0.38	0.250	5,051	0.92	0.210	61	1.26	0.641	420
P, % DM	0.32	0.069	7,423	0.12	0.072	5,051	0.89	0.234	62	1.29	0.488	417
Mg, % DM	0.17	0.045	7,411	0.12	0.058	5,033	0.14	0.030	62	0.22	0.071	414
K, % DM	2.53	0.709	7,419	1.41	0.658	5,082	2.60	0.625	62	4.01	1.509	415
Na, % DM	0.07	0.098	1,442	0.08	0.181	2,342	0.74	0.139	47	1.43	0.805	406
Cl, % DM	0.83	0.394	5,311	0.49	0.420	3,704	1.65	0.343	45	3.00	1.151	265
S, % DM	0.19	0.044	7,426	0.11	0.041	5,029	0.24	0.054	45	0.19	0.213	283
Cu, mg/kg DM	9.00	3.483	3,059	6.20	3.886	3,644	1.24	0.614	33	5.14	6.283	236
Fe, mg/kg DM	625	500.0	3,046	224	288.6	3,622	11	8.9	46	74	103.4	256
Mn, mg/kg DM	63	29.5	3,060	50	51.5	3,628	1	0.5	29	3	2.5	229
Zn, mg/kg DM	32	11.1	3,069	17	9.0	3,626	5	2.2	47	39	36.9	250
Mo, mg/kg DM	1.68	1.070	801	1.44	0.854	796						

[a] N = number of samples and SD = standard deviation; a blank cell under mean signifies no data were available while 0 reflects measured values that are below detection; blank N and SD signify corresponding mean value from a single source.

[b] Fractions (A, B, and C) and rate (Kd of B) of rumen disappearance of protein.

[c] Italics signifies calculated value as described in text.

[d] dRUP = intestinal digestibility of rumen-undegradable protein.

[e] ADIP = acid detergent insoluble protein.

[f] NDIP = neutral detergent insoluble protein.

[g] IVNDFD48 = in vitro 48-hour NDF digestibility.

[h] WSC = water-soluble carbohydrate.

[i] Digestible energy standard; italics signifies calculated value as described in text.

TABLE 19-2 AA, TFA, and FA Content of Some Feedstuffs Commonly Fed to Dairy Cattle[a]

Feed Name	Alfalfa Meal	Almond Hulls	Apple Pomace or By-Product, Wet	Bakery By-Product, Bread Waste	Bakery By-Product, Cereal	Bakery By-Product, Cookies
CP, % DM	19.51	5.25	6.42	14.90	9.20	12.94
Arg, % CP	4.04	2.23	4.52	4.74	6.84	4.19
His, % CP	1.87	0.86	1.86	2.61	2.82	1.77
Ile, % CP	3.80	2.35	3.13	4.00	3.15	3.12
Leu, % CP	6.57	4.05	5.58	7.77	6.16	7.12
Lys, % CP	4.40	2.74	3.93	2.91	4.05	1.71
Met, % CP	1.27	0.90	1.38	1.73	1.57	1.83
Phe, % CP	4.40	2.80	3.31	5.44	3.97	4.78
Thr, % CP	3.92	2.70	3.04	3.36	3.26	3.14
Trp, % CP	1.63	1.00	0.88	1.15	1.37	0.88
Val, % CP	4.82	3.53	4.11	4.42	4.50	4.58
TFAs, % DM	1.61	1.26	1.88	4.76	1.95	9.04
C12:0, % TFA	1.17	0.22	0.60			0.49
C14:0, % TFA	0.58	0.05	1.20	5.21	5.21	3.16
C16:0, % TFA	20.10	14.49	26.90	18.86	18.86	15.82
C16:1, % TFA	1.71	0.26	0.60			0.18
C18:0, % TFA	3.35	3.91	3.90	1.02	1.02	9.29
C18:1 trans, % TFA						7.77
C18:1 cis, % TFA	3.02	50.82	7.80	13.90	13.90	26.41
C18:2, % TFA	21.07	23.39	48.80	56.83	56.83	33.51
C18:3, % TFA	44.93	3.25	10.10	3.81	3.81	0.85
Others, % TFA	4.09	3.61	0.10	0.37	0.37	2.53

Feed Name	Bakery By-Product Meal	Barley Grain, Dry, Ground	Barley Grain, Steam Rolled	Barley Hay	Barley Malt Sprouts	Barley Silage, Headed
CP, % DM	12.83	11.81	11.81	10.75	23.88	10.87
Arg, % CP	4.63	4.91	4.91	2.18	4.55	1.04
His, % CP	2.22	2.22	2.22	1.94	1.90	1.21
Ile, % CP	3.43	3.43	3.43	5.50	3.23	3.45
Leu, % CP	6.94	6.76	6.76	6.65	5.71	4.88
Lys, % CP	2.69	3.61	3.61	3.56	4.71	2.35
Met, % CP	1.48	1.67	1.67	1.87	1.48	1.16
Phe, % CP	4.54	5.09	5.09	4.70	3.44	3.42
Thr, % CP	3.06	3.33	3.33	4.13	3.39	2.51
Trp, % CP	1.20	1.20	1.20	1.42	1.27	1.42
Val, % CP	4.35	4.81	4.81	4.13	4.55	4.80
TFAs, % DM	7.68	1.31	1.31	1.40	1.46	2.06
C12:0, % TFA	0.49			0.80		0.80
C14:0, % TFA	3.16	0.32	0.32	5.50	0.32	5.50
C16:0, % TFA	15.82	22.97	22.97	43.40	22.97	43.40
C16:1, % TFA	0.18	0.05	0.05		0.05	
C18:0, % TFA	9.29	1.53	1.53	4.10	1.53	4.10
C18:1 trans, % TFA	7.77					
C18:1 cis, % TFA	26.41	13.54	13.54	7.30	13.54	7.30
C18:2, % TFA	33.51	55.93	55.93	12.30	55.93	12.30
C18:3, % TFA	0.85	4.34	4.34	2.40	4.34	2.40
Others, % TFA	2.53	1.32	1.32	24.20	1.32	24.20

Feed Name	Barley Silage, Mid-Maturity	Barley Silage, Vegetative	Beet Pulp, Dry	Beet Pulp, Dry, Molasses Added	Beet Pulp, Wet	Bermudagrass Hay
CP, % DM	11.36	14.22	9.92	8.91	9.12	10.99
Arg, % CP	1.04	1.04	3.73	3.73	3.73	3.88
His, % CP	1.21	1.21	3.13	3.13	3.13	1.63
Ile, % CP	3.45	3.45	3.49	3.49	3.49	3.32
Leu, % CP	4.88	4.88	5.90	5.90	5.90	6.22
Lys, % CP	2.35	2.35	5.78	5.78	5.78	3.49
Met, % CP	1.16	1.16	1.57	1.57	1.57	1.30

TABLE 19-2   Continued

Feed Name	Barley Silage, Mid-Maturity	Barley Silage, Vegetative	Beet Pulp, Dry	Beet Pulp, Dry, Molasses Added	Beet Pulp, Wet	Bermudagrass Hay
Phe, % CP	3.42	3.42	3.61	3.61	3.61	3.92
Thr, % CP	2.51	2.51	4.46	4.46	4.46	3.60
Trp, % CP	1.42	1.42	1.08	1.08	1.08	1.24
Val, % CP	4.80	4.80	5.54	5.54	5.54	4.51
TFAs, % DM	1.70	2.07	0.63	0.63	0.64	1.25
C12:0, % TFA	0.80	0.80				2.82
C14:0, % TFA	5.50	5.50				1.18
C16:0, % TFA	43.40	43.40	26.66	26.66	26.66	30.30
C16:1, % TFA			0.39	0.39	0.39	1.42
C18:0, % TFA	4.10	4.10	0.89	0.89	0.89	3.99
C18:1 *trans*, % TFA			0.14	0.14	0.14	1.14
C18:1 *cis*, % TFA	7.30	7.30	11.55	11.55	11.55	3.32
C18:2, % TFA	12.30	12.30	49.83	49.83	49.83	18.79
C18:3, % TFA	2.40	2.40	6.35	6.35	6.35	20.75
Others, % TFA	24.20	24.20	4.19	4.19	4.19	16.29

Feed Name	Bermudagrass Silage, Mature	Bermudagrass Silage, Mid-Maturity	Blood Meal, High dRUP	Blood Meal, Low dRUP	Brewers Grains, Dry	Brewers Grains, Wet
CP, % DM	10.27	14.59	96.97	96.97	25.49	28.08
Arg, % CP	3.88	3.88	4.20	4.20	5.80	5.80
His, % CP	1.63	1.63	6.00	6.00	2.28	2.28
Ile, % CP	3.32	3.32	1.08	1.08	4.02	4.02
Leu, % CP	6.22	6.22	12.40	12.40	8.30	8.30
Lys, % CP	3.49	3.49	8.77	8.77	3.55	3.55
Met, % CP	1.30	1.30	1.15	1.15	2.14	2.14
Phe, % CP	3.92	3.92	6.79	6.79	5.54	5.54
Thr, % CP	3.60	3.60	4.55	4.55	3.59	3.59
Trp, % CP	1.24	1.24	1.58	1.58	1.34	1.34
Val, % CP	4.51	4.51	8.32	8.32	5.47	5.47
TFAs, % DM	1.35	1.44	1.31	1.31	8.31	7.61
C12:0, % TFA	1.44	1.44	0.11	0.11	0.14	0.03
C14:0, % TFA	0.50	0.50	1.48	1.48	0.73	0.40
C16:0, % TFA	21.08	21.08	21.62	21.62	26.69	24.49
C16:1, % TFA	1.31	1.31	1.02	1.02	0.20	0.20
C18:0, % TFA	2.42	2.42	21.66	21.66	2.24	1.83
C18:1 *trans*, % TFA			5.13	5.13		
C18:1 *cis*, % TFA	4.86	4.86	26.47	26.47	14.60	11.23
C18:2, % TFA	24.88	24.88	14.89	14.89	48.87	53.82
C18:3, % TFA	32.46	32.46	0.46	0.46	4.57	5.37
Others, % TFA	11.06	11.06	7.16	7.16	1.95	2.63

Feed Name	Brewers Yeast, Dry	Brewers Yeast, Wet	Calcium Soaps	Candy (Not Chocolate) By-Product	Candy By-Product, High Protein	Canola Meal, Solvent Extracted
CP, % DM	50.72	43.32	0.00	2.37	14.63	41.49
Arg, % CP	0.85	0.85		2.25	2.25	5.93
His, % CP	0.45	0.45			1.57	2.66
Ile, % CP	0.71	0.71		3.60	3.60	3.93
Leu, % CP				6.52	6.52	6.92
Lys, % CP	0.65	0.65		2.25	2.25	5.51
Met, % CP	0.34	0.34		1.57	1.57	1.97
Phe, % CP	0.67	0.67		3.82	3.82	4.00
Thr, % CP	0.60	0.60		3.82	3.82	4.43
Trp, % CP	0.13	0.13		0.67	0.67	1.34
Val, % CP	0.81	0.81		5.84	5.84	5.11
TFAs, % DM	0.11	2.34	84.50	0.25	11.11	2.51
C12:0, % TFA			0.20	0.23	6.54	
C14:0, % TFA			1.60	0.35	5.13	0.19

TABLE 19-2   Continued

Feed Name	Brewers Yeast, Dry	Brewers Yeast, Wet	Calcium Soaps	Candy (Not Chocolate) By-Product	Candy By-Product, High Protein	Canola Meal, Solvent Extracted
C16:0, % TFA			50.80	18.55	18.24	9.76
C16:1, % TFA				0.23	0.14	0.90
C18:0, % TFA			4.10	7.94	18.81	2.24
C18:1 trans, % TFA				17.44	9.08	0.61
C18:1 cis, % TFA			35.70	38.75	30.81	45.21
C18:2, % TFA			7.00	15.56	7.78	31.45
C18:3, % TFA			0.20	0.20	0.58	7.71
Others, % TFA	100.00	100.00	0.40	0.74	2.89	1.92

Feed Name	Canola Seed, Ground	Chocolate By-product	Citrus Pulp, Dry	Citrus Pulp, Wet	Cool-Season Grass Hay, Mature	Cool-Season Grass Hay, Mid-Maturity
CP, % DM	23.36	10.00	7.19	8.66	9.23	13.28
Arg, % CP	5.93	2.25	3.72	3.72	4.10	4.10
His, % CP	2.66	1.57	1.70	1.70	1.94	1.94
Ile, % CP	3.93	3.60	2.88	2.88	3.96	3.96
Leu, % CP	6.92	6.52	5.25	5.25	7.39	7.39
Lys, % CP	5.51	2.25	2.72	2.72	4.85	4.85
Met, % CP	1.97	1.57	1.04	1.04	1.64	1.64
Phe, % CP	4.00	3.82	3.62	3.62	4.78	4.78
Thr, % CP	4.43	3.82	2.94	2.94	4.10	4.10
Trp, % CP	1.34	0.67	0.95	0.95	2.09	2.09
Val, % CP	5.11	5.84	3.85	3.85	5.22	5.22
TFAs, % DM	39.46	20.68	1.72	1.72	0.95	1.58
C12:0, % TFA		6.54	0.67	0.67	0.89	0.89
C14:0, % TFA	0.19	5.13	0.56	0.56		
C16:0, % TFA	9.76	18.24	26.85	26.85	15.22	15.22
C16:1, % TFA	0.90	0.14	0.59	0.59	1.48	1.48
C18:0, % TFA	2.24	18.81	4.93	4.93	1.29	1.29
C18:1 trans, % TFA	0.61	9.08	0.05	0.05		
C18:1 cis, % TFA	45.21	30.81	23.30	23.30	2.52	2.52
C18:2, % TFA	31.45	7.78	34.92	34.92	16.62	16.62
C18:3, % TFA	7.71	0.58	6.42	6.42	55.50	55.50
Others, % TFA	1.92	2.89	1.70	1.70	6.49	6.49

Feed Name	Cool-Season Grass Silage	Corn, Ear with Husk and Some Stalk, Ensiled, High Fiber	Corn, Ear with Husk and Some Stalk, Ensiled, Low Fiber	Corn and Cob Meal, Dry	Corn Cobs	Corn Germ
CP, % DM	13.42	7.95	7.83	8.41	2.97	15.42
Arg, % CP	3.06	3.85	3.85	3.30	4.00	6.64
His, % CP	1.66	2.69	2.69	2.79	2.94	2.99
Ile, % CP	3.57	3.46	3.46	3.54	3.50	3.08
Leu, % CP	6.12	12.98	12.98	13.00	12.70	8.22
Lys, % CP	3.28	2.69	2.69	2.60	2.78	4.58
Met, % CP	1.21	1.83	1.83	2.00	2.50	1.78
Phe, % CP	4.37	5.00	5.00	4.50	4.72	4.11
Thr, % CP	3.34	3.56	3.56	3.56	3.59	3.83
Trp, % CP	1.07			0.68	0.69	1.40
Val, % CP	4.89	4.42	4.42	4.74	4.78	4.86
TFAs, % DM	1.84	2.89	3.11	3.26	0.35	16.89
C12:0, % TFA	6.56					
C14:0, % TFA	0.54	0.30	0.30	0.30	1.15	0.71
C16:0, % TFA	16.76	13.60	13.60	13.60	12.69	16.90
C16:1, % TFA	1.67	0.20	0.20	0.20	0.12	0.13
C18:0, % TFA	1.94	1.80	1.80	1.80	1.94	2.04
C18:1 trans, % TFA						
C18:1 cis, % TFA	3.80	26.00	26.00	26.00	25.10	25.17
C18:2, % TFA	19.96	55.10	55.10	55.10	56.25	52.64
C18:3, % TFA	44.30	1.60	1.60	1.60	1.69	1.47
Others, % TFA	4.46	1.40	1.40	1.40	1.05	0.94

TABLE 19-2   Continued

Feed Name	Corn Germ Meal	Corn Gluten Feed, Dry	Corn Gluten Feed, Wet	Corn Gluten Meal	Corn Grain Dry, Coarse Grind	Corn Grain Dry, Fine Grind
CP, % DM	26.14	23.19	23.11	68.52	8.51	8.51
Arg, % CP	6.64	4.58	4.58	3.14	4.75	4.75
His, % CP	2.99	2.94	2.94	2.02	2.88	2.88
Ile, % CP	3.08	2.99	2.99	3.98	3.38	3.38
Leu, % CP	8.22	8.46	8.46	16.35	12.00	12.00
Lys, % CP	4.58	3.08	3.08	1.64	3.00	3.00
Met, % CP	1.78	1.59	1.59	2.38	2.00	2.00
Phe, % CP	4.11	3.48	3.48	6.18	4.88	4.88
Thr, % CP	3.83	3.58	3.58	3.34	3.63	3.63
Trp, % CP	1.40	0.50	0.50	0.54	0.75	0.75
Val, % CP	4.86	4.73	4.73	4.51	4.63	4.63
TFAs, % DM	2.11	3.38	3.09	1.44	3.84	3.84
C12:0, % TFA		0.03	0.31			
C14:0, % TFA	0.71	0.99	0.06	0.22	2.33	2.33
C16:0, % TFA	16.90	23.94	20.87	13.62	13.21	13.21
C16:1, % TFA	0.13	0.20	0.32	0.08	0.12	0.12
C18:0, % TFA	2.04	2.51	4.30	2.17	1.99	1.99
C18:1 *trans*, % TFA			0.13			
C18:1 *cis*, % TFA	25.17	17.19	19.08	22.22	24.09	24.09
C18:2, % TFA	52.64	50.48	49.97	57.46	55.70	55.70
C18:3, % TFA	1.47	3.21	2.90	2.77	1.62	1.62
Others, % TFA	0.94	1.44	2.07	1.47	0.94	0.94

Feed Name	Corn Grain Dry, Medium Grind	Corn Grain, High Moisture, Coarse Grind	Corn Grain High Moisture, Fine Grind	Corn Grain Screenings	Corn Grain, Steam-Flaked	Corn Hominy
CP, % DM	8.51	8.51	8.51	8.56	8.00	10.06
Arg, % CP	4.75	4.75	3.85	5.00	4.75	6.20
His, % CP	2.88	2.88	2.54	2.67	2.88	2.80
Ile, % CP	3.38	3.38	3.38	3.26	3.38	3.20
Leu, % CP	12.00	12.00	11.60	10.12	12.00	9.10
Lys, % CP	3.00	3.00	2.64	3.60	3.00	4.30
Met, % CP	2.00	2.00	2.11	1.86	2.00	1.90
Phe, % CP	4.88	4.88	4.56	4.65	4.88	4.30
Thr, % CP	3.63	3.63	3.68	3.84	3.63	3.80
Trp, % CP	0.75	0.75	0.98	0.81	0.75	0.90
Val, % CP	4.63	4.63	4.90	4.42	4.63	4.80
TFAs, % DM	3.84	3.84	3.57	3.18	3.14	5.38
C12:0, % TFA						
C14:0, % TFA	2.33	2.33	0.26		0.87	2.62
C16:0, % TFA	13.21	13.21	13.57	14.14	12.92	13.96
C16:1, % TFA	0.12	0.12	0.19		0.08	
C18:0, % TFA	1.99	1.99	1.83	2.05	1.86	2.37
C18:1 *trans*, % TFA						
C18:1 *cis*, % TFA	24.09	24.09	25.99	23.72	23.17	24.80
C18:2, % TFA	55.70	55.70	55.08	56.43	58.38	53.38
C18:3, % TFA	1.62	1.62	1.64	1.97	1.82	1.81
Others, % TFA	0.94	0.94	1.44	1.68	0.90	1.05

Feed Name	Corn Silage, Immature	Corn Silage, Mature	Corn Silage, Typical	Corn Stalks, Ensiled, High DM	Corn Stalk, Ensiled, Low DM	Cotton Gin Trash
CP, % DM	7.91	7.47	7.71	5.61	7.00	11.98
Arg, % CP	2.32	2.32	2.32	2.32	2.32	11.40
His, % CP	1.71	1.71	1.71	1.71	1.71	3.32
Ile, % CP	3.41	3.41	3.41	3.41	3.41	3.39
Leu, % CP	8.54	8.54	8.54	8.54	8.54	7.22

TABLE 19-2   Continued

Feed Name	Corn Silage, Immature	Corn Silage, Mature	Corn Silage, Typical	Corn Stalks, Ensiled, High DM	Corn Stalk, Ensiled, Low DM	Cotton Gin Trash
Lys, % CP	2.80	2.80	2.80	2.80	2.80	4.66
Met, % CP	1.59	1.59	1.59	1.59	1.59	1.83
Phe, % CP	3.90	3.90	3.90	3.90	3.90	5.63
Thr, % CP	3.41	3.41	3.41	3.41	3.41	3.81
Trp, % CP	0.73	0.73	0.73	0.73	0.73	1.42
Val, % CP	4.51	4.51	4.51	4.51	4.51	5.00
TFAs, % DM	2.32	2.36	2.35	0.48	0.72	3.14
C12:0, % TFA	0.31	0.31	0.31	0.31	0.31	
C14:0, % TFA	0.46	0.46	0.46	0.46	0.46	0.70
C16:0, % TFA	17.83	17.83	17.83	17.83	17.83	23.13
C16:1, % TFA	0.36	0.36	0.36	0.36	0.36	0.65
C18:0, % TFA	2.42	2.42	2.42	2.42	2.42	3.25
C18:1 trans, % TFA	0.00	0.00	0.00	0.00	0.00	
C18:1 cis, % TFA	19.24	19.24	19.24	19.24	19.24	19.29
C18:2, % TFA	47.74	47.74	47.74	47.74	47.74	51.56
C18:3, % TFA	8.25	8.25	8.25	8.25	8.25	0.53
Others, % TFA	3.40	3.40	3.40	3.40	3.40	0.89

Feed Name	Cottonseed Hulls	Cottonseed Meal	Cottonseed, Whole Linted	Distillers Grains and Solubles, Dried, High Fat	Distillers Grains and Solubles, Dried, Low Fat	Distillers Grains and Solubles, High Protein
CP, % DM	6.97	46.69	23.31	30.20	30.97	38.99
Arg, % CP	11.40	11.57	10.81	4.30	4.30	4.30
His, % CP	3.32	2.72	2.81	2.66	2.66	2.66
Ile, % CP	3.39	3.01	3.17	3.65	3.65	3.65
Leu, % CP	7.22	5.53	5.79	11.67	11.67	11.67
Lys, % CP	4.66	3.97	4.34	2.81	2.81	2.81
Met, % CP	1.83	1.39	1.49	1.98	1.98	1.98
Phe, % CP	5.63	5.27	5.20	4.87	4.87	4.87
Thr, % CP	3.81	3.05	3.21	3.73	3.73	3.73
Trp, % CP	1.42	1.22	1.18	0.80	0.80	0.80
Val, % CP	5.00	4.23	4.34	4.87	4.87	4.87
TFAs, % DM	3.14	3.06	18.26	11.39	7.90	6.56
C12:0, % TFA				0.12	0.12	0.12
C14:0, % TFA	0.70	0.94	0.69	0.14	0.14	0.14
C16:0, % TFA	23.13	25.80	23.91	14.05	14.05	14.05
C16:1, % TFA	0.65	0.52	0.55	0.13	0.13	0.13
C18:0, % TFA	3.25	2.95	2.33	2.39	2.39	2.39
C18:1 trans, % TFA		0.05		0.01	0.01	0.01
C18:1 cis, % TFA	19.29	18.33	15.24	24.57	24.57	24.57
C18:2, % TFA	51.56	50.20	56.48	56.11	56.11	56.11
C18:3, % TFA	0.53	0.30	0.19	1.68	1.68	1.68
Others, % TFA	0.89	0.92	0.61	0.81	0.81	0.81

Feed Name	Distillers Grains and Solubles, Modified Wet	Distillers Grains with Solubles, Wet	Distillers Solubles	Fat, Canola Oil	Fat, Corn Oil	Fat, Cottonseed Oil
CP, % DM	30.28	31.45	22.58			
Arg, % CP	4.30	4.30	4.30			
His, % CP	2.66	2.66	2.66			
Ile, % CP	3.65	3.65	3.65			
Leu, % CP	11.67	11.67	11.67			
Lys, % CP	2.81	2.81	2.81			
Met, % CP	1.98	1.98	1.98			
Phe, % CP	4.87	4.87	4.87			
Thr, % CP	3.73	3.73	3.73			

TABLE 19-2 Continued

Feed Name	Distillers Grains and Solubles, Modified Wet	Distillers Grains with Solubles, Wet	Distillers Solubles	Fat, Canola Oil	Fat, Corn Oil	Fat, Cottonseed Oil
Trp, % CP	0.80	0.80	0.80			
Val, % CP	4.87	4.87	4.87			
TFAs, % DM	8.35	8.31	9.99	88.00	88.00	88.00
C12:0, % TFA	0.30	0.30	0.30	0.10		
C14:0, % TFA	0.25	0.25	0.25	0.10		0.83
C16:0, % TFA	15.00	15.00	15.00	4.36	11.08	25.97
C16:1, % TFA	0.10	0.10	0.10	0.28		0.57
C18:0, % TFA	2.50	2.50	2.50	2.05	1.55	3.00
C18:1 *trans*, % TFA	0.05	0.05	0.05	3.53		
C18:1 *cis*, % TFA	18.00	18.00	18.00	57.28	26.95	20.16
C18:2, % TFA	55.00	55.00	55.00	18.99	58.95	48.93
C18:3, % TFA	8.00	8.00	8.00	7.64	1.10	0.10
Others, % TFA	0.80	0.80	0.80	5.67	0.38	0.44

Feed Name	Fat, Flaxseed Oil	Fat, Lard	Fat, Safflower Oil	Fat, Soybean Oil	Fat, Sunflower Oil	Fat, Tallow
CP, % DM						
Arg, % CP						
His, % CP						
Ile, % CP						
Leu, % CP						
Lys, % CP						
Met, % CP						
Phe, % CP						
Thr, % CP						
Trp, % CP						
Val, % CP						
TFAs, % DM	88.00	88.00	88.00	88.00	88.00	88.00
C12:0, % TFA		0.20		0.11		0.09
C14:0, % TFA	0.16	1.30	0.10	0.11		3.00
C16:0, % TFA	5.74	23.80	10.77	10.83	7.33	24.43
C16:1, % TFA	0.18	2.70		0.14	0.09	3.79
C18:0, % TFA	4.30	13.50	11.97	3.89	10.65	17.92
C18:1 *trans*, % TFA					0.59	3.99
C18:1 *cis*, % TFA	18.88	41.20	14.33	22.82	43.39	41.62
C18:2, % TFA	14.15	10.20	60.63	53.75	35.49	1.09
C18:3, % TFA	55.95	1.00	0.30	8.23	0.79	0.53
Others, % TFA	0.64	6.10	1.90	0.13	1.67	3.54

Feed Name	Feather Meal	Fish Meal	Flaxseed	Flaxseed Meal	Fruit and Vegetable By-Product, Wet	Glycerol
CP, % DM	90.55	69.19	22.79	38.48	13.62	
Arg, % CP	6.56	5.63	9.10	9.10	4.00	
His, % CP	1.21	2.35	2.17	2.17	2.94	
Ile, % CP	4.60	3.89	4.06	4.06	3.50	
Leu, % CP	8.13	6.74	5.94	5.94	12.70	
Lys, % CP	2.60	6.82	4.06	4.06	2.78	
Met, % CP	0.70	2.53	1.76	1.76	2.50	
Phe, % CP	4.77	3.73	4.63	4.63	4.72	
Thr, % CP	4.53	3.89	3.65	3.65	3.59	
Trp, % CP	0.76	0.96	1.48	1.48	0.69	
Val, % CP	7.01	4.59	4.88	4.88	4.78	
TFAs, % DM	7.85	6.44	33.41	3.08	6.13	5.24
C12:0, % TFA	0.34					
C14:0, % TFA	1.09	10.35	0.16	0.16	0.26	
C16:0, % TFA	24.33	28.46	5.74	5.74	13.57	
C16:1, % TFA	6.51	13.01	0.18	0.18	0.19	

TABLE 19-2 Continued

Feed Name	Feather Meal	Fish Meal	Flaxseed	Flaxseed Meal	Fruit and Vegetable By-Product, Wet	Glycerol
C18:0, % TFA	8.27	6.00	4.30	4.30	1.83	
C18:1 *trans*, % TFA	1.09	0.20				
C18:1 *cis*, % TFA	32.51	10.97	18.88	18.88	25.99	
C18:2, % TFA	13.19	1.09	14.15	14.15	55.08	
C18:3, % TFA	0.54	0.96	55.95	55.95	1.64	
Others, % TFA	12.13	28.96	0.64	0.64	1.44	

Feed Name	Grain Screenings, Source Unknown	Grain Sorghum Hay	Grain Sorghum Silage, Mature	Grain Sorghum Silage, Mid-Maturity	Grass–Legume Mixtures, Mix Hay	Grass–Legume Mixtures, Mix Silage
CP, % DM	16.18	8.81	8.22	8.89	12.12	17.68
Arg, % CP	5.00	4.07	4.07	4.07	4.51	3.47
His, % CP	2.67	2.47	2.47	2.47	1.79	1.68
Ile, % CP	3.26	3.91	3.91	3.91	3.78	3.76
Leu, % CP	10.12	13.04	13.04	13.04	6.79	6.24
Lys, % CP	3.60	2.64	2.64	2.64	4.29	3.85
Met, % CP	1.86	1.93	1.93	1.93	1.43	1.29
Phe, % CP	4.65	5.24	5.24	5.24	4.34	4.28
Thr, % CP	3.84	3.59	3.59	3.59	3.99	3.59
Trp, % CP	0.81	1.16	1.16	1.16	1.37	1.01
Val, % CP	4.42	5.00	5.00	5.00	4.88	4.95
TFAs, % DM	2.91	1.36	1.93	1.56	1.37	2.03
C12:0, % TFA		2.86	2.86	2.86	1.15	9.27
C14:0, % TFA		0.89	0.89	0.89	0.62	0.60
C16:0, % TFA	14.14	20.64	20.64	20.64	18.98	17.79
C16:1, % TFA		0.43	0.43	0.43	1.77	1.79
C18:0, % TFA	2.05	2.42	2.42	2.42	2.79	2.65
C18:1 *trans*, % TFA				0.00	0.17	
C18:1 *cis*, % TFA	23.72	10.18	10.18	10.18	1.85	2.93
C18:2, % TFA	56.43	30.37	30.37	30.37	33.21	17.94
C18:3, % TFA	1.97	25.53	25.53	25.53	33.39	41.51
Others, % TFA	1.68	6.68	6.68	6.68	6.08	5.54

Feed Name	Grass–Legume Mixtures, Predominantly Grass, Hay, Mature	Grass–Legume Mixtures, Predominantly Grass, Hay, Mid-Maturity	Grass–Legume Mixtures, Predominantly Grass, Silage	Grass–Legume Mixtures, Predominantly Legume, Hay, Immature	Grass–Legume Mixtures, Predominantly Legume, Hay, Mature	Grass–Legume Mixtures, Predominantly Legume, Silage
CP, % DM	10.85	4.28	14.34	20.35	17.38	20.04
Arg, % CP	4.47	4.47	3.47	4.50	4.50	3.47
His, % CP	1.79	1.79	1.68	1.79	1.79	1.68
Ile, % CP	3.75	3.75	3.76	3.79	3.79	3.76
Leu, % CP	6.76	6.76	6.24	6.81	6.81	6.24
Lys, % CP	4.25	4.25	3.85	4.31	4.31	3.85
Met, % CP	1.43	1.43	1.29	1.43	1.43	1.29
Phe, % CP	4.34	4.34	4.28	4.34	4.34	4.28
Thr, % CP	3.98	3.98	3.59	4.00	4.00	3.59
Trp, % CP	1.36	1.36	1.01	1.38	1.38	1.01
Val, % CP	4.86	4.86	4.95	4.89	4.89	4.95
TFAs, % DM	1.29	1.93	1.98	1.78	1.23	1.99
C12:0, % TFA	1.15	1.15	9.27	1.15	1.15	9.27
C14:0, % TFA	0.62	0.62	0.60	0.62	0.62	0.60
C16:0, % TFA	18.98	18.98	17.79	18.98	18.98	17.79
C16:1, % TFA	1.77	1.77	1.79	1.77	1.77	1.79
C18:0, % TFA	2.79	2.79	2.65	2.79	2.79	2.65
C18:1 *trans*, % TFA	0.17	0.17		0.17	0.17	
C18:1 *cis*, % TFA	1.85	1.85	2.93	1.85	1.85	2.93
C18:2, % TFA	33.21	33.21	17.94	33.21	33.21	17.94
C18:3, % TFA	33.39	33.39	41.51	33.39	33.39	41.51
Others, % TFA	6.08	6.08	5.54	6.08	6.08	5.54

TABLE 19-2   Continued

Feed Name	Legume Hay, Immature	Legume Hay, Mature	Legume Hay, Mid-Maturity	Legume Silage, Immature	Legume Silage, Mid-Maturity	Meat and Bone Meal, Porcine
CP, % DM	21.54	18.11	20.75	22.06	20.47	56.63
Arg, % CP	4.20	4.20	4.20	1.76	1.76	7.01
His, % CP	1.93	1.93	1.93	1.92	1.92	1.58
Ile, % CP	3.92	3.92	3.92	4.15	4.15	2.57
Leu, % CP	6.69	6.69	6.69	6.74	6.74	5.42
Lys, % CP	4.81	4.81	4.81	4.72	4.72	4.58
Met, % CP	1.33	1.33	1.33	1.35	1.35	1.25
Phe, % CP	4.59	4.59	4.59	4.35	4.35	3.13
Thr, % CP	4.03	4.03	4.03	3.83	3.83	2.92
Trp, % CP	1.38	1.38	1.38	1.19	1.19	0.54
Val, % CP	4.97	4.97	4.97	5.08	5.08	3.88
TFAs, % DM	1.54	1.21	1.50	1.98	2.32	7.45
C12:0, % TFA	1.36	1.36	1.36	11.98	11.98	0.08
C14:0, % TFA	0.85	0.85	0.85	0.66	0.66	1.68
C16:0, % TFA	25.01	25.01	25.01	18.81	18.81	29.47
C16:1, % TFA	2.23	2.23	2.23	1.91	1.91	2.41
C18:0, % TFA	4.01	4.01	4.01	3.35	3.35	17.50
C18:1 *trans*, % TFA	0.35	0.35	0.35			1.27
C18:1 *cis*, % TFA	2.43	2.43	2.43	2.05	2.05	40.43
C18:2, % TFA	18.49	18.49	18.49	15.91	15.91	3.70
C18:3, % TFA	36.79	36.79	36.79	38.71	38.71	0.08
Others, % TFA	8.47	8.47	8.47	6.63	6.63	3.38

Feed Name	Millet Hay	Millet Silage	Molasses	Oat Grain, Rolled	Oat Hay	Oat Hulls
CP, % DM	10.74	13.02	9.27	12.17	8.49	5.01
Arg, % CP	4.10	3.06	4.91	6.49	2.18	6.74
His, % CP	1.94	1.66	1.59	1.91	1.94	2.25
Ile, % CP	3.96	3.57	4.44	3.74	5.50	3.57
Leu, % CP	7.39	6.12	3.59	7.16	6.65	7.29
Lys, % CP	4.85	3.28	1.00	3.86	3.56	4.11
Met, % CP	1.64	1.21	0.22	1.70	1.87	1.71
Phe, % CP	4.78	4.37	2.71	4.84	4.70	5.04
Thr, % CP	4.10	3.34	1.57	3.51	4.13	3.41
Trp, % CP	2.09	1.07	0.45	1.49	1.42	1.32
Val, % CP	5.22	4.89	3.36	5.26	4.13	4.96
TFAs, % DM	1.09	1.47	0.00	4.80	1.45	1.82
C12:0, % TFA	2.86	2.86			1.19	
C14:0, % TFA	0.89	0.89		0.87	0.43	
C16:0, % TFA	20.64	20.64	17.99	17.65	16.44	19.72
C16:1, % TFA	0.43	0.43	0.34	0.16	0.48	
C18:0, % TFA	2.42	2.42	3.61	1.32	1.33	2.51
C18:1 *trans*, % TFA					0.06	2.56
C18:1 *cis*, % TFA	10.18	10.18	12.98	34.78	2.53	34.23
C18:2, % TFA	30.37	30.37	54.94	42.01	23.38	37.67
C18:3, % TFA	25.53	25.53	7.46	1.85	49.90	2.40
Others, % TFA	6.68	6.68	2.68	1.38	4.26	0.91

Feed Name	Oat Silage, Immature	Oat Silage, Mid-Maturity	Pea Hay	Pea Silage	Peanut Hay	Peanut Hulls
CP, % DM	18.51	12.92	15.90	17.04	12.01	8.89
Arg, % CP	2.18	2.18	3.87	3.87	3.87	5.70
His, % CP	1.94	1.94	1.69	1.69	1.69	2.20
Ile, % CP	5.50	5.50	3.73	3.73	3.73	3.30
Leu, % CP	6.65	6.65	6.00	6.00	6.00	5.70
Lys, % CP	3.56	3.56	4.48	4.48	4.48	4.10
Met, % CP	1.87	1.87	1.37	1.37	1.37	9.00
Phe, % CP	4.70	4.70	4.18	4.18	4.18	3.50

TABLE 19-2 Continued

Feed Name	Oat Silage, Immature	Oat Silage, Mid-Maturity	Pea Hay	Pea Silage	Peanut Hay	Peanut Hulls
Thr, % CP	4.13	4.13	3.83	3.83	3.83	3.00
Trp, % CP	1.42	1.42	0.93	0.93	0.93	1.00
Val, % CP	4.13	4.13	5.00	5.00	5.00	4.40
TFAs, % DM	2.24	1.77	1.69	1.68	1.31	3.21
C12:0, % TFA	6.56	6.56	0.43	0.43	0.43	
C14:0, % TFA	0.54	0.54	0.28	0.28	0.28	
C16:0, % TFA	16.76	16.76	17.97	17.97	17.97	9.24
C16:1, % TFA	1.67	1.67	0.15	0.15	0.15	0.08
C18:0, % TFA	1.94	1.94	6.71	6.71	6.71	2.32
C18:1 trans, % TFA						
C18:1 cis, % TFA	3.80	3.80	19.74	19.74	19.74	66.61
C18:2, % TFA	19.96	19.96	38.88	38.88	38.88	19.30
C18:3, % TFA	44.30	44.30	12.98	12.98	12.98	2.45
Others, % TFA	4.46	4.46	2.85	2.85	2.85	0.00

Feed Name	Peanut Meal, Expellers	Peanut Skins	Peanuts	Peas	Pineapple Cannery Waste	Potato By-Product Meal
CP, % DM	42.62	16.19	25.55	24.28	7.02	9.99
Arg, % CP	11.01	6.60	11.01	8.69		2.47
His, % CP	2.22	3.30	2.22	2.44		1.84
Ile, % CP	3.21	2.30	3.21	4.13		3.14
Leu, % CP	6.14	5.90	6.14	7.18		5.34
Lys, % CP	3.21	5.50	3.21	7.23		4.21
Met, % CP	1.03	0.90	1.03	0.89		0.95
Phe, % CP	4.81	3.20	4.81	4.79		3.62
Thr, % CP	2.56	2.60	2.56	3.71		3.11
Trp, % CP	1.03	1.00	1.03	0.89		0.67
Val, % CP	3.88	3.00	3.88	4.65		4.40
TFAs, % DM	7.31	18.61	41.24	1.14	0.94	1.78
C12:0, % TFA						0.35
C14:0, % TFA				0.30		0.49
C16:0, % TFA	9.24	9.24	9.24	23.00		12.18
C16:1, % TFA	0.08	0.08	0.08	0.10		0.55
C18:0, % TFA	2.32	2.32	2.32	1.50		10.70
C18:1 trans, % TFA						31.21
C18:1 cis, % TFA	66.61	66.61	66.61	13.50		35.65
C18:2, % TFA	19.30	19.30	19.30	55.90		5.12
C18:3, % TFA	2.45	2.45	2.45	4.30		1.15
Others, % TFA	0.00	0.00	0.00	1.30		2.60

Feed Name	Poultry By-Product Meal	Rice, Grain	Rice Bran	Rice Bran, Defatted	Rice Hulls	Rice Silage, Headed
CP, % DM	65.62	8.11	14.81	18.54	3.69	7.12
Arg, % CP	7.00	8.22	7.74	7.74	7.74	2.18
His, % CP		2.52	2.75	2.75	2.75	1.94
Ile, % CP	4.25	3.92	3.76	3.76	3.76	5.50
Leu, % CP	7.91	8.16	7.14	7.14	7.14	6.65
Lys, % CP	4.41	3.59	4.73	4.73	4.73	3.56
Met, % CP	1.39	2.70	2.18	2.18	2.18	1.87
Phe, % CP		5.15	4.45	4.45	4.45	4.70
Thr, % CP	4.41	3.58	3.88	3.88	3.88	4.13
Trp, % CP		1.29	1.22	1.22	1.22	1.42
Val, % CP	5.94	5.53	5.72	5.72	5.72	4.13
TFAs, % DM	11.78	1.24	12.00	2.16	0.27	1.62
C12:0, % TFA		0.08				6.56
C14:0, % TFA		0.57	0.28	0.28	0.28	0.54
C16:0, % TFA		14.65	17.53	17.53	17.53	16.76
C16:1, % TFA		0.24	0.21	0.21	0.21	1.67

TABLE 19-2   Continued

Feed Name	Poultry By-Product Meal	Rice, Grain	Rice Bran	Rice Bran, Defatted	Rice Hulls	Rice Silage, Headed
C18:0, % TFA		1.54	1.56	1.56	1.56	1.94
C18:1 *trans*, % TFA						
C18:1 *cis*, % TFA		32.53	39.10	39.10	39.10	3.80
C18:2, % TFA		29.05	38.11	38.11	38.11	19.96
C18:3, % TFA		1.21	1.46	1.46	1.46	44.30
Others, % TFA		20.13	1.75	1.75	1.75	4.46

Feed Name	Rice Silage, Vegetative	Rumen-Protected Lysine	Rumen-Protected Methionine	Rye Annual Fresh, Immature	Rye Annual Fresh, Mid-Maturity	Rye Annual Hay, Immature
CP, % DM	8.27			27.50	20.48	22.89
Arg, % CP	2.18			4.10	4.10	4.10
His, % CP	1.94			1.94	1.94	1.94
Ile, % CP	5.50			3.96	3.96	3.96
Leu, % CP	6.65			7.39	7.39	7.39
Lys, % CP	3.56	100		4.85	4.85	4.85
Met, % CP	1.87		100	1.64	1.64	1.64
Phe, % CP	4.70			4.78	4.78	4.78
Thr, % CP	4.13			4.10	4.10	4.10
Trp, % CP	1.42			2.09	2.09	2.09
Val, % CP	4.13			5.22	5.22	5.22
TFAs, % DM	1.47			3.07	2.44	2.53
C12:0, % TFA	6.56			0.84	0.84	4.60
C14:0, % TFA	0.54			0.24	0.24	3.30
C16:0, % TFA	16.76			13.49	13.49	26.20
C16:1, % TFA	1.67					1.70
C18:0, % TFA	1.94			1.07	1.07	5.40
C18:1 *trans*, % TFA						
C18:1 *cis*, % TFA	3.80			2.07	2.07	11.00
C18:2, % TFA	19.96			13.34	13.34	18.40
C18:3, % TFA	44.30			66.49	66.49	9.40
Others, % TFA	4.46			2.46	2.46	20.00

Feed Name	Rye Annual Hay, Mature	Rye Annual, Hay, Mid-Maturity	Rye Annual Silage, Immature	Rye Annual Silage, Mature	Rye Annual Silage, Mid-Maturity	Rye Grain
CP, % DM	7.62	11.99	16.41	8.28	14.43	11.80
Arg, % CP	4.10	4.10	3.06	3.06	3.06	5.00
His, % CP	1.94	1.94	1.66	1.66	1.66	2.34
Ile, % CP	3.96	3.96	3.57	3.57	3.57	3.19
Leu, % CP	7.39	7.39	6.12	6.12	6.12	6.17
Lys, % CP	4.85	4.85	3.28	3.28	3.28	3.62
Met, % CP	1.64	1.64	1.21	1.21	1.21	1.60
Phe, % CP	4.78	4.78	4.37	4.37	4.37	4.36
Thr, % CP	4.10	4.10	3.34	3.34	3.34	3.30
Trp, % CP	2.09	2.09	1.07	1.07	1.07	1.06
Val, % CP	5.22	5.22	4.89	4.89	4.89	4.57
TFAs, % DM	1.01	1.47	1.95	1.25	1.68	1.45
C12:0, % TFA	4.60	4.60	0.66	0.66	0.66	
C14:0, % TFA	3.30	3.30	1.87	1.87	1.87	0.18
C16:0, % TFA	26.20	26.20	20.40	20.40	20.40	15.93
C16:1, % TFA	1.70	1.70	1.19	1.19	1.19	0.59
C18:0, % TFA	5.40	5.40	2.25	2.25	2.25	0.53
C18:1 *trans*, % TFA						
C18:1 *cis*, % TFA	11.00	11.00	5.14	5.14	5.14	16.46
C18:2, % TFA	18.40	18.40	19.12	19.12	19.12	56.32
C18:3, % TFA	9.40	9.40	39.07	39.07	39.07	9.23
Others, % TFA	20.00	20.00	10.30	10.30	10.30	0.76

TABLE 19-2   Continued

Feed Name	Safflower Meal	Sorghum Forage Silage, Immature	Sorghum Forage Silage, Mature	Sorghum Grain, Dry, Ground	Sorghum Grain, Steam-Flaked	Sorghum Hay
CP, % DM	26.21	11.74	11.30	12.48	12.48	10.18
Arg, % CP	8.31	4.07	4.07	3.83	3.83	4.10
His, % CP	2.46	2.47	2.47	2.25	2.25	1.94
Ile, % CP	3.52	3.91	3.91	4.11	4.11	3.96
Leu, % CP	6.23	13.04	13.04	13.11	13.11	7.39
Lys, % CP	3.09	2.64	2.64	2.22	2.22	4.85
Met, % CP	1.48	1.93	1.93	1.74	1.74	1.64
Phe, % CP	4.41	5.24	5.24	5.15	5.15	4.78
Thr, % CP	3.14	3.59	3.59	3.31	3.31	4.10
Trp, % CP	0.93	1.16	1.16	1.20	1.20	2.09
Val, % CP	4.96	5.00	5.00	5.28	5.28	5.22
TFAs, % DM	3.88	1.74	1.44	2.92	2.92	1.24
C12:0, % TFA		2.86	2.86	0.04	0.04	2.86
C14:0, % TFA		0.89	0.89	0.08	0.08	0.89
C16:0, % TFA	5.40	20.64	20.64	17.16	17.16	20.64
C16:1, % TFA		0.43	0.43	0.63	0.63	0.43
C18:0, % TFA	1.60	2.42	2.42	1.65	1.65	2.42
C18:1 *trans*, % TFA						
C18:1 *cis*, % TFA	13.20	10.18	10.18	29.70	29.70	10.18
C18:2, % TFA	79.50	30.37	30.37	48.18	48.18	30.37
C18:3, % TFA	0.30	25.53	25.53	1.61	1.61	25.53
Others, % TFA		6.68	6.68	0.95	0.95	6.68

Feed Name	Sorghum Soybean Silage	Sorghum–Sudangrass Hay	Sorghum–Sudangrass Silage	Soybean Hay	Soybean Hulls	Soybean Meal, Expellers
CP, % DM	11.66	9.81	12.25	20.08	11.88	47.60
Arg, % CP	3.87	4.10	3.06	3.87	5.21	7.29
His, % CP	1.69	1.94	1.66	1.69	2.61	2.62
Ile, % CP	3.73	3.96	3.57	3.73	3.70	4.54
Leu, % CP	6.00	7.39	6.12	6.00	6.30	7.59
Lys, % CP	4.48	4.85	3.28	4.48	6.30	6.12
Met, % CP	1.37	1.64	1.21	1.37	1.09	1.34
Phe, % CP	4.18	4.78	4.37	4.18	3.87	5.05
Thr, % CP	3.83	4.10	3.34	3.83	3.61	3.90
Trp, % CP	0.93	2.09	1.07	0.93	1.34	1.34
Val, % CP	5.00	5.22	4.89	5.00	4.37	4.73
TFAs, % DM	1.81	1.10	1.63	1.91	1.61	6.12
C12:0, % TFA	0.43	2.86	2.86	0.43		
C14:0, % TFA	0.28	0.89	0.89	0.28	1.47	0.07
C16:0, % TFA	17.97	20.64	20.64	17.97	16.22	11.55
C16:1, % TFA	0.15	0.43	0.43	0.15	0.28	0.09
C18:0, % TFA	6.71	2.42	2.42	6.71	7.03	3.71
C18:1 *trans*, % TFA					0.70	1.42
C18:1 *cis*, % TFA	19.74	10.18	10.18	19.74	15.90	18.13
C18:2, % TFA	38.88	30.37	30.37	38.88	42.66	54.77
C18:3, % TFA	12.98	25.53	25.53	12.98	13.11	9.52
Others, % TFA	2.85	6.68	6.68	2.85	2.64	0.75

Feed Name	Soybean Meal, Extruded	Soybean Meal, Solvent Extracted, 48% CP	Soybean Silage	Soybeans, Whole Raw	Soybeans, Whole Roasted	Spelt Grain
CP, % DM	40.43	52.64	17.99	39.98	40.02	12.90
Arg, % CP	7.29	7.29	3.87	7.25	7.25	4.79
His, % CP	2.62	2.64	1.69	2.61	2.61	2.15
Ile, % CP	4.54	4.54	3.73	4.53	4.53	3.45
Leu, % CP	7.59	7.63	6.00	7.58	7.58	6.54
Lys, % CP	6.12	6.16	4.48	6.14	6.14	2.76

TABLE 19-2　Continued

Feed Name	Soybean Meal, Extruded	Soybean Meal, Solvent Extracted, 48% CP	Soybean Silage	Soybeans, Whole Raw	Soybeans, Whole Roasted	Spelt Grain
Met, % CP	1.34	1.38	1.37	1.33	1.33	1.54
Phe, % CP	5.05	5.03	4.18	5.03	5.03	4.45
Thr, % CP	3.90	3.95	3.83	3.89	3.89	2.95
Trp, % CP	1.34	1.38	0.93	1.33	1.33	1.36
Val, % CP	4.73	4.76	5.00	4.72	4.72	4.44
TFAs, % DM	15.08	1.08	2.86	16.99	15.35	1.66
C12:0, % TFA			0.43	0.58	0.00	
C14:0, % TFA	0.07	0.83	0.28	0.20	0.11	0.23
C16:0, % TFA	11.55	17.28	17.97	11.93	11.80	19.50
C16:1, % TFA	0.09		0.15	0.08	0.07	
C18:0, % TFA	3.71	4.45	6.71	4.05	4.30	1.08
C18:1 trans, % TFA	1.42	0.43				
C18:1 cis, % TFA	18.13	13.22	19.74	21.99	23.58	14.66
C18:2, % TFA	54.77	54.16	38.88	52.43	52.36	60.20
C18:3, % TFA	9.52	8.43	12.98	7.59	6.99	4.32
Others, % TFA	0.75	1.20	2.85	1.17	0.79	

Feed Name	Sudangrass Hay, Mature	Sudangrass Hay, Mid-Maturity	Sudangrass Silage, Mature	Sudangrass Silage, Mid-Maturity	Sugarcane Bagasse Hay	Sugarcane Bagasse Silage
CP, % DM	8.08	14.73	9.54	13.37	3.89	4.99
Arg, % CP	4.10	4.10	3.06	3.06	2.83	2.83
His, % CP	1.94	1.94	1.66	1.66	1.00	1.00
Ile, % CP	3.96	3.96	3.57	3.57	2.83	2.83
Leu, % CP	7.39	7.39	6.12	6.12	5.49	5.49
Lys, % CP	4.85	4.85	3.28	3.28	2.83	2.83
Met, % CP	1.64	1.64	1.21	1.21	0.67	0.67
Phe, % CP	4.78	4.78	4.37	4.37	3.50	3.50
Thr, % CP	4.10	4.10	3.34	3.34	2.83	2.83
Trp, % CP	2.09	2.09	1.07	1.07	4.50	4.50
Val, % CP	5.22	5.22	4.89	4.89	3.83	3.83
TFAs, % DM	0.99	1.05	1.53	1.61	0.72	0.91
C12:0, % TFA	2.86	2.86	2.86	2.86	1.19	1.19
C14:0, % TFA	0.89	0.89	0.89	0.89	0.43	0.43
C16:0, % TFA	20.64	20.64	20.64	20.64	16.44	16.44
C16:1, % TFA	0.43	0.43	0.43	0.43	0.48	0.48
C18:0, % TFA	2.42	2.42	2.42	2.42	1.33	1.33
C18:1 trans, % TFA					0.06	0.06
C18:1 cis, % TFA	10.18	10.18	10.18	10.18	2.53	2.53
C18:2, % TFA	30.37	30.37	30.37	30.37	23.38	23.38
C18:3, % TFA	25.53	25.53	25.53	25.53	49.90	49.90
Others, % TFA	6.68	6.68	6.68	6.68	4.26	4.26

Feed Name	Sunflower Meal	Sunflower Seed	Sunflower Silage	Sweet Corn Cannery Waste	Tapioca (Cassava)	Tomato Pomace
CP, % DM	37.01	20.07	13.27	9.80	2.50	19.30
Arg, % CP	8.03	7.99	3.87	2.32	4.00	11.5
His, % CP	2.44	2.49	1.69	1.71	1.60	3.9
Ile, % CP	4.00	3.91	3.73	3.41	3.20	4.1
Leu, % CP	6.22	6.12	6.00	8.54	5.20	7.1
Lys, % CP	3.50	3.70	4.48	2.80	4.00	8
Met, % CP	2.19	2.13	1.37	1.59	1.20	2.3
Phe, % CP	4.50	4.47	4.18	3.90	3.20	5.8
Thr, % CP	3.63	3.59	3.83	3.41	3.60	3.3
Trp, % CP	1.28	1.41	0.93	0.73	0.80	
Val, % CP	4.84	4.78	5.00	4.51	4.00	4.4
TFAs, % DM	1.02	37.20	3.06	3.81	0.48	12.30

TABLE 19-2   Continued

Feed Name	Sunflower Meal	Sunflower Seed	Sunflower Silage	Sweet Corn Cannery Waste	Tapioca (Cassava)	Tomato Pomace
C12:0, % TFA			0.43	0.31		
C14:0, % TFA	0.76	0.10	0.28	0.46		
C16:0, % TFA	11.59	5.20	17.97	17.83	38.50	
C16:1, % TFA		0.10	0.15	0.36		
C18:0, % TFA	4.37	4.10	6.71	2.42		
C18:1 *trans*, % TFA				0.00		
C18:1 *cis*, % TFA	41.93	39.40	19.74	19.24	38.50	
C18:2, % TFA	38.71	47.90	38.88	47.74	13.30	
C18:3, % TFA	0.59	0.40	12.98	8.25	6.60	
Others, % TFA	2.05	2.80	2.85	3.40	3.10	

Feed Name	Triticale Grain	Triticale Hay	Triticale Plus Pea Silage	Triticale Silage, Mature	Triticale Silage, Mid-Maturity	Urea
CP, % DM	12.05	10.33	15.98	14.16	17.78	281.00
Arg, % CP	4.91	3.84	3.84	3.84	3.84	
His, % CP	2.28	2.53	2.53	2.53	2.53	
Ile, % CP	3.25	3.04	3.04	3.04	3.04	
Leu, % CP	6.40	5.86	5.86	5.86	5.86	
Lys, % CP	3.16	1.83	1.83	1.83	1.83	
Met, % CP	1.67	1.31	1.31	1.31	1.31	
Phe, % CP	4.56	4.78	4.78	4.78	4.78	
Thr, % CP	3.07	2.14	2.14	2.14	2.14	
Trp, % CP	1.05	1.03	1.03	1.03	1.03	
Val, % CP	4.30	3.68	3.68	3.68	3.68	
TFAs, % DM	1.55	1.46	2.13	2.48	2.38	
C12:0, % TFA		1.12	1.12	1.12	1.12	
C14:0, % TFA	0.23	0.42	0.42	0.42	0.42	
C16:0, % TFA	19.50	11.42	11.42	11.42	11.42	
C16:1, % TFA		2.23	2.23	2.23	2.23	
C18:0, % TFA	1.08	0.92	0.92	0.92	0.92	
C18:1 *trans*, % TFA						
C18:1 *cis*, % TFA	14.66	1.49	1.49	1.49	1.49	
C18:2, % TFA	59.45	14.53	14.53	14.53	14.53	
C18:3, % TFA	4.32	62.82	62.82	62.82	62.82	
Others, % TFA	0.76	5.06	5.06	5.06	5.06	

Feed Name	Wheat Bran	Wheat Grain, Rolled	Wheat Hay, Headed	Wheat Hay, Vegetative	Wheat Middlings	Wheat Silage, Headed
CP, % DM	17.40	13.49	9.88	10.50	19.10	10.73
Arg, % CP	6.94	4.79	2.02	2.02	6.65	2.02
His, % CP	2.75	2.15	3.60	3.60	2.61	3.60
Ile, % CP	3.19	3.45	4.01	4.01	3.11	4.01
Leu, % CP	6.21	6.54	6.64	6.64	6.09	6.64
Lys, % CP	4.05	2.76	4.21	4.21	3.98	4.21
Met, % CP	1.46	1.54	1.77	1.77	1.49	1.77
Phe, % CP	3.91	4.45	4.24	4.24	3.98	4.24
Thr, % CP	3.19	2.95	4.21	4.21	3.17	4.21
Trp, % CP	1.84	1.36	1.03	1.03	1.43	1.03
Val, % CP	4.82	4.44	5.80	5.80	4.53	5.80
TFAs, % DM	4.02	1.78	1.01	0.89	3.85	1.53
C12:0, % TFA			1.19	1.19		6.56
C14:0, % TFA	0.10	0.23	0.43	0.43	0.10	0.54
C16:0, % TFA	17.05	19.50	16.44	16.44	17.09	16.76
C16:1, % TFA			0.48	0.48	0.12	1.67
C18:0, % TFA	1.08	1.08	1.33	1.33	1.17	1.94
C18:1 *trans*, % TFA			0.06	0.06		
C18:1 *cis*, % TFA	17.81	14.66	2.53	2.53	17.69	3.80
C18:2, % TFA	59.09	60.20	23.38	23.38	57.78	19.96
C18:3, % TFA	4.73	4.32	49.90	49.90	4.71	44.30
Others, % TFA	0.14		4.26	4.26	1.34	4.46

TABLE 19-2   Continued

Feed Name	Wheat Silage, Vegetative	Wheat Straw	Whey, Dry	Whey, Wet
CP, % DM	13.35	4.50	17.83	7.42
Arg, % CP	2.02	2.02	2.75	2.17
His, % CP	3.60	3.60	1.83	1.65
Ile, % CP	4.01	4.01	5.07	5.39
Leu, % CP	6.64	6.64	8.78	9.04
Lys, % CP	4.21	4.21	6.94	7.22
Met, % CP	1.77	1.77	1.40	1.39
Phe, % CP	4.24	4.24	3.23	2.96
Thr, % CP	4.21	4.21	5.46	6.17
Trp, % CP	1.03	1.03		1.57
Val, % CP	5.80	5.80	4.80	5.13
TFAs, % DM	1.42	0.55	5.27	0.97
C12:0, % TFA	6.56	1.19	0.72	0.72
C14:0, % TFA	0.54	0.43	6.75	6.75
C16:0, % TFA	16.76	16.44	35.74	35.74
C16:1, % TFA	1.67	0.48	0.94	0.94
C18:0, % TFA	1.94	1.33	17.81	17.81
C18:1 trans, % TFA		0.06	2.64	2.64
C18:1 cis, % TFA	3.80	2.53	27.08	27.08
C18:2, % TFA	19.96	23.38	6.89	6.89
C18:3, % TFA	44.30	49.90		
Others, % TFA	4.46	4.26	1.42	1.42

[a] AA and individual FA data were provided by Cornell University (Higgs et al., 2015). Because data originated from a single source, the number of samples and standard deviation are not presented in the table.

TABLE 19-3   Composition of Inorganic Mineral Sources and Element Absorption Coefficients for Dairy Cattle on a 100 Percent DM Basis[a,b,c,d,e,f]

Mineral Source	Mineral	%	Absorption Coefficient
CALCIUM SOURCES			
Bone meal (NRC16F1011)			
Primary mineral	Ca	31.0	0.60
Secondary mineral	P	12.9	0.80
Minor mineral 1	Na	5.7	1.00
Minor mineral 2	Fe	2.7	0.01
Minor mineral 3	S	2.5	N/A
Calcium carbonate, $CaCO_3$ (NRC16F1003)			
Primary mineral	Ca	39.4	0.50
Calcium chloride anhydrous, $CaCl_2$ (NRC16F1004)			
Primary mineral	Ca	36.1	0.60
Secondary mineral	Cl	63.9	0.92
Calcium chloride dihydrate, $CaCl_2 \cdot 2H_2O$ (NRC16F1005)			
Primary mineral	Ca	27.5	0.60
Secondary mineral	Cl	48.2	0.92
Calcium hydroxide, $Ca(OH)_2$ (NRC16F1006)			
Primary mineral	Ca	54.1	0.60
Calcium oxide, CaO (NRC16F1009)			
Primary mineral	Ca	71.5	0.33
Calcium phosphate (dibasic), $CaHPO_4$ (NRC16F1007)			
Primary mineral	Ca	22.0	0.60
Secondary mineral	P	19.3	0.75
Minor mineral 1	Fe	1.4	0.01
Calcium phosphate (monobasic), $Ca(H_2PO_4)_2$ (NRC16F1008)			
Primary mineral	Ca	16.4	0.60
Secondary mineral	P	21.6	0.80
Minor mineral 1	Fe	1.6	0.01

TABLE 19-3   Continued

Mineral Source	Mineral	%	Absorption Coefficient
Calcium sulfate dihydrate, $CaSO_4 \cdot 2H_2O$ (NRC16F1010)			
Primary mineral	Ca	23.3	0.60
Secondary mineral	S	23.5	N/A
Dolomite limestone (magnesium) (NRC16F1012)			
Primary mineral	Ca	22.3	0.45
Secondary mineral	Mg	10.0	0.12
Limestone, ground (NRC16F1013)			
Primary mineral	Ca	35.0	0.45
Secondary mineral	Mg	1.0	0.12
Oystershell, ground (NRC16F1014)			
Primary mineral	Ca	38.0	0.50
Phosphate, Curacao (NRC16F1024)			
Primary mineral	Ca	35.1	0.45
Secondary mineral	P	14.1	0.85
Phosphate, defluorinated (NRC16F1025)			
Primary mineral	Ca	32.0	0.45
Secondary mineral	P	18.0	0.65
Minor mineral 1	Na	4.9	1.00
CHLORIDE SOURCES			
Ammonium chloride, NH_4Cl (NRC16F1069)			
Primary mineral	Cl	66.3	0.92
Calcium chloride anhydrous, $CaCl_2$ (NRC16F1004)			
Primary mineral	Cl	63.9	0.92
Secondary mineral	Ca	36.1	0.60
Magnesium chloride hexahydrate, $MgCl_2 \cdot 6H_2O$ (NRC16F1015)			
Primary mineral	Cl	34.9	0.92
Secondary mineral	Mg	12.0	0.27
Potassium chloride, KCl (NRC16F1016)			
Primary mineral	Cl	50.0	0.92
Secondary mineral	K	50.0	1.00
Sodium chloride (salt), NaCl (NRC16F1017)			
Primary mineral	Cl	60.7	0.92
Secondary mineral	Na	39.3	1.00
COBALT SOURCES			
Cobalt carbonate, $CoCO_3$ (NRC16F1038)			
Primary mineral	Co	46.0	N/A
Cobalt carbonate hexahydrate, $CoCO_3 \cdot 6H_2O$ (NRC16F1039)			
Primary mineral	Co	25.9	N/A
Cobalt chloride hexahydrate, $CoCl_2 \cdot 6H_2O$ (NRC16F1040)			
Primary mineral	Co	24.8	N/A
Secondary mineral	Cl	29.8	0.92
Cobalt sulfate heptahydrate, $CoSO_4 \cdot 7H_2O$ (NRC16F1041)			
Primary mineral	Co	21.0	N/A
Secondary mineral	S	11.4	N/A
COPPER SOURCES			
Copper chloride dihydrate, $CuCl_2 \cdot 2H_2O$ (NRC16F1043)			
Primary mineral	Cu	37.2	0.05
Secondary mineral	Cl	41.7	0.92
Copper oxide, CuO (NRC16F1045)			
Primary mineral	Cu	79.9	0.005

TABLE 19-3   Continued

Mineral Source	Mineral	%	Absorption Coefficient
Copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$ (NRC16F1044)			
Primary mineral	Cu	25.5	0.05
Secondary mineral	S	12.8	N/A
IODINE SOURCES			
Calcium iodate, $Ca(IO_3)_2$ (NRC16F1048)			
Primary mineral	I	63.5	N/A
Secondary mineral	Ca	10.0	0.60
Ethylenediamine dihydroiodide (EDDI) (NRC16F1047)			
Primary mineral	I	80.3	N/A
Potassium iodide, KI (NRC16F1031)			
Primary mineral	I	68.8	N/A
Secondary mineral	K	21.0	1.00
IRON SOURCES			
Ferrous carbonate, $FeCO_3$ (NRC16F1051)			
Primary mineral	Fe	38.0	0.10
Ferrous sulfate heptahydrate, $FeSO_4 \cdot 7H_2O$ (NRC16F1049)			
Primary mineral	Fe	21.8	0.20
Secondary mineral	S	12.4	N/A
Ferrous sulfate monohydrate, $FeSO_4 \cdot H_2O$ (NRC16F1050)			
Primary mineral	Fe	32.9	0.20
Secondary mineral	S	18.9	N/A
Iron oxide, FeO (NRC16F1052)			
Primary mineral	Fe	60.0	0.01
MAGNESIUM SOURCES			
Dolomite limestone (magnesium) (NRC16F1012)			
Primary mineral	Mg	10.0	0.12
Secondary mineral	Ca	22.0	0.45
Magnesium carbonate, $MgCO_3$ (NRC16F1018)			
Primary mineral	Mg	30.8	0.23
Magnesium chloride hexahydrate, $MgCl_2 \cdot 6H_2O$ (NRC16F1015)			
Primary mineral	Mg	12.0	0.27
Secondary mineral	Cl	34.9	0.92
Magnesium hydroxide, $Mg(OH)_2$ (NRC16F1019)			
Primary mineral	Mg	41.7	0.23
Magnesium oxide, MgO (NRC16F1020)			
Primary mineral	Mg	56.2	0.23
Secondary mineral	Ca	< 1	0.45
Magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$ (NRC16F1021)			
Primary mineral	Mg	9.8	0.27
Secondary mineral	S	13.3	N/A
MANGANESE SOURCES			
Manganese carbonate, $MnCO_3$ (NRC16F1056)			
Primary mineral	Mn	47.8	0.0015
Manganese chloride, $MnCl_2$ (NRC16F1054)			
Primary mineral	Mn	43.0	0.005
Secondary mineral	Cl	56.3	0.92
Manganese chloride tetrahydrate, $MnCl_2 \cdot 4H_2O$ (NRC16F1055)			
Primary mineral	Mn	27.7	0.005
Secondary mineral	Cl	35.8	0.92

TABLE 19-3   Continued

Mineral Source	Mineral	%	Absorption Coefficient
Manganese oxide, MnO (NRC16F1059)			
Primary mineral	Mn	77.5	0.003
Manganese sulfate monohydrate, $MnSO_4 \cdot H_2O$ (NRC16F1057)			
Primary mineral	Mn	32.5	0.005
Secondary mineral	S	19.0	N/A
Manganese sulfate pentahydrate, $MnSO_4 \cdot 5H_2O$ (NRC16F1058)			
Primary mineral	Mn	22.8	0.005
Secondary mineral	S	13.3	N/A
PHOSPHORUS SOURCES			
Ammonium phosphate (dibasic), $(NH_4)_2HPO_4$ (NRC16F1022)			
Primary mineral	P	20.6	0.80
Minor mineral 1	S	2.2	N/A
Minor mineral 2	Fe	1.2	0.01
Ammonium phosphate (monobasic), $(NH_4)H_2PO_4$ (NRC16F1023)			
Primary mineral	P	24.7	0.80
Minor mineral 1	S	1.5	N/A
Minor mineral 2	Fe	1.7	0.01
Bone meal (NRC16F1011)			
Primary mineral	P	12.9	0.80
Secondary mineral	Ca	31.0	0.60
Minor mineral 1	Na	5.7	1.00
Minor mineral 2	Fe	2.7	0.01
Minor mineral 3	S	2.5	N/A
Calcium phosphate (dibasic), $CaHPO_4$ (NRC16F1007)			
Primary mineral	P	19.3	0.75
Secondary mineral	Ca	22.0	0.60
Minor mineral 1	Fe	1.4	0.01
Calcium phosphate (monobasic), $Ca(H_2PO_4)_2$ (NRC16F1008)			
Primary mineral	P	21.6	0.80
Secondary mineral	Ca	16.4	0.60
Minor mineral 1	Fe	1.6	0.01
Phosphate, Curacao (NRC16F1024)			
Primary mineral	P	14.1	0.85
Secondary mineral	Ca	35.1	0.45
Phosphate, defluorinated (NRC16F1025)			
Primary mineral	P	18.0	0.65
Secondary mineral	Ca	32.0	0.45
Minor mineral 1	Na	4.9	1.00
Phosphate, monosodium ($NaH_2PO_4 \cdot H_2O$) (NRC16F1026)			
Primary mineral	P	22.5	0.90
Secondary mineral	Na	16.7	1.00
Phosphoric acid, H_3PO_4 (NRC16F1027)			
Primary mineral	P	31.6	0.90
Sodium tripolyphosphate, $Na_5P_3O_{10}$ (NRC16F1028)			
Primary mineral	P	25.0	0.75
Secondary mineral	Na	31.0	1.00
POTASSIUM SOURCES			
Potassium bicarbonate, $KHCO_3$ (NRC16F1029)			
Primary mineral	K	39.1	1.00
Potassium carbonate, K_2CO_3 (NRC16F1030)			
Primary mineral	K	56.6	1.00

TABLE 19-3   Continued

Mineral Source	Mineral	%	Absorption Coefficient
Potassium chloride, KCl (NRC16F1016)			
Primary mineral	K	50.0	1.00
Secondary mineral	Cl	50.0	0.92
Potassium sulfate, K_2SO_4 (NRC16F1032)			
Primary mineral	K	41.8	1.00
Secondary mineral	S	17.4	N/A
SELENIUM SOURCES			
Selenite, sodium, Na_2SeO_3 (NRC16F1061)			
Primary mineral	Se	45.6	N/A
Secondary mineral	Na	36.6	1.00
Selenate, sodium decahydrate, $Na_2SeO_4 \cdot 10H_2O$ (NRC16F1062)			
Primary mineral	Se	21.4	N/A
Secondary mineral	Na	12.5	1.00
SODIUM SOURCES			
Sodium bicarbonate, $NaHCO_3$ (NRC16F1033)			
Primary mineral	Na	27.0	1.00
Sodium carbonate monohydrate, $NaCO_3 \cdot H_2O$ (NRC16F1034)			
Primary mineral	Na	37.1	1.00
Sodium chloride (salt), NaCl (NRC16F1017)			
Primary mineral	Na	39.3	1.00
Secondary mineral	Cl	60.7	0.92
Sodium sesquicarbonate dehydrate, $Na_2CO_3 + NaHCO_3 \cdot 2H_2O$ (NRC16F1035)			
Primary mineral	Na	30.5	1.00
SULFUR SOURCES			
Ammonium sulfate, $(NH_4)_2SO_4$ (NRC16F1037)			
Primary mineral	S	24.1	N/A
Calcium sulfate dihydrate, $CaSO_4 \cdot 2H_2O$ (NRC16F1010)			
Primary mineral	S	23.5	N/A
Secondary mineral	Ca	23.3	0.60
Magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$ (NRC16F1021)			
Primary mineral	S	13.3	N/A
Secondary mineral	Mg	9.8	0.27
Potassium sulfate, K_2SO_4 (NRC16F1032)			
Primary mineral	S	17.4	N/A
Secondary mineral	K	41.8	1.00
Sodium sulfate, Na_2SO_4 (NRC16F1036)			
Primary mineral	S	10.0	N/A
Secondary mineral	Na	14.3	1.00
ZINC SOURCES			
Zinc carbonate, $ZnCO_3$ (NRC16F1064)			
Primary mineral	Zn	52.1	0.20
Zinc chloride, $ZnCl_2$ (NRC16F1065)			
Primary mineral	Zn	48.0	0.20
Secondary mineral	Cl	52.0	0.92
Zinc oxide, ZnO (NRC16F1066)			
Primary mineral	Zn	78.0	0.16

TABLE 19-3 Continued

Mineral Source	Mineral	%	Absorption Coefficient
Zinc sulfate monohydrate, $ZnSO_4 \cdot H_2O$ (NRC16F1067)			
Primary mineral	Zn	36.4	0.20
Secondary mineral	S	17.7	N/A

[a] DM = 100 percent except phosphoric acid = 75 percent.

[b] Mineral concentrations <1 percent not shown.

[c] N/A = not applicable.

[d] For Mg, absorption coefficients assume 1.2 percent K in diet.

[e] Ash content for all sources is equal to 100 percent except for the following: bone meal = 79 percent; ammonium phosphate (dibasic), $(NH_4)_2HPO_4$ = 36 percent; ammonium phosphate (monobasic), $(NH_4)H_2PO_4$ = 36 percent; ammonium sulfate, $(NH_4)_2SO_4$ = 33 percent.

[f] Feeds containing detectable concentrations of nitrogen have the following CP: bone meal = 13.2 percent; ammonium phosphate (dibasic), $(NH_4)_2HPO_4$ = 115.9 percent; ammonium phosphate (monobasic), $(NH_4)H_2PO_4$ = 70.9 percent; ammonium sulfate, $(NH_4)_2SO_4$ = 134.1 percent; the CP in these feeds is assumed to be 100 percent A fraction.

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20

Model Description and Evaluation

MODEL ABBREVIATIONS AND UNITS

Over time, the nutrition community has developed commonly used abbreviations, some of which are predefined for use in the journals. There is good uniformity relative to abbreviations of proximate nutrients, fatty acids (FAs), amino acids (AAs), vitamins, and minerals. However, because digestion, metabolism, and utilization schemes have generally been developed independently by different groups over time, there is no uniformity in the abbreviations used to describe movement of each nutrient through the animal. While this is not problematic for publications focusing on subgroups of nutrients where the abbreviation scheme does not have to be all-encompassing, it is problematic for development of a model containing all of the essential nutrients. In the absence of a clear, intuitive abbreviation scheme, model development will be more error prone, and identification of model inconsistencies will be more problematic. It also should yield model code that is intuitive to understand so that the code can be adopted by others and maintained over time.

For this work, model abbreviations generally follow the pattern: *Location_Nutrient_Modifier*. A similar scheme was outlined independently of this work by Tedeschi and Fox (2020). This approach is a compromise among prior abbreviation schemes that generally results in abbreviations with similar patterns as the historical ones yet removes some ambiguity and allows for more detailed description. For example, one must denote whether digestible protein is apparently or truly digested and the location of the digestion. The addition of a clear location designation as the first term provides more clarity (e.g., ruminally degraded protein should be designated as *Rum_DigCP* or may be denoted as Rum_dCP). Additional examples include Fd_NDF, Dt_NDF, Rum_DigNDF, Du_NDF, and Fe_NDF to denote neutral detergent fiber (NDF) associated with a feed, the diet, digested in the rumen, flowing at the duodenum, and excreted in feces, respectively.

In the interest of maintaining some historical context, and recognizing the universal use of a number of abbreviations that do not follow the scheme, some of the prior abbreviations (e.g., RDP [rumen-degradable protein] and RUP [rumen-undegradable protein]) have been retained, sometimes yielding inconsistencies in the scheme. Additionally, the common use of D to denote digested, M to denote metabolized, and N to denote net was retained to designate energy and protein flows (e.g., DE, ME, NE, MP, and NP to denote digested energy, metabolizable energy, net energy, metabolizable protein, and net protein, respectively).

For nutrients that are tracked from upstream processes through downstream actions, the abbreviation scheme requires a double location designation that was less uniformly applied throughout this work. For example, ruminally undegraded protein must be tracked to the small intestine (SI), where it is digested to calculate AA flows to and absorption from the SI. Thus, there is a challenge in denoting activity in the intestine on nutrients released from the rumen. Per the scheme, one would generate a variable of the form SI_Rum_UP. However, this is cumbersome, and thus the approach was taken to use the second location within the nutrient name. For example, SI_Rum_UP becomes SI_RUP. The intestinal digestion coefficient for Rum_UP would be *SI_dcRUP*; intestinally digested Rum_UP would be denoted as *SI_DigRUP* and the digested AA associated with RUP as *SI_DigAARUP*. While the scheme of indicating location is not perfect, it does provide the needed range in variable designations without losing name transparency and generally yields intuitive names that should not require excessive use of the abbreviation table for decoding. A deviation of the scheme based on nutrient and metabolite transfers and conversions that provides for clearer specification of transfer from location to location with or without interconversion was described by Li et al. (2019), but the committee did not adopt that approach herein.

Phosphorus presents opportunities for ambiguity given the use of P to also denote protein (i.e., CP [crude protein], MP, etc.). Thus, one must be cautious in assigning and using variables for phosphorus to avoid cross-listing variable names with protein variables or misinterpreting model

output. From the scheme, one might be inclined to assign the abbreviation Ph for phosphorus, but future work could result in the addition of a ruminal pH equation, which would be difficult to distinguish from Ph. There does not seem to be a natural solution to the problem that would also be intuitive.

There has also been ambiguity regarding nutrient concentrations versus nutrient intake. For this work, nutrient intakes are clearly defined by the addition of *In* to the end of the abbreviation (e.g., *Rum_DigCPIn*). The absence of *In* as an appendage denotes a concentration (e.g., percentage). For ingredient and dietary nutrients, the default reference for the concentration is dry matter (DM). Thus, *Rum_DigCP* represents the concentration of ruminally digested CP in dietary DM. If the concentration is relative to another entity (e.g., the concentration of *Rum_DigCP* relative to dietary CP), it is denoted by the addition of an underscore and the reference nutrient (e.g., *Rum_DigCP_CP*). These are expressed as percentages unless specified otherwise.

By extension, one should append "out" to variables denoting flow from the specified location (e.g., Fec_DMout for fecal DM output). That convention was not fully adopted, but it is endorsed for future use. In the absence of that appendage, one would infer that Fec_OM would represent the concentration of fecal organic matter (OM) in fecal DM; however, as specified below, it denotes fecal OM output. These deviations are documented at first use. Despite the committee's best efforts, there are additional deviations from the scheme that the reader will note.

The model was coded with default time units of 1 day and mass units of kg for macronutrients. Where mass or flux was in grams, a "g" was added to the variable to denote the change. Vitamins and mineral units are defined for each nutrient, IU, mg, or g. Thus, body weight (BW) is in kg and DM intake (DMI) and milk output are in kg/d. The AA flows are generally expressed in grams, which is denoted across abbreviations.

Subscripts are used herein to avoid replication of equations across classes. When dealing with diets, there are generally multiple ingredients, and thus a subscript of *f* is used to denote each of the different feeds. In a similar manner, the subscript *a* is used for repetitive AA calculations to denote each individual AA and *fa* to denote individual FAs for purposes of model presentation, although each AA and FA equation is explicitly defined in the model code. Examples of such use include Fd_CP_f=feed CP concentration of feed *f*, Fd_NDFIn_f=intake of NDF from feed *f*, Fd_dcFA_f=the digestibility of FAs in feed *f*, $Fd\_CPB\_CP_f$=the percentage of CP in feed *f* that is contained in the B fraction, *Dt_StIn*=dietary starch intake, *Dt_DEIn*=dietary digestible energy intake, *Dt_ArgIn_g*=dietary arginine intake in g/d, *Du_LeuRUP_g*=the flow of leucine from the rumen in the RUP fraction expressed in g/d, *Body_NPgain*=the net protein gain in body tissue, *Mlk_NEout*=the NE excreted in milk, and *Mlk_Fat_g*=the fat excreted with milk expressed as g/d.

Location and nutrient abbreviations are listed in Table 20-1, and a partial list of equation abbreviations is provided in Table 20-2.

MODEL INPUTS

Inputs required by the model include animal, feed, and environmental factors. The animal and environmental factors are similar to those of the seventh revised edition (NRC, 2001). The feed inputs have been expanded to include more carbohydrate information and the FA composition (only used to output dietary concentrations of specific FAs). Required animal inputs include the following (all inputs are not needed for every animal type and state):

1. Breed (An_Breed, "Holstein," "Jersey," "Other")
2. Current physiological state (An_StatePhys, "Calf," "Heifer," "Dry Cow," "Lactating Cow," "Other")
3. Birth weight (*Fet_BWbrth*, kg)
4. Mature body weight (*An_BWmature*, kg)
5. Current herd 305-day milk true protein production (*An_305RHA_MlkTP*, kg/305d)
6. Body weight (*An_BW*, kg)
7. Body condition score (*An_BCS*, 1 to 5 scale)
8. Age (*An_Age*, days)
9. Parity (*An_Parity*, 0 for calves and heifers, 1 for primiparous, and 2 for multiparous)
10. Gestation length (*An_GestLength*, days)
11. Age at first conception (*An_AgeConcept1st*, days; unused. Available for growth simulations)
12. Days in milk at conception (*An_DIMConcept*, days; unused. Available for full lactation simulations)
13. Day of gestation (*An_GestDay*)
14. Days in milk (*An_LactDay*)
15. Current temperature (*Env_TempCurr*, C⁰)
16. Distance from the paddock to the parlor (*Env_Dist Parlor*, m/trip)
17. The number of one-way trips between housing and the parlor (*Env_TripsParlor*, d^{-1})
18. Total climb (uphill only) each day (*Env_Topo*, m/d, uphill only)
19. Target body frame gain (Frm_Gain_{Target}, kg/d)
20. Frame gain prediction equation (*FrmGain_eqn*; 0 = use Frm_Gain_{Target}, 1 = undefined future prediction equation)
21. Target body reserves gain ($Rsrv\_Gain_{Target}$, kg/d)
22. Reserve gain prediction equation (*RsrvGain_eqn*; 0 = use $Rsrv\_Gain_{Target}$, 1 = undefined future prediction equation)
23. Target milk production (*Trg_MilkProd*, kg/d)
24. Target milk lactose content (*Trg_MilkLacp*, percent)
25. Target milk true protein content (*Trg_MilkTPp*, percent)
26. Target milk fat content (*Trg_MilkFatp*, percent)
27. Milk production prediction equation (*mProd_eqn*, 0 = *Trg_MilkProd*, 1 = NRC, 2021)
28. Milk protein production prediction equation (*mPrt_eqn*, 0 = *Trg_MilkTP*, 1 = NRC, 2021)
29. Milk fat production prediction equation (*mFat_eqn*, 0 = *Trg_MilkFat*, 1 = NRC, 2021)

TABLE 20-1 General Location and Nutrient Abbreviations Used for Constructing
Model Terms

Location	Description	Nutrients	Description
Fd	Feed	Wt	Weight
Dt	Diet	GE	Gross energy
An	Whole animal	DE	Digestible energy
		DEnp	DE minus DE from TP
Rum	Rumen	ME	Metabolizable energy
SI	Small intestine	GasE	Gaseous energy
LI	Large intestine	NE	Net energy
TT	Total digestive tract	DM	Dry matter
Fe	Feces	OM	Organic matter
Ur	Urine	NDF	Neutral detergent fiber
Body	Whole empty body	ForNDF	Forage NDF
Frm	Body frame	ADF	Acid detergent fiber
Rsrv	Body reserves	Lg	Lignin
Gest	Gestation	St	Starch
GrUter	Gravid uterus	rOM	Residual OM
Uter	Uterus plus caruncles	CP	Crude protein
Fet	Fetus	TP	True protein
Mlk	Milk and lactation	MP	Metabolizable protein
Scrf	Scurf	NP	Net protein
Env	Animal environment	CPA	CP A fraction, in situ
		CPB	CP B fraction, in situ
Other abbreviations	CPC	CP C fraction, in situ	
Conc	Concentrates	NPN	Supplemental nonprotein nitrogen
For	Forage	FA	Fatty acids
ForDry	Dry forage	Ash	Ash
ForWet	Wet forage	Arg	Arginine
Past	Pasture	His	Histidine
Dc	Digestibility	Ile	Isoleucine
Dig	Digested	Leu	Leucine
		Lys	Lysine
		Met	Methionine
Minerals and vitamins		Phe	Phenylalanine
Ca	Calcium	Thr	Threonine
P	Total phosphorus	Trp	Tryptophan
Pinorg	Inorganic P	Val	Valine
Porg	Organic P	C12	Lauric acid
Na	Sodium	C14	Myristic acid
Mg	Magnesium	C16	Palmitic acid
K	Potassium	C16.1	Palmitoleic acid
Cl	Chloride	C18.0	Stearic acid
S	Sulfur	C18.1c	Oleic acid
Co	Cobalt	C18.1t	Vaccenic + elaidic acid
Cu	Copper	C18.2	Linoleic acid
Fe	Iron	C18.3	Linolenic acid
I	Iodine	OtherFA	Other FA
Mn	Manganese	VitA	Vitamin A
Mo	Molybdenum	VitD	Vitamin D
Se	Selenium	VitE	Vitamin E
Zn	Zinc	DCAD	Cations—anions

30. Target diet DMI (*Trg_Dt_DMI*, kg/d)
31. Diet DMI equation (*DMIn_eqn*, 0) for specified intake or an integer from 1 to 11 as follows:
    1 = predicted dry feed intake for a calf consuming liquid feed (NRC, 2021)
    2 = predicted for all heifers, animal factors (NRC, 2021)

3 = predicted for all heifers, animal factors, and feed factors (NRC, 2021)
4 = predicted for a Holstein heifer and animal factors, prepartum predicted for a single animal
5 = predicted for a Holstein heifer, animal factors, and diet NDF concentration, prepartum predicted for a single animal

TABLE 20-2   Model Abbreviations for Diet and Digestive Macronutrients

Nutrient	Diet[a]	Diet[a]	Ruminal Outflow	Digested or Absorbed	Feces
	% of DM	kg/d or g/d (_g)			
Dry matter	Dt_DM	Dt_DMIn		An_DigDM	Fe_DM
Water		An_WaIn			
Total forage	Dt_For	Dt_ForIn			
Dry forage	Dt_ForDry	Dt_ForDryIn			
Wet forage	Dt_ForWet	Dt_ForWetIn			
Concentrate	Dt_Conc	Dt_ConcIn			
Pasture	Dt_Pasture	Dt_PastureIn			
Ash	Dt_Ash	Dt_AshIn			
Organic matter	Dt_OM	Dt_OMIn		An_DigOM	Fe_OM
Residual OM	Dt_rOM	Dt_rOMIn		An_DigrOMa	Fe_rOM
Nitrogen					Fe_N_g
Crude protein	Dt_CP	Dt_CPIn	Du_CP	An_DigCP	Fe_CP, Fe_InfCP
True protein	Dt_TP	Dt_TPIn	Du_TP	An_DigTP	
Metabolizable protein	Dt_MP	Dt_MPIn			
Nonprotein N[b]	Dt_NPN	Dt_NPNIn	Du_NPN		
CP equivalent of NPN[b]	Dt_NPNCP	Dt_NPNCPIn	Du_NPNCP		
DM of NPN source[b]	Dt_NPNDM	Dt_NPNDMIn	Du_NPNDM		
Fraction A of CP[c]	Dt_CPA	Dt_CPAIn			
Fraction B of CP	Dt_CPB	Dt_CPBIn			
Fraction C of CP	Dt_CPC	Dt_CPCIn			
Ruminally degraded CP	Dt_RDP	Dt_RDPIn			
Ruminally undegraded CP	Dt_RUP	Dt_RUPIn	Du_RUP	Du_idRUP	Fe_RUP
Microbial CP			Du_MiCP_g	Du_idMiCP_g	Fe_MiCP
Microbial N			Du_MiN_g	Du_idMiN_g	
Microbial TP			Du_MiTP_g	Du_idMiTP_g	
Endogenous CP			Du_CPend_g		Fe_CPend_g
Endogenous N			Du_Nend_g		Fe_Nend_g
Starch	Dt_St	Dt_StIn	Du_St	An_DigSt	Fe_St
NDF	Dt_NDF	Dt_NDFIn	Du_NDF	An_DigNDF	Fe_NDF
Forage NDF	Dt_ForNDF	Dt_ForNDFIn			
ADF	Dt_ADF	Dt_ADFIn			
Lignin	Dt_Lg	Dt_LgIn			
Fatty acids	Dt_FA	Dt_FAIn	Du_FA	An_DigFA	Fe_FA
	Mcal/kg	Mcal/d			
Gross energy	Dt_GE	Dt_GEIn			Fe_GE
Digestible energy	Dt_DE	Dt_DEIn			
Metabolizable energy	Dt_ME	Dt_MEIn			

[a] Nutrient concentrations and intake from individual feeds are denoted using the prefix Fd_ in place of Dt_. An additional set of concentrations and intakes is calculated for the summation of Dt_Xxx + Inf_Xxx labeled as An_Xxx.

[b] NPN refers to nitrogen sources not containing peptide bound or free AAs such as urea or ammonium salts. Values are expressed as N for NPN, in CP equivalents for NPNCP, and as DM for NPNDM.

[c] CPA = the CP escaping from an in situ analysis at time 0; CPC = the CP resistant to ruminal degradation at time infinity; CPC = the CP that degrades as a function of time in the rumen.

6 = predicted for a Holstein × Jersey crossbred heifer and animal factors, prepartum predicted for a single animal

7 = predicted for a Holstein × Jersey crossbred heifer, animal factors, and diet NDF concentration, predicted for a single animal

8 = predicted for a lactating cow using animal factors such as BW and body condition score (BCS) (NRC, 2021)

9 = predicted for a lactating cow using animal and feed factors (NRC, 2021)

10 = predicted for a dry cow (NRC, 2021)

11 = predicted for a dry cow (Hayirli et al., 2003 equation)

32. Age when dry feed is first offered to calves (*An_AgeDryFdStart*, days)

33. Control use of in vitro NDF digestibility predictions (*Use_DNDF_IV*; 0 = do not use in vitro NDF digestibility values, 1 = use in vitro NDF digestibility values to adjust NDF digestibility of forage ingredients, 2 = use in vitro NDF digestibility values to adjust NDF digestibility of all ingredients in the diet)

TABLE 20-3   Model Abbreviations for Diet and Digestive AAs and FAs

Nutrient	Diet[a]	Diet[a]	RUP Outflow	Microbial Outflow	Digested or Absorbed
Amino acids	% of DM			kg/d or g/d (_g)	
Arginine	Dt_Arg	Dt_ArgIn_g	Du_ArgRUP_g	Du_ArgMic_g	Abs_Arg_g
Histidine	Dt_His	Dt_HisIn_g	Du_HisRUP_g	Du_HisMic_g	Abs_His_g
Isoleucine	Dt_Ile	Dt_IleIn_g	Du_IleRUP_g	Du_IleMic_g	Abs_Ile_g
Leucine	Dt_Leu	Dt_LeuIn_g	Du_LeuRUP_g	Du_LeuMic_g	Abs_Leu_g
Lysine	Dt_Lys	Dt_LysIn_g	Du_LysRUP_g	Du_LysMic_g	Abs_Lys_g
Methionine	Dt_Met	Dt_MetIn_g	Du_MetRUP_g	Du_MetMic_g	Abs_Met_g
Phenylalanine	Dt_Phe	Dt_PheIn_g	Du_PheRUP_g	Du_PheMic_g	Abs_Phe_g
Threonine	Dt_Thr	Dt_ThrIn_g	Du_ThrRUP_g	Du_ThrMic_g	Abs_Thr_g
Tryptophan	Dt_Trp	Dt_TrpIn_g	Du_TrpRUP_g	Du_TrpMic_g	Abs_Trp_g
Valine	Dt_Val	Dt_ValIn_g	Du_ValRUP_g	Du_ValMic_g	Abs_Val_g
Fatty acids					
C12:0	Dt_C12	Dt_C12In			An_DigC12
C14:0	Dt_C14	Dt_C14In			An_DigC14
C16:0	Dt_C16	Dt_C16In			An_DigC16
C16:1	Dt_C16.1	Dt_C16.1In			An_DigC16.1
C18:0	Dt_C18	Dt_C18In			An_DigC18
C18:1 trans	Dt_C18.1t	Dt_C18.1tIn			An_DigC18.1t
C18:1 cis	Dt_C18.1c	Dt_C18.1cIn			An_DigC18.1c
C18:2	Dt_C18.2	Dt_C18.2In			An_DigC18.2
C18:3	Dt_C18.3	Dt_C18.3In			An_DigC18.3
Other FA	Dt_FAoth	Dt_FAothIn			An_DigFAoth

[a] Nutrient concentrations and intake from individual feeds are denoted using the prefix Fd_ in place of Dt_. An additional set of concentrations and intakes is calculated for the summation of Dt_Xxx + Inf_Xxx labeled as An_Xxx.

TABLE 20-4   Model Abbreviations for Maintenance Use of Energy and Protein

Nutrient	Maintenance	Feces	Urine	Scurf	Activity	Environment
Digest. energy			Ur_DEout			
Metab. energy	An_MEmUse					
Net energy	An_NEmUse				An_NEm_Act	An_NEm_Env
Net protein		Fe_NPend_g	Ur_NPend_g	Scrf_NP_g		
Nitrogen		Fe_N_g	Ur_Nout_g			
Phosphorus		Fe_P_g	Ur_P_g			
Factor						
Temp						Env_TempCurr
Distance						Env_DistParlor
Topography						Env_Topo
Amino acids						
Arginine		Fe_ArgMet_g	Ur_ArgEnd_g	Scrf_Arg_g		
Histidine		Fe_HisMet_g	Ur_HisEnd_g	Scrf_His_g		
Isoleucine		Fe_IleMet_g	Ur_IleEnd_g	Scrf_Ile_g		
Leucine		Fe_LeuMet_g	Ur_LeuEnd_g	Scrf_Leu_g		
Lysine		Fe_LysMet_g	Ur_LysEnd_g	Scrf_Lys_g		
Methionine		Fe_MetMet_g	Ur_MetEnd_g	Scrf_Met_g		
Phenylalanine		Fe_PheMet_g	Ur_PheEnd_g	Scrf_Phe_g		
Threonine		Fe_ThrMet_g	Ur_ThrEnd_g	Scrf_Thr_g		
Tryptophan		Fe_TrpMet_g	Ur_TrpEnd_g	Scrf_Trp_g		
Valine		Fe_ValMet_g	Ur_ValEnd_g	Scrf_Val_g		

[a] Nutrient concentrations and intake from individual feeds are denoted using the prefix Fd_ in place of Dt_. An additional set of concentrations and intakes is calculated for the summation of Dt_Xxx + Inf_Xxx labeled as An_Xxx.

TABLE 20-5 Model Abbreviations for Total and Productive Use of Energy (Mcal/D), Protein (kg or G/D (_G)), and Fat (kg or G/D (_G))

Nutrient	Total	Production	Growth	Gestation	Milk
Weight	An_BW		Body_Gain	GrUter_Wt Uter_Wt Fet_Wt GrUter_BWgain	Mlk_Prod
Digest. energy					
Metab. energy		An_MEprod_Avail		Gest_Meuse	Mlk_MEout
Net energy	An_NEgain	An_NEprod_Avail		Gest_Neuse	Mlk_NEout Mlk_NE_Milk
Protein			Body_NPgain	Gest_NP_g	Mlk_NP_g MlkNP_Milk
Fat	Body_Fat		Body_Fatgain		Mlk_Fat_g MlkFat_Milk
Amino acids	g/d				
Arginine	An_ArgUse_g		Body_ArgGain_g	Gest_Arg_g	Mlk_Arg_g
Histidine	An_HisUse_g		Body_HisGain_g	Gest_His_g	Mlk_His_g
Isoleucine	An_IleUse_g		Body_IleGain_g	Gest_Ile_g	Mlk_Ile_g
Leucine	An_LeuUse_g		Body_LeuGain_g	Gest_Leu_g	Mlk_Leu_g
Lysine	An_LysUse_g		Body_LysGain_g	Gest_Lys_g	Mlk_Lys_g
Methionine	An_MetUse_g		Body_MetGain_g	Gest_Met_g	Mlk_Met_g
Phenylalanine	An_PheUse_g		Body_PheGain_g	Gest_Phe_g	Mlk_Phe_g
Threonine	An_ThrUse_g		Body_ThrGain_g	Gest_Thr_g	Mlk_Thr_g
Tryptophan	An_TrpUse_g		Body_TrpGain_g	Gest_Trp_g	Mlk_Trp_g
Valine	An_ValUse_g		Body_ValGain_g	Gest_Val_g	Mlk_Val_g
Other factors					
Time			An_AgeDay	An_GestDay	An_LactDay

[a] Nutrient concentrations and intake from individual feeds are denoted using the prefix Fd_ in place of Dt_. An additional set of concentrations and intakes is calculated for the summation of Dt_Xxx + Inf_Xxx labeled as An_Xxx.

TABLE 20-6 Model Abbreviations for Minerals and Vitamins

Nutrient	Diet[a]	Diet[a]	Absorbed	Maint.	Growth	Gestation	Lact.	Required
	% of DM				g/d			
Calcium	Dt_Ca	Dt_CaIn	Abs_CaIn	An_Ca_m	An_Ca_g	An_Ca_y	An_Ca_l	An_Ca_req
Chloride	Dt_Cl	Dt_ClIn	Abs_ClIn	An_Cl_m	An_Cl_g	An_Cl_y	An_Cl_l	An_Cl_req
Magnesium	Dt_Mg	Dt_MgIn	Abs_MgIn	An_Mg_m	An_Mg_g	An_Mg_y	An_Mg_l	An_Mg_req
Phosphorus	Dt_P	Dt_Pin	Abs_Pin	An_P_m	An_P_g	An_P_y	An_P_l	An_P_req
Potassium	Dt_K	Dt_Kin	Abs_Kin	An_K_m	An_K_g	An_K_y	An_K_l	An_K_req
Sodium	Dt_Na	Dt_NaIn	Abs_NaIn	An_Na_m	An_Na_g	An_Na_y	An_Na_l	An_Na_req
Sulfur	Dt_S	Dt_Sin						An_S_req
	mg/kg				mg/d			
Cobalt	Dt_Co	Dt_Coin						An_Co_req
Copper	Dt_Cu	Dt_Cuin	Abs_CuIn	An_Cu_m	An_Cu_g	An_Cu_y	An_Cu_l	An_Cu_req
Iodine	Dt_I	Dt_Iin						An_I_req
Iron	Dt_Fe	Dt_Fein	Abs_FeIn	An_Fe_m	An_Fe_g	An_Fe_y	An_Fe_l	An_Fe_req
Manganese	Dt_Mn	Dt_Mnin	Abs_MnIn	An_Mn_m	An_Mn_g	An_Mn_y	An_Mn_l	An_Mn_req
Selenium	Dt_Se	Dt_Sein						An_Se_req
Zinc	Dt_Zn	Dt_Znin	Abs_ZnIn	An_Zn_m	An_Zn_g	An_Zn_y	An_Zn_l	An_Zn_req
	IU/kg				IU/d			
Vitamin A	Dt_VitA	Dt_VitAin						An_VitA_req
Vitamin D	Dt_VitD	Dt_VitDin						An_VitD_req
Vitamin E	Dt_VitE	Dt_VitEin						An_VitE_req

[a] Nutrient concentrations and intake from individual feeds are denoted using the prefix Fd_ in place of Dt_.

36. A dataframe of dietary ingredients with nutrient contents (*f*, various units)
37. A vector of dietary inclusion percentages ordered to correspond to each ingredient in *f* (*Fd_DMInp*, percentage of DM)
38. A vector of nutrient infusion rates, digestibility coefficients, and the location of the infusions (*i*; various units). Infusion locations are rumen, abomasum or duodenum, or blood. These are read from an external file with all nutrient infusions set to 0 by default
39. A vector of target AA and MP post-absorptive use efficiencies (Eff; range of 0 to 1 for each)
40. A switch denoting the effects of an under-developed rumen in a milk-fed calf on diet digestibility (*RumDevDisc_Clf* = 0 for developed and 1 for under-developed)
41. A switch denoting the use of non-milk based protein sources in milk replacers (*NonMilkCP_ClfLiq* = 0 for all milk proteins and 1 for use of other protein sources)

These inputs are passed to the model and used by the model to predict animal performance, requirements for nutrients, and animal excretion.

For purposes of model application, animals are considered calves until they achieve 16 percent of mature BW, heifers until their first calf, primiparous cows for their first lactation, and multiparous cows thereafter. Cows that are not lactating are denoted as dry cows. Males follow the same designation as calves and heifers and should be considered dry cows when mature.

NUTRIENT SUPPLY MODEL

The model is described in two sections: nutrient supply and nutrient utilization. These sections were written by transcribing the model code from R. As such, it is possible that it is not a faithful reproduction of the code, although every attempt was made to ensure that it was. Should there be differences between the description of the model herein and the actual model code written in R, the latter is more likely to be correct, and the difference reflects a mistake in the transcription. The R code was developed and verified over a 4-year period and thus should generally be the more reliable source, although mistakes are certainly possible.

In general, the supply model starts with the specified dietary ingredients and predicts the supply of nutrients absorbed from the digestive tract.

Water Intake

Water intake (*An_WaIn*, L/d) is predicted for heifers, lactating cows, and dry cows. Calves and other physiological states are undefined. The equations use DMI, dietary sodium (Na) and potassium (K), dietary CP, and the current ambient temperature. The prediction is categorized with one equation for lactating cows and a separate equation for dry cows, which was presumed also to apply to postweaned heifers:

$$\frac{An\_WaIn}{L/d} =$$

An_StatePhys	Equation
Lactating Cow	$-91.1 + 2.93 \times Dt\_DMIn + 0.61 \times Dt\_DM + 2.49 \times Dt\_CP + 0.062 \times \left(\dfrac{Dt\_Na}{0.023} + \dfrac{Dt\_K}{0.039} \times 10\right) + 0.76 \times Env\_Temp_{Curr}$
Dry Cow/Heifer	$1.16 \times Dt\_DMIn + 0.23 \times Dt\_DM + 0.44 \times Env\_Temp_{Curr} + 0.061 \times \left(Env\_Temp_{Curr} - 16.4\right)^2$
Other	Undefined

(Equation 20-1)

Dry Matter Intake

DMI can be specified by the user to match observed or target values (*Trg_DMIn*, kg DM/d) or predicted by the model. Intake concepts underpinning the predictions are summarized in Chapter 2. Eight prediction equations are encoded. These are categorized by physiological state (*An_StatePhys*). The model is set to calculate all intakes regardless of animal age and state. The user must select which intake to use for the remaining calculations. From a model standpoint, it is possible to select a calf equation for use with a lactating cow, but that would obviously not be advised.

In all cases, environmental temperatures exceeding the upper critical temperature (UCT) of the animal have been observed to result in reduced DMI if the exposure is prolonged. However, such stress is only reflected in the calf equations. Intake may also be stimulated when temperatures fall below the lower critical temperature (LCT) for extended periods of time, but that concept is not captured in any of the equations. The UCT and LCT are defined as follows:

$$\frac{LCT}{°C} = \begin{cases} \text{Criteria} & \text{Value} \\ An\_Age < 21d & 15 \\ An\_Age \geq 21d & 5 \end{cases}$$

(Equation 20-2)

The upper critical temperature (UCT, °C) is assumed to be the same for all ages.

$$UCT, °C = 25 \qquad \text{(Equation 20-3)}$$

with the difference between the UCT and LCT being defined as the thermoneutral zone (TMZ).

Calves

A single scheme for predicting calf intake is encoded. Total DMIn for calves is predicted based on Trg_DMIn for liquid (milk and milk replacer) and a predicted dry starter feed intake ($Dt\_DMIn\_Calf_{StrtFeed}$, kg DM/d). The latter is predicted from the user-specified animal BW (An_BW, kg), the ME intake from liquid feeds ($Dt\_MEIn\_CalfLiqFd$, Equation 20-5), a user-specified target BW gain (Trg_BWgain, kg/d), the user-specified age (d) when starter feed is initially offered ($An\_Age_{DryFdStart}$), and the ambient temperature relative to the UCT for calves:

$$\frac{Dt\_DMIn\_Calf_{StrtFd}}{kg/d} =$$

Env_Temp	Equation
$< UCT + 10$	$\dfrac{\left(\begin{array}{l}14.73 \times An\_BW + 18.90 \\ \times Dt\_MEIn\_Calf_{LiqFd} \\ + 73.3 \times \dfrac{An\_Age_{DryFdStart}}{7} \\ + 13.50 \times \left(\dfrac{An\_Age_{DryFdStart}}{7}\right)^2 \\ - 29.61 \times \dfrac{An\_Age_{DryFdStart}}{7} \\ \times Dt\_MEIn\_Calf_{LiqFd} - 652.5\end{array}\right)}{1,000}$
$\geq UCT + 10$	$\dfrac{\left(\begin{array}{l}600.1 \times \left[\begin{array}{l}1+14864 \\ \times e^{\left(-1.553 \times An\_AgeDryFdStart / 7\right)}\end{array}\right]^{-1} \\ + 9.95 \times An\_BW - 130.4 \\ \times Dt\_MEIn\_Calf_{LiqFd}\end{array}\right)}{1,000}$

(Equation 20-4)

Liquid feed ME intake ($Dt\_MEIn\_Calf_{LiqFd}$, Mcal/d) is estimated as 91 percent of the gross energy (GE) values:

$$\frac{Dt\_MEIn\_Calf_{LiqFd}}{Mcal/d} = \sum_{f=1}^{N_{f\_Liq}} \left(\begin{array}{l}Fd\_DMInp_{f\_Liq} \\ \times Trg\_Dt\_DMIn \\ \times Fd\_GE_f \times 0.91\end{array}\right)$$

(Equation 20-5)

with Fd_DMInp representing the proportion of DM provided from each feed, and Fd_GE for liquid calf feed is estimated as

$$\frac{Fd\_GE_f}{Mcal/kg} = \frac{\left[\begin{array}{l}(100 - Fd\_Ash - Fd\_FA - Fd\_CP) \times 4.0 \\ + Fd\_FA \times 9.4 + Fd\_CP \times 5.65\end{array}\right]}{100}$$

(Equation 20-6)

Equation 20-6 may overestimate GE for liquid feeds containing lactose as the enthalpy of lactose is 3.95 rather than 4, but the enthalpy of glycerol is 4.3 Mcal/kg, and it also is given a value of 4. Using enthalpies of specific compounds requires accurate information on the concentrations of those compounds that often will not be available. The error in using an enthalpy of 4 Mcal/kg for all OM that is not CP or FAs should be minor.

Total $DMIn$ is predicted as the summation of that from liquid and dry feeds:

$$\frac{Dt\_DMIn\_Calf}{kg/d} = Dt\_DMIn_{LiqFd} + Dt\_DMIn_{StrtFd} + Dt\_DMIn_{ForFd}$$

(Equation 20-7)

where liquid (Dt_DMIn_{LiqFd}) and forage intakes (Dt_DMIn_{ForFd}) are derived from Trg_DMIn and the specified dietary proportions of each ingredient in those classes of feed:

$$\frac{Dt\_DMIn\_Calf_{LiqFd}}{kg/d} = \sum_{f=1}^{N_{f\_Liq}} \left(\begin{array}{l}Fd\_DMInp_{f\_Liq} \\ \times Trg\_Dt\_DMIn\end{array}\right)$$

(Equation 20-8)

$$\frac{Dt\_DMIn\_Calf_{ForFd}}{kg/d} = \sum_{f=1}^{N_{f\_For}} \left(\begin{array}{l}Fd\_DMInp_{f\_For} \\ \times Trg\_Dt\_DMIn\end{array}\right)$$

(Equation 20-9)

Because the predicted starter intake will not necessarily equal that calculated from the product of Trg_DMIn and Fd_DMInp for starter feeds, the diet proportions of all ingredients will not exactly match those specified by the user when predictions of starter intake are used. When the model is set to use the Trg_Dt_DMIn, starter intake is adjusted to represent the difference between the specified An_DMIn and the specified liquid feed intake.

Heifers

Two general equation types are encoded for heifers from weaning to calving, but there are options for each based on breed and on the handling of the late-gestation predictions. The first set of equations is based solely on animal factors, and the second set uses both diet and animal factors to predict intake. These are subdivided to equations for use across breeds, which are the predictions recommended by the committee. Additional sets for Holsteins and Holstein-by-Jersey crossbreds were also encoded for comparison purposes. Each of these equations was coded to reflect individual animal intakes and mean intakes by a pen of animals during the late-gestation period.

Animal Factor Based

Intake of growing heifers of any breed prior to 3 weeks before calving ($Dt\_DMIn\_Heif1_{FarOff}$, kg/d) can be predicted based on animal factors as

$$Dt\_DMIn\_Heif1_{FarOff} = 0.022 \times An\_BWmature$$
$$\times (1 - e^{-1.54*(An\_BW/An\_BWmature)})$$

(Equation 20-10)

Animal and Diet Factor Based

Intake of growing heifers prior to 3 weeks before calving ($Dt\_DMIn\_Heif2_{FarOff}$, kg/d) was predicted based on feed and animal factors as

$$Dt\_DMIn\_Heif2_{FarOff,}\ kg/d = [0.0226 \times An\_BWmature$$
$$\times (1 - e^{\{-1.47 \times (An\_BW/An\_BWmature)\}})] - [0.082 \times (Dt\_NDF$$
$$- \{23.1 + 56 \times (An\_BW/An\_BWmature)$$
$$- 30.6 \times (An\_BW/An\_BWmature)^2\})]$$

(Equation 20-11)

where Dt_NDF was expressed as a percentage of DM, and BW was in kg. Intake of late-gestation heifers (within 60 days of parturition) can also be predicted using the dry and transition cow intake equations.

Late-Gestation Intake

From 3 weeks prior to calving until calving, the normal decline in intake ($Dt\_DMIn\_BW_{LateGest\_ind}$, percentage of BW) for an individual animal is as described in Chapter 12:

$$\frac{Dt\_DMIn\_BW_{LateGest\_ind}}{\%\ of\ BW} = 1.47 - fDMIn_{NDF}$$
$$\times An\_Wk_{PrePart}$$
$$- 0.035 \times An\_Wk_{PrePart}{}^2$$

(Equation 20-12)

where $fDMIn_{NDF}$ (percentage of BW/Wk) is a function of dietary NDF (Dt_NDF, percentage of DM):

$$fDMIn_{NDF} = 0.365 - 0.0028 \times Dt\_NDF$$

(Equation 20-13)

and $An\_Wk_{PrePart}$ (weeks before calving expressed in negative values) is calculated from the user-specified day of gestation ($An\_DayGest$, d) and the expected gestation length for the selected breed ($An\_DayGestLength$, d):

$$\frac{An\_Wk_{PrePart}}{weeks} = \frac{\left(An\_Day_{Gest} - An\_Day_{GestLength}\right)}{7}$$

(Equation 20-14)

$An\_Wk_{PrePart}$ is limited to the range of −3 to 0, and Dt_NDF is limited to the range of 30 to 55 percent.

Because Equation 20-12 is a nonlinear function of time, the intake of a group of animals is not accurately reflected by setting $An\_Wk_{PrePart}$ to the mean of the group. The function must be integrated over the range in time animals are spending in the pen to achieve a proper group estimate. It is assumed that $An\_Wk_{PrePart}$ reflects the mean weeks before calving for the prefreshening pen if the pen is in steady state, and thus the duration of time ($An\_Wk_{PrePartDurat}$, wk) over which to integrate is 2× the mean:

$$An\_Wk_{PrePartDurat} = An\_Wk_{PrePart} \times 2$$

(Equation 20-15)

Pen intake ($Dt\_DMIn\_BW_{LateGest\_pen}$, percentage of BW) is calculated as the integral from $An\_Wk_{PrePartDurat}$ to 0:

$$\frac{Dt\_DMIn\_BW_{PrePart\_Pen}}{\%\ of\ BW} = \frac{\left(\begin{array}{l} 1.47 \times An\_Wk_{PrePartDurat} \\ - \dfrac{fDMIn_{NDF}}{2} \\ \times An\_Wk_{PrePartDurat}^2 - \dfrac{0.035}{3} \\ \times An\_Wk_{PrePartDurat}^3 \end{array}\right)}{An\_Wk_{PrePartDurat}}$$

(Equation 20-16)

Because the Hayirli et al. (2003) work reflected multiparous animals, total daily intake for individual animals ($Dt\_DMIn\_Heif_{Close\_Ind}$, kg/d) and for groups of animals ($Dt\_DMIn\_Heif_{Close\_Pen}$, kg/d) from 3 weeks before calving until calving is calculated from An_BW using a 12 percent reduction:

$$\frac{Dt\_DMIn\_Heif_{Close\_Ind}}{kg/d} = \frac{Dt\_DMIn\_BW_{PrePart\_Ind}}{100}$$
$$\times An\_BW \times 0.88$$

(Equation 20-17a)

$$\frac{Dt\_DMIn\_Heif_{Close\_Pen}}{kg/d} = \frac{Dt\_DMIn\_BW_{PrePart\_Pen}}{100}$$
$$\times An\_BW \times 0.88$$

(Equation 20-17b)

The model calculates intakes with each adjustment equation, and either approach can be chosen for the R code, but Equation 20-17 is used for late-gestation adjustments by the software. Heifer intake (both Dt_DMIn_Heif1 and $Dt\_DMInHeif2$) for the entire period is thus predicted by selection of the far-off or close-up equations based on the user-specified days before calving. Because the close-up equation is discontinuous with the far-off equation, the exact time before calving where the transition in equations occurs is not specified but rather subject to a minimum test:

$$\frac{Dt\_DMIn\_Heif}{kg/d} =$$

Criteria	Equation
$An\_Wk_{PrePart} < -3$	$Dt\_DMIn\_Heif_{FarOff}$
$An\_Wk_{PrePart} >= -3$	$\min\begin{pmatrix} Dt\_DMIn\_Heif1_{Close}, \\ Dt\_DMIn\_Heif_{FarOff} \end{pmatrix}$

(Equation 20-18)

Nonlactating Cows

DMI for nonlactating, multiparous cows is predicted based on Chapter 12 as

$$\frac{Dt\_DMIn\_Dry_{Ind}}{kg/d} = \frac{Dt\_DMIn\_BW_{PrePart\_Ind}}{100} \times An\_BW$$

(Equation 20-19)

$$\frac{Dt\_DMIn\_Dry_{Pen}}{kg/d} = \frac{Dt\_DMIn\_BW_{PrePart\_Pen}}{100} \times An\_BW$$

(Equation 20-20)

The first equation will predict constant intake for $An\_Wk_{PrePart}$ less than -3, and the second will approach an asymptote a little further away from calving as the late-gestation drop in intake is diluted over longer periods at normal intakes.

Lactating Cows

As for heifers, DMI for lactating cows is predicted based solely on animal factors or a combination of animal and dietary factors. The animal factor equation is

$$\frac{Dt\_DMIn\_Lact1}{kg/d} = (3.7 + 5.7 \times (An\_Parity - 1) + 0.305$$
$$\times Milk\_NEuse_{Target} + 0.022 \times An\_BW$$
$$+ (-0.689 - 1.87 \times (An\_Parity - 1)$$
$$\times An\_BCS)$$
$$\times (1 - (0.212 + 0.136 \times (An\_Parity - 1))$$
$$\times e^{(-0.053 \times An\_DayLact)})$$

(Equation 20-21)

where An_Parity is parity expressed as a real value ranging from 1 to 2 with 1 denoting primiparous and 2 multiparous. For individual animals, this value is obviously binary, but for groups of animals, it reflects the mean for the group. Mlk_NEuse_{Target} (Mcal/d) is the NE output for the desired milk output, which is calculated from user-specified, target milk production (Mlk_Prod_{Target}, kg/d) and composition (Equation 20-217).

As discussed for the dry cow equation, the nonlinear change in predicted intake associated with the exponential term will result in biased predictions for pen intakes when the pen mean days in milk (DIM) is used unless all animals

in the pen have identical DIM. Thus, the equation should be integrated over the time range for the pen for pens containing cattle that are less than 90 DIM, the point where the function reaches a plateau. However, the error will be very small provided all cows are greater than 45 DIM. From calving until 45 DIM, use of the pen mean DIM directly with Equation 20-21 will result in a substantial overprediction of DMIn for the pen.

Lactating cow intake is also predicted from a combination of feed and animal factors as

$$\frac{Dt\_DMIn\_Lact2}{kg/d} = 12.0 - 0.107$$
$$\times Dt\_fNDF + 8.17$$
$$\times \frac{Dt\_ADF}{Dt\_NDF} + 0.0253$$
$$\times ForNDF48\_ForNDF - 0.328$$
$$\times (Dt\_ADF / Dt\_NDF - 0.602)$$
$$\times (ForNDF48\_ForNDF - 48.3)$$
$$+ 0.225 \times Milk\_Prod_{Target} + 0.00390$$
$$\times (ForNDF48\_ForNDF - 48.3)$$
$$\times (Milk\_Prod_{Target} - 33.1)$$

(Equation 20-22)

This equation can be expected to be valid for predictions of cows greater than 60 DIM. Predictions for early lactation likely will not be representative due to the lack of a term describing the lag in DMIn from calving to 60 DIM.

Intake Selection

Dt_DMIn (kg/d) is either specified or predicted by setting An_DMIeqn to the number of the desired equation where a choice of 0 utilizes Dt_DMIn_{Target}.

$$\frac{Dt\_DMIn}{kg/d} =$$

An_DMIeqn	Equation
0	Dt_DMIn_{Target}
1	Dt_DMIn_Calf
2	Dt_DMIn_Heif1
3	Dt_DMIn_Heif2
4	$Dt\_DMIn\_BWLateGest\_ind$
5	$Dt\_DMIn\_BWLateGest\_pen$
6	Dt_DMIn_Dry
7	$Dt\_DMIn\_BWPrePart\_ind$
8	$Dt\_DMIn\_BWPrePart\_Pen$
9	Dt_DMIn_Lact1
10	Dt_DMIn_Lact2

(Equation 20-23)

The choice of physiological state can be used to narrow the selection list for intake equations to those available for the chosen state. For example, if Heifer is chosen as the physiological state, the intake equations offered will be a user-specified intake or intake predicted by Equation 20-10 or 20-11.

The selected DMIn is also expressed as proportions of An_BW or metabolic An_BW ($An\_MBW = An\_BW^{0.75}$):

$$\frac{Dt\_DMIn\_BW}{kg/kg\ \text{BW}} = \frac{Dt\_DMIn}{An\_BW}$$

(Equation 20-24)

$$\frac{Dt\_DMIn\_MBW}{kg/kg\ \text{BW}^{0.75}} = \frac{Dt\_DMIn}{An\_BW^{0.75}}$$

(Equation 20-25)

Dietary Nutrient Concentrations and Intake of Feed Nutrients

The intake of each feed ingredient (f) is calculated from Dt_DMIn and the user-specified dietary DM proportions (kg/kg) for each ingredient:

$$\frac{Fd\_DMIn_f}{kg/d} = Fd\_DMInp_f \times Dt\_DMIn$$

(Equation 20-26)

As fed intake and proportions are calculated based on the user-entered DM content of each ingredient:

$$\frac{Fd\_AFIn_f}{kg/d} = \frac{Fd\_DMIn_f}{Fd\_DM_f/100}$$

(Equation 20-27a)

$$\frac{Fd\_AFInp_f}{kg/kg} = \frac{Fd\_AFIn_f}{\sum_{f=1}^{N_f} Fd\_AFIn_f}$$

(Equation 20-27b)

Some predictions are based on feed type or category. Several of these categories are those used by NRC (2001), but additional categories were added. The list of categories is provided in Table 20-7.

Additionally, wet forage was defined as forages with less than 71 percent DM and dry forages as those with 71 percent DM or greater. Although the ingredients are categorized in the feed library, the designations are converted to dietary percentages (i.e., 100 percent for ingredients in the category and 0 percent for ingredients not in the category). This allows calculation of a proportion of the diet derived from each category as a continuous variable (i.e., 45 percent of the diet is concentrate), which is required for application in prediction equations. The general form of this equation is

$$Dt\_DMIn_c = \sum_{f=1}^{N_f} \left(Dt\_DMIn \times \frac{Fd\_DMInp_f}{100} \times \frac{Fd\_Type_{f,c}}{100} \right)$$

(Equation 20-28)

TABLE 20-7   Feed Categories Used for Model Calculations

Category	Description
Additive	Compounds that do not provide known nutrients
Animal protein	Animal-based protein source
By-product/other	By-product of ingredient processing and other uncategorized ingredients
Calf liquid feed	Liquid calf feed, replacer, or milk
Calf liquid feed	Milk and milk replacer feeds fed in liquid form
Energy source	High-starch, low-protein grains
FA supplement	Supplemental free FA
Fat supplement	Supplemental fat as triacylglycerol
Grain crop forage	Maize and small-grain whole-crop forages
Grass/legume forage	Grass or legume forage
Pasture	Grazed pasture (needed to estimate intake by grazing cows)
Plant protein	Plant-based protein source
Sugar/sugar alcohols	Mono- and disaccharides and glycerol
Vitamin/mineral	Vitamins and minerals

Categories are used to make finding a feed easier and to ensure the proper equations for estimating energy and other variables are used. Users must choose a correct category for accurate results.

where $Fd\_Type_{f,c}$ was equal to 100 for ingredients in category c and 0 for ingredients not in category c.

Additional nutrient-based variables are derived from the base nutrients specified in the feed library. These included

$$\frac{Fd\_ForNDF_f}{\%\ DM} = \left(1 - \frac{Fd\_Conc_f}{100} \right) \times Fd\_NDF_f$$

(Equation 20-29)

$$\frac{Fd\_NDFnf_f}{\%\ DM} = Fd\_NDF_f - Fd\_NDFIP_f$$

(Equation 20-30)

$$\frac{Fd\_CPA_f}{\%\ DM} = Fd\_CP_f \times \frac{Fd\_CPA\_CP_f}{100}$$

(Equation 20-31)

$$\frac{Fd\_CPB_f}{\%\ DM} = Fd\_CP_f \times \frac{Fd\_CPB\_CP_f}{100}$$

(Equation 20-32)

$$\frac{Fd\_CPC_f}{\%\ DM} = Fd\_CP_f \times \frac{Fd\_CPC\_CP_f}{100}$$

(Equation 20-33)

$$\frac{Fd\_NPNCP_f}{\%\ DM} = Fd\_CP_f \times \frac{Fd\_NPN\_CP_f}{100}$$

(Equation 20-34)

$$\frac{Fd\_NPN_f}{\%\ DM} = \frac{Fd\_NPNCP_f}{6.25}$$

(Equation 20-35)

$$\frac{Fd\_NPNDM_f}{\%\ DM} = \frac{Fd\_NPNCP_f}{2.81}$$

(Equation 20-36)

$$\frac{Fd\_TP_f}{\%\ DM} = Fd\_CP_f - Fd\_NPNCP_f$$

(Equation 20-37)

$$\frac{Fd\_N_f}{\%\ DM} = \frac{Fd\_CP_f}{6.25}$$ (Equation 20-38)

Because dietary FAs are specified as an input to the model rather that triglycerides (TGs), the mass of FA present in the feed varies depending on the form of the fat where free FAs are hydrated and thus require no correction while FAs present in TGs are hydrated when cleaved from the TG for further metabolism. Thus, a hydration factor must be specified at the feed level and used to calculate FA content:

$$\frac{Fd\_fHydr\_FA_f}{g/g} = \begin{cases} \begin{array}{c|c} Fd\_Category & Value \\ \hline FA\ Supplement & 1 \\ \hline All\ Other\ Categories & \dfrac{1}{1.06} \end{array} \end{cases}$$

(Equation 20-39)

Intake of individual FAs was also calculated and used to generate dietary intakes of total unsaturated (*Dt_UFAIn*), monounsaturated (*Dt_MUFAIn*), polyunsaturated (*Dt_PUFAIn*), and saturated FAs (*Dt_SatFAIn*):

$$\frac{Dt\_UFAIn}{kg/d} = Dt\_C161In + Dt\_C181tIn + Dt\_C181cIn$$
$$+ Dt\_C182In + Dt\_C183In$$

(Equation 20-40a)

$$\frac{Dt\_MUFAIn}{kg/d} = Dt\_C161In + Dt\_C181tIn + Dt\_C181cIn$$

(Equation 20-40b)

$$\frac{Dt\_PUFAIn}{kg/d} = Dt\_C182In + Dt\_C183In$$

(Equation 20-40c)

$$\frac{Dt\_SatFAIn}{kg/d} = Dt\_FAIn - Dt\_UFAIn$$

(Equation 20-40d)

Residual organic matter (rOM)[1] represents the remainder after subtraction of ash, NDF, St, FA, TP, and NPN DM:

$$\frac{Fd\_rOM_f}{\%\ DM} = 100 - Fd\_Ash_f - Fd\_NDF_f - Fd\_St_f$$
$$- (Fd\_FA_f \times Fd\_fHydr\_FA_f)$$
$$- Fd\_TP_f - Fd\_NPNDM_f$$

(Equation 20-41)

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[1] In other chapters of this report this is written as ROM, which also denotes residual organic matter.

For historical purposes, nonfiber carbohydrate (NFC) is calculated as

$$\frac{Fd\_NFC_f}{\%\ DM} = 100 - Fd\_Ash_f - Fd\_NDF_f - Fd\_TP_f$$
$$- Fd\_NPNDM_f - Fd\_FA_f \times Fd\_fHydr\_FA_f$$

(Equation 20-42)

Nutrient (*Nut*) intakes from each feed are subsequently calculated from the nutrient concentrations and the DMI of each feed as defined by Equation 20-26:

$$\frac{Fd\_Nut(i)In_f}{kg/d} = \frac{Fd\_Nut(i)_f}{100} \times Fd\_DMIn_f$$

(Equation 20-43)

Forage and concentrate intakes are calculated in the same manner. Intake of nutrients expressed as a percentage of the parent nutrient (ForNDF, CP fractions, AA, and FA) is calculated as

$$\frac{Fd\_NutFrac(i)In_f}{\%\ of\ Fd\_Nut} = Fd\_Nut(i)In_f \times \frac{Fd\_NutFrac(i)_f}{100}$$

(Equation 20-44)

Each of the above nutrients is subsequently summed to yield dietary nutrient intakes:

$$\frac{Dt\_Nut(i)In}{kg/d} = \sum_{f=1}^{n} Fd\_Nut(i)In_f$$

(Equation 20-45)

where *n* represents the number of ingredients in the diet. Dietary nutrient concentrations and concentrations of concentrate and forage in the diet are calculated as

$$\frac{Dt\_Nut(i)}{\%\ DM} = \frac{Dt\_Nut(i)In}{Dt\_DMIn} \times 100$$

(Equation 20-46)

Vitamin and mineral concentrations are stoichiometrically adjusted to achieve the units denoted in Table 20-2.

AA intakes (g/d) are calculated as

$$\frac{Fd\_AAt(a)In_f}{g/d} = \frac{Fd\_AAt(a)\_CP_f}{100}$$
$$\times \frac{Fd\_CP_f}{100} \times Fd\_DMIn_f \times 1,000$$

(Equation 20-47)

where *Fd_AAt(a)_CP* represented the true AA content of the ingredient (percentage of CP), which is predicted from feed library values as

TABLE 20-8 Fractional Recovery (*Recaa*, G Observed/G True) and Hydration Factors (G Anhydrous AA/G Hydrated AA) for Adjustment of AA Composition Determined by a Standard 24-Hour Acid Hydrolysis and for Conversion from Protein Bound to Free Forms

Amino Acid	Recovery	Hydration
Arg	0.943	0.8967
His	0.932	0.884
Ile	0.893	0.8628
Leu	0.939	0.8628
Lys	0.938	0.8769
Met	0.952	0.8794
Phe	0.943	0.891
Thr	0.937	0.849
Trp	0.943	0.9118
Val	0.907	0.8464

$$\frac{Fd\_AAt(a)\_CP_f}{\%\,CP} = \frac{Fd\_AA(a)\_CP_f}{RecAA(a)}$$

(Equation 20-48)

a denoted each of the 10 essential AAs, and *RecAA(a)* represented the predicted recovery of each AA during acid hydrolysis (see Table 20-8 and discussion in Chapter 6). Reported AA composition also reflects the addition of water across the peptide bond. AAs are measured in free form and reported as such. Therefore, summation of the complete set of reported AAs derived from a protein evaluation that is corrected for incomplete recovery during hydrolysis would be approximately 115 percent of the mass of the starting protein, the difference being the water added to each AA during hydrolysis. The committee decided to calculate the AA flows in hydrated form despite them generally existing in dehydrated, protein-bound form before digestion. In this manner, the predicted AA flows would be expected to match observed AA flows as determined from AA analyses of the hydrolyzed protein after correction for hydrolysis recovery. As will be discussed later, this will also be the case for exported AA in milk protein and so forth, and thus corrections must be made for hydration change when utilizing absorbed AA for synthesis of those proteins. Hydration factors are reported in Table 20-8.

Dietary intakes (g/d) are calculated by summation of the AA intakes for each ingredient in the diet as for other nutrients:

$$\frac{Dt\_AAt(a)In}{g/d} = \sum_{f=1}^{n} Fd\_AAt(a)In_f$$

(Equation 20-49)

Infusions

Although not within the specific charge of the committee, nutrient infusions are often used in nutrition trials to provide additional information on animal responses to varying nutri-

ent supply, and consideration of those infusions is required to make use of the broadest range in nutrient inputs during model development. Such studies often provide clean, independent evaluations of responses at different entry points, and support for their inclusion was important to better define responses. For example, many studies have been conducted to relate rates of production to incremental changes in a nutrient introduced by infusion (Derrig et al., 1974; Storry et al., 1974; Spires et al., 1975). As these types of trials typically have more power in terms of regression analyses, it was deemed important to accommodate such infusions as possible inputs in the model. The infusion inputs are denoted throughout the model using the abbreviation form *Inf_Xxx*, where *Xxx* denotes the nutrient. Total input was calculated by summation of diet and infusion inputs and denoted using a prefix of "*An_*" in place of "*Dt_*" as demonstrated in the following generic nutrient input and nutrient digestion equations:

$$\frac{An\_XxxIn}{kg/d} = Dt\_XxxIn + Inf\_XxxIn$$

(Equation 20-50)

$$\frac{An\_DigXxxIn}{kg/d} = Dt\_DigXxxIn + Inf\_DigXxxIn$$

(Equation 20-51)

No additional documentation is provided as the variables and their inputs to the model should be self-explanatory.

Ruminal Nutrient Digestion of Neutral Detergent Fiber and Starch

Ruminal digestibility of NDF and starch is predicted as described in Chapter 6:

$$\begin{aligned}
\frac{Rum\_dcNDF}{\%} = \\
-31.9 + 0.721 \times \left(\frac{Dt\_NDFIn + Inf\_NDFIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right) \\
-0.247 \times \left(\frac{Dt\_StIn + Inf\_StIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right) \\
+6.63 \times \left(\frac{Dt\_CPIn + Inf\_CPIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right) \\
-0.211 \times \left(\frac{Dt\_CPIn + Inf\_CPIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \right)^2 \\
-0.387 \times \left[\frac{\left(\frac{Dt\_ADFIn + Inf\_ADFIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right)}{\left(\frac{Dt\_NDFIn + Inf\_NDFIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right)} \right] \times 100 \\
-0.121 \times Dt\_ForWet \\
+1.51 \times \left(Dt\_DMIn + Inf\_DMIn_{Rum} \right)
\end{aligned}$$

(Equation 20-52)

$$\underset{\%}{Rum\_dcSt} = 70.6 - 1.45 \times \left(Dt\_DMIn + Inf\_DMIn_{Rum} \right)$$
$$+ 0.424 \times Dt\_ForNDF$$
$$+ 1.39 \times \left(\frac{Dt\_St + Inf\_StIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right)$$
$$- 0.0219 \times \left(\frac{Dt\_St + Inf\_StIn_{Rum}}{Dt\_DMIn + Inf\_DMIn_{Rum}} \times 100 \right)^2$$
$$- 0.154 \times Dt\_ForWet \qquad \text{(Equation 20-53)}$$

Both digestion coefficients were bounded by 0 and 100 to ensure biological consistency and set to the mean values of 37.6 and 65.6 percent of the parent nutrient, respectively, if missing. NDF and starch (*St*) digested in the rumen (kg/d) are calculated as

$$\underset{kg/d}{Rum\_DigNDFIn} = \frac{Rum\_dcNDF}{100}$$
$$\times \left(Dt\_NDFIn + Inf\_NDFIn_{Rum} \right)$$
$$\text{(Equation 20-54)}$$

$$\underset{kg/d}{Rum\_DigStIn} = \frac{Rum\_dcSt}{100} \times \left(Dt\_StIn + Inf\_StIn_{Rum} \right)$$
$$\text{(Equation 20-55)}$$

NDF and *St* passage from the rumen are calculated by difference:

$$\underset{kg/d}{Du\_NDFPas} = Dt\_NDFIn + Inf\_NDFIn_{Rum}$$
$$- Rum\_DigNDFIn$$
$$\text{(Equation 20-56)}$$

$$\underset{kg/d}{Du\_StPas} = Dt\_StIn + Inf\_StIn_{Rum} - Rum\_DigStIn$$
$$\text{(Equation 20-57)}$$

Diets containing only immature grass are found to result in slightly negative *St* passage, which is trapped and set to 0.

Rumen-Degradable and Undegradable Protein and Amino Acids

RDP and RUP (kg/d) were predicted from in situ data, but the passage rate model used in the prior work was replaced with static estimates for forage and concentrates (see Chapter 6).

$$\underset{kg/d}{Fd\_RUPAIn_f} = \left(Fd\_CPAIn_f - Fd\_NPNCPIn_f \right) \times fCPAdu$$
$$\text{(Equation 20-58)}$$

$$\underset{kg/d}{Fd\_RUPBIn_f} = Fd\_CPBIn_f \times \left(1 - \frac{Rum\_dcCPB_f}{100} \right)$$
$$\text{(Equation 20-59)}$$

$$\underset{\%}{Rum\_dcCPB_f} = 100 - \left(\begin{array}{l} Fd\_For_f \times \dfrac{Kp_{For}}{Fd\_KdRUP_f + Kp_{For}} \\ + Fd\_Conc_f \\ \times \dfrac{Kp_{Conc}}{Fd\_KdRUP_f + Kp_{Conc}} \end{array} \right)$$
$$\text{(Equation 20-60)}$$

$$\underset{kg/d}{Fd\_RUPIn_f} = Fd\_RUPAIn_f + Fd\_RUPBIn_f$$
$$+ Fd\_CPCIn_f + \frac{IntRUP}{refCPIn} \times Fd\_CPIn_f$$
$$\text{(Equation 20-61)}$$

where Kp_{Conc} represented the rate of passage of protein in concentrates from the rumen and is 5.28 percent/h, Kp_{For} represented the rate of passage of protein in forages from the rumen and is 4.87 percent/h, *fCPAdu* represented the fractional escape of the A fraction of CP from the rumen and is 0.064 kg/kg of the A fraction of CP, and *IntRUP* represented the intercept from the regression equation fitted to the ruminal outflow data (see Chapter 6), which is −0.086 kg/d at the diet level. Application of the dietary intercept to feeds within the diet requires scaling. This is achieved by dividing the intercept by the mean CP intake for the ruminal outflow data (*refCPIn*, 3.39 kg/d) and multiplication times the CP intake for each ingredient (*Fd_CPIn_f*). This scaling approach does not exactly match the dietary level calculation as derived, but it is very close (<1 percent error) and preserves the ability to calculate contributions of protein and AA from each feed.

The flow of AA associated with RUP (g/d) is calculated from RUP (*Fd_RUPIn*) and the true AA composition (*Fd_AAt(a)_CP*, g/100 g CP) of each ingredient:

$$\underset{g/d}{Fd\_AARUP(a)In_f} = \frac{Fd\_AAt(a)\_CP_f}{100}$$
$$\times Fd\_RUPIn_f \times 1000$$
$$\text{(Equation 20-62)}$$

Dietary intakes of RUP, RDP, and AA in RUP (kg/d, kg/d, and g/d) are derived by summation across ingredients in the diet (RUP and AARUP) and by difference from CP intake (RDP):

$$\underset{kg/d}{Dt\_RUPIn} = \sum_{f=1}^{n} Fd\_RUPIn_f$$
$$\text{(Equation 20-63)}$$

$$\frac{An\_RUPIn}{kg/d} = Dt\_RUPIn + InfRum\_RUPIn$$

(Equation 20-64)

$$\frac{An\_RUP\_CP}{\%\ of\ CP} = \frac{An\_RUPIn}{Dt\_CPIn + InfRum\_CPIn} \times 100$$

(Equation 20-65)

$$\frac{Dt\_RDPIn}{kg/d} = Dt\_CPIn - Dt\_RUPIn$$

(Equation 20-66)

$$\frac{Dt\_RDP}{\%\ of\ DM} = \frac{Dt\_RDPIn}{Dt\_DMIn} \times 100$$

(Equation 20-67)

$$\frac{Dt\_RDP}{\%\ of\ CP} = \frac{Dt\_RDP}{Dt\_CP} \times 100$$

(Equation 20-68)

The combination of dietary and infused supplies has been denoted with a location of *An* reflecting an overall animal supply:

$$\frac{An\_RDPIn}{kg/d} = Dt\_RDPIn + InfRum\_RDPIn$$

(Equation 20-69)

$$\frac{An\_RDP\_CP}{\%\ of\ CP} = \frac{An\_RDPIn}{Dt\_CPIn + InfRum\_CPIn} \times 100$$

(Equation 20-70)

$$\frac{Dt\_AARUP(a)In}{g/d} = \sum_{f=1}^{n} Fd\_AARUP(a)In_f$$

(Equation 20-71)

$$\frac{An\_AARUP(a)In}{g/d} = Dt\_AARUP(a)In \\ + InfRum\_AARUP(a)In$$

(Equation 20-72)

The proportion of feed AA captured in RUP is also calculated for reporting purposes:

$$\frac{AARUP(a)\_AADt(a)}{\%} = \frac{Dt\_AARUP(a)In}{Dt\_AA(a)In} \times 100$$

(Equation 20-73)

Ruminal Microbial Protein

Microbial nitrogen outflow (*Du_MiN_g*, g/d) from the rumen is predicted using the equations described in Chapter 6.

$$\frac{Du\_MiN\_g}{g/d} = \frac{MiN\_Vm}{1 + \dfrac{MiN\_Km_{rdNDF}}{Rum\_DigNDFIn} + \dfrac{MiN\_Km_{rdSt}}{Rum\_DigStIn}}$$

(Equation 20-74)

where

$$\frac{MiN\_Vm}{g/d} = MiN\_Vm_{Int} + MiN\_Vm_{RDPSlp} \times An\_RDPIn$$

(Equation 20-75)

and $MiN\_Vm_{Int} = 100.8$, $MiN\_Vm_{RDPSlp} = 81.56$, $MiN\_Km_{RDNDF} = 0.0939$, and $MiN\_Km_{rdSt} = 0.0274$. *Rum_DigNDFIn* and *Rum_DigStIn* represent NDF and starch digested in the rumen (kg/d) as predicted from Equation 20-54 and Equation 20-55. *An_RDPIn* represented the total RDP supply (kg/d) as predicted from Equation 20-69; however, the effect was capped to yield no additional responses above 12 percent dietary RDP.

Microbial N flow is converted to CP and true protein (TP) flows (g/d) using static stoichiometric coefficients of 6.25 g of CP/g of N and 0.824 g of TP/g of CP. Maximum microbial CP was set at RDP intake.

$$\frac{Du\_MiCP}{kg/d} = Du\_MiN\_g \times 6.25/1,000$$

(Equation 20-76)

$$\frac{Du\_MiTP}{kg/d} = Du\_MiCP \times 0.824$$

(Equation 20-77)

RDP balance in the rumen was estimated by difference from *An_RDPIn* and *Du_MiCP*:

$$\frac{Rum\_RDPbal}{kg/d} = An\_RDPIn - Du\_MiCP$$

(Equation 20-78)

Ruminal AA outflow associated with microbial flows (g/d) is calculated from the microbial true protein flows as

$$\frac{Du\_AAMic(a)}{g/d} = Du\_MiTP \times 1,000 \times \frac{AA(a)\_MiTP}{100}$$

(Equation 20-79)

where *AA(a)_MiTP* represented the AA composition of microbial true protein (g/100 g; Table 6-2 in Chapter 6) with *a* denoting each of the 10 essential AAs (EAAs).

Endogenous and Total Protein and Amino Acid Flow from the Rumen

Endogenous protein flow (kg/d) from the rumen represented protein and nitrogen (N) secreted into the rumen from N sources that were previously absorbed. As such,

they represent a maintenance cost to the animal and do not reflect a net addition to the nutrient supply. However, for comparisons to observed ruminal outflows of N, protein, and AAs, such flow must be predicted as it is represented in the flow measurements made in the animal. This flux (kg/d) is predicted as a linear function of DMIn:

$$\frac{Du\_EndCP}{kg/d} = \frac{96.1 + 7.54 \times (Dt\_DMIn + InfRum\_DMIn)}{1,000}$$

(Equation 20-80)

The AA flow (g/d) associated with the protein flow is predicted from Equation 20-80 and the AA composition listed in Table 6-5.

$$\frac{Du\_AAEndCP(a)}{g/d} = Du\_EndCP \times 1,000$$
$$\times \frac{AA(a)\_DuEndCP}{100}$$

(Equation 20-81)

Total ruminal N (g/d) and AA outflows (g/d) are predicted as the summation of the RUP, microbial, and endogenous flows:

$$\frac{Du\_NAN}{g/d} = \frac{(Du\_MiCP + An\_RUPIn + Du\_EndCP)}{6.25 \times 1,000}$$

(Equation 20-82)

Nonammonia-nonmicrobial N (NANMN) flows (g/d) are generally reported in the literature and represented in the model as

$$\frac{Du\_NANMN}{g/d} = \frac{(An\_RUPIn + Du\_EndCP)}{6.25 \times 1,000}$$

(Equation 20-83)

Ammonia outflow from the rumen is not explicitly represented in the model; however, the derived passage of RDP at 5 percent of the total RDP supply should contain ammonia given the basis of the difference calculation. Thus, the estimate of *Du_NANMN* given by Equation 20-83 is not a clean representation of the in vivo measurement. It more likely represents nonmicrobial N flow. However, for model evaluation purposes, predictions by Equation 20-83 are compared to reported NANMN flows in the literature.

$$\frac{Du\_AA(a)}{g/d} = Dt\_AARUP(a)In + Inf\_AARUP(a)In$$
$$+ Du\_AAMic(a) + Du\_AAEndP(a)$$

(Equation 20-84)

and the proportion (percent) of AA leaving the rumen as AA is represented as

$$\frac{DuAA(a)\_DtAA(a)}{\%} = \frac{Du\_AA(a)}{Dt\_AA(a)In + Inf\_AA(a)In} \times 100$$

(Equation 20-85)

The flows are summed to yield a total essential AA (EAA) flow:

$$\frac{Du\_EAA}{g/d} = Du\_Arg + Du\_His + Du\_Ile$$
$$+ Du\_Leu + Du\_Lys + Du\_Met$$
$$+ Du\_Phe + Du\_Thr + Du\_Trp + Du\_Val$$

(Equation 20-86)

Because AA flows predicted by Equation 20-84 are expressed as true flows that have been corrected for incomplete recovery during AA analyses, uncorrected flows (g/d) are required for comparison to data reported in the literature. These values are provided solely for comparison purposes and have no other function in the model.

$$\frac{Du\_AA(a)_{24h}}{g/d} = Du\_AA(a) \times RecAA(a)$$

(Equation 20-87)

Total Tract Carbohydrate, Protein, and Fatty Acid Digestion and Absorption

Digestion and absorption of carbohydrates, protein, and FAs from the intestine and the total tract are defined in this section.

Carbohydrate Digestion and Absorption

Total tract starch digestion is based on ingredient-specific, base digestibility constants (*Fd_dcSt_f*, percentage of *St*) from Table 3-1 (in Chapter 3), which are adjusted to reflect a digestibility reduction as *Dt_DMIn* increases:

$$\frac{Fd\_DigSt\_Base_f}{\% \ of \ DM} = Fd\_St_f \times \frac{Fd\_dcSt_f}{100}$$

(Equation 20-88)

$$\frac{Fd\_DigStIn\_Base_f}{kg/d} = \frac{Fd\_DigSt\_Base_f}{100} \times Fd\_DMIn_f$$

(Equation 20-89)

$$\frac{Dt\_DigStIn\_Base}{kg/d} = \sum_{f=1}^{N_f} Fd\_DigStIn\_Base_f$$

(Equation 20-90)

$$\frac{TT\_dcSt\_Base}{\% \ of \ St} = \frac{Dt\_DigStIn\_Base}{Dt\_StIn} \times 100$$

(Equation 20-91)

The base digestibility is subsequently adjusted based on *An_DMIn* as a proportion of BW and centered to 3.5 percent of BW to reflect reduced digestion at high intakes:

$$\frac{TT\_dcSt}{\% \text{ of } St} = TT\_dcSt\_Base - (1.0 \times (An\_DMI\_BW - 0.035)) \times 100$$

(Equation 20-92)

which was bounded by 0 on the low end. Digested starch is subsequently estimated for the specified *An_DMIn* as

$$\frac{Dt\_DigStIn}{kg/d} = Dt\_StIn \times \frac{TT\_dcSt}{100}$$

(Equation 20-93)

$$\frac{Dt\_DigSt}{\% \text{ of } DM} = \frac{Dt\_DigStIn}{Dt\_DMIn} \times 100$$

(Equation 20-94)

Total digested starch was

$$\frac{An\_DigStIn}{kg/d} = Dt\_DigStIn + Inf\_StIn \times \frac{Inf\_ttdcSt}{100}$$

(Equation 20-95)

$$\frac{An\_dcSt}{\% \text{ of } St} = \frac{An\_DigStIn}{Dt\_StIn + Inf\_StIn} \times 100$$

(Equation 20-96)

$$\frac{An\_DigSt}{\% \text{ of } DM} = \left(\frac{An\_DigStIn}{Dt\_DMIn + Inf\_RumDMIn + Inf\_SIDMIn} \right) \times 100$$

(Equation 20-97)

$$\frac{Fe\_St}{kg/d} = Dt\_StIn + Inf\_StIn - An\_DigStIn$$

(Equation 20-98)

True digestibility of rOM (*Dt_dcrOMt*, percentage of rOM) was set at 96.1 percent with an endogenous fecal excretion of 3.43 percent of *Dt_DMIn* (see Chapter 3):

$$\frac{Fd\_DigrOMt_f}{\% \text{ of } DM} = \frac{Fd\_dcrOMt}{100} \times Fd\_rOM_f$$

(Equation 20-99)

$$\frac{Fe\_rOMend}{kg/d} = 0.034 \times Dt\_DMIn$$

(Equation 20-100)

Apparent digested rOM intakes are also estimated for individual feeds as

$$\frac{Fd\_DigrOMa_f}{\% \text{ of } DM} = Fd\_DigrOMt_f - 3.43$$

(Equation 20-101)

$$\frac{Fd\_DigrOMaIn_f}{kg/d} = \frac{Fd\_DigrOMa_f}{100} \times Fd\_DMIn_f$$

(Equation 20-102)

These apparent rOM digestibility equations generate negative estimates for some feeds with very low rOM concentrations, including mineral sources. However, summation across all feeds in the diet generates reliable estimates for the diet given derivation of the source work from dietary observations.

True and apparent digested rOM intakes are thus

$$\frac{Fd\_DigrOMtIn_f}{kg/d} = \frac{Fd\_DigrOMt_f}{100} \times Fd\_DMIn_f$$

(Equation 20-103)

$$\frac{Dt\_DigrOMtIn}{kg/d} = \sum_{f=1}^{N_f} Fd\_DigrOMtIn_f$$

(Equation 20-104)

$$\frac{Dt\_DigrOMaIn}{kg/d} = Dt\_DigrOMtIn - Fe\_rOMend$$

(Equation 20-105)

Infusions were considered as

$$\frac{An\_DigrOMaIn}{kg/d} = Dt\_DigrOMaIn + \left(\begin{array}{c} Inf\_GlcIn + Inf\_AcetIn \\ + Inf\_PropIn + Inf\_ButrIn \end{array} \right) \times (fRum + fSI)$$

(Equation 20-106)

$$\frac{An\_DigrOMa}{\% \text{ of } DM} = \left(\frac{An\_DigrOMaIn}{Dt\_DMIn + InfRum\_DMIn + InfSI\_DMIn} \right) \times 100$$

(Equation 20-107)

$$\frac{An\_dcrOMa}{\% \text{ of } rOM} = \frac{An\_DigrOMaIn}{Dt\_rOMaIn} \times 100 + \left(\begin{array}{c} Inf\_GlcIn + Inf\_AcetIn \\ + Inf\_PropIn + Inf\_ButrIn \end{array} \right) \times (fRum + fSI)$$

(Equation 20-108)

$$\frac{Fe\_rOMout}{kg/d} = An\_rOMIn - An\_DigrOMaIn$$

(Equation 20-109)

Total tract digestion of NDF is calculated in a base state from ingredient lignin concentrations (Fd_dcNDF_Lg, percentage of NDF) or from observed 48-hour in vitro NDF digestibility assessments ($Fd\_dcNDF\_IV_{48h}$, percentage of NDF) and subsequently adjusted to reflect the negative impacts of Dt_St and An_DMIn.

$$\frac{Fd\_dcNDF\_Lg}{\% \text{ of } NDF} = 0.75 \times (Fd\_NDF - Fd\_Lg)$$
$$\times \frac{1 - \left(\frac{Fd\_Lg}{Fd\_NDF}\right)^{0.667}}{Fd\_NDF} \times 100$$

(Equation 20-110)

$$\frac{Fd\_dcNDF\_IV48}{\% \text{ of } NDF} = 12 + 0.61 \times \left(Fd\_dcNDF\_IV_{48h}\right)$$

(Equation 20-111)

A selector (Use_dcNDF_IV) is used to select Equation 20-110 or Equation 20-111 to represent the base digestibility for forages only (1) or for all feeds (2). In both cases, the Lg based prediction is used if an IV value is missing.

$$\frac{Fd\_dcNDF\_Base}{\%} =$$

Use_dcNDF_IV48	Value
0	Fd_dcNDF_Lg
1	$Forage: Fd\_dcNDF\_IV48$ $Concentrate: Fd\_dcNDF\_Lg$
2	$All\ Feeds: Fd\_dcNDF\_IV48$

(Equation 20-112)

The base digested NDF is calculated at an ingredient level, summed to a diet total, and subsequently discounted to reflect the negative impacts of dietary starch concentration and DMIn:

$$\frac{Dt\_DigNDFIn\_Base}{kg/d} = \sum_{f=1}^{N_f} \frac{Fd\_dcNDF\_Base_f}{\times Fd\_NDF_f \times Fd\_DMIn_f}$$

(Equation 20-113)

$$\frac{Dt\_dcNDF\_Base}{\% \text{ of } NDF} = \frac{Dt\_DigNDFIn\_Base}{Dt\_NDFIn} \times 100$$

(Equation 20-114)

$$\frac{Dt\_dcNDF}{\% \text{ of } NDF} =$$
$$\left[\begin{array}{l} \frac{Dt\_dcNDF\_Base}{100} - 1.1 \times \left(\begin{array}{c} An\_DMI\_BW \\ -0.035 \end{array}\right) \\ -0.59 \times \left(\frac{\begin{array}{c} Dt\_StIn + Inf\_StIn_{Rum} \\ + Inf\_StIn_{SI} \end{array}}{\begin{array}{c} Dt\_DMIn + Inf\_DMIn_{Rum} \\ + Inf\_DMIn_{SI} \end{array}} - 0.26\right) \end{array} \right] \times 100$$

(Equation 20-115)

$$\frac{Dt\_DigNDFIn}{kg/d} = \frac{Dt\_dcNDF}{100} \times Dt\_NDFIn$$

(Equation 20-116)

$$\frac{An\_DigNDFIn}{kg/d} = Dt\_DigNDFIn + Inf\_NDFIn_{Rum}$$
$$\times \frac{Dt\_dcNDF}{100}$$

(Equation 20-117)

$$\frac{Dt\_DigNDF}{\% \text{ of } DM} = \frac{Dt\_DigNDFIn}{Dt\_DMIn} \times 100$$

(Equation 20-118)

$$\frac{An\_DigNDF}{\% \text{ of } DM} =$$
$$\left(\frac{An\_DigNDFIn}{Dt\_DMIn + Inf\_DMIn_{Rum} + Inf\_DMIn_{SI}}\right) \times 100$$

(Equation 20-119)

$$\frac{Fe\_NDF}{kg/d} = Dt\_NDFIn - Dt\_DigNDFIn$$

(Equation 20-120)

Protein and Amino Acid Digestion and Absorption

Protein

Because each ingredient has an intrinsic RUP digestibility as defined in the feed library, the total intestinal digestibility of RUP ($Dt\_idRUPIn$, kg/d) must be summed from the individual ingredients:

$$\frac{Fd\_idRUPIn_f}{kg/d} = \frac{Fd\_dcRUP_f}{100} \times Fd\_RUPIn_f$$

(Equation 20-121)

$$\frac{Dt\_idRUPIn}{kg/d} = \sum_{f=1}^{N_f} Fd\_idRUPIn_f$$

(Equation 20-122)

$$\frac{An\_idRUPIn}{kg/d} = Dt\_idRUPIn + InfRum\_idRUPIn$$
$$+ InfSI\_idTPIn$$

(Equation 20-123)

Fecal output of undigested RUP is calculated for each feed (Fe_RUPOut_f, kg/d) from the feed digestibilities and for the diet (Fe_RUPOut, kg/d) by difference.

$$\frac{Fe\_RUPout_f}{kg/d} = Fd\_RUPIn_f \times \left(1 - \frac{Fd\_dcRUP_f}{100}\right)$$

(Equation 20-124)

$$\frac{Fe\_RUPout}{kg/d} = An\_RUPIn + Inf\_TPIn_{SI} - An\_idRUPIn$$

(Equation 20-125)

Intestinal digestibility of microbial protein flowing from the rumen (*Du_idMiCP*, kg/d) is assumed to be 80 percent:

$$\frac{Du\_idMiCP}{kg/d} = Du\_MiCP \times 0.80$$

(Equation 20-126)

and the proportion of digested microbial protein that is true protein is assumed to be 82.4 percent:

$$\frac{Du\_idMiTP}{kg/d} = Du\_idMiCP \times 0.824$$

(Equation 20-127)

Fecal undigested microbial CP output (*Fe_MiCPout*, kg/d) derived from ruminal microbes is calculated by difference:

$$\frac{Fe\_MiCPout}{kg/d} = Du\_MiCP - Du\_idMiCP$$

(Equation 20-128)

and does not include any microbial CP synthesized in the large intestine.

$$\frac{An\_idCPIn}{kg/d} = Dt\_idRUPIn + Du\_idMiCP + Inf\_idCPIn$$

(Equation 20-129)

Total tract apparent and true CP and TP digested (kg/d) and digestibility (percent) are calculated as

$$\frac{An\_DigCPaIn}{kg/d} = Dt\_CPIn + Inf\_CPIn_{Rum} + Inf\_CPIn_{SI} - Fe\_CP$$

(Equation 20-130)

$$\frac{An\_DigTPaIn}{kg/d} = An\_TPIn + Inf\_TPIn_{Rum} + Inf\_TPIn_{SI} - Fe\_CP$$

(Equation 20-131)

$$\frac{An\_dcCPa}{\% \text{ of } CP} = \left(\frac{An\_DigCPaIn}{Dt\_CPIn + Inf\_CPIn_{Rum} + Inf\_CPIn_{SI}}\right) \times 100$$

(Equation 20-132)

$$\frac{An\_DigCPtIn}{kg/d} = An\_RDPIn - Fe\_MiCP + An\_idRUPIn - Fe\_NPend$$

(Equation 20-133)

$$\frac{An\_DigTPtIn}{kg/d} = An\_RDTPIn - Fe\_MiTP + An\_idRUPIn - Fe\_NPend$$

(Equation 20-134)

$$\frac{An\_dcCPt}{\% \text{ of } CP} = \left(\frac{An\_DigCPtIn}{Dt\_CPIn + Inf\_CPIn_{Rum} + Inf\_CPIn_{SI}}\right) \times 100$$

(Equation 20-135)

MP intake (*An_MPIn*, kg/d) and dietary concentration (percentage of DM) are calculated as

$$\frac{An\_MPIn}{kg/d} =$$

Criteria	Value
$An\_StatePhs = \text{“Calf”}$	$An\_TPIn - Fe\_CPout + Fe\_CPend$
$An\_StatePhs \neq \text{“Calf”}$	$An\_idRUPIn + Du\_idMiTP + Inf\_TPIn_{Bld}$

(Equation 20-136)

$$\frac{An\_MP}{\% \text{ of } DM} = \left(\frac{An\_MPIn}{Dt\_DMIn + Inf\_DMIn_{Rum} + Inf\_DMIn_{SI}}\right) \times 100$$

(Equation 20-137)

The choice to express *An_MPIn* relative to only the DM provided in the intestinal tract is arbitrary; it is also logical to calculate it using all sources (i.e., *An_DMIn*).

Although ruminal digestion is calculated for all animal types, the data used for those predictions do not include any from calves, and thus a more empirical, total tract digestibility approach was used for animals in that physiological state. The software is configured to exclude ruminal digestion predictions, and the user should do the same for prediction derived from R code. Additionally, the user should ensure that infused proteins or AA are specified with appropriate digestion coefficients given the form of Equation 20-136.

Fecal CP and N output (*Fe_CPout* and *Fe_Nout*, kg/d) are calculated as

$$\frac{Fe\_CPout}{kg/d} =$$

Criteria	Value
$An\_StatePhs = \text{“Calf”}$ & $An\_CPIn_{ClfLiq} > 0$	$0.05 \times An\_CPIn_{ClfLiq} + 0.25 \times An\_CPIn_{ClfDry}$
$An\_StatePhs \neq \text{“Calf”}$	$\begin{pmatrix} Fe\_RUPout \\ + Fe\_MiCPout \\ + Fe\_CPend \\ + Inf\_CPIn_{SI} \\ - Inf\_idCPIn_{SI} \end{pmatrix}$

(Equation 20-138)

where Dt_CPIn was 95 percent digested for liquid feeds and 75 percent digested for dry feeds fed to calves.

$$\frac{Fe\_Nout}{kg/d} = \frac{Fe\_CPout}{6.25}$$
(Equation 20-139)

$$\frac{Fe\_CPend\_LiqFd}{kg/d} =$$

Criteria	Equation
$NonMilkCP\_ClfLiq = 0$	$0.0119 \times Dt\_DMIn_{LiqFd}$
$NonMilkCP\_ClfLiq = 1$	$0.0344 \times Dt\_DMIn_{LiqFd}$

$$\frac{Fe\_CPend\_DryFd}{kg/d} =$$

Criteria	Equation
$An_PhysState = $ "Calf"	$0.0206 \times Dt\_DMIn_{DryFd}$
$An_PhysState \neq $ "Calf"	$12.0 + 0.12 \times An\_NDF \times (Dt\_DMIn + InfRum\_DMIn + InfSI\_DMIn)$

$$Fe\_CPend = Fe\_CPend\_LiqFd + Fe\_CPend\_DryFd \ kg/d$$
(Equation 20-140)

Fe_CPend was arbitrarily assigned to An_RDP and An_RDP to provide an approximation of the contributions of each to endogenous secretions.

$$\frac{Fe\_EndRDP}{kg/d} = Fe\_CPend \times \frac{An\_RDPIn}{An\_CPIn}$$
(Equation 20-141)

$$\frac{Fe\_EndRUP}{kg/d} = Fe\_CPend \times \frac{An\_RUPIn}{An\_CPIn}$$
(Equation 20-142)

Amino Acids

The intestinally digested EAA arising from each feed is defined as a function of the feed EAA input ($Fd\_AARUPIn(i)_f$, g/d) and the digestibility of the RUP:

$$\frac{Fd\_idAARUPIn(a)_f}{g/d} = Fd\_AARUPIn(a)_f \times \frac{Fd\_dcRUP_f}{100} \times F\_idAARUP(a)$$
(Equation 20-143)

where $F\_idAARUP_{(a)}$ (g/g) represented a factor to adjust the intestinal digestibility of individual AAs relative to that of RUP, and *(a)* represented each EAA. However, as noted in Chapter 6, the data are inadequate at this time to uniquely define such factors, and thus the values are all set to 1.

The total intestinally digested supply of each EAA arising from RUP ($Dt\_idAARUPIn(a)$, g/d) is the sum of that arising from each feed:

$$\frac{Dt\_idAARUPIn(a)}{g/d} = \sum_{i=1}^{N_f} Fd\_idAARUPIn(a)_f$$
(Equation 20-144)

Intestinally digested EAAs derived from microbial protein leaving the rumen ($Du\_idAAMic(a)$, g/d) are calculated from the duodenal microbial AA flows:

$$\frac{Du\_idAAMic(a)}{g/d} = Du\_AAMic(a) \times \frac{SI\_dcMiCP}{100}$$
(Equation 20-145)

The supply of intestinally digested AA is by summation:

$$\frac{Du\_idAAIn(a)}{g/d} = Du\_idAAMic(a) + Dt\_idAARUPIn(a)$$
(Equation 20-146)

$$\frac{An\_idAAIn(a)}{g/d} = Du\_idAAIn(a) + Inf\_idAAIn(a)$$
(Equation 20-147)

Finally, the total absorbed AA, EAA, and nonessential AA (NEAA) supplies were

$$\frac{Abs\_AA(a)\_g}{g/d} = An\_idAAIn(a) + Inf\_AA(a)\_g \times fArt$$
(Equation 20-148)

$$\frac{Abs\_EAA\_g}{g/d} = \sum_{a=1}^{N_{EAA}} Abs\_AA(a)\_g$$
(Equation 20-149)

$$\frac{Abs\_NEAA\_g}{g/d} = An\_MPIn \times 1.15 - Abs\_EAA\_g$$
(Equation 20-150)

where *fArt* is the proportion of infused TP introduced into the blood supply (g/g) defined by Equation 20-147. The factor of 1.15 represents the average mass of hydration when converting protein to free AA. The absorbed supply of each EAA is also expressed as a percentage of the total EAA supply and the MP supply:

$$\frac{Abs\_AA\_EAA(a)}{\% \ of \ EAA} = \frac{Abs\_AA(a)\_g}{Abs\_EAA\_g} \times 100$$
(Equation 20-151)

$$\frac{Abs\_AA\_MP(a)}{\%\ of\ MP} = \frac{Abs\_AA(a)\_g}{An\_MPIn\_g} \times 100$$

(Equation 20-152)

Fatty Acid Digestion and Absorption

Digestibility of total FAs in the total tract is specified in the feed library by ingredient as described in Chapter 4. Missing values are filled with a default value of 73 percent except for ingredients placed in the fat or FA supplement categories, which are set as described in Chapter 4. All ingredients in the concentrate feed category use a digestibility of 81 percent regardless of the library entry when *An_StatePhys* is set to calf. Digested total, unsaturated, monounsaturated, polyunsaturated, saturated, and individual FA intakes (kg/d) are subsequently calculated by ingredient assuming the digestibility of each FA (*fa*) is equivalent to the total as for AA digestibility:

$$\frac{Fd\_DigFAIn_f}{kg/d} = \frac{Fd\_ttdcFA_f}{100} \times \frac{Fd\_FA_f}{100} \times Fd\_DMIn$$

(Equation 20-153)

$$\frac{Fd\_DigFA(fa)In_f}{kg/d} = \frac{Fd\_ttdcFA_f}{100} \times \frac{Fd\_FA(fa)\_FA_f}{100}$$
$$\times \frac{Fd\_FA_f}{100} \times Fd\_DMIn_f$$

(Equation 20-154)

where *FA* represented total FA and *fa* represented each individual FA (C12, C14, C16, C16:1, C18:0, C18:1c, C18:1t, C18:2, C18:3, and other FA). Total and individual digested FA dietary intakes from each ingredient are summed to yield dietary digested FA intakes (kg/d):

$$\frac{Dt\_DigFAIn}{kg/d} = \sum_{f=1}^{N_f} Fd\_DigFAIn_f$$

(Equation 20-155)

$$\frac{Dt\_DigFA(fa)In}{kg/d} = \sum_{f=1}^{N_f} Fd\_DigFA(fa)In_f$$

(Equation 20-156)

Dietary digestibilities (percent) of total and individual FA are calculated as

$$\frac{Dt\_DigFA\_FA}{\%\ of\ FA} = \frac{Dt\_DigFAIn}{Dt\_FAIn} \times 100$$

(Equation 20-157)

$$\frac{Dt\_DigFA(fa)\_FA}{\%\ of\ FA} = \frac{Dt\_DigFA(fa)In}{Dt\_FAIn} \times 100$$

(Equation 20-158)

$$\frac{An\_DigFAIn}{kg/d} = Dt\_DigFAIn + Inf\_DigFAIn$$

(Equation 20-159)

Intake and digestibility of individual FAs were not considered but could easily be added to the model given FA composition and digestibility of the infusate.

$$An\_DigFA\_FA$$
$$\%\ of\ FA$$
$$= \left(\frac{An\_DigFAIn}{Dt\_FAIn + Inf\_FAIn_{Rum} + Inf\_FAIn_{SI}} \right) \times 100$$

(Equation 20-160)

Digested unsaturated (*An_DigUFAIn*, kg/d), monounsaturated (*An_DigMUFAIn*, kg/d), polyunsaturated (*An_DigPUFAIn*, kg/d), and saturated (*An_DigSatFAIn*, kg/d) digested FAs are calculated by summation or difference as

$$\frac{An\_DigUFAIn}{kg/d} = An\_DigC161In + An\_DigC181tIn$$
$$+ An\_DigC181cIn$$
$$+ An\_DigC182In + An\_DigC183In$$

(Equation 20-161a)

$$\frac{An\_DigMUFAIn}{kg/d} = An\_DigC161In + An\_DigC181tIn$$
$$+ An\_DigC181cIn$$

(Equation 20-161b)

$$\frac{An\_DigPUFAIn}{kg/d} = An\_DigC182In + An\_DigC183In$$

(Equation 20-161c)

$$\frac{An\_DigSatFAIn}{kg/d} = An\_DigFAIn - An\_DigUFAIn$$

(Equation 20-161d)

However, only total FA digestibility information is output because biohydrogenation is not modeled. Fecal total FA output is calculated by difference:

$$\frac{Fe\_FAout}{kg/d} = Dt\_FAIn + Inf\_FAIn_{Rum}$$
$$+ Inf\_FAIn_{SI} - Dt\_DigFAIn - Inf\_DigFAIn$$

(Equation 20-162)

Total tract apparent digestibility of total FA is calculated as

$$\frac{An\_dcFA}{\%\ of\ FA} = \frac{\begin{array}{c} Dt\_DigFAIn + Inf\_DigFAIn_{Rum} \\ + Inf\_DigFAIn_{SI} \end{array}}{\begin{array}{c} Dt\_FAIn + Inf\_FAIn_{Rum} \\ + Inf\_FAIn_{SI} \end{array}} \times 100$$

(Equation 20-163)

Fecal output and apparent digestibilities of individual FAs can be calculated in a similar manner, but such data are not

adequately represented in the literature, and thus those equations were not included.

Having predicted fecal outputs of all of the primary OM components, digested OM and fecal output of OM (kg/d) are predicted as

$$\frac{An\_DigOMaIn}{kg/d} = An\_DigNDFIn + An\_DigStIn \\ + An\_DigFAIn + An\_DigrOMaIn \\ + An\_DigCPaIn$$

(Equation 20-164)

$$\frac{An\_DigOMtIn}{kg/d} = An\_DigNDFIn + An\_DigStIn \\ + An\_DigFAIn + An\_DigrOMtIn \\ + An\_DigCPtIn$$

(Equation 20-165)

$$\frac{Fe\_OMout}{kg/d} = Fe\_CPout + Fe\_NDFout + Fe\_Stout \\ + Fe\_rOMout + Fe\_FAout$$

(Equation 20-166)

$$\frac{Fe\_OMend}{kg/d} = Fe\_CPend + Fe\_rOMend$$

(Equation 20-167)

$$\frac{TT\_dcOMa}{\% OM} = \frac{An\_DigOMaIn}{An\_OMIn} \times 100$$

(Equation 20-168)

$$\frac{TT\_dcOMt}{\% OM} = \frac{An\_DigOMtIn}{An\_OMIn} \times 100$$

(Equation 20-169)

Gross, Digestible, and Metabolizable Energy Supply

GE supply (Mcal/d) and dietary concentration (Mcal/kg) of GE are calculated from total nutrient intakes and the heats of combustion for each nutrient (see Table 20-9):

$$\frac{An\_GEIn}{Mcal/d} = (Dt\_NDFIn + Inf\_NDFIn) \times En\_NDF \\ + (Dt\_StIn + Inf\_StIn) \times En\_St \\ + Dt\_rOMIn \times En\_rOM \\ + (Dt\_CFatIn + Inf\_FAIn) \times En\_FA \\ + (Dt\_TPIn + Inf\_CPIn) \times En\_CP \\ + Dt\_NPNCPIn \times En\_NPNCP \\ + Inf\_AcetIn \times En\_Acet + Inf\_PropIn \\ \times En\_Prop + Inf\_ButrIn \times En\_Butr$$

(Equation 20-170)

TABLE 20-9 Heats of Combustion (Mcal/kg) for Digested Nutrients

Nutrient	Variable Name	Enthalpy (Mcal/kg)
rOM	En_rOM	4.0
Starch	En_St	4.23
NDF	En_NDF	4.2
CP	En_CP	5.65
NPN (CP basis)	En_NPNCP	0.89
FA	En_FA	9.4
Acetate	En_Acet	3.48
Propionate	En_Prop	4.96
Butyrate	En_Butr	5.95

$$\frac{An\_GE}{Mcal/kg\ DM} = \frac{An\_GEIn}{An\_DMIn}$$

(Equation 20-171)

DE supply (Mcal/d) and concentrations (Mcal/kg) are calculated from digestible nutrient intakes and heats of combustion for each nutrient class:

$$\frac{An\_DEStIn}{Mcal/d} = An\_DigStIn \times En\_St$$

(Equation 20-172)

$$\frac{An\_DErOMIn}{Mcal/d} = An\_DigrOMaIn \times En\_rOM$$

(Equation 20-173)

$$\frac{An\_DENDFIn}{Mcal/d} = An\_DigNDFIn \times En\_NDF$$

(Equation 20-174)

$$\frac{An\_DECPIn}{Mcal/d} = An\_DigCPaIn \times En\_CP$$

(Equation 20-175)

$$\frac{An\_DENPNCPIn}{Mcal/d} = An\_NPNCPIn \times En\_NPNCP$$

(Equation 20-176)

$$\frac{An\_DETPIn}{Mcal/d} = An\_DECPIn - (An\_DENPNCPIn / \\ En\_NPNCP \times En\_CP)$$

(Equation 20-177)

$$\frac{An\_DEFAIn}{Mcal/d} = An\_DigFAIn \times En\_FA$$

(Equation 20-178)

$$\underset{Mcal/d}{Inf\_DEAcetIn} = Inf\_AcetIn \times En\_Acet$$

(Equation 20-179)

$$\underset{Mcal/d}{Inf\_DEPropIn} = Inf\_PropIn \times En\_Prop$$

(Equation 20-180)

$$\underset{Mcal/d}{Inf\_DEButrIn} = Inf\_ButrIn \times En\_Butr$$

(Equation 20-181)

$$\begin{aligned}\underset{Mcal/d}{An\_DEIn} = {}& An\_DENDFIn + An\_DEStIn \\ & + An\_DErOMIn + An\_DETPIn \\ & + An\_DENPNCPIn + An\_DEFAIn \\ & + Inf\_DEAcetIn + Inf\_DEPropIn \\ & + Inf\_DEButrIn \end{aligned}$$

(Equation 20-182)

$$\underset{Mcal/kg\ DM}{An\_DE} = \frac{An\_DEIn}{An\_DMIn}$$

(Equation 20-183)

The DE supplied from protein is subtracted from the total DE supply, yielding a nonprotein DE (*An_DEInp*, Mcal/d) for use as an energy term in milk protein predictions that contained independent effects of MP or AA:

$$\underset{Mcal/d}{An\_DEInp} = An\_DEIn - An\_DENPNCPIn$$

(Equation 20-184)

NUTRIENT UTILIZATION AND ANIMAL PRODUCTION

The general scheme is to estimate the NE and NP associated with each process (e.g., maintenance and production) and to convert NE or NP to ME or MP using an efficiency coefficient that is abbreviated as K with a subscript denoting the function: Kg, Kl, Km, or Ky for growth, lactation, maintenance, and gestation, respectively. Each is defined in the following sections.

Energy and Amino Acid Use for Milk Production: Milk Protein

Net protein in milk is predicted as outlined in Chapter 6:

$$\begin{aligned}\underset{g/d}{Mlk\_NP\_g} = {}& -97.0 + 1.68 \times Abs\_His\_g \\ & + 0.885 \times Abs\_Ile\_g \\ & + 0.466 \times Abs\_Leu\_g \\ & + 1.15 \times Abs\_Lys\_g \\ & + 1.84 \times Abs\_Met\_g \\ & + 0.0773 \times Abs\_OthAA\_g \\ & - 0.00215 \times EAAb^2 \\ & + 10.79 \times An\_DEInp \\ & - 4.60 \times (An\_DigNDF - 17.06) \\ & - 0.420 \times (An\_BW - 612) \end{aligned}$$

(Equation 20-185)

where *Abs_OthAA* (g/d) represented the sum of Abs_NEAA and absorbed supplies of Arg, Phe, Thr, Trp, and Val:

$$\begin{aligned}\underset{g/d}{Abs\_OthAA\_g} = {}& Abs\_NEAA\_g + Abs\_Arg\_g \\ & + Abs\_Phe\_g + Abs\_Thr\_g \\ & + Abs\_Trp\_g + Abs\_Val\_g \end{aligned}$$

(Equation 20-186a)

Abs_EAAb² represented the sum of squares of the five EAAs with linear terms in the equation:

$$\begin{aligned}\underset{g^2/d}{EAAb2} = {}& Abs\_His\_g^2 + Abs\_Ile\_g^2 \\ & + 0.466 \times Abs\_Leu\_g^2 \\ & + Abs\_Lys\_g^2 + Abs\_Met\_g^2 \end{aligned}$$

(Equation 20-186b)

The presence of a quadratic term in Equation 20-185 provides a significant application challenge. This will be problematic for even average herds as the model approaches the end of its expected usable life span (i.e., 15 years). Milk yield has increased linearly by 90 kg/y in the United States over the past several decades. At a feed DM conversion ratio of 1.5 kg of milk to 1 kg feed DM, this equates to 0.17 kg DMIn/d/y. Thus, over 15 years, one would expect DMIn to increase by 2.5 kg/d. At the mean MP concentration in the data set of 9.94 percent MP, this results in an additional 250 g of MP/d, which approximately equates to an additional 5.75 g absorbed methionine (Met)/d. Using 23 kg DMIn as a starting point for today, MP intake would be predicted to be 2,290 g/d and 52 g of absorbed Met. Adding 250 g of MP/d and 5.7 g absorbed Met/d to the starting point yields future mean supplies of 2,550 g MP/d and 58 g absorbed Met/d, which are 28 and 27 percent above the means for the data set, respectively. Such a Met supply is still below the apex of the quadratic for that term but only slightly so. Thus, the marginal efficiency predicted at those intakes would be very low.

Of greater concern than the average herd are the high genetic merit herds that exist today. Many U.S. commercial herds have average daily milk production of 45 kg/d or greater. To achieve that given a normal lactation curve, groups of animals at peak lactation must have pen averages of 60 kg/d or more with ani-

mals in that pen exceeding 80 kg/d. The maximum production for the data used herein is 53.8 kg/d. From Equation 20-186a, the maximum response to MP intake occurs at 3,087 g/d, which generates approximately 1,450 g milk protein/d depending on the *An_DEInp* value chosen. NRC (2001) predicts a DMIn of 33.6 kg/d for a mature cow weighing 750 kg and producing 60 kg of milk with 3.0 percent protein and 3.5 percent fat. At such a DMI, the MP intake would be 3,087 g/d, the content at the maximum response, which yields a dietary MP concentration of 9.2 percent of DM as compared to the mean concentration of 9.9 percent for the data set. Production above 60 kg/d would not be predicted to benefit from further additions of dietary protein given this plateau. Thus, a group at 70 kg/d would be predicted to require 3,087 g MP/d, as would a group or cows at 80 kg of milk/d. At a predicted DMIn of 37 kg/d for 70 kg of milk, the dietary MP concentration would fall to 8.3 percent of DM and a CP content below 14 percent. Progression to 80 kg of milk/d would further decrease the dietary CP content as DMIn scaled upward. Thus, the plateau must increase as genetic potential improves.

The problem was verified by the committee through exploration of the effect of study age on the quadratic term. When the data were split roughly in half using year 2000 as a dividing point, the plateau for predictions of milk protein was 1,051 g/d at an absorbed EAA supply of 1,673 g EAA/d for the data prior to 2000 when using a DE intake from nonprotein components (DEInp) value of 74 Mcal/d. When fit to the data published after 2000, the derived response was nearly linear with a maximum occurring at 6,415 g of absorbed EAA/d. Adding an age variable to the model with a value of 0 for pre-2000 publications and 1 for the remainder and using it to estimate different EAA and DEInp slope coefficients by age factor across the entire data set resulted in plateau estimates of 1,312 g/d milk protein at 1,559 g EAA/d and 1,854 g/d milk protein at 3,767 g EAA/d for the old and new data, respectively. Thus, both approaches support the concept of a plateau that increases over time, but the estimates are quite different depending on the approach. Attempts to refine the latter approach to yield the change in quadratic value by decade resulted in insignificance for all terms, indicating the model was overparameterized and the results unreliable.

Milk Protein Production Scaling Factors

Given the need to scale the quadratic term to accommodate high genetic merit animals, a method of accomplishing this in a reliable manner was required. This is not a new concept, and it has been visually presented for the effects of energy on milk protein production by Orskov (1992), where energy intake can be expected to scale with genetic potential. A similar response was derived by Hanigan et al. (1998) with respect to the impact of energy supply on milk protein production.

Reproducing the conceptual responses with a quadratic requires derivation of combinations of the linear and quadratic terms that yield the desired response pattern. These can be used to formulate a system of equations that can be solved for a series of plateaus that yield similar responses in the linear range. For demonstration purposes, a simple quadratic MP-based model is used:

$$Mlk\_NP\_g \atop g/d = -0.000114 \times An\_MPIn^2 + 0.681 \times An\_MPIn + 25.8$$

(Equation 20-187)

where *An_MPIn* is expressed in g/d.

From the conceptual framework, it was assumed that a reference point on the lower range of the linear portion of the response could be chosen and that the response in the rescaled equation at that absorbed EAA input would be the same. Furthermore, it can be assumed that the intercept will be the same regardless of scaling. One can define two points on the rescaled curve (actually three if one considers the intercept), which can be used to solve for the two unknown parameters (i.e., the linear and quadratic coefficients). Using the commonly accepted generic terms to define the quadratic in symbolic form, the system of equations is

$$Y_1 = a_1 X^2 + b_1 X + c_1 \quad \text{(Equation 20-188)}$$

$$Ym_1 = c_1 - \frac{b_1^2}{4a_1} \quad \text{(Equation 20-189)}$$

$$Xm_1 = \frac{-b_1}{2a_1} \quad \text{(Equation 20-190)}$$

where *Ym* is the plateau and *Xm* is the EAA concentration yielding that plateau. The subscript of 1 denotes the derived parameters (*a, b, c*) and corresponding predicted value (*Y*) for an existing equation (i.e., Equation 20-187).

The second set of equations defining the rescaled parameters and thus state is represented as

$$Y_2 = a_2 X^2 + b_2 X + c_1 \quad \text{(Equation 20-191)}$$

$$Ym_2 = c_1 - \frac{b_2^2}{4a_2} \quad \text{(Equation 20-192)}$$

$$Xm_2 = \frac{-b_2}{2a_2} \quad \text{(Equation 20-193)}$$

Note that the intercept is common to the two sets of equations. Using the parameters from Equation 20-188, Ym_1 and Xm_1 are defined by Equation 20-189 and Equation 20-190 as 1,043 g/d and 2,987 g/d, respectively. Evaluating Equation 20-188 at 10 percent of Xm_1 (299 g/d; denoted as X_1) yields an estimate for Y_1 of 219 g/d.

$$Y_1 = a_1 X_1^2 + b_1 X_1 + c_1 = 219$$

(Equation 20-194)

Assuming, for example, that the plateau should be doubled to 2,086 g/d (represented by Ym_2) and that the rescaled equation should also predict Y to be 219 g/d at X_1 yields

$$Ym_2 = c_1 - \frac{b_2^2}{4a_2} = 2086$$

(Equation 20-195)

$$Y_2 = a_2X_1^2 + b_2X_1 + c_1 = a_2 299^2 + b_2 299 + c_1 = 219$$

(Equation 20-196)

Assuming the intercept remains unchanged and thus can be removed, Equation 20-195 and Equation 20-196 can be solved algebraically for a and b:

$$a_2 = \frac{2\sqrt{Ym_2^2 - Y_2 Ym_2} - 2Ym_2 + Y_2}{X_1^2}$$

(Equation 20-197)

$$b_2 = -\frac{2\sqrt{Ym_2^2 - Y_2 Ym_2} - 2Ym_2}{X_1}$$

(Equation 20-198)

The resulting rescaled a and b yield a greater plateau with a similar response pattern in the linear portions of the curve (see Figure 20-1).

The above can be adapted to an equation with multiple EAAs, such as Equation 20-185. Expansion of the model demonstrates that the form is a summation of individual quadratics using a common a term across the EAA:

$$Y = a_1V_1^2 + \beta_1V_1 + a_1W_1^2 + \delta_1W_1 + a_1X_1^2 + \gamma_1X_1 + \cdots + c$$

(Equation 20-199)

Given parameter estimates for a_1, β_1, δ_1, γ_1, and c, the maximal response (Ym_1) can be determined by summation of the individual responses and the intercept:

$$Ym_1 = c - \frac{\beta_1^2}{4a_1^2} - \frac{\delta_1^2}{4a_1^2} - \frac{\gamma_1^2}{4a_1^2}$$

(Equation 20-200)

Excluding the intercept, which is assumed to be unaffected by animal potential, the maximal response to each EAA can be represented by the individual terms:

$$Ym_{V1} = \frac{-\beta_1^2}{4a_1} \qquad \text{(Equation 20-201)}$$

$$Ym_{W1} = \frac{-\delta_1^2}{4a_1} \qquad \text{(Equation 20-202)}$$

$$Ym_{X1} = \frac{-\gamma_1^2}{4a_1} \qquad \text{(Equation 20-203)}$$

and the concentrations of each EAA that yields the respective Ym calculated as

$$Vm_1 = \frac{-\beta_1}{2a_1} \qquad \text{(Equation 20-204)}$$
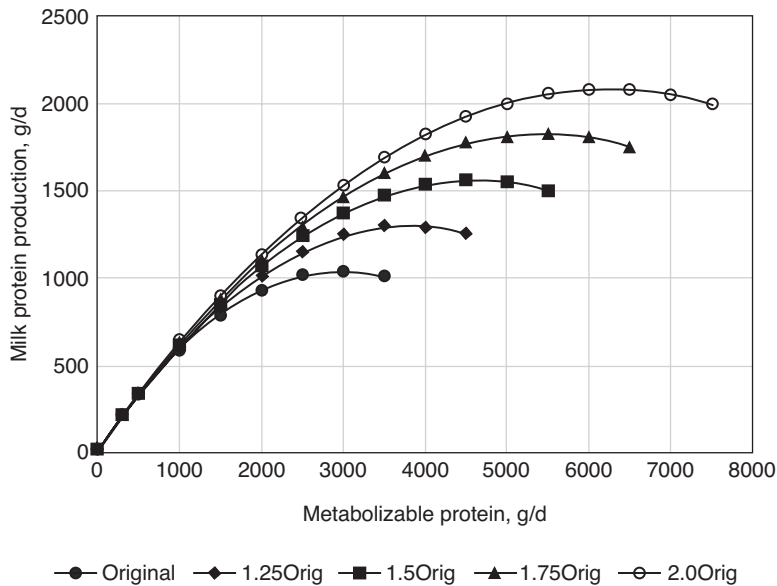
FIGURE 20-1   Example of quadratic scaling using the parameter estimates for Equation 20-187 for milk protein responses to MP intake and Equation 20-197 and Equation 20-198 to calculate rescaled linear and quadratic terms given maximal responses of 1.25, 1.5, 1.75, and 2 times the original maximum.
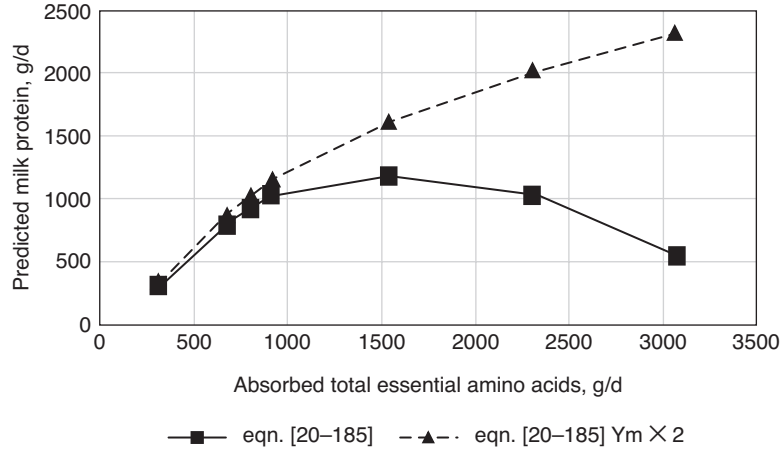
FIGURE 20-2 Predictions of milk protein from Equation 20-185 using the original parameters or after scaling to a plateau that was twice that of the original equation. Individual amino acid and energy concentrations were the observed minimum, first quartile, mean, third quartile, maximum, maximum×1.5, and maximum×2 from the metadata.

$$Wm_1 = \frac{-\delta_1}{2a_1} \qquad \text{(Equation 20-205)}$$

$$Xm_1 = \frac{-\gamma_1}{2a_1} \qquad \text{(Equation 20-206)}$$

Because each term in Equation 20-200 is additive, a new maximal response for each substrate can be set relative to the prior maximums using a common scalar. For example, if the user desires a doubling of the maximal overall rate, then Equations 20-201 to 20-203 would be set to two times their prior maximal values, Equation 20-199 minus the intercept would be evaluated at 10 percent of the maximal concentrations defined by Equations 20-204 to 20-206 using the prior coefficients, and new a_2, β_2, δ_2, and γ_2 would be calculated using Equations 20-197 and 20-198. a_2 would be the same for each AA, but the linear term would vary for each unless they all had identical slopes at the start. Application of the approach is demonstrated in Figure 20-2.

This approach can be applied in the field based on herd characteristics. Ideally, this would be the genetic or genomic profiles for the groups of interest; however, such a scalar would have to be developed. An interim approach is to scale the maximum responses to average herd productivity. Such an approach reflects both genetic potential and management and thus also potentially captures some undefined components of management.

In the model, the scalar is based on an observed 305-day rolling herd average for milk protein ($Mlk\_NP_{RHA(305d)}$). The average DIM for the data set used to derive the model was 136 days, which is close to the midpoint of a 305-day record. Thus, the potential of animals used in the summarized data is reflected in the mean milk protein production of 918 g/d. This equates to 305-day milk protein production of 280 kg. The scalar for application to any herd of animals is thus

$$f_g = 1 + \beta\left(\frac{Mlk\_NP_{RHA(305d)}}{280} - 1\right) \qquad \text{(Equation 20-207)}$$

where $Mlk\_NP_{RHA(305d)}$ is expressed as milk protein per 305 days (kg), and β_g is expected to be 1. Should the scaling prove to be over- or underresponsive, one could adjust β to achieve more or less responsiveness based on observational data, but at this time, a value of 1 should be used.

The above scaling is incorporated into the model, and therefore the model requires a 305-day milk protein production (kg) as an input.

Target milk protein production selection should be assessed for feasibility relative to the maximal milk protein production. Selection of a target that exceeds the scaled maximal production cannot be expected to yield predictions that achieve the target production. Such a monitor is provided as a ratio of Mlk_NP_g over Mlk_NPmax_g:

$$\frac{MlkNP\_MlkNP\max}{g/g} = \frac{Mlk\_NP\_g}{Mlk\_NP\max\_g}$$

(Equation 20-208)

where Mlk_NPmax_g (g/d) represents the maximal production and is calculated as

$$\frac{Mlk\_NP\max\_g}{g/d} = -97.0 + \sum_{a=1}^{N_{EAA}} (mPrtm\_g) + 0.077$$
$$\times Abs\_OthAA\_g$$
$$+ 10.79 \times An\_DEInp - 4.60$$
$$\times (An\_DigNDF - 17.06)$$
$$- 0.420 \times (An\_BW - 612)$$

(Equation 20-209)

and the max response for each EAA was calculated from Equation 20-209 using the rescaled model linear and quadratic EAA parameters:

$$\frac{mPrtmx_{a}\_g}{g/d} = \frac{-(\text{scaled quadratic coefficient})^2}{4(\text{scaled linear coefficient})_{a}}$$

(Equation 20-210)

As production can generally be increased by more than 10 percent for short periods of time by feeding very high-grain diets (prior to development of acidosis-related problems), it can generally be assumed that the genetic potential for milk protein production is greater than expressed production, and thus *MlkNP_MlkNPmax* should generally be less than 0.9, and if not, $Mlk\_NP_{RHA(305d)}$ should be increased to achieve a ratio of 0.9 or lower.

It is expected that Equation 20-185 with appropriate scaling to current herd production will result in the most accurate and precise prediction of milk protein output given its reliance on energy supply plus the availability of five key EAAs. However, milk protein output is also predicted from MP supply after subtraction of MP used for maintenance using an assumed, static target MP efficiency of use provided in Chapter 6. As detailed below, such a prediction is not particularly accurate or precise. The user can also use the *mPrt_eqn* setting to use the *Trg_MilkTPp* as a specified production level. Subsequent equations calculating AA use, AA efficiency, and MP balance are based on the user-specified target in this case.

AA export in milk protein ($AA_{L(a)}$, g/d) is calculated as the product of *Mlk_NP_g* (predicted or user specified) and the composition of milk protein ($AA_{(a)}\_NP$, g hydrated AA/100 g *Mlk_NP*; see Table 6-5).

$$\frac{Mlk\_AA_{(a)}}{g/d} = Mlk\_NP\_g \times \frac{AA_{(a)}\_NP_{Milk}}{100}$$

(Equation 20-211)

where *a* denoted each EAA (arginine [Arg], histidine [His], isoleucine [Ile], leucine [Leu], lysine [Lys], methionine [Met], phenylalanine [Phe], threonine [Thr], tryptophan [Trp], and valine [Val]). As the composition of the AAs is expressed in hydrated (free) form, the summation of all 20 AAs in milk protein would equate to approximately 115 g/100 g of milk TP. The AAs are handled in this form throughout. This correction has been ignored in the past, leading to some bias in the balance of AAs.

MP required for *Mlk_NP* (*An_MPlUse*, kg/d) is calculated as

$$\frac{Mlk\_MPuse}{kg/d} = \frac{Mlk\_NP}{Kl_{MP\_NP}}$$

(Equation 20-212)

where Kl_{Mp_NP} (g/g) is set to the predicted fractional efficiency of MP conversion to protein defined by Equation 20-355. This efficiency reflects the prevailing feeding conditions and thus does not reflect the achievable minimum MP required. Thus, MPuse predicted by Equation 20-212 is not a true reflection of that process.

To address that deficiency and to provide more information, MP to support milk production is also predicted using a static conversion coefficient as for NRC (2001) as a reference point. In this latter case, $Kl_{MP\_NP,Trg}$ was set to 0.69, reflecting the observed target efficiency for conversion of MP to export proteins as outlined in Chapter 6. Such a level of production reflects the average best mix of EAA and energy present in the literature, which may or may not reflect the true biological maximum. However, as it clearly was achieved in the population of data from the literature, it can serve as a minimum achievable target for *Mlk_MPuse* defined by Equation 20-212. Similarly, the efficiency of use of MP for export proteins determined in Equation 20-356 should be expected to be near or exceed 0.68 with ideal diets under ideal production conditions. Additional work is needed to define the true maximums for efficiency of use of MP and each EAA, but in the interim, the observed target efficiencies provided in Chapter 6 can be used as a guide.

$$\frac{Milk\_MPuse_{Trg}}{kg/d} = \frac{Milk\_NP}{Kl_{MP\_NP,\,Trg}}$$

(Equation 20-213)

$$\frac{Kl_{MP\_NP,Trg}}{g/g} = 0.69 \quad \text{(Equation 20-214)}$$

Milk Fat

Given estimates of individual FA intakes, a milk fat prediction was developed and found to have merit. Although likely not as robust at the milk protein prediction, it offers some guidance on expected milk fat production, which is the second most important economic component of milk. Such a prediction allows optimization of diets with consideration of milk component value (protein and fat value) relative to dietary ingredient selection and cost.

The equation was developed using the same methods as for milk protein (i.e., an all-models approach with selection based on statistics and apparent biological validity). It was also cross-validated and evaluated with a selection of field observations. The following equation cross-evaluated well and had a root mean squared error (RMSE) of 205 g/d, representing 18.9 percent of the observed mean with a concordance correlation coefficient (CCC) of 0.62, 1.6 percent of the mean squared error (MSE) segregating in mean bias, and 5.2 percent of the MSE segregating in slope bias.

$$\begin{aligned}\frac{Milk\_Fat\_g}{g/d} &= 453 - 1.42 \times An\_LactDay + 24.52 \\ &\quad \times (Dt\_DMIn - Dt\_FAIn) \\ &\quad + 0.41 \times Dt\_DigC160In \times 1000 + 1.80 \\ &\quad \times Dt\_DigC183In \times 1000 \\ &\quad + 1.45 \times Abs\_Ile\_g + 1.34 \times Abs\_Met\_g\end{aligned}$$

(Equation 20-215)

where *Dt_DMIn* and *Dt_FAIn* represented total DM and FA intakes (kg/d), respectively; *Dt_DigC160In* and *Dt_DigC183In* represented the predicted digested C16:0 and C18:3 intakes (kg/d), respectively; and *Abs_Ile_g* and *Abs_Met_g* represented absorbed Ile and Met (g/d), respectively. *An_LactDay* was capped at a maximum of 375 d reflecting the general range of the data used for derivation of the equation.

The user-specified *Trg_MilkFatp* may also be used to specify the milk fat yield in place of Equation 20-215 by setting the *mFat_eqn* variable to 0 for an input as outlined for milk protein. In that case, all downstream calculations will utilize the specified level of production.

Milk Volume

Although economically unimportant in component-based markets, milk volume is still a benchmark used widely in the industry. The primary osmotic draw determining volume is provided by lactose, with lesser influence from protein and fat. However, because lactose is produced by lactose synthase, which contains alpha-lactalbumin as a required subunit, lactose synthase activity is largely determined by milk alpha-lactalbumin production. Thus, milk protein is a primary determinant of milk volume, with the proportion of alpha-lactalbumin contained in milk protein diverging slightly across animals and breeds, thus creating some variation. Using a similar approach as for milk protein and fat, the best equation to predict milk volume was

$$
\begin{aligned}
\frac{Mik\_Prod_{Hol}}{kg/d} = {} & 4.541 + 11.13 \times Mlk\_NP \\
& + 2.65 \times Mlk\_Fat \\
& + 0.183 \times An\_DEIn - 0.0626 \\
& \times (An\_LactDay - 137) \\
& + 2.766 \times 10^{-4} \times (An\_LactDay - 137)^2 \\
& + 1.603 \times 10^{-6} \\
& \times (An\_LactDay - 137)^3 - 7.397 \times 10^{-9} \\
& \times (An\_LactDay - 137)^4 + 1.57 \\
& \times (An\_Parity - 1)
\end{aligned}
$$

$$
\frac{Mlk\_Prod}{kg/d} = \begin{cases}
\begin{array}{l|c}
Breed & Value \\
\hline
Holstein & Mlk\_Prod_{Hol} \\
Jersey & Mlk\_Prod_{Hol} - 3.40 \\
Other & Mlk\_Prod_{Hol} - 1.53
\end{array}
\end{cases}
$$

(Equation 20-216)

where *Mlk_NP* and *Mlk_Fat* were as predicted above (kg/d); *An_LactDay* and *An_BW* were centered to the mean of 137 days and 612 kg, respectively; and *An_Parity* was represented as a continuous variable reflecting the animal state or the pen average with 1 for primiparous animals and 2 for multiparous animals. Maximum *An_LactDay* was set at 375 d. The predicted volume of milk produced represents the base breed of Holstein. Production was reduced by 3.40 kg/d for Jersey cows and by 1.53 kg/d for cows from breeds other

than Holstein or Jersey. The RMSE was 4.5 kg/d, which was 14.6 percent of the mean observed value with a CCC of 0.75, 0.1 percent of the MSE segregating in mean bias, and 3.3 percent of MSE segregating in slope bias.

The user-specified *Trg_MilkTPp* input variable may also be used to specify milk volume in place of Equation 20-216 by setting the *mProd_eqn* input variable to 0 as outlined for milk protein. In that case, all downstream calculations will utilize the specified level of production.

Milk Energy

If milk lactose is provided as an input, milk energy content (Mcal/kg) is predicted from the target or predicted concentrations of milk fat and milk TP (if milk CP is known, replace 5.85 with 5.5 in Equation 20-215) and the user-provided concentration of milk lactose:

$$
\begin{aligned}
\frac{Milk\_NEp}{Mcal/kg} = {} & 9.29 \times \frac{Milk\_Fat\%}{Milk \times 100} + 5.85 \times \frac{Milk\_Protein\%}{Milk \times 100} \\
& + 3.95 \times \frac{Milk\_Lactose\%_{Target}}{Milk \times 100}
\end{aligned}
$$

(Equation 20-217)

This can be compared to the target milk energy content using user-specified target concentrations:

$$
\begin{aligned}
\frac{Milk\_NEp_{Trg}}{Mcal/kg} = {} & 9.29 \times \frac{Milk\_Fat_{Trg}}{Milk_{Trg}} + 5.85 \\
& \times \frac{Milk\_Protein_{Trg}}{Milk_{Trg}} \\
& + 3.95 \times \frac{Milk\_Lactose_{Trg}}{Milk_{Trg}}
\end{aligned}
$$

(Equation 20-218)

If milk lactose and protein are not available, the energy content can be predicted from milk fat using the equation of Tyrrell and Reid (1965):

$$
\frac{Milk\_NEp_{Trg}}{Mcal/kg} = 0.36 + 9.69 \times \frac{Milk\_Fat_{Trg}}{Milk_{Trg}}
$$

(Equation 20-219)

$$
\frac{Milk\_NEuse_{Trg}}{Mcal/d} = Milk\ NEp_{Trg} \times Milk_{Trg}
$$

(Equation 20-220)

$$
\frac{Milk\_NEuse}{Mcal/d} = Milk\ NEp \times Milk
$$

(Equation 20-221)

$$
\frac{Milk\_MEuse}{Mcal/d} = \frac{Milk\_NE}{Kl_{ME\_NE}}
$$

(Equation 20-222)

where Kl_{ME_NE} reflects the efficiency of conversion of ME to NE for lactation, which was set to 0.66.

$$\frac{Kl_{ME\_NE}}{Mcal/Mcal} = 0.66 \quad \text{(Equation 20-223)}$$

Energy and Amino Acid Use for Gestation

The model of Koong et al. (1975) was used to derive gravid uterine, fetal, and maternal tissue growth parameter estimates by fitting to the observations of Bell et al. (1995) and House and Bell (1993).

$$Q(DayGest) = Q_{DayGest=0} \times e^{\left\{\left(K_{growth} - K_{growth\,decay} \times DayGest\right) \times DayGest\right\}}$$

(Equation 20-224)

where Q represented the quantity of wet weight (kg), energy (Mcal), or protein (kg) at any day of gestation (DayGest); $Q_{DayGest=0}$ was the quantity of each at conception; K_{growth} (d^{-1}) was the initial rate growth in each; and $K_{growth\,decay}$ (d^{-1}) was the decay rate for growth. Initial gravid uterine and uterine weights were as reported by Bell (1995). The resulting model parameters are provided in Table 20-10.

The model was subsequently rearranged to allow the use of calf birth weight (*Fetus_Wt*, kg) and expected or observed gestational length as inputs rather than the quantity of tissue mass at conception. This allowed scaling of the growth curves to accommodate differences in fetal size and gestational length associated with animal size, age, and breed. Additionally, the model was modified to provide for uterine involution postpartum to maintain mass balance in the animal model. AAs arising from involution contributed to the total AA supply as described by Hanigan et al. (2009).

Gravid uterine and uterine (uterus plus caruncles) weights at parturition ($Wt_{(t=parturition)}$, kg) were calculated from calf birth weight (*Fetus_Wt*, kg) and the proportions of each (f_{GrUter_Fetus} and f_{Uter_Fetus}, kg/kg) derived from the fitted model.

$$\frac{GrUter\_Wt_{(t=parturition)}}{kg} = Fetus\_Wt_{(t=parturition)} \times f_{GrUter\_Fetus}$$

(Equation 20-225)

$$\frac{Uter\_Wt_{(t=parturition)}}{kg} = Fetus\_Wt_{(t=parturition)} \times f_{Uter\_Fetus}$$

(Equation 20-226)

For a gestational length of 280 days, $f_{GrUter\_Fetus} = 1.816$ kg/kg and $f_{Uter\_Fetus} = 0.231$ kg/kg.

TABLE 20-10 Nonlinear Regression of Gravid Uterine, Uterine, and Fetal Wet Weights, Protein, and Net Energy on Day of Gestation[a]

Gravid Uterus	Weight, kg		Energy, Mcal		Protein, kg	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Quantity$_{Initial}$	0.674		0.444		0.059	
K$_{Syn}$, d^{-1}	2.43×10^{-2}	1.0×10^{-3}	2.25×10^{-2}	1.3×10^{-3}	2.19×10^{-2}	1.1×10^{-3}
K$_{Syn\,Decay}$	2.45×10^{-5}	4.1×10^{-6}	1.35×10^{-5}	5.0×10^{-6}	1.16×10^{-5}	4.3×10^{-6}
Quantity$_{T=280}$	89.0		88.6		10.9	

Fetus	Weight, kg		Energy, Mcal		Protein, kg	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Quantity$_{Initial}$	0.010		9.2×10^{-3}		1.1×10^{-3}	
K$_{Syn}$, d^{-1}	5.16×10^{-2}	1.1×10^{-3}	4.95×10^{-2}	1.5×10^{-3}	4.82×10^{-2}	1.5×10^{-3}
K$_{Syn\,Decay}$	7.59×10^{-5}	4.5×10^{-6}	6.35×10^{-5}	5.8×10^{-6}	5.85×10^{-5}	6.0×10^{-6}
Quantity$_{T=280}$	49.0		66.2		8.14	

Maternal Tissue	Weight,[b] kg		Energy, Mcal		Protein, kg	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Quantity$_{Initial}$	0.204					
K$_{Syn}$, d^{-1}	2.42×10^{-2}	4.0×10^{-5}				
K$_{Syn\,Decay}$	3.53×10^{-5}	1.6×10^{-7}				
Quantity$_{T=280}$	11.3		15.3[c]		1.88[c]	

[a] Data used for model fitting were those reported by Bell et al. (1995) and House and Bell (1993). The model was fit using the nls function of the lme4 package of R (ver. 3.5.1).

[b] Derived from the regression model of Bell (1995).

[c] Estimated as (uterus + caruncle weights) × fetal energy or protein at *DayGest* = 280. Gestational length of 280 was chosen based on observed Holstein gestational lengths.

The derived tissue weights at parturition were used to predict gravid uterine weight and growth rates at any point of the gestational period

$$
\frac{GrUter\_Wt\_Gest}{kg} =
$$
$$
= GrUter\_Wt_{(t=parturition)}
$$
$$
\times e^{-\left(K_{GrUterSyn} - K_{GrUterSynDecay} \times DayGest\right) \times \left(LengthGest - DayGest\right)}
$$

(Equation 20-227)

$$
\frac{GrUter\_Wt\_PP}{kg} = Uter\_Wt_{(t=parturition)} \times e^{-K_{UterDeg} \times DayLact}
$$

(Equation 20-228)

$$
\frac{GrUter\_Wt}{kg} =
$$

Criteria	Value, kg/kg BW
$0 < DayGest \leq Gestation\ Length$	$GrUter\_Wt\_Gest$
$0 < DayLact < 100$	$GrUter\_Wt\_PP$
$An\_AgeDay < 240$	0
Otherwise	0.204

(Equation 20-229)

The K_{Syn} and $K_{SynDecay}$ used in Equations 20-227 and 20-228 are those listed in Table 20-10. Equation 20-228 represents the involution of the uterine tissue after parturition, where $K_{UterDeg}$ is assumed to be 0.2; $K_{UterDeg}$ is not known with certainty, but a value of 0.2 results in essentially complete involution by day 21 of lactation and resulted in expected blood AA concentrations postcalving (Hanigan et al., 2009). Tissue protein and AA released by such involution are a relatively small contributor to overall AA balance in the postpartum period (202 g NP/d on day 1 postpartum for a 50-kg calf), and thus halving or doubling $K_{UterDeg}$ would marginally change NP contributions from maternal gestational tissue postpartum.

A nonpregnant BW (An_BW_{NPr}, kg) is calculated from the observed BW and the predicted gravid uterine weight:

$$
\frac{An\_BW_{NPr}}{kg} = An\_BW - GrUter\_Wt
$$

(Equation 20-230)

Daily rates of wet tissue deposition (kg/d) are derived from Equations 20-227 and 20-228 as

$$
\frac{GrUter\_Wt_{Gain}\_Gest}{kg/d}
$$
$$
= \left(K_{GrUterSyn} - K_{GrUterSynDecay} \times DayGest\right)
$$
$$
\times GrUter\_Wt
$$

(Equation 20-231)

$$
\frac{GrUter\_Wt_{Gain\_PP}}{kg/d} = -K_{UterDeg} \times Uter\_Wt
$$

(Equation 20-232)

$$
\frac{GrUter\_Wt_{Gain}}{kg/d} =
$$

Criteria	Value
$0 < DayGest < Gestation\ Length$	$GrUter\_Wt_{Gain}\_Gest$
$0 < DayLact < 100$	$GrUter\_Wt_{Gain}\_PP$
Otherwise	0

(Equation 20-233)

Finally, gestational (*Gest*) requirements for NE (Mcal/d), ME (Mcal/d), and NP (g/d) deposition are calculated from the rate of change in gravid uterine tissue mass using the concentrations of energy (0.950 Mcal/kg) and CP (123 g/kg) in the final gravid uterus at parturition, as listed in Table 20-10, and the fraction of TP in CP (0.86 g/g):

$$
\frac{Gest\_NEgain}{Mcal/d} = GrUter\_Wt_{Gain} \times 0.950
$$

(Equation 20-234)

$$
\frac{Gest\_NPgain\_g}{g/d} = GrUter\_Wt_{Gain} \times 123 \times 0.86
$$

(Equation 20-235)

The conversion of ME to NE (Ky_{ME_NE}, Mcal/Mcal) in support of gestation was set at 0.14 when *Gest_NEgain* is positive (during gestation), which was derived from Ferrell et al. (1976). Efficiency was set to 0.89 for postpartum uterine regression:

$$
\frac{Ky_{ME\_NE}}{Mcal/Mcal} =
$$

Criteria	Value
$Gest\_NE_{gain} \geq 0$	0.14
$Gest\_NE_{gain} < 0$	0.89

(Equation 20-236)

ME use in support of gestation is subsequently calculated from *Gest_NE* as

$$
\frac{Gest\_MEuse}{Mcal/d} = \frac{Gest\_NE_{gain}}{Ky_{ME\_NE}}
$$

(Equation 20-237)

The same criteria and strategy are used for calculation of *Gest_MPuse* required from *Gest_NP_g*, where Ky_{MP_NP} is set to 0.33 when *Gest_NPgain* was positive as for NRC (2001) and to 1 when *Gest_NPgain* was negative during postpartum regression.

$$
\frac{Ky_{MP\_NP}}{g/g} =
$$

Criteria	Value
$Gest\_NP_{gain} \geq 0$	0.33
$Gest\_NP_{gain} < 0$	1

(Equation 20-238)

$$\frac{Gest\_MPuse\_g}{g/d} = \frac{GrUter\_NP_{gain}}{Ky_{MP\_NP}}$$

(Equation 20-239)

Gestational metabolizable AA requirements (g/d) are calculated based on the AA composition of gravid uterine protein ($AA_{(i)}\_NP_{GrUter}$), which was assumed to be equal to body protein (see Table 6-5):

$$\frac{Gest\_AA_{(i)}}{g/d} = Gest\_MP \times \frac{AA_{(i)}\_NP_{GrUter}}{100}$$

(Equation 20-240)

As the NE and NP values are accumulated over the full gestation, the ratios represent the average composition of the tissue over the entire pregnancy. This approach ensures that the accumulated energy and protein in the gravid uterus are reflected at parturition. However, as the composition changes slightly as gestation progresses, there are small errors of prediction of nutrient deposition rates in the middle of the gestational period, but these errors will compensate provided the pregnancy proceeds to full term. This approach will also slightly underestimate energy and protein release from the involuting uterus as the energy and protein composition of the uterus is greater than the gravid uterus. Both of these errors are also quite small and lack biological significance relative to the overall use of energy and AAs in the animal.

Energy and Amino Acid Use for Growth and Body Reserves

Because gut fill, fetal tissue weight, and body composition are determined at slaughter and used to calculate gain of water, ash, fat, and protein, it is useful to predict mass of each of those components, but these relationships are not well defined for all physiological states and thus remain incomplete.

Gut fill as a proportion of BW ($GutFill\_BW$, kg/kg) is determined based on relative BW and dietary intake of starter and milk (Dt_DMIn_{Milk}, kg/d) and calf starter ($Dt\_DMIn_{Starter}$, kg/d):

$$\frac{GutFill\_BW}{kg/kg\ BW} =$$

Criteria	Value
$Dt\_DMIn_{Milk} > 0$ & $Dt\_DMIn_{Starter} \le 0.1$	0.06
$Dt\_DMIn_{Milk} > 0$ & $Dt\_DMIn_{Starter} > 0.1$	0.07
$Dt\_DMIn_{Milk} > 0$ & $Dt\_DMIn_{Starter} \le 0.1$ & $An\_BW > 0.16 \times An\_BW_{Mature}$	0.09
$Dt\_DMIn_{Milk} = 0$ & $Dt\_DMIn_{Starter} > 0.1$ & $An\_BW > 0.16 \times An\_BW_{Mature}$	0.15
$An\_Parity > 0$	0.18

(Equation 20-241)

Actual gut fill is calculated from An_BW_{NPr} and subtracted from An_BW and An_BW_{NPr} to determine empty BW (EBW) (An_EBW, kg) for each:

$$\frac{An\_EBW}{kg} = An\_BW - An\_GutFill$$

(Equation 20-242)

$$\frac{An\_EBW_{NPr}}{kg} = An\_BW_{NPr} - An\_GutFill$$

(Equation 20-243)

$$\frac{An\_EBW_{Mature}}{kg} = An\_BW_{Mature} \times 0.82$$

(Equation 20-244)

EBW is adjusted to a standard BCS of 3.0, assuming the mass of a unit of BCS is a function of the BW of the animal:

$$\frac{BW\_BCS}{kg/BCS} = 0.094 \times An\_BW$$

(Equation 20-245)

$$\frac{An\_EBW_{NPr3}}{kg} = An\_EBW_{NPr} + \{(3 - BCS) \times BW\_BCS \times BW\}$$

(Equation 20-246)

Composition of Gain

The model uses the specified An_BW and target body gain for frame (Frm_Gain_{Target}, kg/d) and reserves ($Rsrv\_Gain_{Target}$, kg/d) to calculate body weight gain by summation:

$$\frac{An\_BWgain}{kg/d} = Frm\_Gain + Rsrv\_Gain$$

(Equation 20-247)

and to predict energy, protein, and AA utilization for gain. The model also calculates energy and MP allowable rates of gain.

To accommodate potential future predictions of Frm_Gain and $Rsrv\_Gain$, these inputs were passed to a general variable so that a prediction selection scheme can be used as for milk production.

$$\frac{Frm\_Gain}{kg/d} = Frm\_Gain_{Target}$$

(Equation 20-248)

$$\frac{Rsrv\_Gain}{kg/d} = Rsrv\_Gain_{Target}$$

(Equation 20-249)

Selectors were included among the model inputs to control such future frame and reserve gain predictions but not implemented in the code. To use the selectors, Equations 20-248 and 20-249 can be replaced with if statements to select among predictions as for milk NP or DMIn code.

Empty frame gain (kg/d) is calculated by subtraction of gut fill, assuming fill is proportional to body and frame weights. Calf empty frame gain was calculated, assuming gut fill was 9 percent of frame gain.

$$\frac{Frm\_Gain_{Empty}}{kg/d} =$$

Criteria	Value
$Dt\_DMIn_{ClfLiq} > 0$ & $Dt\_DMIn_{ClfDry} > 0$	$Frm\_Gain \times 0.91$
otherwise	$Frm\_Gain \times (1 - GutFill\_BW)$

(Equation 20-250)

Empty reserves gain was assumed to be equal to reserves gain (i.e., no gut fill changes).

$$\frac{Rsrv\_Gain_{Empty}}{kg/d} = Rsrv\_Gain$$

(Equation 20-251)

$$\frac{An\_EBW\_Gain}{kg/d} = Frm\_Gain_{Empty} + Rsrv\_Gain_{Empty}$$

(Equation 20-252)

The composition of gain (g of fat or protein/g gain) is specified as

$$\frac{FatGain\_FrmGain}{kg/kg} =$$

Criteria	Value
$An\_BW \leq 0.16 \times An\_BW_{Mature}$	$0.0786 + 0.0370 \times An\_REGain$
$An\_BW > 0.16 \times An\_BW_{Mature}$ & $Parity = 0$	$\left(0.067 + 0.375 \times \dfrac{An\_BW}{An\_BW_{Mature}}\right) \times \dfrac{An\_EBW_{Gain}}{An\_BW_{Gain}}$

(Equation 20-253)

$$\frac{FatGain\_RsrvGain}{kg/kg} = 0.622$$

(Equation 20-254)

$$\frac{NPgain\_FrmGain}{kg/kg} = \left(0.201 - 0.081 \times \frac{An\_BW}{An\_BW_{Mature}}\right) \times 0.85 \times 0.86$$

(Equation 20-255)

$$\frac{NPgain\_RsrvGain}{kg/kg} = 0.068 \times 0.86$$

(Equation 20-256)

Fat, NP, water, and ash gains are calculated as

$$\frac{Body\_FatGain}{kg/d} = FatGain\_FrmGain \times Frm\_Gain_{Empty} + FatGain\_RsrvGain \times Rsrv\_Gain_{Empty}$$

(Equation 20-257)

$$\frac{Body\_NPGain}{kg/d} = NPgain\_FrmGain \times Frm\_Gain + NPgain\_RsrvGain \times Rsrv\_Gain$$

(Equation 20-258)

$$\frac{Body\_NPGain\_g}{g/d} <- Body\_NPGain \times 1000$$

(Equation 20-259)

$$\frac{Body\_AAGain\_g_{(a)}}{g/d} = Body\_NPGain\_g \times \frac{AA_{(a)}\_NP_{Body}}{100}$$

(Equation 20-260)

$$\frac{Body\_NonFatGain}{kg/d} = An\_EBW\_Gain - Body\_FatGain$$

(Equation 20-261)

$$\frac{Body\_AshGain}{kg/d} = 0.056 \times Body\_NonFatGain$$

(Equation 20-262)

$$\frac{Body\_WaterGain}{kg/d} = 0.729 \times Body\_NonFatGain$$

(Equation 20-263)

Except for calves, body fat and NP gain are used to estimate retained energy captured in gain (An_REGain, Mcal/d).

$$\frac{An\_REGain}{Mcal/d} =$$

Criteria	Value
$An\_BW \leq 0.16 \times An\_BW_{Mature}$	$An\_EBWgain^{1.10} \times An\_EBW^{0.205}$
$An\_BW > 0.16 \times An\_BW_{Mature}$	$9.4 \times Body\_FatGain + 5.55 \times Body\_CPgain$

(Equation 20-264)

Conversion of *An_REGain* to *An_NELGain* factors was

$$
\frac{Kg_{NEL\_RE}}{Mcal/Mcal} = \begin{cases}
\begin{array}{|l|c|}
\hline
\textit{Criteria} & \textit{Value} \\
\hline
An\_BW \leq 0.16 \\ \times An\_BW_{Mature} & 1.0 \\
\hline
An\_BW > 0.16 \\ \times An\_BW_{Mature} \ \& \ Parity = 0 & 0.89 \\
\hline
Parity > 0 \ \& \ Mlk\_Prod > 0 & 1.12 \\
\hline
Parity > 0 \ \& \ Mlk\_Prod = 0 & 0.89 \\
\hline
\end{array}
\end{cases}
$$

(Equation 20-265)

The conversion of ME to NEL for support of maintenance and lactation (*Km_ME_NE* and *Kl_ME_NE*, Mcal/Mcal) were set to 0.66. The efficiency of conversion of ME to RE for frame gain (*Kf_ME_RE*) was defined as

$$
\frac{Kf_{ME\_RE}}{Mcal/Mcal} =
$$

$$
\begin{array}{|l|l|}
\hline
\textit{Criteria} & \textit{Value} \\
\hline
An\_StatePhys = \text{``Calf''} & (0.56 \times Dt\_DMIn_{ClfLiq}) \\
 & + (1.1376 \times (An\_DE \times 0.93) \\
 & \times Dt\_DMIn_{ClfLiq} \\
 & / Dt\_DMIn) + (-(0.1198 \\
 & \times (An\_DE \times 0.93)) \\
 & + (0.0076 \times (An\_DE \times 0.93)) \\
 & - 1.2979) / (An\_DE \times 0.93) \\
 & \times Dt\_DMIn_{ClfDry} / Dt\_DMIn \\
\hline
An\_BW > 0.16 \\ \times An\_BW_{Mature} & 0.63 \\
\hline
Parity > 0 \\ \& Mlk\_Prod > 0 & 0.66 \\
\hline
Parity > 0 \\ \& Mlk\_Prod = 0 & 0.66 \\
\hline
\end{array}
$$

(Equation 20-266)

The efficiency of conversion of ME to RE in support of reserve gains (*Kr_ME_RE*) was:

$$
\frac{Kr_{ME\_RE}}{Mcal/Mcal} =
$$

$$
\begin{array}{|l|c|}
\hline
\textit{Criteria} & \textit{Value} \\
\hline
An\_BW > 0.16 \times An\_BW_{Mature} \\ \text{and } An\_MilkProd = 0 & 0.60 \\
\hline
An\_MilkProd > 0 \text{ and } Rsrv\_Gain > 0 & 0.75 \\
\hline
0.86 An\_MilkProd > 0 \text{ and} \\ Rsrv\_Gain < 0 & 0.89 \\
\hline
\end{array}
$$

(Equation 20-267)

The overall conversion efficiency for ME to RE is thus the weighted average of *Kf_+ME_RE* and *Kr_ME_RE*. ME required for NE gain (*An_MEgain*, Mcal/d) is calculated as

$$
\frac{Body\_MEuse}{Mcal/d} = An\_NEGain_{Rsrv} / Kr\_ME\_RE
$$
$$
+ \frac{An\_NEGain_{Frm}}{Kf_{ME\_RE}}
$$

(Equation 20-268)

The energy content of BW change (*NEgain_BWgain*, Mcal/kg) is calculated as

$$
\frac{NEgain\_BWgain}{Mcal/kg} = \frac{An\_NEgain_{Frm} + An\_NEgain_{Rsrv}}{An\_BWgain}
$$

(Equation 20-269)

MP required for the gain in NP (*Body_MPuse*, Mcal/d) is calculated as

$$
\frac{Body\_MPuse}{kg/d} = \frac{An\_NPgain}{Kg_{MP\_NP}}
$$

(Equation 20-270)

where Kg_{MP_NP} (g/g) represents the average observed efficiency of conversion of MP to NP in support of protein gain, which was

$$
\frac{Kg_{MP\_NP}}{g/g} =
$$

$$
\begin{array}{|l|l|}
\hline
\textit{Criteria} & \textit{Value} \\
\hline
An\_StatePhys = Calf & 0.70 - 0.532 \times \\
 & (An\_BW/An\_BW\_mature) \\
 & \times 0.86 \\
\hline
An\_Parity = 0 \text{ and} \\ An\_BW\_empty / \\ An\_BW_{mature}\_empty > 0.12 & 0.64 - 0.3 \times \\ An\_EBW/An\_EBW_{mature}) \\ \times 0.86 \\
\hline
An\_Parity > 0 & Trg\_MP\_NP \times 0.86 \\
\hline
\end{array}
$$

(Equation 20-271)

Kg_{MP_NP} should not be viewed as the maximal achievable efficiency. The AA composition of MP affects efficiency and that was not considered in model development. Minimum efficiency was set at 0.39.

Energy and Amino Acid Use for Maintenance: Energy

Maintenance costs for animals that are not subjected to environmental stress (An_NEmUse$_{NS}$, Mcal/d) are calculated from BW and age as

$$An\_NEmUse_{NS} \atop Mcal/d} =$$

Criteria	Value
Calves: $Dt\_DMIn_{LiquidFeed} > 0$	$0.0769 \times An\_EBW^{0.75}$
Calves: $Dt\_DMIn_{LiquidFeed} = 0$	$0.097 \times An\_BW^{0.75}$
Heifers: Dt_DMIn_{Milk} $= 0 \, \& \, Parity = 0$	$0.10 \times An\_BW^{0.75}$
Cows: $Parity > 0$	$0.10 \times An\_BW_{NPr\_3}^{0.75}$

(Equation 20-272)

When the mean environmental temperature (T, ^{0}C) is outside of the TNZ (defined by the lower and upper critical temperatures, Equation 20-2 and Equation 20-3), additional maintenance costs occur. These are calculated as

$$An\_NEmUse_{Env} \atop Mcal/d} =$$

Criteria	Value
$LCT < T < UCT$	0
$T < LCT$ $\& An\_BW < 100$	$0.00201 \times (LCT - T)$ $\times An\_BW^{0.75}$
$T > UCT$ $\& An\_BW < 100$	$0.00201 \times (T - UCT)$ $\times An\_BW^{0.75}$

(Equation 20-273)

Some locomotion costs are intrinsic to the above maintenance requirements. For cows, maintenance costs were largely derived from metabolic chamber work, and thus that cost includes the energy associated with standing, eating and drinking, and moving from standing to recumbency and the reverse. In many cases, the cows may have also been in early gestation, resulting in some gestational cost being included in maintenance. Calf and heifer values have more typically been derived from comparative slaughter techniques, with animals moving from stalls to feed and water, and thus reflective of a confinement system. Energy required for activity above those levels should be included as additional maintenance costs. Ignoring the costs of locomotion in a confinement operation perhaps slightly underestimates maintenance when pens are located a long distance from the parlor, but the error is likely very small. It is more important to consider activity in pasture conditions where the distance walked and the topography can result in significant increases in energy expenditures.

The cost of locomotion for grazing activity (*An_NEmUse_{Graze}*, Mcal/d) is detected and calculated based on the inclusion of feeds categorized as pasture in the ration:

$$An\_NEmUse_{Graze} \atop Mcal/d} =$$

Criteria	Value
$\dfrac{Dt\_PastIn}{Dt\_DMIn} < 0.005$	0
$\dfrac{Dt\_PastIn}{Dt\_DMIn} \geq 0.005$	$0.0075 \times An\_BW^{0.75}$ $\times \dfrac{(600 - 12 \times Dt\_PastSupplIn)}{600}$

(Equation 20-274)

where *Dt_PastIn* and *Dt_PastSupplIn* are the consumption of pasture and nonpasture DM (kg/d). This cost is for flat topography (i.e., no hills).

Additional locomotion costs (*An_NEmUse_{Parlor}*, Mcal/d) are calculated from the round-trip distance from the barn or paddock to the parlor (*Env_DistParlor*, m), the number of milkings, and animal size. As for grazing activity, this cost is estimated for a flat surface.

$$\frac{An\_NEmUse_{Parlor}}{Mcal/d} = 0.00035 \times \frac{Env\_DistParlor}{1000}$$
$$\times Env\_TripsParlor \times An\_BW$$

(Equation 20-275)

The cost associated with elevation change while grazing and in transit to and from milking is calculated from the daily total climb while grazing and during transit between the milking parlor and the barn or paddock (*Env_TopParlor*, m) and animal size. Climb only considers the meters of uphill climb (i.e., the fall is not subtracted as the latter has little locomotion cost).

$$\frac{An\_NEmUse_{Topo}}{Mcal/d} = 0.0067 \times \frac{Env\_TopoParlor}{1,000} \times An\_BW$$

(Equation 20-276)

Distance walked during the entire day could also be estimated from step activity recorded by activity monitors. Estimates for meters of climb (without consideration of fall) based on loose categories for topography are as follows:

Hilliness	Vertical Distance Climbed
Mild	50 m/d
Moderate	200 m/d
Severe	500 m/d

Climb could also be estimated from distance traveled and satellite imaging data if one assumes random movement in the pasture and channeled movement to and from the milking parlor, or it could be calculated directly from animal movements using the global positioning system.

Total activity costs are derived by summation:

$$\frac{An\_NEmUse_{Act}}{Mcal/d} = An\_NEmUse_{Graze} + An\_NEmUse_{Parlor} + An\_NEmUse_{Topo}$$

(Equation 20-277)

and the adjusted NEm is

$$\frac{An\_NEmUse}{Mcal/d} = An\_NEmUse_{NS} + An\_NEmUse_{Env} + An\_NEmUse_{Act}$$

(Equation 20-278)

ME requirements for maintenance (An_MEm, Mcal/d) are calculated from An_NEm using a conversion efficiency (Km, Mcal/Mcal) as

$$\frac{An\_MEmUse}{Mcal/d} = \frac{An\_NEm}{Km_{ME\_NE}}$$

(Equation 20-279)

The efficiency used varies by animal state, with calves consuming only liquid being

$$Km_{ME\_NE(ClfLiq)} = 0.718$$

(Equation 20-280)

and calves consuming only dry feed as

$$Km_{ME\_NE(ClfDry)} = 1.1104 - (0.0946 \times Dt\_ME) + (0.0065 \times Dt\_ME^2) - \frac{0.7783}{Dt\_ME}$$

(Equation 20-281)

These two partial efficiencies were weighted by the amount of liquid and dry feed consumed to yield the overall maintenance efficiency for calves. Heifers and cows used constant efficiencies regardless of diet type:

$$\frac{Km_{ME\_NE}}{Mcal/Mcal} =$$

Criteria	Value
$An\_StatePhys =$ "Calf" and $An\_ME_{ClfDry} > 0$ and $Dt\_DMIn\_ClfLiq = 0$	Km_NE
$An\_StatePhys =$ "Calf" and $Dt\_DMIn\_ClfLiq = 0$	0.69
$An\_StatePhys \neq$ "Calf" & $An\_Parity = 0$	0.63
$An\_Parity > 0$	0.66

(Equation 20-282)

Protein

Net CP and TP depositions in scurf ($Scrf\_CP\_g$ and $Scrf\_NP\_g$, g/d respectively) are defined as

$$\frac{Scrf\_CP\_g}{g/d} = 0.20 \times An\_BW^{0.60}$$

(Equation 20-283)

$$\frac{Scrf\_TP\_g}{g/d} = Scrf\_CP\_g \times 0.86$$

(Equation 20-284)

$$\frac{Scrf\_TP}{kg/d} = Scrf\_TP\_g/1,000$$

(Equation 20-285)

and the losses of individual AA in scurf TP as a function of the concentration of each AA in scurf protein ($AA_{(i)}\_NP_{Scrf}$; see Table 6-5) as

$$\frac{Scrf\_AA_{(i)}\_g}{g/d} = \frac{Scrf\_NP\_g \times AA_{(i)}\_NP_{Scrf}}{100}$$

(Equation 20-286)

Urinary endogenous N losses (Ur_Nend, g/d) include contributions from urea, 3-methyl-histidine (3MH), endogenous purine derivatives (PDs), creatinine (Creatn), creatine (Creat), and hippuric acid:

$$\frac{Ur\_Nend\_Urea\_g}{g/d} = 0.010 \times An\_BW$$

(Equation 20-287)

$$\frac{Ur\_Nend\_Creatn\_g}{g/d} = 0.00946 \times An\_BW$$

(Equation 20-288)

$$\frac{Ur\_Nend\_Creat\_g}{g/d} = Ur\_Nend\_Creatn\_g \times 0.37$$

(Equation 20-289)

$$\frac{Ur\_Nend\_PD\_g}{g/d} = 0.0271 \times An\_BW^{0.75}$$

(Equation 20-290)

$$\frac{Ur\_Nend\_3MH\_g}{g/d} = (7.82 + 0.55 \times An\_BW) \times (3 \times 14)/169/1,000$$

(Equation 20-291)

$$\frac{Ur\_Nend\_sum\_g}{g/d} = (Ur\_Nend\_Urea\_g + Ur\_Nend\_Creatn\_g + Ur\_Nend\_Creat\_g + Ur\_Nend\_PD\_g + Ur\_Nend\_3MH\_g)/(1 - 0.46)$$

(Equation 20-292)

$$Ur\_Nend\_Hipp\_g \atop g/d = Ur\_Nend\_sum\_g \times 0.46$$

(Equation 20-293)

The urinary endogenous N losses are also approximated by summation of the above as

$$Ur\_Nend\_g \atop g/d = 0.053 \times An\_BW$$

(Equation 20-294)

the latter being used for downstream calculations. Net endogenous TP loss in urine is assumed to be 16 percent N, and all of *Ur_Nend* is assumed to have derived from TP/AAs:

$$Ur\_NPend\_g \atop g/d =$$

Criteria	Equation
$An\_StatePhys =$ "*Calf*"	$2.75 \times An\_BW^{0.50}$
$An\_StatePhys \neq$ "*Calf*"	$Ur\_Nend\_g \times 6.25$

(Equation 20-295)

$$Ur\_NPend \atop kg/d = Ur\_NPend\_g \times 0.001$$

(Equation 20-296)

The fraction of urinary endogenous loss arising only from metabolizable EAA (*Ur_EAAend*, g/d; i.e., urea and 3-methyl-histidine) is predicted as

$$Ur\_EAAend\_g \atop g/d = 0.010 \times An\_BW \times 6.25$$

(Equation 20-297)

and the loss of individual AA as urinary endogenous TP is a function of the concentration of each AA in body protein (see Table 6-5) plus an adjustment for the loss of 3-meythl-histidine:

$$\frac{Ur\_AAend_{(i)}\_g}{g/d} = \frac{Ur\_EAAend\_g \times AA_{(i)}\_NP_{Gain}}{100}$$

(Equation 20-298)

where *i* represented the individual AA excepting His, which included the contribution of 3-methyl-histidine:

$$\frac{Ur\_AAend_{(His)}\_g}{g/d} = \frac{Ur\_NP\_g \times AA_{(His)}\_NP_{Gain}}{100} + Ur\_Nend\_3MH\_g \times \frac{169}{3 \times 14}$$

(Equation 20-299)

NP loss as fecal endogenous (*Fe_Nend*, g/d; and *Fe_NPend*, g/d; also referred to as metabolic fecal) are defined from dietary NDF concentrations and DMIn as

$$\frac{Fe\_CPend}{kg/d} = \frac{\left(12.0 + 0.12 \times An\_NDF\right) \times \left(\begin{array}{c} Dt\_DMIn + InfRum\_DMIn \\ + InfSI\_DMIn \end{array}\right)}{1,000}$$

(Equation 20-300)

$$Fe\_Nend \atop kg/d = Fe\_CPend \times 0.16$$

(Equation 20-301)

and 73 percent of the *Fe_CPend* is assumed to be TP:

$$Fe\_NPend\_g \atop g/d = Fe\_CPend \times 0.73$$

(Equation 20-302)

Use of AA for fecal endogenous losses is calculated from the NP flow and the composition of that protein ($AA_{(i)}\_NP_{MFP}$; see Table 6-5):

$$\frac{Fe\_AAend_{(i)}\_g}{g/d} = \frac{Fe\_NP\_g \times AA_{(i)}\_NP_{MFP}}{100}$$

(Equation 20-303)

Total net maintenance NP and MP use for export proteins is by summation:

$$An\_NPmUse\_g = Scrf\_NP\_g + Fe\_NPend\_g + Ur\_NPend\_g$$

(Equation 20-304)

$$\frac{An\_MPmUse\_g}{g/d} = \frac{\left(An\_NPmUse\_g - Ur\_NPend\_g\right)}{Km_{MP\_NP}} + Ur\_NPend\_g$$

(Equation 20-305)

where Km_{MP_NP} represents the target efficiency of conversion of MP to maintenance NP (assumed to be equal to that of export proteins):

$$Km_{MP\_NP} \atop g/g = 0.69 \quad \text{(Equation 20-306)}$$

Ur_NPend_g is assumed to be equal to MP use as the AAs representing that cost are transferred directly from blood to urine without a conversion loss.

Metabolizable Energy Supply

Having determined urinary N and gaseous energy losses, ME intake and dietary concentrations can be calculated by difference,

$$An\_MEIn \atop Mcal/d =$$

Criteria	Value
$An\_StatePhys =$ "Calf" and $Dt\_DMIn\_ClfLiq > 0$	$An\_DEIn\_base\_ClfLiq \times 0.96 + An\_DEIn\_base \_ClfDry \times K\_DE\_ME_{ClfDry}$
$An\_StatePhys \neq$ "Calf"	$An\_DEIn - An\_GasEIn -Ur\_DEIn$

(Equation 20-307)

where An_DEIn_base = the DE unadjusted for $DMIn$ and $K\_DE\_ME_{ClfDry}$ is the efficiency of conversion of DE to ME

$$K\_DE\_ME_{ClfDry} \atop mcal/mcal =$$

Criteria	Equation
$An\_StatePhys =$ "Calf" and $Dt\_DMIn_{ClfLiq} > 0.015 \times An\_BW$ and $RumDevDisc\_Clf = 1$	0.93
$An\_StatePhys =$ "Calf" and $RumDevDisc\_Clf = 1$	0.93×0.90

Urinary N (Ur_N_g, g/d) and energy (Ur_DEIn, Mcal/d) losses are defined as

$$\frac{Ur\_N\_g}{g/d} = \frac{\left(\begin{array}{c} An\_CPIn \times 1,000 - Fe\_CP\_g \\ - Scrf\_NP\_g \\ -Fec\_NP_{Metab}-g - Milk\_NP\_g \\ -Body\_NP_{gain}-g \\ -Gest\_NP\_g \end{array} \right)}{6.25}$$

(Equation 20-308)

$$\frac{Ur\_DEIn}{Mcal/d} = 0.0143 \times Ur\_N\_g$$

(Equation 20-309)

From Equation 20-308, it can be seen that NP use must be calculated before Ur_N_g can be defined. Thus, in the case of the model, the entire ME section must be placed after the sections defining NP use (below). The equations are placed here for organizational reasons.

Gaseous energy (GasE) losses (Mcal/d) are predicted from nutrient intakes and concentrations:

$An\_GasEOut = \atop Mcal/d$

Criteria	Value
Calves: $An\_BW \leq 0.16 \times An\_BW_{Mature}$	0
Heifers: $An\_BW > 0.16 \times AnBWMature$ and $An\_Parity = 0$	$-0.038 + 0.051 \times An\_GEIn + 0.0091 \times An\_NDF$
DryCow: $AnParity > 0$ and $An\_MilkProd = 0$	$\begin{array}{c} 0.69 + 0.053 \\ \times An\_GEIn - 0.07 \\ \times \left(\dfrac{Dt_{FAIn} + InfRum_{FAIn}}{Dt_{DMIn} + InfRum_{DMIn}} \right) \times 100 \end{array}$
Lactating Cow:	$\begin{array}{c} 0.294 \times (Dt\_DMIn \\ + InfRum\_DMIn) \\ -0.347 \\ \times \left(\begin{array}{c} Dt\_FA \\ + InfRum\_FAIn / \\ InfRum\_DMIn \\ \times 100 \end{array} \right) \end{array}$
$An\_MilkProd > 0$	$+ 0.0409 \times An\_DigNDF$

(Equation 20-310)

GasE loss was reduced by 5 percent if monensin is included in the diet:

$$An\_GasEOut \atop Mcal/d =$$

Criteria	Value
No monensin	$An\_GasEOut$
Dietary monensin	$An\_GasEOut \times 0.95$

(Equation 20-311)

$An\_GasEOut$ was converted to units of g/d and L/d assuming 55.6 MJ/kg, 4.184 MJ/Mcal, and 1,497 L/kg:

$$\frac{CH4out\_g}{g/d} = \frac{An\_GasEOut}{55.5/4.184} \times 1,000$$

(Equation 20-312)

$$\frac{CH4out\_L}{L/d} = \frac{CH4out\_g}{1,000} \times 1,497$$

(Equation 20-313)

$$\frac{CH4g\_Milk}{g/kg} = \frac{CH4out\_g}{Mlk\_Prod}$$

(Equation 20-314)

$$\frac{CH4L\_Milk}{L/kg} = \frac{CH4out\_L}{Mlk\_Prod}$$

(Equation 20-315)

$$\frac{An\_ME}{Mcal/kg\ DM} = \frac{An\_MEIn}{An\_DMIn}$$
(Equation 20-316)

$$\frac{AnME\_DE}{Mcal/Mcal} = \frac{An\_MEIn}{An\_DEIn}$$
(Equation 20-317)

The above equations apply for weaned animals of all ages. Alternative energy supply equations are used for milk-fed calves.

Energy, Protein, and Amino Acid Balance

Total protein exported from the body and body growth (*An_NPxprt*, g/d) is calculated by summation:

$$\frac{An\_NPxprt\_g}{g/d} = Milk\_NP\_g + BodyNPgain\_g \\ + Scrf\_NP\_g + Fe\_NPend\_g \\ + Ur\_NPend\_g$$
(Equation 20-318)

Total NP use for production as

$$\frac{An\_NPprod\_g}{g/d} = An\_NPmilk\_g + Gest\_NPgain\_g$$
(Equation 20-319)

$$\frac{AnNPxprt\_g}{g/d} = An\_NPxprt\_g \times 0.16$$
(Equation 20-320)

Total AA use as

$$\frac{An\_AAuse_{(i)}\_g}{g/d} = Gest\_AA_{(i)}\_g + Mlk\_AA_{(i)}\_g \\ + Bod\_AAgain_{(i)}\_g \\ + Scrf\_AA_{(i)}\_g + Fe\_AAend_{(i)}\_g \\ + Ur\_AAend_{(i)}\_g$$
(Equation 20-321)

And total MP use as

$$\frac{An\_MPuse\_g}{g/d} = Gest\_MPuse\_g + Milk\_MPuse\_g \\ + Body\_MPuse\_g \\ + Scrf\_MPuse\_g + Fe\_MPenduse\_g \\ + Ur\_MPenduse\_g$$
(Equation 20-322)

$$\frac{An\_Nuse\_g}{g/d} = An\_NPuse\_g \times 0.16$$
(Equation 20-323)

NE and ME available for production (Mcal/d) are calculated as

$$\frac{An\_NEprod_{Avail}}{Mcal/d} = An\_NEIn - An\_NEmUse$$
(Equation 20-324)

$$\frac{An\_MEprod_{Avail}}{Mcal/d} = An\_MEIn - An\_MEmUse$$
(Equation 20-325)

Energy balance (Mcal/d) is calculated as the supply minus all use:

$$\frac{An\_MEbal}{Mcal/d} = An\_MEIn - An\_MEmUse - Body\_MEuse \\ - Gest\_MEuse - Milk\_MEuse$$
(Equation 20-326)

$$\frac{An\_NEbal}{Mcal/d} = An\_NEIn - An\_NEmUse - An\_NEgain \\ - Gest\_NEuse - Milk\_NEuse$$
(Equation 20-327)

$$\frac{An\_NPbal\_g}{g/d} = An\_MPIn\_g - \frac{Gest\_NP\_g}{Ky_{MP\_NP}} \\ - \frac{Mlk\_NP\_g}{Kx_{MP\_NP}} \\ - \frac{Body\_NPgain\_g}{Kg_{MP\_NP}} - \frac{Scrf\_NP\_g}{Kx_{MP\_NP}} \\ - \frac{Fe\_NPend\_g}{Kx_{MP\_NP}} - Ur\_NPend\_g$$
(Equation 20-328)

$$\frac{An\_AAbal_{(i)}\_g}{g/d} = Abs\_AA_{(i)}\_g - Gest\_AA_{(i)}\_g \\ - Mlk\_AA_{(i)}\_g \\ - Body\_AAgain_{(i)}\_g - Scrf\_AA_{(i)}\_g \\ - Fe\_AAend_{(i)}\_g - Ur\_AAend_{(i)}\_g$$
(Equation 20-329)

Allowable Production Estimates

Energy and protein available for production can be partitioned to gain, milk, or gestation, although the latter would seem to be largely prescribed within normal ranges and thus was ignored for these scenarios. Based on conservation of mass principles, one can rearrange Equations 20-327 and 20-328 to isolate each productive function and estimate how much production is allowed by the energy and protein supplies given constant rates for the other functions and target efficiencies for MP to NP conversion. Obviously, nutrient availability is not the sole determinant of production, and thus these predictions should be considered guides at best. Response surface-based predictions such as for *Mlk_NP* and *Mlk_Fat* are better reflections

of expected changes in production and should be used as an expectation guide rather than these historical simplifications.

Based on mass balance, ME available for gain ($An\_MEavail_{gain}$, Mcal/d) is

$$\frac{An\_MEavail_{Gain}}{Mcal/d} = An\_MEIn - An\_MEmUse \\ - Gest\_MEuse - Milk\_MEuse$$
(Equation 20-330)

Energy allowable rates of body gain (Body_$Gain_{ME\_Allow}$, kg BW/d) with *Frm* and *Rsrv* gain proportioned to body gain according to *Frm* and *Rsrv* gain input proportion are calculated as

$$\frac{Body\_Gain_{NE\_Allow}}{kg/d} = \frac{An\_MEavail_{Gain}}{Kg_{ME\_RE} \times NEgain\_BWgain}$$
(Equation 20-331)

Managing body condition of lactating animals is important to avoid negative impacts on productivity and animal health, and thus it is useful to know how long it would take on a given diet to achieve a 1-unit change in BCS assuming all of the extra energy was channeled into body reserves ($An\_BCS\_1\Delta\_d$). This is estimated in days, assuming that a 1-unit change is equivalent to 9.8 percent of *An_BW*:

$$\frac{BodyFat\_BCS}{kg/BCS\ Unit} = An\_BW \times 0.098$$
(Equation 20-332)

$$\frac{An\_BCS\_1\Delta\_d}{d} = \frac{BodyFat\_BCS}{Body\_Gain_{ME\_Allow}}$$
(Equation 20-333)

The rate of MP allowable body gain (*Body_Gain$_{MP\_Allow}$*, kg BW/d) can be estimated from the MP supply (g/d) assuming the composition of the EAA in the MP is appropriately matched to animal needs and that the conversion of MP to NP in support of milk (if the animal is lactating) is at the target efficiency.

$$\frac{An\_MPavail_{Gain}}{kg/d} = An\_MPIn - Gest\_MPuse \\ - Milk\_MPuse_{Trg} - Scrf\_MPuse \\ - Fe\_NPenduse - Ur\_NPenduse$$
(Equation 20-334)

$$\frac{Body\_Gain_{MP\_Allow}}{kg/d} = \frac{An\_MPavail_{Gain}}{Kg_{MP\_NP} \times GainNP\_Gain}$$
(Equation 20-335)

Using the same approach, energy (*Milk$_{NE\_Allow}$*, kg/d) and NP allowable milk (*Milk$_{MP\_Allow}$*, kg/d) can be predicted subject to the same assumptions:

$$\frac{An\_MEavail_{Milk}}{Mcal/d} = An\_MEIn - An\_MEmUse \\ - Body\_MEuse - Gest\_MEuse$$
(Equation 20-336)

$$\frac{An\_MPavail_{Milk}\_g}{kg/d} = An\_MPIn - Gest\_MPuse \\ - Body\_MPuse - Scrf\_MPuse \\ - Fe\_MPenduse - Ur\_MPenduse$$
(Equation 20-337)

$$\frac{Milk_{ME\_Allow}}{kg/d} = \frac{An\_MEavail_{Milk}}{Kl_{ME\_NE} \times MilkNE\_Milk}$$
(Equation 20-338)

$$\frac{Milk_{MP\_Allow}}{kg/d} = \frac{An\_MPavail_{Milk}}{Kl_{MP\_NP,Trg} \times Trg\_MilkTPp/100}$$
(Equation 20-339)

The predicted milk protein content of milk could be used instead of the user-entered target value in Equation 20-339.

Manure

$$\frac{Man\_Out}{kg\ Wet/d} = -28.3 + 3.6 \times An\_DMIn + 12.4 \times Dt\_K$$
(Equation 20-340)

$$\frac{Man\_VolSold}{kg/d} = Fe\_OM - Dt\_LgIn + \frac{Ur\_Nout\_g}{0.16 \times 1,000}$$
(Equation 20-341)

$$\frac{Man\_Nout\_g}{g/d} = Ur\_Nout\_g + Fe\_N\_g + Scrf\_N\_g$$
(Equation 20-342)

Manure output of the macro- and microminerals with predictions for absorption was calculated by difference from dietary intakes (*Dt_XxIn*) and retained in product (*An_Xx_prod*):

$$\frac{Man\_XxOut}{g/d} = Dt\_XxIn - An\_Xx\_prod$$
(Equation 20-343)

where *Xx* refers to each of the individual minerals. The total manure content of these macro- and microminerals was by summation:

$$\frac{Man\_MacroMinOut\_g}{g/d} = Man\_Ca\_out + Man\_P\_out \\ + Man\_Mg\_out \\ + Man\_K\_out + Man\_Na\_out \\ + Man\_Cl\_out$$
(Equation 20-344)

$$\frac{Man\_MicroMinOut\_mg}{mg/d} = Man\_CuOut + Man\_FeOut + Man\_MnOut + Man\_ZnOut$$
(Equation 20-345)

Water, Energy, Protein, and Amino Acid Efficiencies

Water use and fractional efficiencies were calculated as

$$\frac{Man\_WaOut}{kg/d} = Man\_Out - Fe\_OM - \frac{Ur\_Nout\_g}{0.45 \times 1,000} - \frac{Man\_MacroMinOut\_g}{1,000} - \frac{Man\_MicroMinOut\_mg}{1,000,000}$$
(Equation 20-346)

$$\frac{An\_WaOut_{Insens}}{kg/d} = An\_WaIn - Mlk\_Prod - Man\_WaOut$$
(Equation 20-347)

$$\frac{WaIn\_Milk}{kg/kg} = \frac{An\_WaIn}{Mlk\_Prod}$$
(Equation 20-348)

$$\frac{ManWa\_Milk}{kg/kg} = \frac{Man\_WaOut}{Mlk\_Prod}$$
(Equation 20-349)

The fractional efficiencies of energy transfers are calculated as

$$\frac{AnDE\_AnGE}{Mcal/Mcal} = \frac{An\_DEIn}{An\_GEIn}$$
(Equation 20-350)

$$\frac{AnNE\_AnDE}{Mcal/Mcal} = \frac{An\_NEIn}{An\_DEIn}$$
(Equation 20-351)

$$\frac{AnNE\_AnME}{Mcal/Mcal} = \frac{An\_NEIn}{An\_MEIn}$$
(Equation 20-352)

The fractional efficiencies of use of dietary and MP are calculated as

$$\frac{AnNP\_DtCP}{g/g} = \frac{An\_NPuse}{An\_CPIn}$$
(Equation 20-353)

$$\frac{AnNP\_AnMPa}{g/g} = \frac{An\_NPuse}{An\_MPIn}$$
(Equation 20-354)

$$\frac{AnNP\_AnMPt}{g/g} = \frac{\left(Milk\_NP\_g + Body\_NPgain\_g + Scrf\_NP\_g + Fe\_NPend\_g + \dfrac{Gest\_NP\_g}{Ky_{MP\_NP}} \right)}{An\_MPIn - Ur\_NPend}$$
(Equation 20-355)

$$\frac{AnNPexprt\_DtCP}{g/g} = \frac{An\_NPexprt}{An\_CPIn}$$
(Equation 20-356)

$$\frac{AnNPexprt\_AnMP}{g/g} = \frac{An\_NPexprt}{An\_MPIn}$$
(Equation 20-357)

$$\frac{MilkNP\_AnCP}{g/g} = \frac{Milk\_NP}{An\_CPIn}$$
(Equation 20-358)

$$\frac{MilkNP\_AnMP}{g/g} = \frac{Milk\_NP}{An\_MPIn}$$
(Equation 20-359)

$$\frac{BodyNPgain\_AnMP}{g/g} = \frac{Body\_NPgain}{An\_MPIn}$$
(Equation 20-360)

$$\frac{GestNP\_AnMP}{g/g} = \frac{Gest\_NP}{An\_MPIn}$$
(Equation 20-361)

Because the fractional conversion of MP to endogenous urinary NP is assumed to be 1, it is subtracted from *An_NP* and from *An_MPIn* in Equation 20-355 to reflect its direct conversion. The full use in support of gestation was also considered as the inefficiency of gestation NP deposition occurs primarily outside of the animal. One might argue the use of a similar adjustment for the remaining equations utilizing *An_MPIn*, but such adjustments were not undertaken, and consideration of those values should be adjusted accordingly. The value predicted by Equation 20-355 is used for comparison to the target efficiency in Chapter 6.

The fractional efficiencies of use of dietary and metabolizable AA are calculated as

$$\frac{AnAA_{(a)}\_DtAA_{(a)}}{g/g} = \frac{An\_AAuse_{(a)}}{Dt\_AAIn_{(a)}}$$
(Equation 20-362)

The efficiencies of individual absorbed EAA use for export protein plus retained in body protein are calculated for comparison to the target efficiencies listed in Chapter 6.

$$\frac{AnAAxprt_{(a)}\_AbsAAt_{(a)}}{g/g} = \frac{\begin{pmatrix} Mlk\_AA_{(a)}\_g \\ + Bod\_AAgain_{(a)}\_g \\ + Scrf\_AA_{(a)}\_g \\ + Fe\_AAend_{(a)}\_g \end{pmatrix}}{\begin{array}{c} Abs\_AA_{(a)} - Ur\_AAend_a \\ -(Gest\_AA(a)\_g/Ky_{MP\_NP}) \end{array}}$$
(Equation 20-363)

$$\frac{AnAAa_{(a)}\_AbsAA_{(a)}}{g/g} = \frac{An\_AAuse_{(a)}}{Abs\_AA_{(a)}}$$
(Equation 20-364)

$$\frac{MilkAA_{(a)}\_DtAA_{(a)}}{g/g} = \frac{Milk\_AA_{(a)}}{Dt\_AAIn_{(a)}}$$
(Equation 20-365)

$$\frac{MilkAA_{(a)}\_AbsAA_{(a)}}{g/g} = \frac{Milk\_AA_{(a)}}{Abs\_AA_{(a)}}$$
(Equation 20-366)

$$\frac{BodyAA_{(a)}\_AbsAA_{(a)}}{g/g} = \frac{Body\_AAgain_{(a)}}{Abs\_AA_{(a)}}$$
(Equation 20-367)

$$\frac{GestAA_{(a)}\_AbsAA_{(a)}}{g/g} = \frac{Gest\_AA_{(a)}}{Abs\_AA_{(a)}}$$
(Equation 20-368)

$$\frac{UrNout\_DigNIn}{g/g} = \frac{Ur\_Nout\_g}{An\_DigCPtIn \times 1,000/6.25}$$
(Equation 20-369)

As for MP the fractional efficiency of AA conversion to export and body gain protein was calculated (Equation 20-363) based on the assumption that AAs transferred to endogenous urinary AAs were at an efficiency of 1 and that total gestation use operates at a lower efficiency, and these efficiencies should be compared to the target efficiencies provided in Chapter 6.

A number of additional fractional efficiencies are also calculated for display in reports but not presented here.

Vitamin and Mineral Supply and Use

Absorption coefficients (ACs) have been assessed for the macrominerals and for some of the microminerals. For these, absorbed supplies (*Xx_absMinIn*) of minerals are defined for each ingredient (*Fd_absMinIn*) and summed to yield daily intakes (*An_absMinIn*), which are compared to requirements for absorbed minerals (*An_Min_req*). For the remainder of the microminerals and for the vitamins, requirements are defined in terms of dietary intakes (*Xx_VMIn*). In most cases, requirements are defined factorially for maintenance (*An_VM_m*), growth (*An_VM_gr*), lactation (*An_VM_l*), and gestation (*An_VM_y*) and summed to yield an overall requirement (*An_VM_req*), and the balance (*An_VM_bal*) of absorbed or dietary and required is calculated. The units for all driving variables and the variable definitions are provided in the prior sections. The vitamin and mineral calculations are grouped by class.

For those minerals based on absorbed supplies (calcium [Ca], phosphorus [P], magnesium [Mg], sodium [Na], potassium [K], chloride Cl⁻, cobalt [Co], copper [Cu], iron [Fe], manganese [Mn], and zinc [Zn]), the ACs (*Fd_acXx*, g/g) are specified by feed in the feed library. These are used below as specified except in the case of calves (*An_StatePhys*="Calf"), where requirements for some minerals are based on dietary concentrations. In those cases, absorbability was set to a value of 1 to allow use of a common framework across physiological states. This approach should not be construed to imply that absorbability of those minerals by calves is complete. For those based on absorbability, the feed-specific values were generally not used for unweaned calves (*An_StatePhys*="Calf" and *Dt_DMIn_ClfLiq*>0) in lieu of the following dietary mineral absorption coefficients: Ca=1.0 for liquid feeds and 0.60 for dry feeds, P=1.0 for liquid feeds and 0.75 for dry feeds, Mg=1.0 for liquid feeds and 0.26 for dry feeds, K=1.0, Na=1.0, Cl=1 for liquid feeds and 0.92 for dry feeds, Cu=0.10, Fe=0.10, Mn=0.005, and Zn=0.20.

Macrominerals

Calcium, g/d

Ca supply is predicted as

$$\frac{Fd\_absCaIn_f}{g/d} = Fd\_CaIn_f \times Fd\_acCa_f$$
(Equation 20-370)

$$\frac{Abs\_CaIn}{g/d} = \sum_{f=1}^{N_f} Fd\_absCaIn_f$$
(Equation 20-371)

Calcium required by calves consuming liquid feed is calculated as

$$\frac{An\_Ca\_Clf}{g/d} = \frac{\begin{pmatrix} 0.0127 \times An\_EBW \end{pmatrix} \\ + \begin{pmatrix} 14.4 \times An\_EBW^{-0.139} \times An\_EBWgain \end{pmatrix}}{0.73}$$
(Equation 20-372)

Ca requirements for weaned calves, heifers, and cows are calculated as

$$\frac{Fe\_Ca\_m}{g/d} = 0.9 \times An\_DMIn$$
(Equation 20-373)

$$An\_Ca\_g \atop g/d = (9.83 \times An\_BWmature^{0.22} \times An\_BW^{-0.22})$$
$$\times An\_BWgain$$

(Equation 20-374)

$$An\_Ca\_y \atop g/d =$$
$$\begin{pmatrix} 0.0245 \times e^{(0.05581-0.00007 \times An\_GestDay) \times An\_GestDay} - 0.0245 \\ \times e^{(0.05581-0.00007 \times (An\_GestDay-1)) \times (An\_GestDay-1)} \end{pmatrix}$$
$$\times \frac{An\_BW}{715}$$

(Equation 20-375)

The lactation requirement for absorbed Ca can be predicted from milk volume or from milk protein production.

$$An\_Ca\_l \atop g/d =$$

Criteria	Value
An_Milk Prod = 0	0
An_Milk Prod > 0	An_kCa_l × Trg_Milk Prod
or	
MilkNP_Milk > 0	(0.295 + 0.239 × MlkNP_Milk) × An_Milk Prod

(Equation 20-376)

where An_kCal_l (g Ca/L of milk) is defined by breed:

$$An\_kCa\_l \atop g/L =$$

Breed	Value
Jersey	1.17
Holstein	1.03
Other	1.17

(Equation 20-377)

$$An\_Ca\_req \atop g/d =$$

Criteria	Value
An_StatePhys = "Calf" and Dt_DMIn_ClfLiq > 0	An_Ca_Clf
else	Fe_Ca_m + An_Ca_g + An_Ca_y + An_Ca_l

(Equation 20-378)

$$An\_Ca\_bal \atop g/d = Abs\_CaIn - An\_Ca\_req$$

(Equation 20-379)

Ca captured in animal product is calculated as

$$An\_Ca\_prod \atop g/d = Abs\_Ca\_y + An\_Ca\_l + An\_Ca\_g$$

(Equation 20-380)

Phosphorus, g/d

$$Fd\_absPIn_f \atop g/d = Fd\_PIn_f \times Fd\_acPtot_f$$

(Equation 20-381)

$$Abs\_PIn \atop g/d = \sum_{f=1}^{N_f} Fd\_absPIn_f$$

(Equation 20-382)

$$An\_P\_Clf \atop g/d = \frac{0.0118 \times An\_EBW + (5.85 \times An\_EBW^{-0.027} \times An\_EBWgain)}{0.65}$$

(Equation 20-383)

$$Ur\_P\_m = 0.0006 \times An\_BW$$

(Equation 20-384)

$$Fe\_P\_m \atop g/d =$$

Parity	Equation
0	0.8 × An_DMIn
1+	1.0 × An_DMIn

(Equation 20-385)

$$An\_P\_m \atop g/d = Ur\_P\_m + Fe\_P\_m$$

(Equation 20-386)

$$An\_P\_g \atop g/d = \left(1.2 + \left(4.635 \times An\_BWmature^{0.22} \times An\_BW^{-0.22}\right)\right)$$
$$\times An\_BWgain$$

(Equation 20-387)

$$An\_P\_y \atop g/d =$$
$$\begin{pmatrix} 0.02743 \times e^{\left(\times (An\_GestDay) \times (An\_GestDay)\right)} - 0.02743 \\ \times e^{\left((0.05527 - 0.000075 \times (An\_GestDay - 1)) \times (An\_GestDay - 1)\right)} \end{pmatrix}$$
$$\times \frac{An\_BW}{715}$$

(Equation 20-388)

$$\frac{An\_P\_l}{g/d} = \left(0.48 + 0.13 \times MlkNP\_Milk \times 100\right) \\ \times An\_MilkProd \qquad \text{(Equation 20-389)}$$

or if milk protein is not known:

$$\frac{An\_P\_l}{g/d} = 0.09 \times An\_Milk\ Prod$$

(Equation 20-390)

$$\frac{An\_P\_req}{g/d} = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline An\_StatePhys \\ = \text{"Calf" and} & An\_P\_Clf \\ Dt\_DMIn\_ClfLiq > 0 \\ \hline & An\_P\_m \\ & + An\_P\_g \\ else & + An\_P\_y \\ & + An\_P\_l \end{array} \end{cases}$$

(Equation 20-391)

$$\frac{An\_P\_bal}{g/d} = Abs\_PIn - An\_P\_req$$

(Equation 20-392)

$$\frac{An\_P\_prod}{g/d} = An\_P\_y + An\_P\_g + An\_P\_l$$

(Equation 20-393)

$$\frac{Fe\_P\_g}{g/d} = Dt\_PIn - An\_P\_l - An\_P\_gr - Ur\_P\_m$$

(Equation 20-394)

Magnesium, g/d

As discussed in Chapter 7 (Minerals), absorption of magnesium (Mg) from inorganic mineral sources is affected by dietary K content. Thus, Mg provided by ingredients in the vitamin/mineral category was summed and an absorption coefficient for Mg was calculated based on Dt_K:

$$\frac{Dt\_acMg}{g/d} =$$

Criteria	Value
An_StatePhys = "Calf" and Dt_DMIn_ClfLiq > 0	1
An_StatePhys = "Calf" and Dt_DMIn_ClfLiq = 0	0.26

$$else \qquad \frac{\left(\begin{array}{c} 44.1 - 5.42 \\ \times \ln(Dt\_K \times 10) - 0.08 \\ \times \dfrac{Dt\_Mg_{Vitamin/Mineral}}{Dt\_Mg} \times 100 \end{array} \right)}{100}$$

(Equation 20-395)

and used to estimate absorbed Mg intake:

$$\frac{Abs\_MgIn}{g/d} = Dt\_acMg \times Dt\_MgIn$$

(Equation 20-396)

$$\frac{An\_Mg\_Clf}{g/d} = \frac{\begin{array}{c} 0.0035 \times An\_EBW \\ + \left(0.60 \times An\_EBW^{-0.036} \times An\_EBWgain\right) \end{array}}{0.30}$$

(Equation 20-397)

$$\frac{Ur\_Mg\_m}{g/d} = 0.0007 \times An\_BW$$

(Equation 20-398)

$$\frac{Fe\_Mg\_m}{g/d} = 0.3 \times An\_DMIn$$

(Equation 20-399)

$$\frac{An\_Mg\_m}{g/d} = Ur\_Mg\_m + Fe\_Mg\_m$$

(Equation 20-400)

$$\frac{An\_Mg\_g}{g/d} = 0.45 \times An\_BWgain$$

(Equation 20-401)

$$\frac{An\_Mg\_y}{g/d} = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ \hline An\_GestDay > 190 & 0.3 \times \dfrac{An\_BW}{715} \end{array} \end{cases}$$

(Equation 20-402)

$$\frac{An\_Mg\_l}{g/d} = 0.11 \times An\_MilkProd$$

(Equation 20-403)

$$An\_Mg\_req \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline An\_StatePhys \\ = \text{``Calf''} \ and & An\_Mg\_Clf \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline & An\_Mg\_m \\ & + An\_Mg\_g \\ else & + An\_Mg\_y \\ & + An\_Mg\_l \end{array} \end{cases}$$

(Equation 20-404)

$$\frac{An\_Mg\_bal}{g/d} = Abs\_MgIn - An\_Mg\_req$$

(Equation 20-405)

$$\frac{An\_Mg\_prod}{g/d} = An\_Mg\_g + An\_Mg\_y + An\_Mg\_l$$

(Equation 20-406)

Sodium, g/d

$$\frac{Fd\_absNaIn_f}{g/d} = Fd\_NaIn_f \times Fd\_acNa_f$$

(Equation 20-407)

$$\frac{Abs\_NaIn}{g/d} = \sum_{f=1}^{N_f} Fd\_absNaIn_f$$

(Equation 20-408)

$$\frac{An\_Na\_Clf}{g/d} = \frac{\begin{array}{c} 0.00637 \times An\_EBW \\ + \left(1.508 \times An\_EBW^{-0.045} \times An\_EBWgain\right) \end{array}}{0.24}$$

(Equation 20-409)

$$\frac{Fe\_Na\_m}{g/d} = 1.45 \times An\_DMIn$$

(Equation 20-410)

$$\frac{An\_Na\_g}{g/d} = 1.4 \times An\_BWgain$$

(Equation 20-411)

$$An\_Na\_y \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ \hline An\_GestDay > 190 & 1.4 \times \dfrac{An\_BW}{715} \end{array} \end{cases}$$

(Equation 20-412)

$$\frac{An\_Na\_l}{g/d} = 0.4 \times An\_MilkProd$$

(Equation 20-413)

$$An\_Na\_req \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline An\_StatePhys \\ = \text{``Calf''} \ and & An\_Na\_Clf \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline & Fe\_Na\_m \\ & + An\_Na\_g \\ else & + An\_Na\_y \\ & + An\_Na\_l \end{array} \end{cases}$$

(Equation 20-414)

$$\frac{An\_Na\_prod}{g/d} = An\_Na\_g - An\_Na\_y - An\_Na\_l$$

(Equation 20-415)

$$\frac{An\_Na\_bal}{g/d} = Abs\_NaIn - An\_Na\_req$$

(Equation 20-416)

Chloride, g/d

$$\frac{Fd\_absClIn_f}{g/d} = Fd\_ClIn_f \times Fd\_acCl_f$$

(Equation 20-417)

$$\frac{Abs\_ClIn}{g/d} = \sum_{f=1}^{N_f} Fd\_absClIn_f$$

(Equation 20-418)

$$\frac{An\_Cl\_Clf}{g/d} = 0.8 \times \frac{\begin{array}{c} 0.00637 \times An\_EBW \\ + \left(\begin{array}{c} 1.508 \times An\_EBW^{-0.045} \\ \times An\_EBWgain \end{array}\right) \end{array}}{0.24}$$

(Equation 20-419)

$$\frac{Fe\_Cl\_m}{g/d} = 1.11 \times An\_DMIn$$

(Equation 20-420)

$$\frac{An\_Cl\_gr}{g/d} = 1.0 \times An\_BWgain$$

(Equation 20-421)

$$An\_Cl\_y \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ \\ An\_GestDay > 190 & 1.0 \times \dfrac{An\_BW}{715} \end{array} \end{cases}$$

(Equation 20-422)

$$\frac{An\_Cl\_l}{g/d} = 1.0 \times An\_Milk\,Prod$$

(Equation 20-423)

$$An\_Cl\_req \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline \begin{array}{c} An\_StatePhys \\ = \text{``Calf''} \; and \\ Dt\_DMIn\_ClfLiq > 0 \end{array} & An\_Cl\_Clf \\ \\ else & \begin{array}{l} Fe\_Cl\_m \\ + An\_Cl\_g \\ + An\_Cl\_y \\ + An\_Cl\_l \end{array} \end{array} \end{cases}$$

(Equation 20-424)

$$\frac{An\_Cl\_prod}{g/d} = An\_Cl\_g - An\_Cl\_y - An\_Cl\_l$$

(Equation 20-425)

$$\frac{An\_Cl\_bal}{g/d} = Abs\_ClIn - An\_Cl\_req$$

(Equation 20-426)

Potassium, g/d

$$\frac{Fd\_absKIn_f}{g/d} = Fd\_KIn_f \times Fd\_acK_f$$

(Equation 20-427)

$$Abs\_KIn = \sum_{f=1}^{N_f} Fd\_absKIn_f$$

(Equation 20-428)

$$\frac{An\_K\_Clf}{g/d} = \frac{0.0203 \times An\_EBW + \left(1.14 \times An\_EBW^{-0.048} \times An\_EBWgain\right)}{0.13}$$

(Equation 20-429)

$$Ur\_K\_m \atop g/d = \begin{cases} \begin{array}{c|c} Trg\_MilkProd & Equation \\ \hline > 0 & 0.07 \times An\_BW \\ 0 & 0.2 \times An\_BW \end{array} \end{cases}$$

(Equation 20-430)

$$\frac{Fe\_K\_m}{g/d} = 2.5 \times An\_DMIn$$

(Equation 20-431)

$$\frac{An\_K\_m}{g/d} = Ur\_K\_m + Fe\_K\_m$$

(Equation 20-432)

$$\frac{An\_K\_g}{g/d} = 2.5 \times An\_BWgain$$

(Equation 20-433)

$$An\_K\_y \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ \\ An\_GestDay > 190 & 1.03 \times \dfrac{An\_BW}{715} \end{array} \end{cases}$$

(Equation 20-434)

$$\frac{An\_K\_l}{g/d} = 1.5 \times An\_MilkProd$$

(Equation 20-435)

$$An\_K\_req \atop g/d = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline \begin{array}{c} An\_StatePhys = \text{``Calf''} \\ and \; Dt\_DMIn\_ClfLiq > 0 \end{array} & An\_K\_Clf \\ \\ else & \begin{array}{l} An\_K\_m \\ + An\_K\_g \\ + An\_K\_y \\ + An\_K\_l \end{array} \end{array} \end{cases}$$

(Equation 20-436)

$$\frac{An\_K\_prod}{g/d} = An\_K\_g - An\_K\_y - An\_K\_l$$

(Equation 20-437)

$$\frac{An\_K\_bal}{g/d} = Abs\_KIn - An\_K\_req$$

(Equation 20-438)

Sulfur, g/d

$$\frac{An\_S\_req}{g/d} = 2.0 \times An\_DMIn$$

(Equation 20-439)

$$\frac{An\_S\_bal}{g/d} = Dt\_SIn - An\_S\_req$$

(Equation 20-440)

Microminerals

Cobalt, mg/d

$$Abs\_CoIn \atop mg/d = \begin{cases} \begin{array}{c|c} \text{Criteria} & \text{Value} \\ \hline An\_StatePhys = \text{``Calf'' and} & \\ Dt\_DMIn\_ClfLiq > 0 & 0 \\ \hline else & Dt\_CoIn \end{array} \end{cases}$$

(Equation 20-441)

$$\frac{An\_Co\_req}{mg/d} = 0.2 \times An\_DMIn$$

(Equation 20-442)

$$\frac{An\_Co\_bal}{mg/d} = Abs\_CoIn - An\_Co\_req$$

(Equation 20-443)

Copper, mg/d

$$Fd\_acCu_f = \begin{cases} \begin{array}{c|c} An\_StatePhys & \text{Value} \\ \hline An\_StatePhys = \text{``Calf'' and} & 1 \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline An\_StatePhys = \text{``Calf'' and} & 0.1 \\ Dt\_DMIn\_ClfLiq = 0 & \\ \hline else & Fd\_acCu_f \end{array} \end{cases}$$

(Equation 20-444)

$$\frac{Fd\_absCuIn_f}{mg/d} = Fd\_CuIn_f \times Fd\_acCu_f$$

(Equation 20-445)

$$\frac{Abs\_CuIn}{mg/d} = \sum_{f=1}^{N_f} Fd\_absCuIn_f$$

(Equation 20-446)

$$\frac{An\_Cu\_Clf}{mg/d} = \frac{0.0145 \times An\_BW + (2.5 \times An\_BWgain)}{0.5}$$

(Equation 20-447)

$$\frac{An\_Cu\_m}{mg/d} = 0.0145 \times An\_BW$$

(Equation 20-448)

$$\frac{An\_Cu\_g}{mg/d} = 2.0 \times An\_BWgain$$

(Equation 20-449)

$$An\_Cu\_y \atop mg/d =$$

$$\begin{cases} \begin{array}{c|c} \text{Criteria} & \text{Equation} \\ \hline An\_GestDay < 90 & 0 \\ 90 <= An\_GestDay <= 190 & 0.0003 \times An\_BW \\ An\_GestDay > 190 & 0.0023 \times An\_BW \end{array} \end{cases}$$

(Equation 20-450)

$$\frac{An\_Cu\_l}{mg/d} = 0.04 \times An\_MilkProd$$

(Equation 20-451)

$$An\_Cu\_req \atop mg/d = \begin{cases} \begin{array}{c|c} \text{Criteria} & \text{Equation} \\ \hline \begin{array}{c} An\_StatePhys \\ = \text{``Calf'' and} \\ Dt\_DMIn\_ClfLiq > 0 \end{array} & An\_Cu\_Clf \\ \hline else & \begin{array}{c} An\_Cu\_m \\ + An\_Cu\_g \\ + An\_Cu\_y \\ + An\_Cu\_l \end{array} \end{array} \end{cases}$$

(Equation 20-452)

$$\frac{An\_Cu\_prod}{g/d} = An\_Cu\_g + An\_Cu\_y + An\_Cu\_l$$

(Equation 20-453)

$$\frac{An\_Cu\_bal}{g/d} = Abs\_CuIn - An\_Cu\_req$$

(Equation 20-454)

Iodine, mg/d

$$An\_I\_req \atop mg/d =$$

$$\begin{cases} \begin{array}{c|c} \text{Criteria} & \text{Equation} \\ \hline \begin{array}{c} An\_StatePhys = \text{``Calf''} \\ \text{and } Dt\_DMIn\_ClfLiq > 0 \end{array} & 0.8 \times An\_DMIn \\ \hline else & \begin{array}{c} 0.216 \times An\_BW^{0.528} \\ + 0.1 \times Trg\_MilkProd \end{array} \end{array} \end{cases}$$

(Equation 20-455)

$$\frac{An\_I\_bal}{mg/d} = Dt\_IIn - An\_I\_req$$

(Equation 20-456)

Iron, mg/d

$$Fd\_acFe_f = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline An\_StatePhys = \text{"Calf" and} & 1 \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline else & Fd\_acFe_f \end{array} \end{cases}$$

(Equation 20-457)

$$\frac{Fd\_absFeIn_f}{mg/d} = Fd\_FeIn_f \times Fd\_acFe_f$$

(Equation 20-458)

$$\frac{Abs\_FeIn}{mg/d} = \sum_{f=1}^{N_f} Fd\_absFeIn_f$$

(Equation 20-459)

$$\frac{An\_Fe\_Clf}{mg/d} = \frac{34 \times An\_BWgain}{0.25}$$

(Equation 20-460)

$$An\_Fe\_g = 34 \times An\_BWgain$$

(Equation 20-461)

$$\frac{An\_Fe\_y}{mg/d} = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ An\_GestDay > 190 & 0.025 \times An\_BW \end{array} \end{cases}$$

(Equation 20-462)

$$\frac{An\_Fe\_l}{mg/d} = 1.0 \times An\_MilkProd$$

(Equation 20-463)

$$\frac{An\_Fe\_req}{mg/d} = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_StatePhys & \\ = \text{"Calf" and} & An\_Fe\_Clf \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline else & An\_Fe\_g \\ & + An\_Fe\_y \\ & + An\_Fe\_l \end{array} \end{cases}$$

(Equation 20-464)

$$\frac{An\_Fe\_prod}{g/d} = An\_Fe\_g + An\_Fe\_y + An\_Fe\_l$$

(Equation 20-465)

$$\frac{An\_Fe\_bal}{mg/d} = Abs\_FeIn - An\_Fe\_req$$

(Equation 20-466)

Manganese, mg/d

$$Fd\_acMn_f = \begin{cases} \begin{array}{c|c} An\_StatePhys & Value \\ \hline An\_StatePhys & \\ = \text{"Calf" and} & 1 \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline An\_StatePhys & \\ = \text{"Calf" and} & 0.05 \\ Dt\_DMIn\_ClfLiq = 0 & \\ \hline else & Fd\_acMn_f \end{array} \end{cases}$$

(Equation 20-467)

$$\frac{Fd\_absMnIn_f}{mg/d} = Fd\_MnIn_f \times Fd\_acMn_f$$

(Equation 20-468)

$$\frac{Abs\_MnIn}{mg/d} = \sum_{f=1}^{N_f} Fd\_absMnIn_f$$

(Equation 20-469)

$$\frac{An\_Mn\_m}{mg/d} = 0.0026 \times An\_BW$$

(Equation 20-470)

$$\frac{An\_Mn\_Clf}{mg/d} = \frac{0.0026 \times An\_BW + \left(0.7 \times An\_BWgain\right)}{0.01}$$

(Equation 20-471)

$$\frac{An\_Mn\_g}{mg/d} = 0.7 \times An\_BWgain$$

(Equation 20-472)

$$\frac{An\_Mn\_y}{mg/d} =$$
$$\begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ An\_GestDay > 190 & 0.00042 \times An\_BW \end{array} \end{cases}$$

(Equation 20-473)

$$\frac{An\_Mn\_l}{mg/d} = 0.03 \times An\_MilkProd$$

(Equation 20-474)

$$An\_Mn\_req \atop mg/d = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_StatePhys \\ = \text{``Calf''} \text{ and} & An\_Mn\_Clf \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline & An\_Mn\_m \\ & + An\_Mn\_g \\ else & + An\_Mn\_y \\ & + An\_Mn\_l \end{array} \end{cases}$$

(Equation 20-475)

$$An\_Mn\_prod \atop g/d = An\_Mn\_g + An\_Mn\_y + An\_Mn\_l$$

(Equation 20-476)

$$An\_Mn\_bal \atop mg/d = Abs\_MnIn - An\_Mn\_req$$

(Equation 20-477)

Selenium, mg/d

$$An\_Se\_req \atop mg/d = 0.3 \times An\_DMIn$$

(Equation 20-478)

$$An\_Se\_bal \atop mg/d = Dt\_SeIn - An\_Se\_req$$

(Equation 20-479)

Zinc, mg/d

$$Fd\_acZn_f = \begin{cases} \begin{array}{c|c} Criteria & Value \\ \hline An\_StatePhys = \text{``Calf''} \text{ and} & 1 \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline An\_StatePhys = \text{``Calf''} \text{ and} & 0.20 \\ Dt\_DMIn\_ClfLiq = 0 & \\ \hline else & Fd\_acZn_f \end{array} \end{cases}$$

(Equation 20-480)

$$Fd\_absZnIn_f \atop mg/d = Fd\_ZnIn_f \times Fd\_acZn_f$$

(Equation 20-481)

$$Abs\_ZnIn \atop mg/d = \sum_{f=1}^{N_f} Fd\_absZnIn_f$$

(Equation 20-482)

$$An\_Zn\_Clf \atop mg/d = \frac{2.0 \times An\_DMIn + (24 \times An\_BWgain)}{0.25}$$

(Equation 20-483)

$$An\_Zn\_m \atop mg/d = 5.0 \times An\_DMIn$$

(Equation 20-484)

$$An\_Zn\_g \atop mg/d = 24 \times An\_BWgain$$

(Equation 20-485)

$$An\_Zn\_y \atop mg/d = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_GestDay <= 190 & 0 \\ An\_GestDay > 190 & 0.017 \times An\_BW \end{array} \end{cases}$$

(Equation 20-486)

$$An\_Zn\_l \atop mg/d = 4.0 \times An\_MilkProd$$

(Equation 20-487)

$$An\_Zn\_req \atop mg/d = \begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_StatePhys \\ = \text{``Calf''} \text{ and} & An\_Zn\_Clf \\ Dt\_DMIn\_ClfLiq > 0 & \\ \hline & An\_Zn\_m \\ & + An\_Zn\_g \\ else & + An\_Zn\_y \\ & + An\_Zn\_l \end{array} \end{cases}$$

(Equation 20-488)

$$An\_Zn\_prod \atop g/d = An\_Zn\_g + An\_Zn\_y + An\_Zn\_l$$

(Equation 20-489)

$$An\_Zn\_bal \atop mg/d = Abs\_ZnIn - An\_Zn\_req$$

(Equation 20-490)

Vitamins

Vitamin A, IU/d

$$An\_VitA\_req \atop IU/d =$$

$$\begin{cases} \begin{array}{c|c} Criteria & Equation \\ \hline An\_MilkProd <= 35\ kg/d & 110 \times An\_BW \\ \hline An\_MilkProd > 35\ kg/d & \begin{array}{l} 110 \times An\_BW + 1,000 \\ \times (An\_MilkProd - 35) \end{array} \end{array} \end{cases}$$

(Equation 20-491)

$$\frac{An\_VitA\_bal}{IU/d} = Dt\_VitAIn - An\_VitA\_req$$

(Equation 20-492)

Vitamin D, IU/d

$$\frac{An\_VitD\_req}{IU/d} =$$

Criteria	Equation
$An\_MilkProd = 0 \; kg/d$	$32 \times An\_BW$
$An\_MilkProd > 0 \; kg/d$	$40 \times An\_BW$

(Equation 20-493)

$$\frac{An\_VitD\_bal}{IU/d} = Dt\_VitDIn - An\_VitD\_req$$

(Equation 20-494)

Vitamin E, IU/d

$$\frac{An\_VitE\_req}{IU/d} =$$

Criteria	Equation
$An\_MilkProd = 0 \; \& \; An\_Parity >= 1$	$2.0 \times An\_BW$
$An\_GestDay \geq 259 \; \& \; An\_Preg = 1$	$3.0 \times An\_BW$
Otherwise	$0.8 \times An\_BW$

(Equation 20-495)

$$\frac{An\_VitE\_bal}{IU/d} = Dt\_VitEIn - An\_VitE\_req$$

(Equation 20-496)

If animals are grazing, the vitamin E requirement is reduced by 50 IU/kg of pasture DM intake. The contribution is capped at the total vitamin E requirement.

MODEL EVALUATIONS

A large data set was assembled from the literature for use in model fitting primarily for protein and fat digestion and for derivation of milk protein, milk fat, and milk production predictions. Those data were also used for model testing. Thus, evaluations of protein and fat digestion and milk production presented in this chapter are not independent of the derivation data. However, extensive evaluations of those predictions using cross-evaluation techniques were conducted during derivation, which helps ensure the equations will perform equally well with independent data. Predictions of DMIn and carbohydrate digestion were developed from independent data, and thus the evaluations of those predictions presented below are independent of the derivation data.

The evaluations are summarized and presented as root RMSE and its partition into mean and slope bias as described by Bibby and Toutenberg (1977) and the CCC as described by Lin (1989). Unless specified otherwise, evaluations were not adjusted for random study effects.

Fitting and Evaluation Data

The protein and fat digestion data encompassed 1,149 treatment means from 275 publications. The primary selection criteria for data gathering were (1) ruminal outflow, (2) total tract FA digestibility, (3) infusions of AAs or proteins, (4) rumen-protected AA feeding trials, and (5) mammary arteriovenous difference studies. The data are primarily from mature, lactating animals (N = 1,023), but some observations were collected from dry cows and young growing animals, although the minimum animal size was 319 kg of BW. A summary of the animal characteristics for the data is provided in Table 20-11, and a summary of the diets is provided in Table 20-12. The resulting predictions of digestibility and absorption are provided in Table 20-12.

Dry Matter Intake and Ruminal Outflow

Residual analyses for predictions of lactating cow DMIn and ruminal outflow of N, starch, and NDF are presented in Table 20-13. DMI of lactating cows based on animal factors only was predicted with a small mean bias and moderate slope bias, whereas the prediction based on animal and feed factors had a larger mean bias but no slope bias. However, the

TABLE 20-11 A Summary of the Animal Characteristics for the Evaluation Data Used

	BW, kg	DMIn, kg/d	Milk, kg/d	Lactose, %	Protein, %	Fat, %	Days in Milk	Parity
N	932	1,149	1,023	421	934	926	1,023	1,149
Mean	602	20.0	29.9	4.65	3.05	3.52	134	0.95
Median	600	20.2	30.2	4.78	3.1	3.57	138	1
SD	58.2	4.18	8.57	0.78	0.46	0.68	51.8	0.22
Minimum	319	5.81	0	0	0	0	0	0
Maximum	788	31.8	53.8	5.34	3.9	5.62	344	1
1. Quartile	571	17.5	24.6	4.66	2.94	3.24	106	1
3. Quartile	636	22.9	36.3	4.86	3.23	3.8675	153	1
Skewness	−0.30	−0.33	−0.57	−5.54	−4.92	−2.22	0.39	−4.14
Kurtosis	2.05	0.41	1.14	30.2	30.6	10.5	1.24	15.2

TABLE 20-12   A Summary of the Dietary Characteristics for the Evaluation Data[a]

Variable	Mean	Median	SD	Min	Max	1. Quart	3. Quart	Skewness	Kurtosis
Dt_DMIn, kg/d	20.0	20.2	4.2	5.8	31.8	17.5	22.9	−0.33	0.41
Dt_ForWet	40.3	44.8	21.4	0.0	100.0	30.0	55.0	−0.51	−0.14
Dt_ForDry	11.3	0.0	16.8	0.0	99.7	0.0	17.5	1.77	3.02
Dt_Conc	48.4	50.0	12.5	0.0	100.0	40.0	55.4	−0.41	1.93
Dt_Ash	7.55	7.40	1.97	4.00	22.47	6.31	8.40	2.93	17.73
Dt_NDF	32.7	31.9	6.1	14.6	54.3	28.4	35.9	0.65	0.62
Dt_ForNDF_NDF, % of NDF	74.0	76.6	12.9	0.0	100.0	66.2	83.0	−0.83	1.10
Dt_ADF	20.0	19.3	4.2	7.2	39.0	17.2	22.0	0.91	1.64
Dt_Lg	3.65	3.45	0.96	1.57	7.65	2.94	4.22	0.84	0.59
Dt_St	26.7	27.3	8.5	1.1	53.0	21.4	32.6	−0.41	0.40
Dt_rOM	13.6	13.5	5.2	−5.8	42.4	10.7	16.5	0.28	2.41
Dt_CP	16.5	16.6	2.5	7.7	26.2	15.1	18.0	−0.08	1.27
Dt_TP	16.1	16.3	2.7	7.7	26.2	14.6	17.8	−0.14	0.64
Dt_NPNCP	0.37	0.00	0.91	0.00	7.10	0.00	0.00	3.78	17.81
Dt_NPN	0.06	0.00	0.15	0.00	1.14	0.00	0.00	3.78	17.81
Dt_CPA_CP, % of CP	37.0	35.5	8.3	18.2	81.8	31.2	41.4	1.12	2.34
Dt_CPB_CP, % of CP	53.9	54.5	9.0	13.0	74.0	49.2	59.8	−0.68	0.89
Dt_CPC_CP, % of CP	8.88	8.49	2.77	3.63	29.85	7.00	10.50	1.34	4.96
Dt_FA	3.39	2.85	1.53	0.77	15.22	2.30	4.36	1.64	5.80
Dt_C12	0.052	0.038	0.051	0.003	1.004	0.015	0.075	6.45	105.43
Dt_C14	0.041	0.034	0.035	0.005	0.413	0.015	0.052	2.71	14.52
Dt_C16	0.666	0.456	0.450	0.118	3.574	0.385	0.815	2.24	6.48
Dt_C161	0.034	0.018	0.041	0.005	0.527	0.013	0.033	3.77	24.37
Dt_C180	0.157	0.074	0.208	0.010	2.420	0.057	0.156	4.11	27.00
Dt_C181t	0.023	0.006	0.044	0.000	0.540	0.003	0.019	4.29	29.34
Dt_C181c	0.758	0.547	0.593	0.020	7.546	0.402	1.005	3.04	20.89
Dt_C182	1.235	1.165	0.534	0.129	4.573	0.884	1.449	1.21	2.56
Dt_C183	0.333	0.300	0.185	0.056	2.273	0.211	0.415	3.65	28.20
Dt_OtherFA	0.113	0.077	0.110	0.027	1.333	0.063	0.127	6.04	55.25
Dt_ArgIn, g/d	164	159	59	30	338	124	202	0.33	−0.20
Dt_HisIn, g/d	80	80	26	21	183	61	98	0.19	−0.07
Dt_IleIn, g/d	139	139	43	34	269	107	168	0.17	−0.24
Dt_LeuIn, g/d	274	277	89	73	621	213	331	0.35	0.51
Dt_LysIn, g/d	153	152	54	29	298	113	193	0.12	−0.56
Dt_MetIn, g/d	54	54	17	14	114	41	64	0.35	0.23
Dt_PheIn, g/d	160	161	49	38	311	121	193	0.18	−0.01
Dt_ThrIn, g/d	129	129	39	33	251	99	155	0.10	−0.23
Dt_TrpIn, g/d	40	40	13	9	87	30	48	0.31	0.38
Dt_ValIn, g/d	173	176	51	43	344	133	208	0.16	0.00

[a] Values are expressed as a percentage of DM unless specified otherwise. The variables are those specified for the model. N = 1,149.

TABLE 20-13   Residual Analyses for Predictions of DMI of Lactating Animals and Ruminal Outflow of Nitrogen (N), Starch, and NDF[a]

Variable Equation	DMIn Equation 20-21	DMIn Equation 20-22	Du_MicN Equation 20-74	Du_NANMN Equation 20-83	Du_NAN Equation 20-82	Du_St Equation 20-57	Du_NDF Equation 20-56
Units	kg/d	kg/d	g/d	g/d	g/d	kg/d	kg/d
N	951	951	596	587	585	178	319
Observed mean	20.6	20.6	278	205	487	2.66	3.76
Predicted mean	20.8	22.3	269	203	474	2.31	3.98
CCC	0.71	0.54	0.51	0.56	0.74	0.54	0.43
RMSE	2.50	3.29	81	68	88	1.18	1.03
RMSE, % mean	12.1	15.9	29.2	33.1	18.0	44.3	27.3
Mean bias, % MSE	0.4	26.8	1.2	0.1	2.0	8.8	4.7
Slope bias, % MSE	9.0	3.2	2.1	2.1	1.6	0.1	0.0
Mean bias	−0.2	−1.7	8.9	2.2	12.3	0.3	−0.2
Slope bias	0.31	0.26	0.22	−0.17	0.11	0.05	−0.01
P, mean bias	0.06	0.00	0.01	0.44	0.00	0.00	0.00
P, slope bias	0.0001	0.0001	0.0003	0.0004	0.002	0.61	0.88

[a] Errors of prediction for ruminal outflow of AA are presented in Table 20-14. The CCC for these ranged from 0.43 to 0.59 with very minimal or no slope bias and small proportions of mean bias.

TABLE 20-14   Residual Analyses for Predictions of Ruminal Outflow of AAs (g/d) by Equation 20-87

Variable	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
N	216	221	221	221	223	220	221	221	221
Observed mean	119	57.4	122	225	158	48.6	130	124	138
Predicted mean	130	60.7	135	229	184	54.5	141	132	150
CCC	0.54	0.51	0.49	0.60	0.45	0.43	0.57	0.58	0.52
RMSE	32.8	18.1	36.2	62.5	56.6	17.4	34.8	31.1	40.3
RMSE, % mean	27.7	31.5	29.8	27.7	35.9	35.7	26.7	25.1	29.1
Mean bias, % MSE	11.9	3.3	13.9	0.4	21.8	11.5	10.3	7.1	8.6
Slope bias, % MSE	0.2	2.2	0.1	0.1	0.0	0.0	0.0	0.8	0.0
Mean bias	−11.3	−3.3	−13.5	−4.0	−26.5	−5.9	−11.2	−8.3	−11.8
Slope bias	−0.06	−0.18	−0.05	0.04	−0.02	−0.01	0.01	0.11	0.02
P, mean bias	0.00	0.01	0.00	0.34	0.00	0.00	0.00	0.00	0.00
P, slope bias	0.45	0.03	0.60	0.60	0.83	0.95	0.92	0.16	0.81

TABLE 20-15   Residual Analyses for Predictions of Fecal Nutrient Output (kg/d)

Variable	Fe_CP	Fe_NDF	Fe_St	Fe_FA	Fe_OM
Equation	Equation 20-138	Equation 20-120	Equation 20-98	Equation 20-162	Equation 20-166
N	458	412	203	121	448
Observed mean	1.08	3.24	0.451	0.340	5.58
Predicted mean	0.89	3.20	0.666	0.312	5.00
CCC	0.53	0.77	0.27	0.80	0.71
RMSE	0.29	0.56	0.45	0.093	1.04
RMSE, % mean	26.8	17.4	99.8	27.4	18.7
Mean bias, % MSE	40.9	0.3	22.8	9.0	30.9
Slope bias, % MSE	9.8	0.2	22.2	4.4	0.1
Mean bias	0.2	0.0	−0.2	0.0	0.6
Slope bias	0.51	0.03	−0.65	0.16	0.02
P, mean bias	0.00	0.26	0.00	0.00	0.00
P, slope bias	0.0001	0.41	0.0001	0.02	0.49

former had greater precision with an RMSE of 12.4 percent and a CCC of 0.70 versus 17.3 and 0.56, respectively, for the latter equation, indicating that the feed factors are introducing more variation than explaining.

Predictions of ruminal outflow of N had CCC from 0.5 to 0.75 with minimal mean bias but significant slope bias as may be expected given that they were fitted to the same data used to derive them. Predictions of ruminal outflow of *St* and NDF had a CCC of 0.54 and 0.41, respectively, with *St* flow underpredicted (excessive ruminal degradation) and NDF flow overpredicted (inadequate ruminal degradation). These latter two are independent evaluations as the source equations were developed from other data.

Fecal Output

Errors of prediction for fecal nutrient excretion are summarized in Table 20-15. The CCC for predictions of fecal CP, NDF, and FA ranged from 0.49 to 0.81. There was positive mean and slope bias for fecal CP predictions with output underpredicted by 0.20 kg/d on average and the underprediction becoming greater (positive slope on the residuals) as predicted output increased. This is consistent with an over-

estimate of the digestibility of RUP or microbial CP, which results in a larger absolute error as flow through the system increases. Thus, it seems that one or both of the intestinal protein digestibility estimates are too great. However, predictions of fecal CP also require predictions of endogenous protein flows, including incorporation of blood urea into microbial protein in the large intestine. Endogenous flows may not be well captured by the predictions, and thus it is unclear if the problem is solely due to intestinal digestibility. Fecal NDF was predicted without mean or slope bias and a CCC of 0.77 and an RMSE of 17.7 percent.

Fecal starch predictions had a CCC of 0.19. The lower predictability for this variable at least in part reflects the high extent of digestion of the nutrient. The model is attempting to predict relatively small fractional outputs from large inputs, and the variance in estimates will be large relative to the output and thus low apparent predictability of the output itself. However, the negative mean bias and negative slope bias suggest the opposite of that observed for fecal CP (i.e., the intestinal digestibility is too low and should be increased). The needed increase should be even greater than indicated by these evaluations if one considers that starch outflow from the rumen was underpredicted in the model.

TABLE 20-16   Residual Analyses for Predictions of Milk and Milk Component Production

Variable Equation	Protein Equation 20-185	Fat Equation 20-215	Milk Equation 20-216	$Milk_{ME\_Allow}$ Equation 20-338	$Milk_{MP\_Allow}$ Equation 20-339
Units	g/d	g/d	kg/d	kg/d	kg/d
N	935	935	935	935	935
Observed mean	929	1,098	30.9	30.9	30.9
Predicted mean	930	1,114	31.0	34.7	32.6
CCC	0.75	0.62	0.75	0.67	0.64
RMSE	133	188	4.50	7.50	6.80
RMSE, % mean	14.4	17.1	14.6	24.4	22.1
Mean bias, % MSE	0.0	0.7	0.1	24.5	5.9
Slope bias, % MSE	3.1	0.0	3.3	37.9	28.6
Mean bias	−1.1	−16.0	−0.1	−3.7	−1.7
Slope bias	0.16	0.02	0.16	−0.45	−0.43
P, mean bias	0.54	0.01	0.38	0.00	0.00
P, slope bias	0.0001	0.51	0.0001	0.0001	0.0001

Because of lack of data when nutrients are summed to determine fecal rOM, only 14 observations were available in the literature, and no meaningful estimate of variance is possible. Fecal FAs were predicted with good accuracy and precision with a CCC of 0.81 and an RMSE of 26.6 percent. There was significant mean and slope bias, with the slope bias having a greater effect. The slope bias was positive, indicating that digestibility is slightly overpredicted.

The overall quality of the predictions is reflected in the CCC of 0.82 for OM output and the lack of slope bias for that variable. There was mean bias of 0.58 kg/d, indicating OM excretion is underpredicted on average. As the two major mean biases were contributed by protein and starch with opposing and nearly equal errors, it is unclear what the source of this error is. The RMSE is relatively modest at 18.6 percent, indicating, as does the CCC, that the predictions track with observed data fairly well despite the mean bias. Although there may be a mean bias in the predictions of energy supply to the animal associated with the overprediction of OM digestibility, the bias should remain constant and the relative differences among diets well captured.

Milk and Milk Component Production

A summary of the accuracy and precision of predictions of milk, milk protein, and milk fat production is provided in Table 20-16. Milk production is predicted empirically from predicted protein and fat production and has a CCC of 0.75 with an RMSE of 14.6 percent with minor mean and slope bias. These are all large improvements over the ME and MP allowable milk productions using the scheme of NRC (2001). In particular, the slope bias for the NRC (2001) approach is very severe, as has been identified previously. Although these predictions are provided by the model and the software, users are strongly encouraged not to make use of those predictions as they clearly are very biased. Milk protein and fat production were not previously predicted, and thus the new predictions are a substantial step forward with a CCC of 0.75 and

0.62 and an RMSE of 14.4 and 17.1 percent, respectively. However, it is important to note that all of these predictions were derived from the evaluations, and thus additional independent evaluations are required. In addition, the upper range in milk and milk component yields does not extend to the levels achieved by high-producing herds today; thus, in many situations, results are extrapolated beyond the range of the data.

MODEL VARIANCE RELATIVE TO ANIMAL PERFORMANCE AND DIET SPECIFICATIONS

A number of factors contribute to the precision or imprecision of model predictions relative to animal performance on a specified diet. These factors include errors of measurement of inputs and outputs, biological variation among animals and groups of animals, and variation in environmental stress. Because these factors also contribute to variation in the data used to define and parameterize the model, predictions by the model are subject to uncertainty. Knowing the contribution of each of the major components to system uncertainty allows one to estimate the uncertainty in each part given the other system components. Such knowledge can be used to determine when diet reformulation is required and whether animal performance has deviated significantly from the expected production. Although assessing the variation in each part of the system would require a major effort, it is possible to estimate the likely minimum variance in each from the variance measured with best practices and the designed precision of the measurement systems.

The sources of variation that affect the predictions can be divided into eight general categories reflecting the different components of the feeding and animal system. These sources are associated with (1) assessments of the nutrient composition of ingredients used to construct the diet, (2) assessments of the amount of each ingredient in the diet, (3) assessments of the amount of diet consumed, (4) assessments of the size of the animal, (5) assessments of animal perfor-

TABLE 20-17   Standard Deviation for Analyses of Repeated Samplings of Feed Grains and By-Products Reported by St-Pierre and Weiss (2015)

Nutrient	Whole Cottonseed	Dry Corn	High-Moisture Corn	Dried Distillers Grains	Soybean Meal	Wet Brewers Grains	Concentrate[a]
DM	2.11	1.96	6.14	1.9	0.81	1.98	1.38
Ash	0.55	0.58	0.33	0.91	0.56	0.58	0.57
CP	1.68	0.54	0.73	3.57	1.41	4.09	0.975
Starch		1.49			0.49		0.99
NDF	4.14	2	5.36	2.56	1.67	3.98	1.835
Lignin		4.73			3.69		4.21
Ca	0.091	0.026	0.034	0.043	0.096	0.112	0.061
K	0.092	0.055	0.057	0.285	0.18	0.049	0.118
Mg	0.046	0.018	0.015	0.09	0.033	0.031	0.026
Na		0.015	0.015	0.081	0.053	0.023	0.034
P	0.085	0.038	0.036	0.147	0.051	0.088	0.045
Cu	3.69	2.22	1.33	2.2	2.71	4.89	2.465
Fe	38.2	29.16	39.92	23.2	41.7	67.1	35.43
Mn	9.8	4.39	3.07	7.25	6.01	15.3	5.2
Zn	10.8	8.45	5.84	18.4	6.07	22.3	

[a] An average of corn and soybean meal.

mance, (6) genetic and epigenetic variation in the animals, (7) environmental effects, and (8) the model. The sum of this variation contributes to deviations in animal behavior relative to model predictions on farm and in the laboratory, and the first seven elements contribute to model uncertainty in terms of structure and parameter estimates (St-Pierre, 2016). The first five sources of variation can be assessed and used to explore their impact on model performance. The last three sources are much more difficult to assess individually, but they can be assessed in aggregate by difference from the overall observed variance given knowledge of the variance in the first five elements. This is the approach that was taken by the committee using the literature data summarized in Chapter 6.

Variation associated with measurement of nutrient composition of the source ingredients and the impact of that variance on animal performance have been explored (St-Pierre and Weiss, 2015). In that work, the authors assessed the variance of analyses of multiple samples over a 12-month period and over multiple farms of six grain and by-product ingredients. A summary of that information is provided in Table 20-17. Those standard deviations include farm-to-farm variance, month-to-month variance, and sampling and analytical variance.

Users need to be aware of the variation associated with both sampling and analyses when formulating and evaluating diets. The feed composition tables (see Chapter 19) include standard deviations, but that variance includes more sources than sampling and analytical. It is important to stress the need for representative sampling on farm or in the research laboratory. A biased sample will always result in biased nutrient composition no matter how precise the assay method.

Variation in measurements of ingredient inclusion in the diet and the amount of the diet consumed by animals is sub-

ject to the precision of the weight-recording devices used, the accuracy of the operator in loading ingredients and diet delivery, and the estimates of the DM content of the ingredients. These would be impossible to know retrospectively for each of the publications reported in the literature, but they can be assessed on farm, and loading and delivery variance should be assessed and monitored. The precision of estimates of milk production and composition is similarly subject to the precision of the measuring and analytical equipment with the assumption the overall mean is not biased.

Biological variation derives from genetic diversity among animals, which may be categorized and assessed by animal function. There is diversity among animals in DMIn, ruminal fermentation, intestinal nutrient digestibility, efficiency of transfer from absorbed nutrients to product, and nutrient excretion. Variation in the environmental conditions under which animals are housed also contributes by altering DMIn and the efficiency of animal function.

Because the model structure and parameter estimates described herein were derived as a number of discrete components, and because the uncertainty of predicted values relative to observed values reflects the combination of measurement error, biological variation, and model uncertainty, it is not possible to directly estimate model uncertainty. However, one can derive animal plus model variation by difference from total variation if the other sources of uncertainty are identified and subtracted. While the resulting estimate is a combination of biological variation, environmental effects, and model uncertainty, it remains useful as it reflects the uncertainty of predictions applied across groups of animals and thus the expected variation in overall model predictions when applied to random groups of animals.

Using the variance estimates from Table 20-17 and Table 20-18 and those listed above to introduce normally dis-

TABLE 20-18 Standard Deviations for Analyses of Duplicate Samplings of a Feed Conducted by a Commercial Feed Analysis Laboratory in the United States[a]

	Units	TMR	Corn Silage	Grasses	Legumes	Mixed Forages	Small Grains Forage	Sorghum Sudan
N		34	1264	121	1043	515	284	62
DM (manual)	% AF	1.11	3.96	4.31	5.21	1.90	1.09	1.41
DM (NIR)	% AF	0.68	0.31	0.47	0.34	0.31	0.57	0.90
Ash	% DM	0.07	0.17	0.28	1.10	0.41	0.79	0.87
CP	% DM	0.32	0.37	0.74	1.45	0.84	0.11	0.12
Total amino acids	% DM		0.54	0.70	1.16	0.48	0.16	0.16
Starch	% DM	2.14	6.16	0.15	3.03	1.21	0.95	1.66
Ethanol Sol. CHO	% DM	0.17	0.12	0.53	0.28	0.31	0.50	0.23
Water Sol. CHO	% DM	0.20	0.13					
NDF	% DM	2.14	3.31	1.90	4.94	4.25	0.91	0.55
NDF (ash free)	% DM	2.06	3.15	1.82	4.59	4.57	1.05	0.85
ADF	% DM	1.33	1.81	0.24	1.65	0.83	0.82	0.33
Lignin	% DM	0.071	0.066	0.071	0.096	0.128	0.094	0.026
dNDFr30	% DM	0.96	1.25	0.48	1.46	3.75	0.90	0.54
dNDFr48	% DM		1.24	1.44	1.72	1.35	1.32	0.89
dNDFr120	% DM	0.30	1.27	1.03	1.67	1.46	1.37	0.76
dNDFr240	% DM	0.66	1.05	0.75	1.64	1.60	1.29	0.62
Crude fat	% DM	0.052	0.028	0.012	0.020	0.015	0.006	0.004
Total fatty acids	% DM	0.032	0.015	0.007	0.007	0.004	0.007	0.003
Linoleic	% TFA	0.60	4.20	1.01	5.73	4.45	0.86	1.09
Linolenic	% TFA	1.10	4.98	1.18	9.92	10.27	2.34	2.77
Myristic	% TFA	0.006	0.010	0.005	0.019	0.013	0.003	0.003
Oleic	% TFA	0.40	1.51	0.35	2.54	2.28	0.23	0.29
Palmitic	% TFA	0.51	0.50	0.63	1.60	1.11	0.37	0.48
Stearic	% TFA	0.006	0.010	0.030	0.026	0.024	0.033	0.015
Ca	% DM		0.0002	0.0071	0.0073	0.0061	0.0023	0.0014
K	% DM		0.0046	0.0201	0.0258	0.0264	0.0238	0.0307
Mg	% DM		0.00009	0.00025	0.00042	0.00025	0.00018	0.00036
P	% DM		0.00005	0.00011	0.00012	0.00016	0.00009	0.00008
S	% DM		0.000018	0.000088	0.000169	0.000083	0.000025	0.000021

[a] Kindly provided by Rock River Laboratories, Inc. Data represent analyses of random duplicate samplings of a single ingredient by each technician each day from March 2018 through October 2019.

tributed, random error, a population of 200 observations was generated for each diet in the literature data summarized in Table 20-11. The source population was reduced to those observations reporting milk, milk protein, and milk fat, yielding 935 treatment means and 187,000 observations when replicated with input and milk measurement variance. A summary of the variance in milk output is provided in Table 20-19.

Predictions of variation in diet specifications to achieve a given level of production were determined using the same data with the variance associated with measurements of milk production and composition removed, and the variance due to model structure plus animal and environmental variation added. In this manner, the simulated data represented the expected production from each diet as specified with model plus animal and environmental variation. Because a range of diets can result in the same level of production due to the additive nature of the key driving nutrients contributing to milk production, the data were filtered to select observations within each source diet that were plus or minus 1 standard

error (SE) from the mean for milk, milk protein, or milk fat production. Variance was calculated for a full range of model factors using the selected observations from each source diet, and the resulting population standard deviation (SD) was reduced to a mean SD across the source diets. This ensured that dietary differences among diets did not influence the variance estimates, yielding a pure estimate of the expected variance for each model input given a diet and a level of milk production. The process was repeated for animal group sizes of 1, 4, 8, 16, 32, 64, 128, and 256 animals. A group size of 1 represents the individual animal, 4 was used for the majority of the studies in the evaluation data, 8 and 16 reflect the range of group sizes typically used for production trials, and the remaining group size encompasses the typical range in group sizes on farm. These estimates are provided in Table 20-20.

Although variance introduced into model inputs to generate the estimates in Table 20-20 was randomly and independently distributed, the model creates covariance in the responses, and thus the estimates are overspecified to

TABLE 20-19   Variance Estimates for Observed Milk Production and Predicted Milk
Production with Input and Milk Measurement Variance

	Milk	Milk Protein	Milk Protein	Milk Fat	Milk Fat
Observed	kg/d	g/d	%	g/d	%
Mean	31.0	935	3.03	1,100	3.59
Variance	53.7	46,936	0.046	65,058	0.27
SD	7.33	217	0.21	255	0.52
Predicted					
Mean	30.29	907	2.98	1,100	3.65
Variance	27.7	31,532	0.034	37,374	0.20
SD	5.27	178	0.19	193	0.45
Model + animal + environment[a]					
Mean	0.75	28.2	0.044	0.58	−0.066
Variance	25.9	15,404	0.012	27,684	0.072
SD	5.09	124	0.11	166	0.27

[a] Model plus animal plus environmental variation was derived by difference.

TABLE 20-20   Model Input Variance for a Specified Level of Production[a]

Group Size	Milk								
	Dt_DMIn	Dt_NDF	Dt_DEIn	Dt_St	Dt_CP	Dt_CPA	Dt_CPB	Dt_CPC	Dt_FA
	kg/d				% DM				
1	0.18	9.27	3.92	12.99	3.83	1.33	2.92	0.41	1.25
4	0.18	9.19	3.84	12.83	3.81	1.32	2.92	0.40	1.24
8	0.18	8.91	3.75	12.40	3.71	1.29	2.84	0.39	1.19
16	0.17	8.48	3.63	11.84	3.59	1.25	2.75	0.38	1.16
32	0.17	8.20	3.57	11.54	3.57	1.24	2.75	0.37	1.13
64	0.16	8.27	3.42	11.45	3.51	1.22	2.72	0.38	1.12
128	0.16	7.43	3.30	9.75	3.10	1.07	2.44	0.32	1.02
256	0.15	7.07	3.37	9.09	3.00	1.03	2.37	0.32	1.06

	Milk Protein								
	Dt_DMIn	Dt_NDF	Dt_DEIn	Abs_His	Abs_Ile	Abs_Leu	Abs_Lys	Abs_Met	Abs_Thr
	kg/d	% DM	Mcal/d				g/d		
1	0.19	9.10	3.78	10.35	21.37	33.24	29.55	8.97	18.65
4	0.18	9.05	3.76	10.37	21.29	33.21	29.45	8.93	18.58
8	0.18	8.82	3.64	10.31	21.11	32.75	29.19	8.74	18.41
16	0.18	8.68	3.55	9.93	20.77	31.88	28.57	7.97	18.03
32	0.17	8.22	3.44	9.46	20.04	30.37	27.27	7.56	17.33
64	0.17	8.12	3.26	9.13	19.22	29.29	26.38	7.18	16.73
128	0.16	7.91	3.19	8.87	19.00	28.56	25.60	7.09	16.31
256	0.15	7.46	3.18	8.52	17.94	26.58	24.18	6.56	15.30

	Milk Fat								
	Dt_DMIn	Dt_NDF	Dt_FA	Dt_C16	Dt_C18.0	Dt_C18.1t	Dt_C18.1c	Dt_C18.2	Dt_C18.3
	kg/d				% DM				
1	0.18	9.28	1.26	0.306	0.107	0.022	0.45	0.47	0.16
4	0.18	9.19	1.26	0.306	0.107	0.022	0.45	0.47	0.16
8	0.18	9.13	1.26	0.306	0.106	0.021	0.45	0.47	0.16
16	0.18	8.89	1.20	0.290	0.100	0.020	0.43	0.45	0.15
32	0.17	8.67	1.17	0.282	0.098	0.019	0.41	0.44	0.15
64	0.17	8.36	1.10	0.264	0.095	0.018	0.39	0.42	0.14
128	0.16	8.06	1.09	0.254	0.094	0.018	0.39	0.41	0.14
256	0.15	7.96	1.11	0.275	0.106	0.019	0.39	0.40	0.13

[a] Variance was estimated for each diet and averaged across diets for varying group sizes.

TABLE 20-21 Partial Correlations Between Milk Production and Selected Nutrient Inputs[a]

	Milk	Milk Protein	Milk Fat
An_DEIn	0.22	0.18	0.26
Dt_NDFIn	0.34	0.49	0.18
Dt_LgIn	0.06	0.04	0.17
Dt_StIn	0.31	0.47	0.13
Dt_rOMIn	0.21	0.41	0.04
Dt_CPAIn	−0.03	0.01	−0.02
Abs_His_g	−0.01	0.03	0.02
Abs_Ile_g	0.02	0.05	0.01
Abs_Leu_g	−0.03	−0.05	−0.05
Abs_Lys_g	0.13	0.09	−0.01
Abs_Met_g	0.16	0.18	0.25
Abs_Thr_g	−0.01	0.03	0.03
Dt_FAIn	0.03	0.15	−0.03
Dt_C16In	0.09	0.03	0.10
Dt_C18.3In	0.08	−0.01	0.18

[a] Values were calculated from simulation data that were ±1 SE (group size = 256) from the mean predicted values for each of milk, milk protein, and milk fat.

varying degrees. For example, milk fat predictions use total FA, C16:0, C18:3, absorbed Ile, and absorbed Met as drivers of output. Thus, a reduction in one of these inputs could be offset by an increase in one of the others. Therefore, one must also consider the correlations and covariance among outputs and the driving factors. Partial correlations between milk production and the primary driving nutrients for production were calculated and are presented in Table 20-21.

MODEL APPLICATION IN R

The model described above is coded in R as a function that can be applied to a table of data using the mapply or lapply functions of R, or if the data set is large, the mcmapply or parLapply functions can be used to process individual observations in parallel. Model output from the function is gathered into a list of matrices or dataframes that are returned to the R working environment. The lists can be consolidated into a set of dataframes or matrices and merged with the observed data to evaluate model performance.

The model code and scripts to run the five observations are provided at the National Academies Press website (see https://www.nap.edu/catalog/25806), and in the C:\NASEM\ NASEM-Dairy-8\script folder after installation of the software.

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21

Nutrient Requirement Tables

Tables 21-1 to 21-3 are intended to be used as a general guide to compare the expected nutrient concentrations of diets fed to meet the minimum requirements of dairy cattle at varying stages of maturity, lactation stages, growth rates, milk production, and milk composition. The nutrient concentrations are based on the required amounts of each nutrient divided by the predicted dry matter intake (DMI) using prediction equations presented in Chapter 2 (Dry Matter Intake) for heifers and milking cows, Chapter 10 (Nutrient Requirements of the Young Calf), and Chapter 12 (Dry and Transition Cows).

Requirements for each nutrient in calves, heifers, dry cows, and milking cows are based on the calculated requirements for each nutrient provided in the individual chapters corresponding to that nutrient. Energy and protein requirements for growth are presented in Chapter 11 (Growth). Dietary rumen-undegradable protein (RUP) was estimated as the difference between total dietary crude

protein (CP) and rumen-degradable protein where dietary CP is based on the expected ratio of metabolizable protein to total dietary CP. There is no specific RUP requirement as the metabolizable protein supply would depend on the microbial protein produced from rumen fermentation and RUP supplied by individual ingredients in the diet. For energy and protein, provisions for mobilization and replenishment of body reserves during the lactation cycle have been included.

Deviations from expected DMIs will result in different nutrient concentrations required to meet the animal's nutrient requirements. In addition, nutrient requirements for metabolic fecal excretion and availability of some nutrients are driven by both DMI and the amount and composition of the individual feed ingredients within the diet. Thus, the most accurate estimates of the actual nutrient concentrations needed to meet the animal's requirements are best depicted by using the diet software.

TABLE 21-1 Predicted Nutrient Concentrations (DM Basis) Needed to Meet the Nutrient Requirements for Holstein Cattle at Varying Stages of Lactation and Ages of Maturity

| | Growing Calves and Heifers | | | | | | Dry Cows[a] Days Prepartum | | | Lactating Cows by Parity (Body Weight) and Days in Milk[a] | | | | |
| | | | | | | | | | | First (570 kg) | | Mature (700 kg) | | |
Age, days	30	100	225	350	475	600	60–21d1	<21d	Days-in-Milk	15	150	20	100	200
Age, days	30	100	225	350	475	600	60–21d1	<21d	Milk, kg	33	39	53	55	43
BW, kg	65	120	230	330	420	530	740	740	Fat %	3.9	3.6	3.7	3.5	3.8
Growth Rate, kg/d	0.7	0.7	0.9	0.8	0.7	0.9	0.1	0.1	Protein %	3.1	3.0	2.8	2.8	3.3
Dry matter intake, kg/d	1.4	3.9	6.6	8.5	9.8	11.0	13.9	12.3		20.8	23.9	25.8	29.4	27.4
ME, Mcal/kg	3.68	2.26	2.09	1.95	1.92	2.12	1.93	2.25		2.39	2.61	2.58	2.73	2.60
NE_L, Mcal/kg	—	—	—	—	—	—	1.28	1.49		1.58	1.72	1.70	1.80	1.73
Rumen-degradable protein, %	—	10.0	10.0	10.0	10.0	10.0	10.0	10.0		10.0	10.0	10.0	10.0	10.0
Rumen-undegradable protein, %	—	6.6	4.4	2.6	1.7	2.7	1.9	4.3		6.2	7.0	7.5	7.4	7.5
Crude protein, %	21.0	16.6	14.4	12.6	11.7	12.7	11.9	14.3		16.2	16.0	17.5	17.4	17.5
Metabolizable protein, %	16.5	9.5	8.1	6.8	6.1	14.0	5.2	6.7		9.8	9.8	10.9	10.2	10.1
NDF, min %	—	25–33	25–33	25–33	25–33	25–33	25–33	25–33		25–33	25–33	25–33	25–33	25–33
Forage NDF, min %	—	19–25	19–25	19–25	19–25	19–25	19–25	19–25		19–25	19–25	19–25	19–25	19–25
Starch max, % (varies)	—	15–20	15–20	15–20	15–20	15–20	15–20	15–20		22–30	22–30	22–30	22–30	22–30
Macrominerals, %														
Ca	0.59	0.78	0.58	0.44	0.37	0.39	0.31	0.39		0.57	0.57	0.64	0.60	0.58
P	0.45	0.32	0.26	0.21	0.18	0.19	0.19	0.21		0.35	0.35	0.39	0.37	0.35
Mg	0.15	0.14	0.12	0.12	0.12	0.10	0.13	0.14		0.17	0.17	0.18	0.18	0.17
K	1.00	0.51	0.52	0.54	0.56	0.60	0.62	0.69		1.03	0.97	1.10	1.00	0.99
Na	0.35	0.17	0.16	0.16	0.15	0.16	0.16	0.17		0.21	0.21	0.23	0.22	0.21
Cl	0.28	0.14	0.14	0.13	0.13	0.13	0.13	0.14		0.29	0.30	0.34	0.32	0.29
S	—	0.20	0.20	0.20	0.20	0.20	0.20	0.20		0.20	0.20	0.20	0.20	0.20
DCAD-S mEq/kg min	—	39	42	45	50	60	66	−100		148	130	157	135	137
Trace minerals, mg/kg														
Cu	5	16	16	15	15	17	18	19		9	8	10	8	10
Co	—	0.20	0.20	0.20	0.20	0.20	0.20	0.20		0.20	0.20	0.20	0.20	0.20
I	0.78	0.69	0.58	0.54	0.53	0.54	0.51	0.54		0.46	0.42	0.47	0.42	0.41
Fe	90	61	46	32	24	28	13	15		16	16	21	19	16
Mn	50	49	44	40	38	43	38	43		28	26	31	28	27
Se	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		0.3	0.3	0.3	0.3	0.3
Zn	70	47	41	36	34	35	30	32		57	58	66	62	61
Vitamins, IU/kg														
Vitamin A	5,218	3,390	3,829	4,265	4,698	5,288	5,850	6,630		3,021	2,796	3,687	3,303	3,103
Vitamin D	1,518	924	1,044	1,163	1,281	1,442	1,595	1,810		1,099	954	1,085	952	1,021
Vitamin E	86	49	25	28	31	35	85	181		22	19	22	19	20

[a]Energy and protein requirements for dry and lactating cows have been adjusted for growth (0.19 and 0.1 kg/d) for first versus mature cows and changes in energy reserves (−0.36, −1.00, 0.20, −1.70, 0.21, and 0.21 kg/d) for the respective groups beginning with dry cows at less than 21 days prepartum. Days pregnant were set at 10, 60, and 110 for cows at 100, 150, and 200 DIM.

TABLE 21-2 Predicted Nutrient Concentrations (DM Basis) Needed to Meet the Nutrient Requirements for Jersey Cattle at Varying Stages of Lactation and Ages of Maturity

| | Growing Calves and Heifers | | | | | | Dry Cows[a] Days Prepartum | | | Lactating Cows by Parity (Body Weight) and Days in Milk[a] | | | | |
| | | | | | | | | | | First (425 kg) | | Mature (520 kg) | | |
	30	100	225	350	475	600	60–21d1	<21d	Days in Milk	15	150	20	100	200
Age, days	30	100	225	350	475	600	60–21d1	<21d	Milk, kg	22	27	35	37	31
BW, kg	45	90	175	245	310	400	555	555	Fat %	4.9	4.9	5.0	4.8	4.8
Growth Rate, kg/d	0.5	0.6	0.7	0.6	0.7	0.7	0.06	0.06	Protein %	3.9	3.7	3.5	3.5	3.7
Dry matter intake, kg/d	1.0	3.0	5.1	6.5	7.4	8.3	10.4	9.4		16.5	19.4	20.5	23.5	21.9
ME, Mcal/kg	3.69	2.41	2.16	2.02	2.13	2.25	2.04	2.19		2.41	2.72	2.67	2.80	2.68
NE$_L$, Mcal/kg	—	—	—	—	—	—	1.36	1.44		1.59	1.79	1.76	1.85	1.78
Rumen-degradable protein, %	—	10.0	10.0	10.0	10.0	10.0	10.0	10.0		10.0	10.0	10.0	10.0	10.0
Rumen-undegradable protein, %	—	7.8	4.4	3.1	3.1	2.9	1.8	3.7		6.8	7.6	8.0	8.0	7.6
Crude protein, %	22.9	17.8	14.4	13.1	13.1	12.9	11.8	13.7		16.8	17.6	18	18	17.6
Metabolizable protein, %	18.2	10.6	8.2	7.2	6.9	6.6	5.5	6.1		10.2	10.0	11.1	10.5	10.2
NDF, min %	—	25–33	25–33	25–33	25–33	25–33	25–33	25–33		25–33	25–33	25–33	25–33	25–33
Forage NDF, min %	—	19–25	19–25	19–25	19–25	19–25	19–25	19–25		19–25	19–25	19–25	19–25	19–25
Starch max, % (varies)	—	15–20	15–20	15–20	15–20	15–20	15–20	15–20		22–30	22–30	22–30	22–30	22–30
Macrominerals, %														
Ca	0.75	0.84	0.58	0.44	0.43	0.39	0.31	0.38		0.56	0.56	0.63	0.60	0.57
P	0.55	0.34	0.26	0.21	0.20	0.19	0.21	0.23		0.33	0.34	0.37	0.35	0.34
Mg	0.15	0.14	0.12	0.12	0.12	0.10	0.13	0.14		0.16	0.16	0.17	0.17	0.16
K	1.20	0.50	0.52	0.53	0.56	0.60	0.62	0.68		0.96	0.89	1.01	0.92	0.93
Na	0.43	0.17	0.16	0.16	0.16	0.16	0.16	0.17		0.20	0.20	0.21	0.21	0.20
Cl	0.34	0.14	0.14	0.13	0.13	0.13	0.12	0.14		0.27	0.27	0.31	0.29	0.27
S	—	0.20	0.20	0.20	0.20	0.20	0.20	0.20		0.20	0.20	0.20	0.20	0.20
DCAD-S, mEq/kg min	—	39	40	43	50	60	66	−100		133	114	140	119	124
Trace minerals, mg/kg														
Cu	6	17	15	15	16	17	18	19		9	8	9	8	8
Co	—	0.20	0.20	0.20	0.20	0.20	0.20	0.20		0.20	0.20	0.20	0.20	0.20
I	1.08	0.77	0.64	0.61	0.60	0.61	0.58	0.62		0.45	0.41	0.46	0.41	0.41
Fe	110	68	46	32	32	29	13	15		13	14	17	16	14
Mn	60	52	44	39	42	44	38	43		25	23	28	25	25
Se	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		0.3	0.3	0.3	0.3	0.3
Zn	84	49	41	36	36	35	30	32		53	54	59	56	53
Vitamins, IU/kg														
Vitamin A	6,084	3,286	3,745	4,162	4,592	5,273	5,850	6,510		2,836	2,405	2,796	2,520	2,616
Vitamin D	1,770	896	1,021	1,135	1,252	1,438	1,595	1,774		1,031	875	1,017	884	951
Vitamin E	90	48	25	28	30	35	85	177		21	17	20	18	19

[a] Energy and protein requirements for dry and lactating cows have been adjusted for growth (0.14 and 0.06 kg/d) for first versus mature cows and changes in energy reserves (−0.24, −0.75, 0.15, −1.28, 0.16, and 0.16 kg/d) for the respective groups beginning with dry cows at less than 21 days prepartum. Days pregnant were set at 10, 60, and 110 for cows at 100, 150, and 200 DIM.

TABLE 21-3 Predicted Protein and Amino Acid Requirements for First-Lactation and Mature Holstein and Jersey Cows at Varying Days in Milk

		Holstein					Jersey				
Parity (Body Weight, kg)		First (570 kg)		Mature (700 kg)			First (425 kg)		Mature (525 kg)		
Days in milk		15	150	20	100	200	15	150	20	100	200
Milk, kg/d		33	39	53	55	43	22	27	35	37	31
Milk protein, %		3.06	3.02	2.76	2.83	3.26	3.90	3.70	3.49	3.46	3.69
Milk protein, g/d		1,010	1,178	1,463	1,557	1,402	858	999	1,222	1,280	1,144
Dry matter intake, kg/d		20.8	23.9	25.8	29.4	27.4	16.5	19.4	20.5	23.5	21.9
Protein intake, g/d		3,370	4,063	4,515	5,116	4,795	2,772	3,414	3,690	4,230	3,854
Rumen degradable, g/d		2,080	2,390	2,580	2,940	2,740	1,650	1,940	2,050	2,350	2,190
Rumen undegradable, g/d		1,290	1,673	1,935	2,176	2,055	1,122	1,474	1,640	1,880	1,664
Net protein, g/d		1,462	1,667	2,005	2,141	1,968	1,207	1,382	1,644	1,749	1,586
Metabolizable protein, g/d (eff. = 0.69)		2,034	2,333	2,802	2,999	2,757	1,686	1,942	2,306	2,457	2,228
Target Absorbed Essential Amino Acids	Target Efficiency	Target Absorbed Amino Acids, g/d									
Histidine	0.75	53	61	73	79	72	39	54	50	67	61
Isoleucine	0.69	108	126	152	164	149	88	113	117	142	127
Leucine	0.74	182	211	254	273	249	137	183	179	229	206
Lysine	0.70	154	179	216	232	212	120	162	156	202	183
Methionine	0.70	49	57	69	74	68	39	52	52	66	59
Phenylalanine	0.60	113	131	157	169	154	87	114	115	143	129
Threonine	0.60	104	120	143	155	142	80	107	103	133	121
Tryptophan	0.63	25	29	35	38	34	19	26	25	32	29
Valine	0.85	120	139	168	181	165	96	127	127	159	143

Appendix A

Statement of Task

A committee will prepare a report that reviews the scientific literature on the nutrition of dairy cattle and updates the nutrient requirements contained in the 2001 NRC publication *Nutrient Requirements of Dairy Cattle*. The report will contain a comprehensive analysis of recent research on the feeding and nutrition of dairy cattle, including research on the amounts of amino acids, lipids, fiber, carbohydrates, minerals, vitamins, and water needed by preweanling, growing, reproducing, and lactating dairy cattle.

The committee will evaluate new information to improve the accuracy of predicting animal performance from nutrient input and of predicting nutrient input when animal performance is known. Consideration will be given to variables that may affect nutrient requirements, such as environmental conditions and type of production system. The report will also address the effects of mycotoxins and other toxins; recent research on energy utilization by dairy cattle; the composition of feed ingredients, mineral supplements, and feed additives routinely fed to dairy cattle; coproducts from the biofuels industry; and the accuracy of estimating energy in forages using the NRC prediction equation.

New information about nutrient metabolism and utilization, antioxidants, effects of feed grain processing on starch rate and site of digestion, feed additives that alter rumen metabolism and postabsorptive metabolism, and rumen and metabolic modifiers will be included. The committee will also review nutritional and feeding strategies that minimize nutrient losses in manure and reduce greenhouse gas production and include a discussion of the effect of feeding on the nutritional quality and safety of dairy products. Future areas of needed research will be identified. Depending on the extent of new information available, an update of the current computer model to calculate nutrient requirements may be developed. Appropriate testing and analysis of any new model will be conducted.

Appendix B

Acronyms and Abbreviations

AA	amino acid
AC	absorption coefficient
ACP	acyl carrier protein
ADF	acid detergent fiber
ADG	average daily gain
ADH	antidiuretic hormone
ADIP	acid detergent insoluble protein
ADL	acid detergent lignin
ADP	apparently digestible protein
AEA	apparent efficiency of IgG absorption
AI	Adequate Intake
AMS	automatic milking system
AOAC	Association of Official Analytical Chemists
BCS	body condition score
BHBA	β-hydroxybutyric acid
BRIX	unit of measurement of sugar concentration in an aqueous solution
BSE	bovine spongiform encephalopathy; "mad cow disease"
BUN	blood urea N
BV	biological value
BW	body weight
CCK	cholecystokinin
CF	crude fat
CJD	Creutzfeldt–Jakob disease
CLA	conjugated linoleic acid
CoA	coenzyme A
CP	crude protein
CV	coefficient of variation
DA	displaced abomasum
DCAD	diet cation–anion difference calculated with Na, K, and Cl
DCAD-S	dietary cation–anion difference calculated with Na, K, Cl, and S
dCP	digested crude protein
DDGS	dried distillers grains with solubles
DE	digestible energy
DEI	digestible energy intake
DEInp	nonprotein DEI

dFA	digested fatty acid
DFM	direct-fed microbial
DIM	days in milk
DM	dry matter
DMI	dry matter intake
DMT1	divalent metal transporter 1
dNDF	digested NDF
DOM-1	de-epoxy DON
DON	deoxynivalenol/vomitoxin
DRI	Dietary Reference Intake
dROM	digested residual organic matter
dRUP	intestinally digested rumen-undegraded protein
dSt	digested starch
EAA	essential amino acid
EAR	estimated average requirement
EBG	empty body gain
EBW	empty body weight
EBWtr	empty body water
ECW	extracellular fluid water
EDDI	ethylenediamine dihydriodide
EMPS	efficiency of microbial protein synthesis
EN	endogenous N
EO	essential oil
eROM	endogenous ROM
EUCP	endogenous urinary CP
FA	fatty acid
FAD	flavin adenine dinucleotide
FFM	fat-free matter
FMN	flavin mononucleotide
fNDF	forage NDF
fNDFD	digestibility of forage NDF measured in vitro or in situ
FPCM	fat- and protein-corrected milk
FPstarter	time relative to first offer of starter
FWI	free water intake
GasE	gaseous energy
GE	gross energy
GEI	gross energy intake
GHG	greenhouse gas
GM	genetically modified
GPx/GSHpx	glutathione peroxidases
HCl	hydrochloric acid
HCN	prussic acid/hydrocyanic acid
HMBi	isopropyl ester of HMTBA
HMTBA/HMB	DL-2-hydroxy-4-methylthiobutanoate
HP	heat production
ICW	intracellular fluid water
Ig	immunoglobulin
iNDF	indigestible NDF
IU	international unit
IV	iodine value

IVNDFD	in vitro NDF digestibility
IVNDFD48	IVNDFD after 48-hour incubation
IVSD	in vitro starch digestibility
k_d	degradation rate
k_n	rate of passage
LB	large breed
LCFA	long-chain fatty acid
LDA	left displacement of abomasum
MatBW	mature body weight
MBW	metabolic body weight
MCFA	medium-chain fatty acid
MCP	microbial crude protein
ME	metabolizable energy
MEg	ME for gain
MEI	ME intake
MEiLD	ME intake from liquid diet
MEm	ME requirement for maintenance
MFD	milk fat depression
MFP	metabolic fecal protein
MicN	microbial N
MilkE	milk net energy
MIR	mid-infrared
Mn-SOD	manganese superoxide dismutase
MNU	microbial N derived from urinary urea-N
MOS	maltooligosaccharides, oligosaccharides containing mannose
MP	metabolizable protein
MPY	milk protein yield
MR	milk replacer
MSE	mean squared error
MSPE	mean squared prediction error
MTL	maximum tolerable level
MUFA	monounsaturated fatty acid
MUN	milk urea N
MW	molecular weight
MY	mean milk yield
NAN	nonammonia nitrogen
NANMN	nonammonia-nonmicrobial N
NDF	neutral detergent fiber
NDIP	neutral detergent insoluble protein
NDSC	neutral detergent soluble carbohydrates
NDSF	neutral detergent soluble fiber
NEAA	nonessential amino acid
NEFA	nonesterified fatty acid
NEL	net energy for lactation
NEm	net energy for maintenance
NFC	nonfiber carbohydrate
NFFS	nonforage fiber sources
nfNDF	nonforage NDF
NP	net protein
NPN	nonprotein nitrogen

APPENDIX B|	
OM	organic matter
PA	pasture availability
paNDF	physically adjusted NDF
PD	purine derivatives
pdNDF	potentially digestible NDF
PDV	portal-drained viscera
PEM	polioencephalomacia
peNDF	physically effective NDF
PSPS	Penn State Particle Separator
PTH	parathyroid hormone
PUFA	polyunsaturated fatty acid
qPCR	quantitative polymerase chain reaction
RDA	Recommended Dietary Allowance
RDP	rumen-degradable protein
RE	retained energy
RH	relative humidity
RMSE	root mean squared error
RMSEP	root mean squared error of prediction
ROM	residual organic matter
ROS	reactive oxygen species
RP	rumen protected
RUP	rumen-undegradable protein
SB	small breed
SCC	somatic cell count
SFDMI	solid feed DMI
sNPNCPE	supplemental nonprotein nitrogen on a crude protein-equivalent basis
Ta	air temperature
TBW	total body water
TCA	tricarboxylic acid
TDN	total digestible nutrients
TDS	total dissolved solids
TFA	total fatty acid
THI	temperature humidity index
TMR	total mixed ration
TP	true protein
TRx	thioredoxin reductases
TSE	transmissible spongiform encephalopathy
TSH	thyroid-stimulating hormone
UE	urinary energy
UFA	unsaturated fatty acid
UL	tolerable upper intake level
UN	urinary nitrogen
USP	United States Pharmacopeia
vCJD	variant CJD
VFA	volatile fatty acid
VOC	volatile organic compound
WDG	wet distillers grains
WSC	water-soluble carbohydrates

Appendix C

Committee Member Biographies[1]

Richard A. Erdman (*Co-Chair*) is a Professor of animal sciences in the Animal and Avian Sciences Department at the University of Maryland. Dr. Erdman's research focuses primarily on the nutrition of the dairy cow with an emphasis on energy metabolism and the effects of nutrition on milk components. He was born and raised on a dairy farm near Fort Atkinson, Wisconsin. Dr. Erdman joined the Dairy Science Department at the University of Maryland as an assistant professor in 1979. He served as the department chair at Maryland from 1999 to 2007, where he provided administrative leadership to a department consisting of 22 faculty, 28 staff members, 240 undergraduate students, and 40 graduate students. He has taught undergraduate and graduate courses in applied nutrition, energy metabolism, and animal production systems. He has served as a major Professor to more than 25 graduate students who hold positions in industry and academia. Dr. Erdman has received several awards, including the American Feed Industries Award for Dairy Nutrition Research in 1996. He was a member of the National Research Council committee that prepared the 2001 report on the *Nutrient Requirements of Dairy Cattle*. Dr. Erdman received his BS in animal science at the University of Wisconsin–River Falls and an MS and a PhD in animal nutrition at the University of Kentucky.

William P. Weiss (*Co-Chair*) is a Professor of and an Extension Specialist in animal science at The Ohio State University. His main research areas include factors affecting digestibility and nutrient excretion by dairy cows, energy metabolism in dairy cows, the relationships between minerals and vitamins and the health of dairy cows, statistical and chemical evaluation of feedstuffs, and the effects of diet variability on productivity of dairy cows and profitability of dairy farms. Dr. Weiss was given the American Feed Industry Award, the Applied Dairy Nutrition Award, and the Pioneer Forage Research Award by the American Dairy Association and has served as

a Diplomate of the American College of Animal Nutrition since 2010. He was named a Fellow of the American Dairy Science Association in 2015. Dr. Weiss was a member of the National Research Council (NRC) Committee on Animal Nutrition from 1997 to 2001. He also was a member of the NRC committee that prepared the 2001 report on the *Nutrient Requirements of Dairy Cattle*. Dr. Weiss earned his BS in animal science and MS in dairy cattle nutrition at Purdue University and his PhD in dairy cattle nutrition at The Ohio State University.

Michael S. Allen is a University Distinguished Professor of dairy cattle nutrition at Michigan State University. His primary areas of expertise include the digestion kinetics of fiber and starch, the effects of type and temporal supply of metabolic fuels (glucose, lactate, amino acids) on energy intake and partitioning, grouping strategies, metabolic diseases, fiber requirements, evaluation of forage (and feedstuff) quality, and production response to supplemental fat for lactating cows. He has earned many awards, including the American Feed Industry Association Nutrition Award, the Nutrition Professional's Applied Dairy Nutrition Award, and the Pioneer Forage Award from the American Dairy Science Association. Dr. Allen earned his BS in agriculture and MS and PhD in dairy nutrition from Cornell University, and he conducted his postdoctoral work at the U.S. Department of Agriculture-US Dairy Forage Research Center.

Louis Armentano is a Professor of dairy science and nutrition at the University of Wisconsin–Madison. His main interests are in ruminant nutrition and intermediary metabolism as it affects lactating dairy cows, as well as quantitative techniques of whole-animal and system-specific nutrient fluxes. Dr. Armentano's past research focused on liver metabolism and the use of by-product feeds in dairy rations. Dr. Armentano's current research is focused on the role of specific dietary fatty acids and their differential effect on milk production, energy balance, and milk fatty acid composition. He is also actively involved in describing and

[1] The committee member biography information was current at the time the committee was formed in 2014.

measuring feed efficiency in dairy herds, as well as improving feed efficiency through genetic and genomic selection and also by other management practices. Dr. Armentano received his BS in animal science from Cornell University, MS in nutrition from North Carolina State University, and PhD in nutritional physiology from Iowa State University.

James K. Drackley is a Professor of animal sciences at the University of Illinois at Urbana-Champaign. Dr. Drackley's current research program centers on dry period nutrition and metabolism of transition cows, as well as aspects of calf nutrition and management. Previous research areas included liver metabolism, dietary fat utilization, and lipid metabolism in ruminants. He grew up on a dairy farm in southwestern Minnesota and received his BS in dairy production and his MS in dairy science from South Dakota State University. Dr. Drackley joined the faculty of the University of Illinois in 1989 after receiving his PhD in nutritional physiology from Iowa State University. Dr. Drackley has taught courses in ruminant nutrition, energy nutrition, and lipid metabolism. He has published extensively and has trained 42 graduate students. He has received local and national awards for his research and teaching, including the 2002 American Feed Industry Award for Excellence in Dairy Cattle Nutrition Research and the 2007 Nutrition Professionals, Inc. Applied Dairy Nutrition Award from the American Dairy Science Association (ADSA). Dr. Drackley has long been active in the ADSA, including service on the Board of Directors, President of the Midwest Branch, and Chair of the Production Division.

Jeffrey L. Firkins is a Professor in the Department of Animal Sciences at The Ohio State University. His areas of expertise include bioactive properties of milk and quantitative analyses of kinetic models. Dr. Firkins's research studies the interface between nutrition and microbiology to enhance the conversion of dietary protein into microbial protein and reduce enteric methane production; the interactions of physical, chemical, and microbiological processes related to fiber and starch degradation, passage, and biohydrogenation in dairy cattle; and the quantitative prediction of protein and carbohydrate digestion and microbial protein production in dairy cattle. Dr. Firkins is a member of the American Dairy Science Association (ADSA), and he was awarded the ADSA Nutrition Professionals Applied Dairy Nutrition Award in 2003, received the ADSA American Feed Industry Association Dairy Nutrition Research Award in 2012, and was elected as an ADSA Fellow in 2020. Dr. Firkins received his BS in animal science and MS and PhD in ruminant nutrition from the University of Illinois.

Mary Beth Hall is a Research Animal Scientist at the U.S. Dairy Forage Research Center with the U.S. Department of Agriculture's (USDA's) Agricultural Research Service. Her current research includes evaluating and developing methods for analyzing nutritionally relevant carbohydrates in feeds, determining the differences in and factors affecting ruminal

fermentation products among nonfiber carbohydrates, and identifying the direct and interaction effects of protein degradability and nonfiber carbohydrate type on dairy cattle lactation performance. Dr. Hall also has experience in the use and structure of models, various in vitro fermentation techniques for feed evaluation, practical dairy cattle nutrition, and the application of ruminant nutrition principles to captive herbivores. Before joining the USDA, she was an Associate Professor at the University of Florida, working in dairy nutrition extension and research. Dr. Hall earned her BS in animal science from Cornell University, MS in animal science from Virginia Polytechnic Institute and State University, and PhD in animal science from Cornell University.

Mark D. Hanigan is the David R. and Margaret Lincicome Professor of Dairy Science in the College of Agriculture and Life Sciences at Virginia Polytechnic Institute and State University. His areas of expertise include animal nutrition, nutrient metabolism, and metabolic modeling. Dr. Hanigan's research interests include experimental and modeling work focused on amino acid and protein metabolism, volatile fatty acid metabolism, phosphorus digestion, lipid deposition in dairy and beef cattle, the regulation of energy and nitrogen metabolism in the ruminant, and the resulting impact on the environment. He is a member of the American Dairy Science Association and the American Society for Nutrition. Dr. Hanigan received his BS in dairy science from Iowa State University, and MS in animal science and PhD in nutrition from the University of California, Davis (UC Davis), and he conducted his postdoctoral work in the Department of Biochemistry and Biophysics at UC Davis.

Ermias Kebreab is a Professor of animal science, the Sesnon Endowed Chair of Sustainable Animal Agriculture in the Department of Animal Science, and the Associate Dean of global engagement in the College of Agricultural and Environmental Sciences at the University of California, Davis. He conducts research on reducing the impact of animal agriculture, particularly dairy cattle, on the environment by reducing greenhouse gas emissions and nutrient loading. Dr. Kebreab uses a range of mathematical modeling techniques and experimentation to address environmental and nutrition-related issues. Some of the awards received in recognition of Dr. Kebreab's work include the 2006 Young Scientist Award from the Canadian Society of Animal Science, the 2008 Early Career Achievement Award, and the 2014 Ruminant Nutrition Award from the American Society of Animal Science. Dr. Kebreab received his BS in biology from the University of Asmara (Eritrea) and an MS in integrative biology and a PhD in ecological modeling from the University of Reading (United Kingdom).

Paul J. Kononoff is a Professor of dairy nutrition in the Department of Animal Science at the University of Nebraska–Lincoln. His research focuses on feed characterization and the

relationships between fiber quality and ruminal fermentation in lactating dairy cattle. He has studied how manipulations of forage particle size and cell wall lignification affect feed efficiency, milk production, milk composition, and rumen fermentation. His research has also examined ration formulation methods that seek to maximize the inclusion of corn milling coproducts while sustaining normal milk production and composition. As a dairy nutritionist, Dr. Kononoff has assisted the dairy industry as a Technical Support Specialist for Renaissance Nutrition (Roaring Spring, Pennsylvania) and as a Project Director of the Ruminant Feed Analysis Consortium (Durham, New Hampshire). Dr. Kononoff is a member of the American Dairy Science Association and the Nebraska State Dairy Association. He served as an Editor for the *Journal of Dairy Science* and the *Canadian Journal of Animal Science*. Dr. Kononoff currently serves as the Editor-in-Chief for the *Journal of Dairy Science*. He received his BS and MS in animal science from the University of Saskatchewan and his PhD in animal science from The Pennsylvania State University.

Hélène Lapierre is a Research Scientist of animal metabolism in the Research and Development Centre at Agriculture and Agri-Food Canada and an Adjunct Professor in the Department of Animal Science at Université Laval. Her areas of expertise include the efficiency of nutrient use in the production of milk and milk components; factors affecting release or utilization of nutrients by the gut, the liver, and the mammary gland; and improvement of the transfer of protein from dairy rations into milk protein in order to lower milk production costs and reduce nitrogenous excreta in the environment. Dr. Lapierre is a member of the Canadian Society of Animal Science and the American Dairy Science Association (ADSA). She was the recipient of the American Feed Industry Association Dairy Nutrition Research Award from ADSA in 2016. Dr. Lapierre earned her BS and MS in agriculture from the Université Laval and PhD in biology from the University of Sherbrooke. She conducted postdoctoral work at the U.S. Department of Agriculture's Agricultural Research Service's Beltsville Agricultural Research Center in Maryland.

Michael J. VandeHaar is a Professor of dairy nutrition and physiology in the Department of Animal Science at Michigan State University. His research involves dairy cattle nutrition and physiology with the intent of improving heifer growth and mammary development and increasing the efficiency of protein production in the dairy industry. For the past 4 years, Dr. VandeHaar has been Project Director of a large U.S. Department of Agriculture–funded project, "Genomic Selection and Herd Management for Improved Feed Efficiency of the Dairy Industry." A major part of the project is to develop a database of feed intake and milk output on 8,000 cows with genotypes. This database will be used to develop genomic breeding values for feed efficiency and to develop and test nutritional models. Dr. VandeHaar is a member of the National Animal Nutrition Program NRSP-9 modeling subcommittee. In 2013, he was awarded the highest honor for dairy scientists in nutrition, the American Feed Industry Association Award. Dr. VandeHaar earned his BA from Dordt College, Iowa, and MS and PhD from Iowa State University. He conducted his postdoctoral work at the University of North Carolina.