

5 Protein and Amino Acids

Dietary protein generally refers to crude protein (CP), which is defined for feedstuffs as the nitrogen (N) content $\times 6.25$. The definition is based on the assumption that the average N content of feedstuffs is 16 g per 100 g of protein. The calculated CP content includes both protein and nonprotein N (NPN). Feedstuffs vary widely in their relative proportions of protein and NPN, in the rate and extent of ruminal degradation of protein, and in the intestinal digestibility and amino acid (AA) composition of ruminally undegraded feed protein. The NPN in feed and supplements such as urea and ammonium salts are considered to be degraded completely in the rumen.

IMPORTANCE AND GOALS OF PROTEIN AND AMINO ACID NUTRITION

Ruminally synthesized microbial CP (MCP), ruminally undegraded feed CP (RUP), and to a much lesser extent, endogenous CP (ECP) contribute to passage of metabolizable protein (MP) to the small intestine. Metabolizable protein is defined as the true protein that is digested postruminally and the component AA absorbed by the intestine. Amino acids, and not protein per se, are the required nutrients. Absorbed AA, used principally as building blocks for the synthesis of proteins, are vital to the maintenance, growth, reproduction, and lactation of dairy cattle. Presumably, an ideal pattern of absorbed AA exists for each of these physiologic functions. The *Nutrient Requirements of Poultry* (National Research Council, 1994) and the *Nutrient Requirements of Swine* (National Research Council, 1998) indicate that an optimum AA profile exists in MP for each physiologic state of the animal and this is assumed to be true for dairy animals.

The goals of ruminant protein nutrition are to provide adequate amounts of rumen-degradable protein (RDP) for optimal ruminal efficiency and to obtain the desired animal productivity with a minimum amount of dietary CP. Optimizing the efficiency of use of dietary CP requires selection

of complementary feed proteins and NPN supplements that will provide the types and amounts of RDP that will meet, but not exceed, the N needs of ruminal microorganisms for maximal synthesis of MCP, and the types and amounts of digestible RUP that will optimize, in so far as possible, the profile and amounts of absorbed AA. As discussed later, research indicates that the nutritive value of MP for dairy cattle is determined by its profile of essential AA (EAA) and probably also by the contribution of total EAA to MP. Improving the efficiency of protein and N usage while striving for optimal productivity is a matter of practical concern. Incentives include reduced feed costs per unit of lean tissue gain or milk protein produced, a desire for greater and more efficient yields of milk protein, creation of space in the diet for other nutrients that will enhance production, and concerns of waste N disposal. Regarding milk protein production, research indicates that content (and thus yield) of milk protein can be increased by improving the profile of AA in MP, by reducing the amount of “surplus” protein in the diet, and by increasing the amount of fermentable carbohydrate in the diet.

Major Differences from Previous Edition

In 1985, the Subcommittee on Nitrogen Usage in Ruminants (National Research Council, 1985) expressed protein requirements in units of absorbed protein. Absorbed protein was defined as the digestible true protein (i.e., digestible total AA) that is provided to the animal by ruminally synthesized MCP and feed protein that escaped ruminal degradation. This approach was adopted for the previous edition of this publication (National Research Council, 1989). The absorbed protein method introduced the concept of degraded intake CP (DIP) and undegraded intake CP (UIP). Mean values of ruminal undegradability for common feeds, derived from in vivo and in situ studies using sheep and cattle, were reported. This factorial approach for estimating protein requirements recognized the three fates of dietary protein (fermentative digestion

in the reticulo-rumen, hydrolytic/enzymatic digestion in the intestine, and passage of indigestible protein with feces) and separated the requirements of ruminal microorganisms from those of the host animal. However, a fixed intestinal digestibility of 80 percent for UIP was used, no consideration was given to the contribution of endogenous CP to MP, and no consideration was given to the AA composition of UIP or of absorbed protein.

Some differences exist in terminology. To be consistent with the current edition of *Nutrient Requirements of Beef Cattle* (National Research Council, 1996), and to avoid implications that proteins are absorbed, the term MP replaces absorbed protein. To be consistent with the *Journal of Dairy Science*, the terms DIP and UIP are replaced with RDP and RUP, respectively.

The primary differences between the protein system of this publication and that used in the previous edition relate to predicting nutrient supply. Microbial CP flows are predicted from intake of total tract digestible organic matter (OM) instead of net energy intake. The regression equation considers the variability in efficiency of MCP production associated with apparent adequacy of RDP. A mechanistic system developed from in situ data is used for calculating the RUP content of feedstuffs. Insofar as regression equations allow, the system considers some of the factors (DMI, percentage of concentrate feeds in diet DM, and percentage NDF in diet DM) that affect rates of passage of undigested feed and thus the RUP content of a feedstuff. The system is considered to be applicable to all dairy animals with body weights greater than 100 kg and that are fed for early rumen development. To increase the accuracy of estimating the contribution of the RUP fraction of individual feedstuffs to MP, estimates of intestinal digestibility have been assigned to the RUP fraction of each feedstuff (range = 50 to 100). Endogenous protein and NPN also are considered to contribute to passage of CP to the small intestine. Endogenous CP flows are calculated from intake of DM. And finally, regression equations are included that predict directly the content of each EAA in total EAA of duodenal protein and flows of total EAA. Flows of digestible EAA and their contribution to MP are calculated. Dose-response curves that relate measured milk protein content and yield responses to changes of predicted percentages of digestible Lys and Met in MP are presented. The dose-response relationships provide estimates of model-determined amounts of Lys and Met required in MP for optimal utilization of absorbed AA for milk protein production. The inclusion of equations for predicting passage of EAA to the small intestine along with assignment of RUP digestibility values that are unique to individual feedstuffs brings awareness to differences in nutritive value of RUP from different feedstuffs and should improve the prediction of animal responses to substitution of protein sources.

PROTEIN

Chemistry of Feed Crude Protein

Feedstuffs contain numerous different proteins and several types of NPN compounds. Proteins are large molecules that differ in size, shape, function, solubility, and AA composition. Proteins have been classified on the basis of their 3-dimensional structure and solubility characteristics. Examples of classifications based on solubility would include globular proteins [albumins (soluble in water and alkali solutions and insoluble in salt and alcohol), globulins (soluble in salt and alkali solutions and sparingly soluble or insoluble in water and insoluble in alcohol), glutelins (soluble only in alkali), prolamines (soluble in 70 to 80 percent ethanol and alkali and insoluble in water, salt, and absolute alcohol), histones (soluble in water and salt solutions and insoluble in ammonium hydroxide)] and fibrous proteins [e.g., collagens, elastins, and keratins (insoluble in water or salt solutions and resistant to digestive enzymes)] (Orten and Neuhaus, 1975; Rodwell, 1985; Van Soest, 1994). Globular proteins are common to all feedstuffs whereas fibrous proteins are limited to feeds of animal and marine origin. Albumins and globular proteins are low molecular weight proteins. Prolamines and glutelins are higher molecular weight proteins and contain more disulfide bonds. Generally, feeds of plant origin contain all of the globular proteins but in differing amounts. For example, cereal grains and by-product feeds derived from cereal grains contain more glutelins and prolamines whereas leaves and stems are rich in albumins (Blethen et al., 1990; Sniffen, 1974; Van Soest, 1994). A sequential extraction of 38 different feeds with water, dilute salt (0.5 percent NaCl), aqueous alcohol (80 percent ethanol), and dilute alkali (0.2 percent NaOH) indicated that the classic protein fractions (albumins, globulins, prolamines, and glutelins) plus NPN accounted for an average of 65 percent of total N (Blethen et al., 1990). The unaccounted for, insoluble N would include protein bound in intact aleurone granules of cereal grains, most of the cell-wall associated proteins, and some of the chloroplasmic and heat-denatured proteins that are associated with NDF (Van Soest, 1994). Among the feeds that were evaluated, those with the highest percentage of insoluble protein (> 40 percent of CP) were forages, beet pulp, soy hulls, sorghum, dried brewers grains, dried distillers grains, fish meal, and meat and bone meal (Blethen et al., 1990).

Feedstuffs also contain variable amounts of low molecular weight NPN compounds. These compounds include peptides, free AA, nucleic acids, amides, amines, and ammonia. Nonprotein N compounds generally are determined as the N remaining in the filtrate after precipitation of the true protein with either tungstic or trichloroacetic acid (Licitra et al., 1996). Grasses and legume forages contain the highest and most variable concentrations of

NPN. Most of the reported concentrations of NPN in CP of grasses and legume forages are within the following ranges: fresh material (10B15%), hay (15B25%), and silage (30B65%) (Fairbairn et al., 1988; Garcia et al., 1989; Grum et al., 1991; Hughes, 1970; Krishnamoorthy et al., 1982; Messman et al., 1994; Van Soest, 1994; Xu et al., 1996). Hays and especially silages contain higher amounts of NPN than the same feed when fresh because of the proteolysis that occurs during wilting and fermentation. The proteolysis that occurs in forages during wilting and ensiling is a result of plant and microbial proteases and peptidases. Plant proteases and peptidases are active in cut forage and are considered to be the principal enzymes responsible for the conversion of true protein to NPN in hays and ensiled feeds (Fairbairn et al., 1988; Van Soest, 1994). Rapid wilting of cut forages and conditions that promote rapid reductions in pH of ensiled feeds slow proteolysis and reduce the conversion of true protein to NPN (Garcia et al., 1989; Van Soest, 1994). The NPN content of fresh forage is composed largely of peptides, free AA, and nitrates (Van Soest, 1994). Fermented forages have a different composition of NPN than fresh forages. Fermented forages have higher proportional concentrations of free AA, ammonia, and amines and lower concentrations of peptides and nitrate (Fairbairn et al., 1988; Van Soest, 1994). The NPN content of most non-forage feeds is 12 percent or less of CP (Krishnamoorthy et al., 1982; Licitra et al., 1996; Van Soest, 1994; Xu et al., 1996).

Mechanism of Ruminal Protein Degradation

The potentially fermentable pool of protein includes feed proteins plus the endogenous proteins of saliva, sloughed epithelial cells, and the remains of lysed ruminal microorganisms. The mechanism of ruminal degradation has been reviewed (Broderick et al., 1991; Broderick, 1998; Cotta and Hespell, 1984; Jouany, 1996; Jouany and Ushida, 1999; Wallace, 1996; Wallace et al., 1999). In brief, all of the enzymatic activity of ruminal protein degradation is of microbial origin. Many strains and species of bacteria, protozoa, and anaerobic fungi participate by elaborating a variety of proteases, peptidases, and deaminases (Wallace, 1996). The liberated peptides, AA, and ammonia are nutrients for the growth of ruminal microorganisms. Peptide breakdown to AA must occur before AA are incorporated into microbial protein (Wallace, 1996). When protein degradation exceeds the rate of AA and ammonia assimilation into microbial protein, peptide and AA catabolism leads to excessive ruminal ammonia concentrations. Some of the peptides and AA not incorporated into microbial protein may escape ruminal degradation to ammonia and become sources of absorbed AA to the host animal.

Bacteria are the principal microorganisms involved in protein degradation. Bacteria are the most abundant micro-

organisms in the rumen (10^{10-11} /ml) and 40 percent or more of isolated species exhibit proteolytic activity (Broderick et al., 1991; Cotta and Hespell, 1984; Wallace, 1996). Most bacterial proteases are associated with the cell surface (Kopečný and Wallace, 1982); only about 10 percent of the total proteolytic activity is cell free (Broderick, 1998). Therefore, the initial step in protein degradation by ruminal bacteria is adsorption of soluble proteins to bacteria (Nugent and Mangan, 1981; Wallace, 1985) or adsorption of bacteria to insoluble proteins (Broderick et al., 1991). Extracellular proteolysis gives rise to oligopeptides which are degraded further to small peptides and some free AA. Following bacterial uptake of small peptides and free AA, there are five distinct intracellular events: (1) cleavage of peptides to free AA, (2) utilization of free AA for protein synthesis, (3) catabolism of free AA to ammonia and carbon skeletons (i.e., deamination), (4) utilization of ammonia for resynthesis of AA, and (5) diffusion of ammonia out of the cell (Broderick, 1998).

The bacterial population that is responsible for AA deamination has been of considerable interest. Amino acid catabolism and ammonia production in excess of bacterial need wastes dietary CP and reduces efficiency of use of RDP for ruminant production. For many years it was assumed that deamination was limited to the large number of species of bacteria that had been identified to produce ammonia from protein or protein hydrolyzates (Wallace, 1996). However, this assumption was challenged by Russell and co-workers (Chen and Russell, 1988, 1989; Russell et al., 1988) who concluded that the deaminative activity of these bacteria was too low to account for rates of ammonia production usually observed in vivo or in vitro with mixed cultures. Their efforts led to the eventual isolation of a small group of bacteria that had exceptionally high deaminative activity and that used AA as their main source of carbon and energy (Russell et al., 1988; Paster et al., 1993). As a result of these and other studies, it is now accepted that AA deamination by bacteria is carried out by a combination of numerous bacteria with low deaminative activity and a much smaller number of bacteria with high activity (Wallace, 1996). Of particular interest has been the observation that the growth of some of these bacteria with high deaminating activity is suppressed by the ionophore, monensin (Chen and Russell, 1988, 1989; Russell et al., 1988).

Protozoa also are active and significant participants in ruminal protein degradation. Protozoa are less numerous than bacteria in ruminal contents (10^{5-6} /ml) but because of their large size, they comprise a significant portion of the total microbial biomass in the rumen (generally less than 10 percent but sometimes as high as 50 percent) (Jouany, 1996; Jouany and Ushida, 1999). Several differences exist between protozoa and bacteria in their metabolism of protein. First, they differ in feeding behavior. Instead of forming a complex with feeds, protozoa ingest

particulate matter (bacteria, fungi, and small feed particles). Bacteria are their principal source of ingested protein (Jouany and Ushida, 1999). As a result of this feeding behavior (i.e., ingestion of food), protozoa are more active in degrading insoluble feed proteins (e.g., soybean meal or fish meal) than more soluble feed proteins (e.g., casein) (Hino and Russell, 1987; Jouany, 1996; Jouany and Ushida, 1999). Ingested proteins are degraded within the cell to yield a mixture of peptides and free AA; the AA are incorporated into protozoal protein. Proteolytic specific activity of protozoa is higher than that of bacteria (Nolan, 1993). A second difference between protozoa and bacteria is that while both actively deaminate AA, protozoa are not able to synthesize AA from ammonia (Jouany and Ushida, 1999). Thus, protozoa are net exporters of ammonia and because of this, defaunation decreases ruminal ammonia concentrations (Jouany and Ushida, 1999). And lastly, protozoa release large amounts of peptides and AA as well as peptidases into ruminal fluid. This is the result of significant secretory processes and significant autolysis and death (Coleman, 1985; Dijkstra, 1994). Jouany and Ushida (1999) suggest that excreted small peptides and AA can represent 50 percent of total protein ingested by protozoa. Other studies indicate that 65 percent or more of protozoal protein recycles within the rumen (Ffoulkes and Leng, 1988; Punia et al., 1992).

Much less is known about the involvement of fungi in ruminal protein catabolism. Currently, anaerobic fungi are considered to have negligible effects on ruminal protein digestion because of their low concentrations in ruminal digesta (10^{3-4} /ml) (Jouany and Ushida, 1999; Wallace and Monroe, 1986).

Kinetics of Ruminal Protein Degradation

Ruminal degradation of dietary feed CP is an important factor influencing ruminal fermentation and AA supply to dairy cattle. RDP and RUP are two components of dietary feed CP that have separate and distinct functions. Ruminally degraded feed CP provides a mixture of peptides, free AA, and ammonia for microbial growth and synthesis of microbial protein. Ruminally synthesized microbial protein typically supplies most of the AA passing to the small intestine. Ruminally undegraded protein is the second most important source of absorbable AA to the animal. Knowledge of the kinetics of ruminal degradation of feed proteins is fundamental to formulating diets for adequate amounts of RDP for rumen microorganisms and adequate amounts of RUP for the host animal.

Ruminal protein degradation is described most often by first order mass action models. An important feature of these models is that they consider that the CP fraction of feedstuffs consists of multiple fractions that differ widely in rates of degradation, and that ruminal disappearance of

protein is the result of two simultaneous activities, degradation and passage. One of the more complex of these models is the Cornell Net Carbohydrate Protein System (CNCPS) (Sniffen et al., 1992). In this model, feed CP is divided into five fractions (A, B₁, B₂, B₃, and C) which sum to unity. The five fractions have different rates of ruminal degradation. Fraction A (NPN) is the percentage of CP that is instantaneously solubilized at time zero, which is assumed to have a degradation rate (k_d) of infinity; it is determined chemically as that proportion of CP that is soluble in borate-phosphate buffer but not precipitated with the protein denaturant, trichloroacetic acid (TCA) (Figure 5-1). Fraction C is determined chemically as the percentage of total CP recovered with ADF (i.e., ADIN) and is considered to be undegradable. Fraction C contains proteins associated with lignin and tannins and heat-damaged proteins such as the Maillard reaction products (Sniffen et al., 1992). The remaining B fractions represent potentially degradable true protein. The amounts of each of these 3 fractions that are degraded in the rumen are determined by their fractional rates of degradation (k_d) and passage (k_p); a single k_p value is used for all fractions. Fraction B₁ is that percentage of total CP that is soluble in borate-phosphate buffer and precipitated with TCA. Fraction B₃ is calculated as the difference between the portions of total CP recovered with NDF (i.e., NDIN) and ADF (i.e., fraction C). Fraction B₂ is the remaining CP and is calculated as total CP minus the sum of fractions A, B₁, B₃, and C. Reported ranges for the fractional rates of degradation for the three B fractions are: B₁ (120–400 %/h), B₂ (3–16 %/h), and B₃ (0.06–0.55 %/h). The RDP and RUP values (percent of CP) for a feedstuff using this model are computed using the equations

$$\begin{aligned} \text{RDP} = & A + B_1 [k_d B_1 / (k_d B_1 + k_p)] \\ & + B_2 [k_d B_2 / (k_d B_2 + k_p)] \\ & + B_3 [k_d B_3 / (k_d B_3 + k_p)] \end{aligned}$$

and

$$\begin{aligned} \text{RUP} = & B_1 [k_p / (k_d B_1 + k_p)] \\ & + B_2 [k_p / (k_d B_2 + k_p)] \\ & + B_3 [k_p / (k_d B_3 + k_p)] + C. \end{aligned}$$

This model is used in Level II of the *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) report.

The most used model to describe in situ ruminal protein degradation divides feed CP into three fractions (A, B, and C). Fraction A is the percentage of total CP that is NPN (i.e., assumed to be instantly degraded) and a small amount of true protein that rapidly escapes from the in situ bag because of high solubility or very small particle size. Fraction C is the percentage of CP that is completely undegradable; this fraction generally is determined as the feed CP remaining in the bag at a defined end-point of degradation. Fraction B is the rest of the CP and includes the proteins

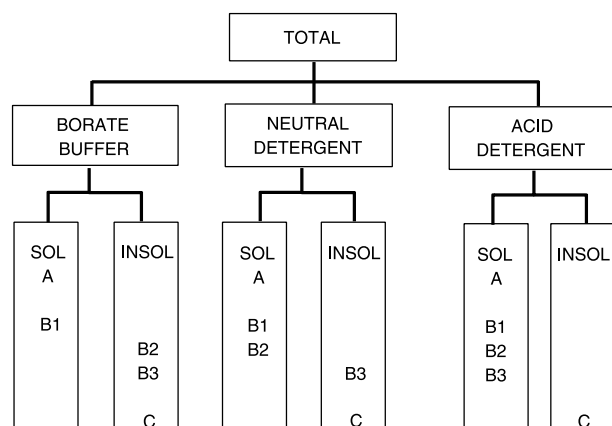


FIGURE 5-1 Analyses of crude protein fractions using borate-phosphate buffer and acid detergent and neutral detergent solutions (Roe et al., 1990; Sniffen et al., 1992).

that are potentially degradable. Only the B fraction is considered to be affected by relative rates of passage; all of fraction A is considered to be degraded and all of fraction C is considered to pass to the small intestine. The amount of fraction B that is degraded in the rumen is determined by the fractional rate of degradation that is determined in the study for fraction B and an estimate of fractional rates of passage. The RDP and RUP values for a feedstuff (percent of CP) using this model are computed using the equations $RDP = A + B [k_d / (k_d + k_p)]$ and $RUP = B [k_p / (k_d + k_p)] + C$. This simple model has been the most widely used model for describing degradation and ruminal escape of feed proteins (e.g., AFRC, 1984; National Research Council, 1985; Ørskov and McDonald, 1979). It is noted that data obtained from in situ, in vitro, and enzymatic digestions generally fit a model that divides feed CP into these fractions (Broderick et al., 1991) and that most of the in situ data used to validate results obtained with cell-free proteases have been obtained using this model (Broderick, 1998). As discussed later, it is this model in conjunction with in situ derived data that is used for predicting ruminal protein degradability in this edition.

Numerous factors affect the amount of CP in feeds that will be degraded in the rumen. The chemistry of feed CP is the single most important factor. The two most important considerations of feed CP chemistry are: (1) the proportional concentrations of NPN and true protein, and (2) the physical and chemical characteristics of the proteins that comprise the true protein fraction of the feedstuff. Nonprotein N compounds are degraded so quickly in the rumen ($>300\%/h$) that degradation is assumed to be 100 percent (Sniffen et al., 1992). However, this is not an entirely correct assumption because degradability is truly related to rate of passage. For example, assuming a k_p of $2.0\%/h$ and a k_d of $300\%/h$, then $\text{degradation} = 3.00 / (3.00 + 0.02) = 0.993$ or 99.3 percent, and not 1.00 or 100 percent. Feedstuffs that contain high concentrations of NPN in CP

contribute little RUP to the host animal. When dairy cattle are fed all-forage diets, measurements of passage of non-ammonia, non-microbial N (i.e., RUP-N plus endogenous N) often are less than 30 percent of N intake (Beever et al., 1976, 1987; Holden et al., 1994a; Van Vuuren et al., 1992). In contrast to NPN, which is assumed to be completely degraded, the rates of degradation of proteins are highly variable and result in variable amounts of protein being degraded in the rumen. For example, the range in k_d given in Tables 15-2a,b are 1.4 for Menhaden fish meal to 29.2 for sunflower meal. Assuming a k_p for each feed of 7.0 percent, the range in degradabilities of the B fraction would be 16.7 to 80.7 percent. Some characteristics of proteins shown to contribute to differences in rates of degradation are differences in 3-dimensional structure, differences in intra- and inter-molecular bonding, inert barriers such as cell walls, and antinutritional factors.

Differences in 3-dimensional structure and chemical bonding (i.e., cross-links) that occur both within and between protein molecules and between proteins and carbohydrates are functions of source as well as processing. These aspects of structure affect microbial access to the proteins, which apparently is the most important factor affecting the rate and extent of degradation of proteins in the rumen. Proteins that possess extensive cross-linking, such as the disulfide bonding in albumins and immunoglobulins or cross-links caused by chemical or heat treatment, are less accessible to proteolytic enzymes and are degraded more slowly (Ferguson, 1975; Hurrell and Finot, 1985; Mahadevan et al., 1980; Mangan, 1972; Nugent and Mangan, 1978; Nugent et al., 1983; Wallace, 1983). Proteins in feathers and hair are extensively cross-linked with disulfide bonds and largely for that reason, a considerable amount of the protein in feather meal is in fraction C (Tables 15-2a,b). Similarly, a considerable portion of the protein in meat meal and meat and bone meal is in fraction C. Proteins in meat meal and meat and bone meal may contain considerable amounts of collagen that has both intramolecular and intermolecular cross-links (Orten and Neuhaus, 1975). In contrast, a majority of the protein in menhaden fish meal is in fraction B but the fractional rate of degradation of fraction B is slower than in other protein supplements (Tables 15-2a,b). Heat used in the drying of fish protein was shown to induce the formation of disulfide bonds (Opstvedt et al., 1984). Heat processing also coagulates protein in meat products which makes it insoluble (Bendall, 1964; Boehme, 1982), and cooling of the products causes a random relinkage of chemical bonds which shrinks the protein molecules (Bendall, 1964). Collectively, these effects of heating and cooling of proteins decrease microbial access and make the proteins more resistant to ruminal degradation.

Other factors affecting the ruminal degradability of feed protein include ruminal retention time of the protein, microbial proteolytic activity, and ruminal pH. The effect

of these factors on the kinetics of ruminal protein degradation have been reviewed (Broderick et al., 1991; National Research Council, 1985).

Nitrogen Solubility vs. Protein Degradation

Several commercial feed testing laboratories in the United States provide at least one measurement of N solubility for feedstuffs. Although recognized that N solubility in a single solvent is not synonymous with CP degradation in the rumen, the general absence of alternatives other than using "book values" for RUP (e.g., National Research Council, 1985) left little else to help nutritionists ensure that adequate but not excessive amounts of RDP were fed. Solubility measurements have been useful for ranking feeds of similar types for ruminal CP degradability. This is because of the positive relationship that exists between N solubility and degradation within similar feedstuffs (e.g., Beever et al., 1976; Laycock and Miller, 1981; Madsen and Hvelplund, 1990; Stutts et al., 1988). Many studies have indicated that changing N solubility by adding or removing NPN supplements, by changing method of forage preservation, or processing conditions of protein supplements affects animal response (e.g., Aitchison et al., 1976; Crish et al., 1986; Lundquist et al., 1986). Several different solvents have been used. At present, the most common procedure is incubation in borate-phosphate buffer (Roe et al., 1990). This method has gained in popularity because it is used for determining the A and B₁ nitrogen fractions in the CNCPS (Sniffen et al., 1992).

Although a high correlation exists between N solubility in a single solvent and protein degradability for similar feedstuffs, the same does not exist across classes of feedstuffs. For example, Stern and Satter (1984) reported a correlation of 0.26 between N solubility and in vivo protein degradation in the rumen of 34 diets that contained a variety of N sources. Madsen and Hvelplund (1990) also reported a poor relationship between N solubility and in vivo degradation of CP when used over a range of feedstuffs. There appear to be several reasons for these poor relationships. First, as indicated in the section "Chemistry of Feed Crude Protein", the proteins that are extracted by a solvent depend not only on the chemistry of the proteins but also on the composition of the solvent. For that reason, different solvents provide different estimates of CP solubility (Cherney et al., 1992; Crawford et al., 1978; Crooker et al., 1978; Lundquist et al., 1986; Stutts et al., 1988). Second, soluble proteins are not equally susceptible to degradation by rumen enzymes. Among the pure soluble proteins, casein is degraded rapidly whereas serum albumin, ovalbumin, and ribonuclease A are degraded much slower (Annison, 1956; Mahadevan et al., 1980; Mangan, 1972). Mahadevan et al. (1980) also observed that soluble proteins from soybean meal, rape-

seed meal, and fish meal were degraded at different rates with rates of degradation for all three supplements being intermediate between those for albumins and casein. Therefore, structure as well as solubility determines degradability. Third, as indicated in the section "Mechanism of Ruminal Protein Degradation", solubility is not a prerequisite to degradation. As an example, Mahadevan et al. (1980) observed that soluble and insoluble proteins of soybean meal were hydrolyzed in vitro at almost identical rates. Because bacteria attach to insoluble proteins and because protozoa engulf feed particles, insoluble proteins need not enter the soluble protein pool before attack by microbial proteases. And last, soluble proteins that are not yet degraded may leave the rumen faster than insoluble proteins. This is because of a more likely association of soluble protein with the liquid fraction of ruminal contents. For example, Hristov and Broderick (1996) observed that although feed NAN in the liquid phase of ruminal contents was only 12 percent of total ruminal feed NAN, 30 percent of the feed NAN that escaped the rumen flowed with the liquids. This indicates a disproportional escape of soluble proteins.

In conclusion, a change in N solubility in a single solvent appears to be a more useful indicator of a change in protein degradation when applied to different samples of the same feedstuff than when used to compare different feedstuffs that differ in chemical and physical properties. Clearly, the relationship between solubility and degradability is the highest when most of the soluble N is NPN (Sniffen et al., 1992).

Microbial Requirements for N Substrates

Peptides, AA, and ammonia are nutrients for the growth of ruminal bacteria; protozoa cannot use ammonia. Estimates of the contribution of ammonia versus preformed AA to microbial protein synthesis by the mixed rumen population have been highly variable (Wallace, 1997). Studies using N¹⁵ ammonia or urea infused into the rumen or added as a single dose demonstrated that values for microbial N derived from ammonia ranged from 18 to 100 percent (Salter et al., 1979). The N¹⁵ studies of Nolan (1975) and Leng and Nolan (1984) indicated that 50 percent or more of the microbial N was derived from ammonia and the rest from peptides and AA. The mixed ruminal microbial population has essentially no absolute requirement for AA (Virtanen, 1966) as cross-feeding among bacteria can meet individual requirements. However, researchers have observed improved microbial growth or efficiency when peptides or AA replaced ammonia or urea as the sole or major source of N (Cotta and Russell, 1982; Russell and Sniffen, 1984; Griswold et al., 1996). Maeng and Baldwin (1976) reported increased microbial yield and growth rate on 75% urea + 25% AA-N as compared to

100% urea. Microbial requirements for N substrates of ammonia-N, AA, and peptides can also be affected by the basal diet and may explain some of the variability in the above experiments.

There is evidence that AA and especially peptides are stimulatory in terms of both growth rate and growth yield for ruminal microorganisms growing on rapidly degraded energy sources (Argyle and Baldwin, 1989; Chen et al., 1987; Cruz Soto et al., 1994; Russell et al., 1983). However, when energy substrates are fermented slowly, stimulation by peptides and AA does not always occur. Chikunya et al. (1996) demonstrated that when peptides were supplied with rapidly or slowly degraded fiber, microbial growth was enhanced only if the fiber was degraded rapidly. Russell et al. (1992) indicated that microorganisms fermenting structural carbohydrates require only ammonia as their N source while species degrading nonstructural carbohydrate sources will benefit from preformed AA.

Recent experiments (Wallace, 1997) have confirmed the earlier results of Salter et al. (1979) showing that the proportion of microbial N derived from ammonia varies according to the availability of N sources. The minimum contribution to microbial N from ammonia was 26 percent when high concentrations of peptides and AA were present, with a potential maximum of 100 percent when ammonia was the sole N source. Griswold et al. (1996) examined the effect of isolated soy protein, soy peptides, individual AA blended to profile soy protein, and urea on growth of microorganisms in continuous culture. Griswold et al. (1996) demonstrated that N forms other than ammonia are needed not only for maximum microbial growth but also as NPN for adequate ruminal fiber digestion.

Many reports of the uptake of C¹⁴-AA and peptides have indicated that mixed microbial populations preferentially took up peptides rather than free AA (Cooper and Ling, 1985; Prins et al., 1979). However, Ling and Armstead (1995) found that free AA were the preferred form of AA incorporated by *S. bovis*, *Selenomonas ruminantium*, *Fibrobacter succinogenes* and *Anaerovibrio lipolytica*, whereas peptides were preferred only by *P. ruminicola*. *P. ruminicola* can comprise greater than 60 percent of the total flora in sheep fed grass silage (Van Gylswyk, 1990). In other studies where an AA preference was exhibited, the preference may have been the result of specific dietary conditions where *P. ruminicola* numbers were lower. Wallace (1996) demonstrated that AA deamination is carried out by two distinct bacterial populations, one with low activity and high numbers and the other with high activity and low numbers. *P. ruminicola* occurs in high numbers but has low deaminase activity.

Jones et al. (1998) investigated the effects of peptide concentrations in microbial metabolism in continuous culture fermenters. The basal diet contained 17.8 percent CP, 46.2 percent NSC, and 32.9 percent NDF. Peptides

replaced urea as a N source at levels of 0, 10, 20 and 30 percent of total N, a urea-molasses mixture represented 8.6, 7.0, 4.9, and 2.9 percent of DM with increasing peptide and glucose replacement. Digestion of DM and CP and microbial CP production were affected quadratically by peptide addition; the highest values for each variable occurred at 10 percent peptide addition. Fiber digestion decreased linearly with increasing peptide addition. Reduced ammonia-N concentrations appeared to be the cause of reduced microbial CP production and reduced fiber digestion at levels of peptides greater than 10 percent of total N. The efficiency of conversion of peptide N to microbial CP increased with increasing peptides; however, there was no change in grams of microbial N produced per kilogram of OM digested. Jones et al. (1998) suggested that with diets containing high levels of NSC, excessive peptide concentrations relative to that of ammonia can depress protein digestion and ammonia concentrations, limit the growth of fiber-digesting microorganisms, and reduce ruminal fiber digestion and microbial protein production. Microorganisms that ferment NSC produce and utilize peptides at the expense of ammonia production from protein and other N sources (Russell et al., 1992). It should be noted that in continuous culture systems, protozoa can be washed out in the first few days of operation.

Animal Responses to CP, RDP, and RUP

LACTATION RESPONSES

Crude protein. A data set of 393 means from 82 protein studies was used to evaluate the milk and milk protein yield responses to changes in the concentration of dietary CP (Table 5-1). The descriptive statistics for the data set are presented in Table 5-2. When CP content of diets change, the relative contribution of protein from different sources also change so this evaluation is confounded with source of protein and concentrations of RDP and RUP. Overall, milk yield increased quadratically as diet CP concentrations increased. The regression equation obtained was:

$$\text{Milk yield} = 0.8 \times \text{DMI} + 2.3 \times \text{CP} - 0.05 \times \text{CP}^2 - 9.8 \quad (r^2 = 0.29)$$

where milk yield and dry matter intake (DMI) are kilograms/d and CP is percent of diet DM.

Dry matter intake was included in the regression to account indirectly for some of the differences among studies such as basal milk production and BW. Dry matter intake accounted for about 60 percent and CP about 40 percent of non-random variation. Assuming a fixed DMI (there was no correlation between intake and CP percent in this data set), the maximum milk production was obtained at 23 percent CP. The marginal response to

TABLE 5-1 Studies Used to Evaluate Milk and Milk Protein Yield Responses to Changes in the Concentration of Dietary Crude Protein

Annexstad et al. (1987)	Henderson et al. (1985)	McCormick et al. (1999)
Aharoni et al. (1993)	Henson et al. (1997)	McGuffey et al. (1990)
Armentano et al. (1993)	Higginbotham et al. (1989)	Nakamura et al. (1992)
Atwal et al. (1995)	Hoffman and Armentano (1988)	Owen and Larson (1991)
Baker et al. (1995)	Hoffman et al. (1991)	Palmquist and Weiss (1994)
Bertrand et al. (1998)	Holter et al. (1992)	Palmquist et al. (1993)
Blauwiel and Kincaid (1986)	Hongerholt and Muller (1998)	Polan et al. (1997)
Blauwiel et al. (1990)	Howard et al. (1987)	Polan et al. (1985)
Bowman et al. (1988)	Huyler et al. (1999)	Powers et al. (1995)
Broderick (1992)	Jaquette et al. (1986)	Robinson and Kennelly (1988b)
Broderick et al. (1990)	Jaquette et al. (1987)	Robinson et al. (1991b)
Bruckental et al. (1989)	Kaim et al. (1983)	Roseler et al. (1993)
Canfield et al. (1990)	Kaim et al. (1987)	Santos et al. (1998a,b)
Casper et al. (1990)	Kalscheur et al. (1999a,b)	Sloan et al. (1988)
Chen et al. (1993)	Kerry and Amos (1993)	Spain et al. (1995)
Christensen et al. (1993a, b)	Khorasani et al. (1996a)	Voss et al. (1988)
Crawley and Kilmer (1995)	Kim et al. (1991)	Wattiaux et al. (1994)
Cunningham et al. (1996)	King et al. (1990)	Weigel et al. (1997)
De Gracia et al. (1989)	Klusmeyer et al. (1990)	Wheeler et al. (1995)
DePeters and Bath (1986)	Komaragiri and Erdman (1997)	Windschitl (1991)
Dhiman and Satter (1993)	Lees et al. (1990)	Wohlt et al. (1991)
Garcia-Bojalil et al. (1998a)	Leonard and Block (1988)	Wright (1996)
Grant and Haddad (1998)	Lundquist et al. (1986)	Wu et al. (1997)
Grings et al. (1991)	Macleod and Cahill (1987)	Wu and Satter (2000)
Grings et al. (1992a)	Manson and Leaver (1988)	Zimmerman et al. (1992)
Grummer et al. (1996)	Mantysaari et al. (1989)	Zimmerman et al. (1991)
Hadsell and Sommerfeldt (1988)	McCarthy et al. (1989)	

TABLE 5-2 Descriptive Statistics for Data Set Used to Evaluate Animal Responses to CP and RDP

Variable	N	Mean	Std. Dev.
Milk, kg/d	393	31.4	6.1
Milk protein yield, g/d	360	972	153
Dry matter intake, kg/d	393	20.2	3.4
CP, % of dry matter	393	17.1	2.6
RDP, % of dry matter	172	10.7	1.8
RUP, % of dry matter	172	6.2	1.4

increased dietary CP (first derivative of the CP components of the regression equation) is: $2.3 - 0.1 \times \text{CP}$. Therefore, increasing dietary CP one percentage unit from 15 to 16 percent would be expected to increase milk yield an average of 0.75 kg/d and increasing CP one percentage unit from 19 to 20 percent would be expected to increase milk yield by 0.35 kg/d. Although milk production may be increased by feeding diets with extremely high concentrations of CP, the economic and environmental costs must be compared with lower CP diets. The marginal response obtained from this data set was similar to that obtained by Roffler et al. (1986). With their equation, increasing dietary CP from 14 to 18 percent would result in an increase of 2.1 kg/d of milk and with the equation above the expected increase is 2.8 kg/d.

Dietary CP was not correlated ($P > 0.25$) with milk protein percent, but was correlated weakly ($r = 0.14$; $P < 0.01$) with milk protein yield (because of the relationship of dietary CP with milk yield). The regression equation was:

milk protein yield (g/d) = $17.7 \times \text{DMI} + 55.6 \times \text{CP} - 1.26 \times \text{CP}^2 + 31.8$ ($r^2 = 0.19$) where DMI is kilograms/day and CP is percent of diet DM. Maximum yield of milk protein was obtained at 22 percent CP (essentially the same as for milk yield) and the marginal response is equal to $55.63 - 2.52 \times \text{CP}$ where CP is a percent of diet DM.

Rumen degradable and undegradable protein. A regression approach also was used to evaluate lactation responses to concentrations of RDP and RUP in the dietary DM. To evaluate lactation responses to RDP in diet DM, 38 studies with 206 treatment means were selected in which diets varied in content of RDP (Table 5-3). All diets were entered into this edition's model for predicted concentrations of RDP and RUP in diet DM. As expected, concentrations of RDP and RUP (as percentages of diet DM) were correlated with concentrations of dietary CP (RDP; $r = 0.78$, $P < 0.001$; RUP, $r = 0.53$, $P < 0.001$), therefore it is not possible to separate effects of total CP from those of RDP or RUP. A regression equation for milk yield with RDP and RUP (both as percent of DM) was derived to overcome the problems associated with the correlation between CP and RDP and RUP (the correlation between RDP and RUP was not significant ($r = -0.11$, $P > 0.05$)). Dietary RDP and RUP were calculated using the model described in this publication based on values in the data set described above. The regression equation also included DMI for the reasons explained above. The regression equation (Figure 5-2) was:

$$\text{Milk} = -55.61 + 1.15 \times \text{DMI} + 8.79 \times \text{RDP} - 0.36 \times \text{RDP}^2 + 1.85 \times \text{RUP} \quad (r^2 = 0.52)$$

TABLE 5-3 Studies Used to Evaluate Milk Yield Responses to Changes in the Concentration of Dietary Ruminally Degraded Protein

Annexstad et al. (1987)	Grings et al. (1992)	King et al. (1990)
Armentano et al. (1993)	Grummer et al. (1996)	Komaragiri and Erdman (1997)
Baker et al. (1995)	Ha and Kennelly (1984)	Leonard and Block (1988)
Barney et al. (1981)	Harris et al. (1992)	Mantysaari et al. (1989)
Bertrand et al. (1998)	Henson et al. (1997)	McGuffey et al. (1990)
Blauwiel et al. (1990)	Higginbotham et al. (1989)	Palmquist and Weiss (1994)
Casper et al. (1990)	Hoffman et al. (1991)	Roseler et al. (1993)
Christensen et al. (1993a,b)	Holter et al. (1992)	Santos et al. (1998a,b)
Cunningham et al. (1996)	Hongerholt and Muller (1998)	Wattiaux et al. (1994)
Dhiman and Satter (1993)	Kalscheur et al. (1999a)	Weigel et al. (1997)
Garcia-Bojalil et al. (1998a)	Khorasani et al. (1996b)	Windschitl (1991)
Grant and Haddad (1998)	Kim et al. (1991)	Wu and Satter (2000)
Grings et al. (1991)		

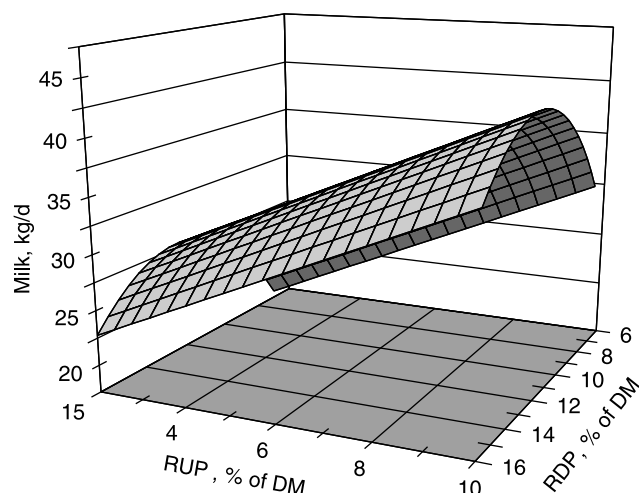


FIGURE 5-2 Response surface for data set described in “Animal Responses to CP, RDP, and RUP” section. Maximum milk yield occurred at 12.2 percent RDP (percent of diet DM). Dry matter intake was held constant at 20.6 kg/day.

where DMI and milk are kilograms/day, and RDP and RUP are percent of diet DM. Based on that equation, maximum milk yield occurred (DMI and RUP held constant) when RDP equaled 12.2 percent of diet DM, and the marginal change in milk to increasing RDP was $8.79 - 0.72 \times \text{RDP}$. The quadratic term for RUP was not significant and was removed from the model. Milk yield increase linearly to RUP at the rate of 1.85 kg for each percentage unit increase in RUP.

In comparison this edition's model estimates an average RDP requirement of 10.2 percent for this data set. Predicted milk yield (using the above regression equation) at 10.2 percent RDP (DMI and RUP held constant mean values of the data set of 20.6 kg/d DMI and 6.2 percent, respectively) is 31.7 kg/d and 33.2 kg/d when RDP is 12.2 percent. A portion of the discrepancy between model predicted requirement for RDP and regression predicted maximal milk production may be caused by the positive correlation between RDP and DM intake ($\text{DMI} = 14.4 + 0.58$

$\times \text{RDP}$; $r = 0.35$, $P < 0.001$). Based on that regression, an increase in 2 percentage units of RDP (i.e., 10.2 to 12.2 percent) would increase DMI by about 1.1 kg/d. Based on this edition's requirements (assumed 72 percent TDN), an increase of about 2 kg/d of milk is expected from that change in DMI. Increasing dietary RDP above model predicted requirements may result in increased DM intake.

A similar shaped function (data not shown) was obtained when milk protein yield was regressed on dietary RDP and RUP:

$$\begin{aligned} \text{Milk protein} = & -1.57 + 0.0275 \times \text{DMI} + 0.223 \\ & \times \text{RDP} - 0.0091 \times \text{RDP}^2 + 0.041 \\ & \times \text{RUP} \quad (r^2 = 0.51) \end{aligned}$$

where milk protein and DMI are kilograms per day and RDP and RUP are percentages of dietary DM. Maximum milk protein yield occurred at 12.2 percent RDP (the same as milk yield). Milk protein yield increased linearly with increasing dietary RUP.

Santos et al. (1998b) published a comprehensive review of the effects of replacing soybean meal with various sources of RUP on protein metabolism (29 published comparisons) and production (127 published comparisons). Santos et al. (1998b) reported that in 76 percent of the metabolism studies, higher RUP decreased MCP flows to the small intestine. Supplementation with RUP usually did not affect flow of total EAA, and RUP supplementation usually did not increase or actually decreased flow of lysine to the duodenum. Supplementation of RUP increased milk production in only 17 percent of the studies and heat-treated or chemically-treated soybean meal or fish meal were the most likely RUP supplements to cause increased milk production (Santos et al., 1998b). When studies were combined, cows fed diets with treated soybean meal ($P < 0.03$) or fish meal ($P < 0.01$) produced statistically more milk than cows fed soybean meal. Cows fed other animal proteins (blood, feather, meat meals) or corn gluten meal produced similar or numerically less milk than cows fed soybean meal (Santos et al., 1998b). See additional discussion in Chapter 16.

The regression equations derived above for milk and milk protein yield responses to dietary CP, RDP, and RUP should be interpreted and used cautiously in view of low r^2 values. A more sophisticated statistical analysis (e.g., controlling for trial effects, adjusting for variances within trials, etc.) would probably yield different and more accurate coefficients.

EFFECTS ON REPRODUCTION

Protein in excess of lactation requirements has been shown to have negative effects on reproduction. Several workers have reported that feeding diets containing 19 percent or more CP in diet DM lowered conception rates (Bruckental et al., 1989; Canfield et al., 1990; Jordan and Swanson, 1979; McCormick et al., 1999). Others have observed that cows fed 20–23 percent CP diets (as compared to 12–15 percent CP) had decreased uterine pH, increased blood urea, and altered uterine fluid composition (Jordan et al., 1983; Elrod and Butler, 1993). In a majority of the studies reviewed by Butler (1998), plasma progesterone concentrations in early lactation cows were lower when diets contained 19–20 percent CP vs. lower concentrations of CP.

In a review of protein effects on reproduction, Butler (1998) concluded that excessive amounts of either RDP or RUP could be responsible for lowered reproductive performance. However, intakes of “digestible” RUP in amounts required to adversely affect reproduction without a coinciding surplus of RDP would be uncommon. In most of the studies reviewed by Butler (1998), excessive RDP rather than excessive RUP was associated with decreased conception rates. Canfield et al. (1990) showed that feeding diets containing RUP to meet requirements while feeding RDP in excess of requirements resulted in decreased conception rates. Garcia-Bojalil et al. (1998b) reported that RDP fed in excess (15.7 percent of DM) of recommendations decreased the amount of luteal tissue in ovaries of early lactation cows.

Although most studies have indicated an adverse effect on reproductive performance of feeding high CP diets, others indicate no effect of diet CP on reproduction. Carroll et al. (1988) observed no differences in pregnancy rate or first service conception rates of dairy cows fed 20 percent CP and 13 percent CP diets. Howard et al. (1987) reported no difference in fertility between cows in second and greater lactation fed 15 percent CP or 20 percent CP diets.

There are many theories as to why excess dietary CP decreases reproductive performance (Barton, 1996a, 1996b; Butler, 1998; Ferguson and Chalupa, 1989). The first theory relates to the energy costs associated with metabolic disposal of excess N. To the extent that additional energy may be required for this purpose, this energy may be taken from body reserves in early lactation to support

milk production. Delayed ovulation (e.g., Beam and Butler, 1997; Staples et al., 1990) and reduced fertility (Butler, 1998) have been associated with negative energy status. Another effect of negative energy status is decreased plasma progesterone concentrations (Butler, 1998).

Another theory is that excessive blood urea N (BUN) concentrations could have a toxic effect on sperm, ova, or embryos, resulting in a decrease in fertility (Canfield et al., 1990). High BUN concentrations have also been shown to decrease uterine pH and prostaglandin production (Butler, 1998). High BUN may also reduce the binding of leutinizing hormone to ovarian receptors, leading to decreases in serum progesterone concentration and fertility (Barton, 1996a). Ferguson and Chalupa (1989) reported that by-products of N metabolism may alter the function of the hypophyseal-pituitary-ovarian axis, therefore decreasing reproductive performance. And last, high levels of circulating ammonia may depress the immune system and, therefore, may result in a decline in reproductive performance (Anderson and Barton, 1988).

Milk urea nitrogen (MUN) and blood urea nitrogen (BUN) are both indicators of urea production by the liver. Milk urea N concentrations greater than 19 mg/dl have been associated with decreased fertility (Butler et al., 1995). Likewise, BUN concentrations greater than 20 mg/dl have been linked with reduced conception rates in lactating cows (Ferguson et al., 1988). Bruckental et al. (1989) found that BUN levels increased when diet CP was increased from 17 to 21.6 percent and pregnancy rate decreased by 13 percentage units. In a case study, Ferguson et al. (1988) observed that cows with BUN levels higher than 20 mg/dl were three times less likely to conceive than cows with lower BUN concentrations. Although high BUN concentrations have been associated with decreased reproductive performance, others have reported no adverse effects on pregnancy rate, services per conception, or days open with BUN levels above 20 mg/dl (Oldick and Firkins, 1996).

Studies by Carroll et al. (1987) and Howard et al. (1987) indicate that maintaining a strict reproductive management protocol can reduce the negative effects of excess protein intake on reproduction. Barton (1996a) demonstrated that an intense reproductive program could be used to reach reproductive success regardless of diet CP level or plasma urea N concentrations. These studies highlight the idea that dietary protein is just one of many things that have an effect on reproductive performance. Protein intake, along with other factors such as reproductive management, energy status, milk yield, and health status all have an effect on reproductive performance in dairy cattle.

Synchronizing Ruminant Protein and Carbohydrate Digestion: Effects on Microbial Protein Synthesis

Microbial protein synthesis in the rumen depends largely on the availability of carbohydrates and N in the rumen.

Bacteria are capable generally of capturing the majority of ammonia that is released in the rumen from AA deamination and the hydrolysis of NPN compounds. However, dietary conditions often occur in which the rate of ammonia release in the rumen exceeds the rate of uptake by ruminal bacteria. Examples of such conditions would include a surplus of RDP or a lack of available energy (Maeng et al., 1997). This asynchronous release of ammonia and energy in the rumen results in inefficient utilization of fermentable substrates and reduced synthesis of MCP. A variety of studies have focused on increasing the efficiency of microbial protein synthesis by manipulating dietary components (Aldrich et al., 1993a; Hoover and Stokes, 1991; Herrera-Saldana et al., 1990; Maeng et al., 1976). Excellent reviews describe the relationship between ruminal protein and carbohydrate availability and its impact on MCP synthesis in the rumen (Hoover and Stokes, 1991; Clark et al., 1992; Stern et al., 1994; Dewhurst et al., 2000).

Several studies indicate that synchronizing for rapid fermentation with fast degradable starch and protein sources stimulates greater synthesis or efficiency of synthesis of MCP. Herrera-Saldana et al. (1990) reported that MCP passage to the duodenum of lactating cows was highest (3.00 kg/d) when starch and protein degradability were synchronized for fast rates of digestion (barley and cottonseed meal). Flows of MCP were lower when the primary fermentable carbohydrate and protein sources were either synchronized for slow degradability (milo and brewer's dried grains; 2.14 kg/d) or asynchronous (barley and brewer's dried grains or milo and cottonseed meal; 2.64 and 2.36 kg/d, respectively). Efficiency of MCP synthesis (MCP/kg of truly digested OM) followed similar trends as MCP passage to the duodenum. Aldrich et al. (1993b) formulated diets to contain high and low concentrations of rumen-available nonstructural carbohydrates (HRANSC and LRANSC) and high and low concentrations of rumen-available protein (HRAP and LRAP) using high moisture shelled corn vs. coarse ground, dry ear corn and canola meal vs. blood meal, respectively. Flow of MCP to the duodenum was highest (1.64 kg/d) with HRANSC/HRAP and lowest (1.34 kg/d) with HRANSC/LRAP, flows were intermediate (1.46 and 1.48 kg/d) for the two LRANSC diets. Similar to the findings of Herrera-Saldana et al. (1990), efficiencies of synthesis of MCP were highest with the HRANSC/HRAP diet. Stokes et al. (1991a) reported that diets formulated to contain 31 or 39 percent NSC and 11.8 or 13.7 percent RDP in diet DM supported greater MCP synthesis than a diet containing 25 percent NSC and 9 percent RDP. Diets formulated to be synchronous vs. asynchronous in ruminal digestion rates of carbohydrate and protein have also increased flows and efficiency of synthesis of MCP in sheep (Sinclair et al., 1993, 1995). In the study by Sinclair et al. (1995), diets were similar in carbohydrate source (barley) and were either synchronous

with rapeseed meal (diet A) or asynchronous with urea (diet B). The efficiency of MCP synthesis was 11–20 percent greater in sheep given diet A vs. diet B.

Numerous other studies have reported higher MCP passage (in vivo or in continuous culture) when either the NSC level was increased or more degradable carbohydrates were substituted for those less degradable (McCarthy et al., 1989; Spicer et al., 1986; Stokes et al., 1991a; Stern et al., 1978) or when RDP in diet DM was increased (Cecava et al., 1991; Hussein et al., 1991; McCarthy et al., 1989; Stokes et al., 1991b). A review of 16 studies indicated that MCP flow to the duodenum was increased by an average of 10 percent when slowly degradable sources of starch (e.g., corn grain) were replaced by more rapidly degraded starch (e.g., barley) (Sauvant and van Milgen, 1995). However, there was no effect of differences in rate of starch degradation on the efficiency of conversion of ruminally digested OM to MCP. Lykos et al. (1997) evaluated diets formulated to have similar rates of RDP with three rates (6.04, 6.98, and 7.94%/h) of NSC degradation in the rumen. Concentrations of RDP and NSC in diet DM were held constant across treatments. Rates of NSC degradation were achieved primarily by replacing cracked corn with ground high moisture corn. Flow of MCP to the duodenum tended to be the highest with the highest rate of NSC degradation. Efficiency of conversion of ruminally digested OM to MCP was increased as ruminal NSC availability increased, demonstrating the importance of timing of available energy to the ruminal microorganisms.

Studies evaluating the importance of providing a gradual or even supply (vs. an uneven supply) of energy and N substrates to ruminal microorganisms are limited. Henning et al. (1993) investigated this issue in cannulated sheep fed both at maintenance and at a higher level of nutrition. Treatments consisted of a soluble carbohydrate mixture (maltose, dextrose and maltotriose) and a soluble N mixture (urea and sodium caseinate). Providing an even supply of energy increased passage of MCP and efficiency of MCP synthesis when the maintenance diet was fed but only tended to increase efficiency of MCP synthesis when the more adequate diet was fed. In contrast, the even supply of N increased passage of MCP only when the more adequate diet was fed. The results indicate that merely improving the degree of synchronization between energy and N release rates in the rumen does not necessarily increase microbial cell yield and that a gradual or even release of energy and possibly N as well are also important.

Synchronizing rates of ruminal degradation of carbohydrates and protein may have a more pronounced effect in animals having high rates of ruminal passage (e.g., high DMI). Newbold and Rust (1992) observed in batch culture that a temporary restriction of supplies of either N or carbohydrate reduced subsequent bacterial growth rate. However, given the same total supply of nutrients, bacterial

concentrations recovered after 12 h of incubation to concentrations observed prior to restriction of nutrient supplies. This suggests that microbial cells in the rumen are able to handle periods of nutrient shortage. These results were confirmed by the *in vitro* studies of Van Kessel and Russell (1997). However, when midlactation dairy cows were provided diets that varied in rumen degradable OM and CP, or fed at different feeding frequencies, no differences were observed in MCP production or microbial efficiency (Shabi et al., 1998).

The importance of providing a synchronized vs. an unsynchronized supply of N substrates to the mixed ruminal microbial population on ruminal protein and carbohydrate synchrony is unclear. Of particular interest is the identification of factors that affect efficiency of bacterial uptake of ammonia and alpha-amino N. Hristov et al. (1997) investigated the effect of different levels of carbohydrates and simultaneous provision of ammonia and alpha-amino N (AA and peptides) on the utilization of ammonia and alpha-amino N by ruminal microorganism *in vitro*. Rumen inoculum was incubated with five concentrations (0, 1, 5, 15, and 30 g/L) of carbohydrate (75 percent mixed sugars and 25 percent soluble starch) and five N sources (ammonia, free AA, ammonia plus free AA, peptides, and ammonia plus peptides). The ammonia pool in all treatments was labeled with $(^{15}\text{NH}_4)_2\text{SO}_4$. Observations included: (1) increased uptake and incorporation of ammonia into microbial N from all N treatments with increasing carbohydrate level, (2) a preference for rumen microbes to use alpha-amino N as compared to ammonia N, and (3) increased uptake of AA and peptides with added ammonia. It is concluded that the efficiency of use of ammonia and alpha-amino N by rumen microbes is not constant and is influenced by the availability (or balance) of energy, ammonia, and alpha-amino N.

Others have found that higher NSC or RDP in diet DM does not always support greater microbial growth. The extent to which ammonia is captured as MCP is affected by various factors such as diet type, ruminal fermentation characteristics, and DMI. Therefore, it should not be surprising that several studies conducted to evaluate the effect of synchronizing carbohydrate and protein degradation in the rumen observed no effects on MCP synthesis, efficiency of MCP synthesis, or no carbohydrate by protein interaction effects on MCP passage (Casper et al., 1999; Cecava et al., 1991; Feng et al., 1993; Hussein et al., 1991; McCarthy et al., 1989; Scollan et al., 1996; Stokes et al., 1991b).

The major nutrients required by rumen microbes are carbohydrates and proteins, but the most suitable sources and quantities needed to support maximum growth have not been determined. Although peptides, AA, and ammonia all may serve individually as sources of N for mixed ruminal microbes, the total population achieves the highest

growth rate on mixtures of all three sources. Based on data from both *in vitro* and *in vivo* studies, there is general agreement that rate of digestion of carbohydrates is the major factor controlling the energy available for microbial growth (Hoover and Stokes, 1991).

It is possible to alter the synchronization of protein and carbohydrate, either by changing dietary ingredients or by altering the relative times of feeding ingredients (Shabi et al., 1998). However it is not possible to identify whether an increase in MCP synthesis by feeding different ingredients (Herrera-Saldana et al., 1990; Aldrich et al., 1993a; Sinclair et al., 1993, 1995) is an effect of synchrony or a factor associated with the manipulation of the ingredients (level and type) themselves (Dewhurst et al., 2000).

In summary, it is well documented that the kinetics of carbohydrate and protein degradation varies widely according to feed source, its chemical composition, and method of processing. The available literature indicates that when rumen fermentation is normal, there is little additional benefit of altering carbohydrate and protein degradation rates, or their level of synchrony, on microbial protein synthesis.

Ruminally Protected Proteins

"Rumen protected" has been defined by the Association of American Feed Control Officials (Noel, 2000) as "a nutrient(s) fed in such a form that provides an increase in the flow of that nutrient(s), unchanged, to the abomasum, yet is available to the animal in the intestine." Thus, rumen protected proteins are protein-containing feeds that have been treated or processed in ways to decrease ruminal protein degradability and increase the content of digestible RUP. Most research has focused on oilseeds and oilseed meals. Rumen protected proteins, as well as protein supplements that have an inherent high rate of ruminal escape, are important in dairy cattle nutrition because of the low content of digestible RUP in most feedstuffs. Reliance on feed proteins with a high content of digestible RUP is greatest in high producing cows when most or all of the forage is provided by high quality grasses and legumes. In these situations, the basal diet often contains adequate or more than adequate amounts of RDP but is deficient in RUP. Thus, protein supplementation should be limited to high RUP-containing feedstuffs to avoid large excesses of RDP.

Many methods have been investigated to decrease the rate and extent of ruminal degradation of feed proteins. Most of the methods have involved the use of heat, chemical agents, or a combination of heat and chemical agents (Kaufmann and Lüpping, 1982; Satter, 1986; Broderick et al., 1991; Schwab, 1995). The challenge has been to identify treatments or processing conditions that increase digestible RUP to an extent that justifies the cost of the treatment,

and in the case of the first three methods, with minimal loss of AA.

Heat processing is the most used treatment in North America. Heat processing decreases rumen protein degradability by denaturation of proteins and by the formation of protein-carbohydrate (Maillard reactions) and protein-protein cross-links. Commercial methods that rely solely on heat (dry or in combination with added moisture) include cooker-expeller processing of oilseeds, additional heat treatment of solvent extracted oilseed meals, roasting, extrusion, pressure toasting, and micronization of legume seeds, and expander treatment of cereal grains and protein supplements. Studies of ruminal degradation of protein of heat processed feedstuffs using the in situ approach indicate reductions in fraction A, increases in fractions B and C, and decreases in the fractional rates of degradation of the B fraction (Goelema et al., 1999; Prestløkken, 1999; Wang et al., 1999).

Careful control of heating conditions is required to optimize the content of digestible RUP (Schwab, 1995a). Under-heating results in only a small increase in digestible RUP. Over-heating of feeds (i.e., heat-damaged protein) reduces the intestinal digestibility of RUP through the formation of indigestible Maillard products and protein complexes (Van Soest, 1994). Over-heating also causes significant absolute losses of lysine, cystine, and arginine (Parsons et al., 1992; Barneveld et al., 1994a; Dale, 1996). Among those AA, lysine is the most sensitive to heat damage and undergoes both destruction and decreased availability (Weiss et al. 1986a,b; Barneveld et al., 1994b,c; Nakamura et al., 1994b). Optimal conditions of heat processing are generally considered to be those which significantly decrease ruminal protein degradability without adverse effects on postruminal digestion or significant losses of AA. However, combined measurements of RUP with measurements (or estimates) of intestinal-available lysine in RUP indicates that some loss of chemically determined available lysine is needed to achieve the heat treatment of oilseeds and oilseed meals that maximizes postruminal available lysine (Broderick and Craig, 1980; Craig and Broderick, 1981; Faldet et al., 1991; Faldet et al., 1992a,b). The relationships between heat input and concentrations of RDP, RUP, indigestible RUP, and digestible RUP have been described (Satter, 1986).

Chemical treatment of feed proteins can be divided into three categories: (1) chemicals that combine with and introduce cross-links in proteins (e.g., aldehydes), (2) chemicals that alter protein structure by denaturation (e.g., acids, alkalis, and ethanol), and (3) chemicals that bind to proteins but with little or no alteration of protein structure (e.g., tannins) (Broderick et al., 1991; Schwab, 1995a). For a variety of reasons, often including less than desired levels of effectiveness, use of chemical agents as the sole treatment for increasing the RUP content

of feed proteins has not received commercial acceptance. A more effective approach involving "chemical" agents has been to combine chemical and heat treatments. An example of this approach is the addition of lignosulfonate, a byproduct of the wood pulp industry that contains a variety of sugars (mainly xylose), to oilseed meals before heat treatment. The combined treatments enhance nonenzymatic browning (Maillard reactions) because of the enhanced availability of sugar aldehydes that can react with protein (Broderick et al., 1991; Schwab, 1995a).

Successful use of rumen protected proteins and other proteins that have a high ruminal escape requires consideration of AA composition and knowledge of the content and intestinal digestibility of the RUP fraction.

Predicting Passage of Microbial Protein

Ruminally synthesized microbial protein typically supplies a majority of the AA flowing to the small intestine of growing cattle (Titgemeyer and Merchen, 1990b) and dairy cows (Clark et al., 1992). Microbial protein is the protein of the ruminal bacteria, protozoa, and fungi that pass to the small intestine. Bacteria provide most of the microbial protein leaving the rumen. Protozoa contribute significantly to the microbial biomass of ruminal contents. However, because they are more extensively recycled in the rumen than bacteria (Ffoulkes and Leng, 1988; Leng et al., 1986; Punia et al., 1992), protozoa do not contribute to postruminal protein supply in proportion to their contributions to the total microbial biomass in the rumen.

In the 1989 *Nutrient Requirements of Dairy Cattle* publication, bacterial crude protein production (BCP) in lactating dairy cows was predicted from net energy intake using the equation: $BCP = 6.25 (-30.93 + 11.45 NE_L)$. For growing animals, BCP was predicted from TDN intake using the equation: $BCP = 6.25 (-31.86 + 26.12 TDN)$. These equations were adapted from the 1985 National Research Council's report *Ruminant Nitrogen Usage*.

The most recent *Nutrient Requirements of Beef Cattle* report (National Research Council, 1996) adopted two different strategies in predicting microbial protein production in the rumen. In Level I of the beef model (National Research Council, 1996), BCP was estimated to be 130 grams per kilogram TDN intake with a downward adjustment for diets containing less than 40 percent forage, an unlikely circumstance for growing dairy heifers. Level II of the beef model (National Research Council, 1996) used an adaptation of the Cornell Net Carbohydrate and Protein System to predict BCP in both growing and mature beef cattle.

Using the range in TDN requirements for growing heifers from Table 6-2 in *Nutrient Requirements of Dairy Cattle* (1989), TDN intake would range from 1.82 to 8.80 kg/day. The implied range in BCP production per unit of

TDN would be 53 to 140 g BCP/kg of TDN. The calculated variation in microbial efficiency is due to the negative intercept in the original 1985 National Research Council equation (National Research Council, 1985). The adjustment to a constant 130 g BCP/kg of TDN presented in *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) appears more reasonable. Burroughs et al. (1974) proposed a value of 104.4 for microbial amino acids. Assuming 80 percent microbial amino acids in microbial N, this would correspond to a factor of 130.5 (104.4/0.8) for MCP. However, validation of this was nearly impossible because of the lack of reported data specific to growing dairy heifers in the literature. There are considerable data in the beef cattle literature but unfortunately, most of these reports were in animals fed high concentrate diets that would be atypical of those fed to growing replacement heifers and bulls.

There is a wealth of published data on MCP production, particularly in lactating dairy cows at high feed intakes, which has been published since the 1985 National Research Council's report on *Ruminant Nitrogen Usage*. Several methods were considered for predicting MCP production in the lactating dairy cow. Figure 5-3 shows the relationship between NE_L intake and microbial N flows using a data set (Table 5-4) consisting of 334 treatment means from published literature since 1985 and collected from lactating and dry cows. Superimposed on Figure 5-3 is a prediction line using the 1989 lactating dairy cow equation. Although the previous edition of *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) equation performed reasonably well at intakes of less than 30 Mcal of NE_L , microbial N flow was consistently

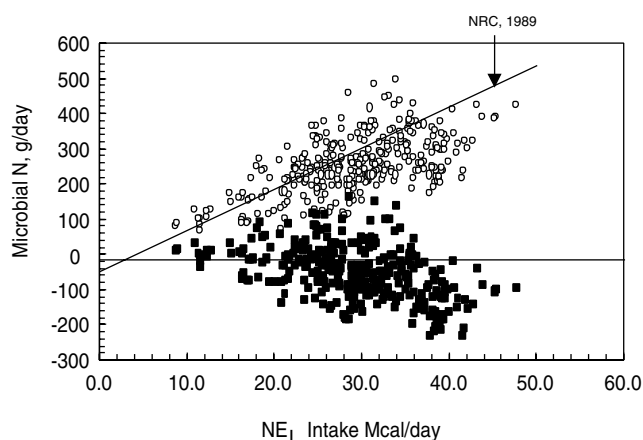


FIGURE 5-3 Plot of observed (open circles) and residuals (squares) for measured microbial N flow (g/day) versus estimated NE_L intake in lactating and dry dairy cows. The National Research Council, 1989, line is the predicted line where microbial N = $-30.93 + 11.45 NE_L$. At high levels of NE_L intake, microbial N production is over-predicted.

over-predicted at high NE_L intakes which are more common in today's higher producing cows. The 1985 equation was based on cows fed NE_L intakes ranging from 5 to 29 Mcal/day. The maximal NE_L intake in that data set is equivalent to only about 3 times maintenance intake for a 600 kg dairy cow. To overcome this problem, the literature data set (Table 5-4) was used to develop new microbial N prediction equations.

Several different prediction variables were evaluated including both linear and quadratic effects of DM, OM, and NE_L intakes. Although addition of quadratic terms did correct for over prediction at high feed intake, the standard error of prediction for individual treatment means was high (61 g N) and no regression equation had an r^2 of more than 0.39. Alternatively, equations used in Level II of the beef model (National Research Council, 1996) were tested on a smaller subset of data with similar results where microbial N flow was again over-predicted at high feed intake with no improvement in overall prediction error. Measured rumen fermentable OM obtained from the literature data set was an even poorer predictor of microbial N with a standard error of prediction of 67 g N.

Within the literature data set (Table 5-4), there was a large range in measured efficiencies of microbial protein synthesis (12-54 g microbial N/kg rumen fermented OM). The wide range in measured efficiencies of microbial protein synthesis explains why fermented OM was a poor predictor of microbial N passage to the duodenum. Because of the variability in efficiency of microbial protein synthesis, it was concluded that systems driven by fermented energy alone or by indirect indicators of fermented energy such as TDN or NE_L would not be accurate enough to predict passage of microbial N to the duodenum unless at least some of the variability was accounted for in efficiency of microbial protein synthesis.

An important factor affecting efficiency of microbial protein synthesis is the relative availability of N for fermentation. Apparent ruminal N balance is an indirect indicator of N availability for microbial protein synthesis. Where balance is positive, N from dietary RDP is in excess of N captured as microbial N and there is a net loss of N from the rumen to the animal tissues. Where apparent ruminal N balance is negative, there is a net gain of N in the rumen indicating inadequate N from RDP for microbial protein synthesis and a net gain from recycling of N from the animal tissues to the rumen. Figure 5-4 shows the relationship between observed microbial efficiency and apparent ruminal N balance using the literature data set where the microbial efficiency (g microbial N/kg truly fermented OM) was equal to $29.74 - 0.30 ARND$ ($r^2 = 0.41$, $SEy = 6.5$). The equation suggests a microbial efficiency of 29.74 g N/kg truly fermented OM at an apparent ruminal N digestibility of zero.

TABLE 5-4 Studies Used to Determine the Relationship Between NE_L Intake and Passage of Microbial Protein to the Small Intestine of Lactating Dairy Cows

Aldrich et al. (1993b)	Klumsmeier et al. (1991b)	Robinson and Sniffen (1985)
Arieli et al. (1993)	Klumsmeier et al. (1990)	Robinson et al. (1991a)
Armentano et al. (1986)	Kung et al. (1983)	Robinson et al. (1997)
Benchaa et al. (1994a)	Lu et al. (1988)	Robinson et al. (1994)
Benchaa et al. (1991)	Lykos et al. (1997)	Robinson et al. (1985)
Benchaa et al. (1994b)	Lynch et al. (1991)	Rode and Satter (1988)
Blauwiekel et al. (1997)	Mabjeesh et al. (1996)	Rode et al. (1985)
Calsamiglia et al. (1995b)	Mabjeesh et al. (1997)	Santos et al. (1984)
Cameron et al. (1991)	Madsen (1986)	Sarwar et al. (1991)
Chan et al. (1997)	Mansfield and Stern (1994)	Schwab et al. (1992a)
Christensen et al. (1993b)	McCarthy et al. (1989)	Schwab et al. (1992b)
Christensen et al. (1996)	Merchen and Satter (1983)	Seymour et al. (1992)
Cunningham et al. (1993)	Moller (1985)	Song and Kennelly (1989)
Cunningham et al. (1994)	Murphy et al. (1987)	Stensig and Robinson (1997)
Cunningham et al. (1996)	Narasimhalu et al. (1989)	Stern et al. (1983)
Doreau et al. (1991)	Ohajuruka et al. (1991)	Stern et al. (1985)
Erasmus et al. (1992)	Oldham et al. (1979)	Stokes et al. (1991b)
Erasmus et al. (1994b)	Oliveira et al. (1995)	Tamminga et al. (1979)
Espindola et al. (1997)	O'Mara et al. (1997b)	Teller et al. (1992)
Feng et al. (1993)	Overton et al. (1995)	Tice et al. (1993)
Herrera-Saldana et al. (1990)	Palmquist et al. (1993)	van Vuuren et al. (1992)
Holden et al. (1994a)	Pantoja et al. (1995)	Waltz et al. (1989)
Joy et al. (1997)	Pantoja et al. (1994)	Weisbjerg et al. (1992)
Kalscheur et al. (1997a)	Pena et al. (1986)	Windschitl and Stern (1988)
Kalscheur et al. (1997b)	Pires et al. (1997)	Yang et al. (1997)
Khorasani et al. (1996a)	Poore et al. (1993)	Yoon and Stern (1996)
King et al. (1990)	Prange et al. (1984)	Zerbini et al. (1988)
Klumsmeier et al. (1991a)	Putnam et al. (1997)	Zhu et al. (1997)

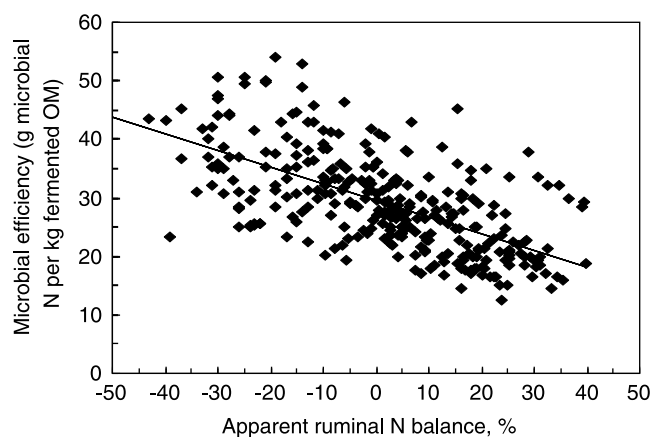


FIGURE 5-4 Relationship between measured efficiency of microbial protein synthesis (g microbial N/kg rumen fermented OM) and apparent ruminal N balance (microbial efficiency = $29.74 - 0.30$ apparent ruminal N digestibility percent, $r^2 = 0.41$, $P < 0.001$, $S_y = 6.49$, $n = 306$).

The *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) report assumed a net recycling of 15 percent of dietary N intake or an apparent ruminal N balance of -15 percent. The average apparent ruminal N balance in the literature data set was plus 1.0 percent suggesting that on average net recycling of N to the rumen was zero. If under practical circumstances, ruminal N balance ranges from $+20$ to -20 percent, efficiency of microbial protein synthesis would vary from 24 to 36 g N/kg of

OM fermented in the rumen and would have a major impact on estimated microbial protein production.

The implication is that as availability of N increases in relation to fermented OM, efficiency of microbial protein synthesis decreases. If ruminal N availability is relatively high compared to fermented OM, then output of microbial N per unit of fermented OM decreases, indicating that microbial utilization of N and energy becomes uncoupled and energy utilization for microbial protein synthesis becomes less efficient because the excess N is not used by the rumen microbes (Clark et al., 1992). Systems for predicting microbial N production as fixed linear functions are likely to over predict microbial protein production, particularly at high intakes of ruminally fermented OM. This would be true regardless of whether microbial N was predicted directly from ruminally fermented OM or indirectly using total tract digestible OM (TTDOM) intake or energy intake as an indicator of ruminally fermented OM.

The 1989 *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) report assumed an efficiency of use of apparent ruminally degraded N (RDP) of 0.9. If N recycling is set to zero, then net RDP required would be $1.11 \times$ microbial N. The mean RDP to microbial N ratio (RDP:MN) in the data set was 1.18 or about 1.2. Although deficits in RDP for microbial N synthesis can be made up through N recycling, the impact of low RDP availability on rumen fermentation is not well understood

nor could it be defined using the current literature data set. Therefore, the mean RDP to microbial N ratio of 1.18 was used to define RDP requirements assuming an apparent ruminal N balance of zero.

Ruminally fermented OM is not practical to use as a direct index of available energy for microbial growth as there are not adequate means by which rumen fermentability of an individual feedstuff or diet can be predicted. Previously cited techniques for predicting TDN offered a more practical indirect indicator of ruminally fermented OM. This is similar to the use of NE_L intake in *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) publication. In a summary of experiments with dairy cows fed diets containing as much as 7 percent of added dietary fat, rumen fermentability of the diet was reduced by an amount equivalent to the amount of fat added to the diet and total microbial N production was unaffected (Erdman, 1995). Because the increase in efficiency of microbial protein synthesis was due to a reduction in fermented OM and not an increase in microbial N synthesis, TTDOM was used as an indirect indicator of fermentable energy. This can be calculated by adjusting the contribution of fat to TDN by a factor of 1.25 where: $TTDOM = TDN - [(EE - 1) \times 1.25]$. The factor of 1.25 corresponds to the increase in energy content of absorbed ether extract (EE) versus other dietary components and EE is adjusted downward to account for the 1 percent dietary EE of non-fatty acid origin.

To correct for differences in microbial efficiency due to availability of RDN in relation to microbial N, the microbial efficiency values were adjusted in the literature data set using the equation ($\text{g microbial N/kg of TTDOM} = 32.78 - 8.29 \text{ RDN:MN}$, $r^2 = 0.35$, $P < 0.001$, $S_y = 4.8$, $n = 270$). The microbial N yields adjusted to a common RDN:MN availability of 1.2 were then regressed against TTDOM. The results are shown in Figure 5-5.

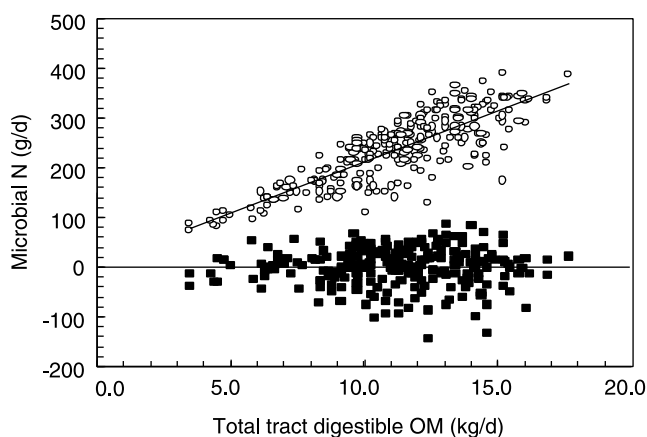


FIGURE 5-5 Plot of adjusted (open circles) and residuals (squares) for measured microbial N (g/d) versus measured total tract digestible OM (kg/d). (Microbial N = 21.03 total tract digestible OM. $r^2 = 0.69$, $P < 0.001$, $S_y = 38.1$, $n = 266$).

Microbial N flow corrected to 1.2 RDN:MN was related linearly to TTDOM at all levels of TTDOM intakes. This was also true for the relationship with both NE_L and TDN intake. Calculated intercepts were not different from zero and regression coefficients using zero intercepts were 21.03, 20.32, and 8.21 g microbial N per kilogram TTDOM, per kilogram TDN, and per Mcal NE_L , respectively. Each equation had a standard error of prediction of 38 g. If coefficients were converted to a microbial CP basis ($N \times 6.25$), corresponding coefficients would be 131, 127, and 51 g respectively. The coefficient (127) for TDN is identical to the adapted Burrough's value (130.5) and the value (130) used in Level I of the *Nutrient Requirements of Beef Cattle* report (National Research Council, 1996) suggesting that a common value (130) could be used for both growing animals and lactating dairy cows. In this volume, 130 g of microbial CP/kg discounted TDN is used to estimate microbial protein synthesis. Because there is no intercept in these equations, the microbial protein and net absorbed protein values can be assigned to individual feeds, which was not possible in the *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) report.

In summary, it is assumed that the yield of MCP is 130 g/kg of TDN (discounted) intake and that the requirement for RDP is $1.18 \times \text{MCP yield}$. Therefore, yield of MCP is calculated as $0.130 \times \text{TDN (discounted TDN, see Chapter 2)}$ when RDP intake exceeds $1.18 \times \text{MCP yield}$. When RDP intake is less than $1.18 \times \text{TDN-predicted MCP}$, then MCP yield is calculated as 0.85 of RDP intake ($1.00/1.18 = 0.85$).

Predicting Passage of Rumen Undegradable Feed Protein

The values for RUP reported in the previous edition of *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) were based on in vivo and in situ estimates from cattle and sheep and in many cases represented few observations. Subsequent to the *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) publication, a wealth of data has been published that have provided estimates of RUP concentrations in feedstuffs. Approaches have included in vivo, in situ, and in vitro (enzymatic, inhibitor, nitrogen solubility and protein fractionation, continuous culture fermentation, gel electrophoresis, and near-infrared reflectance spectroscopy) techniques (Hoffman et al., 1999; Michalet-Doreau and Ould-Bah, 1992; Nocek, 1988; Stern et al., 1997). Although often used as the standard by which other methods are evaluated, the in vivo approach requires the use of cannulated animals and, therefore, is subject to errors associated with cannula placement and the use of microbial and digesta flow markers.

The in situ procedure has emerged as the most widely used approach for estimating RUP (Stern et al., 1997) and

is used in this edition. The procedure has been modified and adopted in several countries (Lindberg, 1985; Michalet-Doreau and Ould-Bah, 1992; Nocek, 1988; Stern et al., 1997; Vanzant et al., 1998). Adherence to guidelines for standardizing factors known to affect the results (Michalet-Doreau and Ould-Bah, 1992; Nocek, 1988; Stern et al., 1997) have increased considerably the reproducibility of the measurements within and among laboratories.

As described in the section "Kinetics of Ruminal Protein Degradation", the in situ procedure can be used to identify and quantify at least three N fractions which commonly are referred to as the A, B, and C fractions, and the rate of degradation (Kd) of fraction B. Fraction A includes NPN, rapidly solubilized protein, and protein in particles of smaller size than the porosity of the Dacron polyester or nylon bags into which the feedstuff is placed during incubation in the rumen. The different forms of N in fraction A cannot be separated by using the in situ procedure, nor can the rate be determined at which fraction A is degraded. Fraction C is estimated by a defined end-point of degradation, which corresponds to the lowest percent residual beyond which no further ruminal degradation occurs (Nocek and English, 1986). Different approaches have been described to combine estimates of the Kd of fraction B with rates of passage (Kp) from the rumen to estimate RUP (see Michalet-Doreau and Ould-Bah, 1992; Stern et al., 1997; and Bach et al., 1998, for review). The portion of fraction B determined not to be degraded, plus fraction C, is assumed to be RUP. Important assumptions with the in situ method are that "disappearance" from the bag is synonymous with degradation and that any N that has disappeared from the bag, including N associated with rapidly degradable proteins that are likely to be hydrolyzed as peptides (Broderick and Wallace, 1988), has been degraded and can be used by ruminal microorganisms.

In situ data from 190 cattle experiments were reviewed. The experiments involved 1326 individual feedstuff observations. Most of the publications were published between 1988 and 1998. Experiments involving sheep were not used because rumen degradation kinetics have been shown to differ between sheep and cows (Sebek and Everts, 1999; Siddons and Paradine, 1983; Prigge et al., 1984; Uden and Van Soest, 1984). Rarely were all three fractions reported, and sometimes Kd was not reported. In cases of incomplete information, the data were discarded unless enough information was provided to solve for the missing parameter by using either of the two equations, $RDP = A + B[Kd/(Kd + Kp)]$ or $RUP = B[Kp/(Kp + Kd)] + C$. For observations in which no C fraction was reported, but the sum of the A and B fractions was less than 100, the residual was considered to be the C fraction. In the majority of observations where the protein fractions and Kd were esti-

mated by using the model of Ørskov and McDonald (1979), or the linear approach of Mathers and Miller (1981), the sum of the A and B fractions equaled 100 (i.e., B and C were "lumped" together and Kd was for the "B + C" fractions). In general, those data were considered acceptable if a small to negligible C fraction could be expected (e.g., most energy feeds, unprocessed oil-seeds, or unprocessed oil-seed meals). However, for forages or for feedstuffs that were heat processed, or feedstuffs where a moderate to large C fraction could be expected (e.g., blood meal, corn gluten meal), if the sum of the A and B fractions equaled 100, then those data were not used. In situations in which an assumed value for Kp was needed to calculate RDP, RUP, or a missing N fraction, an assumed rate of 5 %/h was used. If needed and not reported, RDP was calculated as $100 - RUP$ and RUP was calculated as $100 - RDP$. Some authors included a lag term for model-fitting procedures. However, lag was not considered for purposes of solving for missing information.

Of the total 1326 feedstuff observations, 801 observations from 170 experiments (Table 5-5) were considered acceptable for inclusion into the feed library (Tables 15-2a,b). Most of the rejected data were of feedstuffs that were either experimental in nature or uncommon to North America. Other reasons for not accepting data included clear deviations from recommended procedures, reported estimates of protein fractions that exceeded 100% of CP, or no reported C fraction when one would be expected.

A number of diet-related factors such as ruminal pH, frequency of feeding, particle size, and Kp can affect the estimates of Kd (see reviews by Lindberg, 1985; Michalet-Doreau and Ould-Bah, 1992; Nocek, 1988; Vanzant et al., 1998). However, sufficient data were not available to allow for more than one set of Kd values to be summarized for those factors. The RDP or RUP fraction of CP can be calculated for each feedstuff by the two equations:

$$RDP = A + B[Kd/(Kd + Kp)] \quad (5-1)$$

where:

$$\begin{aligned} RDP &= \text{RDP of the feedstuff, percentage of CP} \\ A &= \text{Fraction A, percentage of CP} \\ B &= \text{Fraction B, percentage of CP} \\ Kd &= \text{rate of degradation of the B fraction, \% / h} \\ Kp &= \text{rate of passage from the rumen, \% / h} \end{aligned}$$

$$RUP = B[Kp/(Kd + Kp)] + C \quad (5-2)$$

where:

$$\begin{aligned} RUP &= \text{RUP of the feedstuff, percentage of CP} \\ B &= \text{Fraction B, percentage of CP} \\ Kd &= \text{rate of degradation of the B fraction, \% / h} \\ Kp &= \text{rate of passage from the rumen, \% / h} \\ C &= \text{Fraction C, percentage of CP} \end{aligned}$$

The sum of RDP plus RUP equals 100%.

TABLE 5-5 Studies Reporting In Situ Determined Estimates of N Fractions and Rates of Protein Degradations That Were Used in Preparing This Edition

Akayezu et al. (1997)	Herrera-Saldana et al. (1990)	Robinson et al. (1991a)
Aldrich et al. (1996)	Hoffman et al. (1993)	Robinson et al. (1991b)
Alexandrov (1998)	Hongerholt and Muller (1998)	Robinson and Kennelly (1988a)
Antoniewicz et al. (1995)	Hristov (1998)	Robinson and Kennelly (1988b)
Arieli and Adin (1994)	Hristov and Sandev (1998)	Robinson and McNiven (1993)
Arieli et al. (1989)	Ibrahim et al. (1995)	Robinson and McNiven (1994)
Arieli et al. (1995)	Janicki and Stallings (1988)	Robinson and McQueen (1994)
Armentano et al. (1997)	Jones-Endsley et al. (1997)	Romagnolo et al. (1994)
Armentano et al. (1993)	Keady and Steen (1996)	Rooke et al. (1985)
Armentano et al. (1983)	Keady et al. (1994)	Schroeder et al. (1996)
Armentano et al. (1986)	Kenelly et al. (1988)	Seymour and Polan (1986)
Balde et al. (1993)	Khalili et al. (1994)	Sicilano-Jones, J. L., Personal communication.
Barney, N. C., Personal communication.	Khalili et al. (1992)	Sievert and Shaver (1993)
Batajoo and Shaver (1998)	Khorasani et al. (1996a)	Singh et al. (1995)
Beauchemin et al. (1997)	Khorasani et al. (1994a, b)	Song and Kennelly (1989)
Beckers et al. (1995)	Khorasani et al. (1992)	Stallings et al. (1991)
Beever et al. (1986)	Khorasani et al. (1993)	Stanford et al. (1996)
Ben Salem et al. (1993)	Kibelolaud et al. (1993)	Steg et al. (1994)
Berzaghi et al. (1997)	Kirkpatrick and Kennelly (1987)	Stutts et al. (1988)
Bohnert et al. (1998)	Klover et al. (1998)	Subuh et al. (1994)
Boila and Ingalls (1992)	Kowalski et al. (1997)	Susmel et al. (1993)
Boila and Ingalls (1994)	Lehman et al. (1995)	Susmel et al. (1991)
Brown and Pate (1997)	Lu et al. (1988)	Susmel et al. (1990)
Calsamiglia et al. (1995b)	Lykos and Varga (1995)	Tammenga et al. (1991)
Carey et al. (1993)	Maiga et al. (1997)	Valentine and Bartsch (1988)
Caton et al. (1994)	Makoni et al. (1991)	van der Aar et al. (1984)
Cecava, M. J., Personal communication.	Manyuchi et al. (1992)	van der Koelan et al. (1992)
Coblentz et al. (1999)	Marshall et al. (1993)	Vanhatalo et al. (1995)
Coblentz et al. (1997)	McKinnon et al. (1995)	van Vuuren et al. (1989)
Coblentz et al. (1998)	McNiven et al. (1994)	van Vuuren et al. (1992)
Cody et al. (1990)	Michalet-Doreau and Cerneau (1991)	van Vuuren et al. (1991)
Cozzi et al. (1995)	Michalet-Doreau and Nozière (1998)	van Vuuren et al. (1993)
Cozzi et al. (1993)	Michalet-Doreau and Ould-Bah (1992)	Vanzant et al. (1996)
Cozzi and Polan (1994)	Mir et al. (1993)	Varvikko and Vanhatalo (1992)
Cushnahan et al. (1995)	Mir et al. (1992)	Vasquez-Anon et al. (1993)
Dawson and Mayne (1997)	Mupeta et al. (1997)	Vieira et al. (1997)
Dawson and Mayne (1998)	Murphy and Kennelly (1987)	Vik-Mo (1989)
Deacon et al. (1988)	Murphy et al. (1993)	von Keyserlingk and Mathison (1993)
Deacon et al. (1988)	Mustafa et al. (1996)	von Keyserlingk and Mathison (1989)
Denham et al. (1989)	Mustafa et al. (1997)	von Keyserlingk et al. (1996)
DePeters and Bath (1986)	Napoli and Santini (1989)	Walhain et al. (1992)
DeVisser et al. (1998)	Negi et al. (1988)	Waltz and Stern (1989)
England et al. (1997)	Nocek et al. (1979)	Waltz et al. (1989)
Erasmus (1993)	Nocek and Grant (1987)	Wanderly et al. (1999)
Erdman et al. (1986)	Olson et al. (1994)	Wang et al. (1997)
Erdman and Vandersall (1983)	O'Mara et al. (1997a, b)	Wattiaux et al. (1994)
Erdman et al. (1987)	O'Mara et al. (1998)	Wen-Shyg et al. (1995)
Erickson et al. (1986)	Petit et al. (1994)	Windschitl and Stern (1988)
Faldet et al. (1991)	Petit and Tremblay (1992)	Xu et al. (1996)
Ganesh and Grieve (1990)	Peyraud et al. (1997)	Yan et al. (1998)
Givens et al. (1997)	Piepenbrink and Schingoethe (1998)	Yang et al. (1997)
Goelema et al. (1998)	Pires et al. (1997)	Yang et al. (1996)
Gordon and Peoples (1986)	Polan et al. (1997)	Yang et al. (1999)
Grings et al. (1991)	Polan et al. (1998)	Yong-Gang et al. (1994)
Grings et al. (1992a)	Powers et al. (1995)	Yoon et al. (1996)
Grings et al. (1992b)	Prakash et al. (1996)	Zerbini and Polan (1985)
Ha and Kennelly (1984)	Rioux et al. (1995)	

The use of the equations presented above requires for each feedstuff an estimate of the rate of passage (K_p) from

the rumen. For the purpose of developing equations that would predict rates of passage, 275 experiments were

reviewed in which estimates of K_p were reported for a variety of feedstuffs. Three equations were developed and have been adopted for use in this publication:

Equation for estimating K_p of wet forages (i.e., silages and fresh forages)

$$K_p = 3.054 + 0.614X_1$$

where:

K_p = rate of passage from the rumen, %/h

X_1 = DMI, percentage of BW

Equation for estimating K_p of dry forages

$$K_p = 3.362 + 0.479X_1 - 0.007X_2 - 0.017X_3$$

where:

K_p = rate of passage from the rumen, %/h

X_1 = DMI, percentage of BW

X_2 = concentrate, percentage of diet DM

X_3 = NDF of feedstuff, percentage of DM

Equation for estimating K_p of concentrates

$$K_p = 2.904 + 1.375X_1 - 0.020X_2$$

where:

K_p = rate of passage from the rumen, %/h

X_1 = DMI, percentage of BW

X_2 = concentrate, percentage of diet DM

The equations were derived from experiments in which rare earth elements were used as K_p markers. Studies involving Cr-mordanted feeds and Cr-mordanted NDF were not used to estimate K_p of feeds. No significant independent variables could be identified for predicting K_p of concentrates when the data set included these studies. The subcommittee recognized that intrinsic properties of feedstuffs, such as particle size and density, functional specific gravity, and processing of grains are not considered by the equations. Those factors, in addition to others (e.g., ruminal pH, feeding frequency, and use of ionophores) (see reviews by Owens and Goetsch, 1986 and Firkins et al., 1998), could not be considered because data are too sparse to make adjustments for those factors. Nonetheless, data from which the equations were developed for estimating K_p are diverse with respect to DMI (2.7 to 26.8 kg/d), body weight (120 to 745 kg), DMI as percentage of body weight (0.8 to 4.4%), concentrate in dietary DM (0 to 85%), and represent estimates of K_p obtained in growing, lactating, and nonlactating cattle.

Standardized methods have been proposed (AFRC, 1992; Lindberg, 1985; Madsen et al., 1995; Michalet-Doreau and Ould-Bah, 1992; Nocek, 1988; Ørskov, 1982; Vanzant et al., 1998; Wilkerson et al., 1995) for the in situ procedure of estimating RUP of feedstuffs. Those reviews agree generally about most procedural aspects, but the

committee deemed it necessary to augment the recommendations in those reviews to foster a more complete reporting of data such that future summaries possibly may account for factors (e.g., ruminal pH, DMI) that may affect estimates of Kd. The recommendations by the committee are shown in Table 5-6.

The committee encourages the development and acceptance of an alternative method for quantifying N fractions and Kd that can be adopted by commercial feed testing laboratories for estimating RUP of feedstuffs. Chemical approaches are the most attractive for quantifying N fractions in feedstuffs because those procedures can be performed under routine laboratory conditions. The most sophisticated approach described to date is the use of the detergent system developed by Goering and Van Soest (1970) for analysis of carbohydrates in conjunction with extraction with borate phosphate buffer (Krisnamoorthy et al., 1982; Fox et al., 1990; Chalupa et al., 1991; Sniffen et al., 1992). As discussed previously, this method partitions CP into five fractions (A, B1, B2, B3, and C) according to rates of ruminal degradation and is the method that is used in the CNCPS (Sniffen et al., 1992). Protein degradability is calculated on the basis of pool size and rates of degradation of protein fractions in combination with ruminal passage rate.

Digestibility of Rumen Undegradable Feed Protein

The previous edition of *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) recognized that intestinal digestion of feed proteins may differ. However, because of the lack of sufficient data at the time, a constant digestibility value of 80 percent was used for RUP of all feedstuffs. This value was selected because it approximated the average calculated true absorption of both nonammonia-N and RUP as measured in vivo (see Tables 13 and 14 in *Nutrient Requirements of Dairy Cattle* 1989 report). The current edition of *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) also assumes that all RUP is 80 percent digestible.

Other feeding standards have attempted to account for differences in RUP digestibility among feedstuffs. However, the approaches have differed. For example, it is assumed in the UK Metabolizable Protein System (Webster, 1987) that acid detergent insoluble nitrogen (ADIN) is both undegradable in the rumen and indigestible in the small intestine. The equation of Webster et al. (1984) was adopted in that publication to predict digestible RUP from ADIN values [$\text{g/kg DM} = 0.90 (\text{RUP N-ADIN})/\text{RUP N}$]. However, more recent data raise concerns about the appropriateness of using ADIN to predict RUP digestibility. Although a good relationship between ADIN and N indigestibility has been demonstrated for most forages (Goer-

TABLE 5-6 Recommended Procedures and Reporting Details for a Standardized In Situ Procedure for Measuring Ruminal Degradability of Protein in Dairy Cattle^a

Item	Recommendation
<i>Diet</i>	
Type	Similar to that of desired application. Report ingredient and chemical composition (minimum of DM, CP, NDF, and ash)
Feeding level	Similar to that of desired application; report DMI and ruminal pH
Feeding frequency	2 times/d if not fed for <i>ad libitum</i> DMI
<i>Evaluated feedstuff</i>	
Chemical composition	Report (minimum) DM, CP, NDF, and ash
Physical characteristics	Report specifics about processing of feedstuffs (e.g., steam-flaked, 0.39 kg/L; heated, 150 °C, 3 h)
Sample processing	2-mm screen size (Wiley mill)
<i>Bag</i>	
Material	Polyester
Pore size	40 to 60 μ
<i>Incubation procedure</i>	
Number of animals	2; report BW
Number of days	2
Number of replications	1
Presoaking	Recommended
Ruminal position	Ventral rumen
Insertion/removal	Remove simultaneously
Incubation times, h	0, 2, 4, 8, 16, 24, and 48 (include 72 for forages). Report time zero washout so a lag time can be calculated.
Rinsing	Machine (5 times at 1 min/rinse)
Standard substrate	Recommended
<i>Microbial correction</i>	Required
<i>Mathematic model</i>	Non-linear

^a Adapted and modified from AFRC, 1992; Lindberg, 1985; Madsen et al., 1995; Michalet-Doreau and Ould-Bah, 1992; Nocek, 1988; Ørskov, 1982; Vanzant et al., 1998; Wilkerson et al., 1995.

ing et al., 1972; Yu and Thomas, 1976) and other feeds that were not heat processed (Waters et al., 1992), others have reported that ADIN is partially digestible and that a poor relationship exists between ADIN and N digestibility in nonforage plant protein sources that have been subjected to heat treatment (e.g., Nakamura et al., 1994a; Rogers et al., 1986; Cleale et al., 1987; Weiss et al., 1989; Harty et al., 1998; Waters et al., 1992). In each of the latter studies, the evaluated feedstuffs were distiller's products and other grain-byproducts that had been subjected to sufficient heat and moisture to induce the Maillard reactions and thus have "added" ADIN. These data indicate that much of the ADIN from these products is digestible but it is not clear whether this involves ruminal digestion, postruminal digestion, or both. Nakamura et al. (1994b) confirmed that significant amounts of ADIN in heat-damaged corn gluten meal and distillers grains were digestible but that the absorbed N from the heat-damaged protein was not used for growth by lambs and cattle. Waters et al. (1992) also confirmed the findings of Van Soest et al. (1987) that high tannin feeds bind protein in the gut which appears as ADIN in feces. The result was a high negative mean value (–89 percent) for apparent digestibility of ADIN in digestibility trials with sheep in which diets contained high tannin feeds. In contrast, diets that contained distillers products resulted in high positive values (62 percent) for ADIN digestibility whereas diets consisting only of "conventional"

feeds resulted in a mean digestibility value for ADIN of 2 percent (Waters et al., 1992). Observations such as these indicate that ADIN is probably a useful indicator of non-usable N but that it may not be useful for estimating digestibility of RUP. In the French PDI System (Jarrige, 1989), variable digestibility values for RUP (0.25 to 0.95) are assigned to feedstuffs. Digestibility values were calculated from results of digestibility experiments with sheep using the assumption that the between-feed differences in fecal N excretion per unit of DMI results from indigestible dietary protein.

Other methods for estimating the intestinal digestibility of RUP include in vivo procedures, nonruminant animal bioassay, the in situ mobile nylon bag technique, and in vitro techniques (e.g., lysine availability test and enzymatic methods) (Stern et al., 1997). Although used as the standard by which other methods are evaluated, the in vivo approach requires the use of cannulated animals and is subject to inherent animal variation and errors associated with cannula placement and the use of microbial and digesta flow markers. The most widely used approach for estimating the true intestinal digestibility of the RUP fraction of feedstuffs is the mobile bag technique. Although requiring the need for ruminally and duodenally cannulated animals, the technique is relatively easy and it provides a more direct and physiologic approach than the use of ADIN. Using this method, small amounts of washed,

ruminally undegraded feed residues are placed in bags. The bags are then usually preincubated in a pepsin/HCl solution for 1 to 3 h, inserted into the duodenum of cannulated ruminants, and then recovered either from an ileal cannula or (more typically because of convenience) from the feces. A comparison of ileal and fecal recovery of mobile bags provides similar estimates of RUP digestibility (Beckers et al., 1996; Boila and Ingalls, 1994, 1995; Hvelplund, 1985; Jarosz et al., 1994; Moshtaghi Nia and Ingalls, 1995; Todorov and Griginov, 1991; Vanhatalo and Ketoja, 1995). Recovered bags are washed thoroughly to remove endogenous and other contaminating protein and analyzed for N or AA content. Therefore, estimates of RUP digestibility obtained using this technique are considered to be estimates of true rather than apparent digestibility. Factors that can potentially affect the accuracy of the estimates of intestinal digestibility obtained using the mobile bag technique have been reviewed (Beckers et al., 1996; Stern et al., 1997) and a standardized procedure for its use has been recommended (Madsen et al., 1995). Studies have indicated good correlation between results from fecal collection of bags and in vivo intestinal CP digestion (Hvelplund, 1985; Todorov and Griginov, 1991).

Calsamiglia and Stern (1995) developed a three-step in vitro procedure that provides an alternative to the use of intestinally cannulated ruminants for estimating intestinal digestibility of the RUP fraction of feed proteins. The procedure consists of: (1) incubating ruminally undegraded feed residues for 1 h in 0.1N HCl solution containing 1 g/L of pepsin, (2) neutralizing the mixture with 1N NaOH and a pH 7.8 phosphate buffer containing pancreatin followed by a 24-h incubation, and (3) precipitation of undigested proteins with a 100 percent (wt/vol) trichloroacetic acid solution. Pepsin-pancreatin digestion of protein is calculated as TCA-soluble N divided by the amount of N in the sample (Dacron bag residue) used in the assay. The authors reported an excellent correlation ($r = 0.91$) with in vivo estimates of intestinal CP digestion when using ruminally undegraded feed residues from 16-h ruminal incubations.

To arrive at estimates of RUP digestibility for this publication, 54 studies were summarized (Table 5-7). The mobile bag technique with recovery of the bags from the feces was used in 48 studies and the in vitro procedure of Calsamiglia and Stern (1995) was used in 6 studies. Porosity of bag material used in the mobile bag technique studies ranged from 9 to 53 μm . Comparative data within studies in which the effect of bag pore size on protein digestibility was measured indicated that digestibility tended to increase slightly with increasing pore size. Beckers et al. (1996) obtained digestibility values of 87 and 92 percent, 72 and 75 percent, and 64 and 69 percent for ruminal residues of soybean meal, wheat bran, and meat and bone meal when pore size was 10 and 43 μm , respectively. Hvelplund (1985) obtained values of 95 and 97 percent, 87 and 87 percent,

and 74 and 75 percent for residues of soybean meal, coconut cakes, and rapeseed meal when pore size was 9 and 22 μm . Porosities of 40 to 53 μm were used in all but twelve studies identified for this data set. Mobile bags containing the ruminal residues were preincubated in a pepsin/HCl solution before placement in the duodenum in 75 percent of the studies. Studies not employing pepsin/HCl preincubation were retained in the data set because comparative data in studies that have evaluated the importance of pepsin/HCl preincubation indicate that it is not a necessary step when the mobile bag technique includes preincubation of feeds in the rumen (Vanhatalo et al., 1995; Voigt et al., 1985). For feeds in which data were limited or did not exist, the values reported by Jarriige (1989) in Table 13.3 of *Ruminant Nutrition: Recommended Allowances and Feed Tables* were used. The mean values used in this revision (Tables 15-2a,b) are rounded to the nearest 5 percentage units to emphasize the lack of precision involved in arriving at mean values.

Predicting Passage of Endogenous Protein

Predicted passage of protein to the small intestine in the previous *Nutrient Requirements of Dairy Cattle* publication (National Research Council, 1989) was assumed to originate entirely from ruminally synthesized microbial protein and RUP. However, research indicates that endogenous protein N also contributes to N passage to the duodenum and maybe should be considered in models designed to predict passage of protein to the small intestine. Sources of endogenous protein that may contribute to duodenal protein include: (1) mucoproteins in saliva, (2) epithelial cells from the respiratory tract, (3) cellular debris from the sloughing and abrasion of the epithelial tissue of the mouth, esophagus, and the reticulo-rumen, (4) cellular debris from the sloughing and abrasion of the epithelial tissue of the omasum and abomasum, and (5) enzyme secretions into the abomasum. Significant amounts of the first three sources of endogenous protein probably are degraded by ruminal microorganisms, and therefore do not contribute in their entirety to protein passage to the small intestine.

Attempts to measure passage of endogenous protein N to the small intestine of ruminants are limited because of the difficulty of being able to distinguish endogenous N from microbial N and feed N in duodenal digesta. Several different approaches have been used. One approach has been to measure the flow of nonammonia-N (NAN) through the rumen and abomasum when cows and steers were nourished totally on volatile fatty acids infused into the rumen. Using this approach, Ørskov et al. (1986) obtained mean flows of NAN from the rumen of two non-lactating, pregnant Holstein cows (650 and 700 kg) of 8.3 g/d or 51 mg/kg $\text{BW}^{0.75}$; for two steers (307 and 405 kg), the flows were 5.1 g/d or 58.2 mg/kg $\text{BW}^{0.75}$. Ørskov et al.

TABLE 5-7 Published Studies That Were Summarized for the Purpose of Arriving at Estimates of Intestinal Digestibility of the RUP Fraction of Feedstuffs

Antoniewicz et al. (1992)	Hvelplund et al. (1994)	Prestløkken (1999)
Arieli et al. (1989)	Hvelplund et al. (1991)	Rae and Smithard (1985)
Beckers et al. (1996)	Jarosz et al. (1994)	Rooke (1985)
Boila and Ingalls (1994)	Kendall et al. (1991)	Steg et al. (1994)
Boila and Ingalls (1995)	Kibelolaud et al. (1993)	Todorov and Girginov (1991)
Calsamiglia and Stern (1995)	Kopečný et al. (1998)	Vanhatalo et al. (1995)
Calsamiglia et al. (1995a)	Liu et al. (1994)	Vanhatalo and Ketoja (1995)
Cros et al. (1992a)	Maiga et al. (1996)	Vanhatalo and Varvikko (1995)
Cros et al. (1992b)	Masoero et al. (1994)	Vanhatalo et al. (1996)
Dakowski et al. (1996)	Mhgeni et al. (1994)	van Straalen and Huisman (1991)
Deacon et al. (1988)	Moshtaghi Nia and Ingalls (1992)	van Straalen et al. (1993)
de Boer et al. (1987)	Moshtaghi Nia and Ingalls (1995)	van Straalen et al. (1997)
Erasmus et al. (1994a)	Mupeta et al. (1997)	Varvikko and Vanhatalo (1992)
Frydrych (1992)	Mustafa et al. (1998)	Volden and Harstad (1995)
Goelema et al. (1998)	O'Mara et al. (1997a)	von Keyserlingk et al. (1998)
Hindle et al. (1995)	Palmquist et al. (1993)	Walhain et al. (1992)
Howie et al. (1996)	Pereira et al. (1998)	Wang et al. (1999)
Hvelplund (1985)	Piepenbrink and Schingoethe (1998)	Weisbjerg et al. (1996)

(1986) used the same approach with growing cattle and lambs but measured flows of NAN through both the rumen and abomasum. In this experiment with four steers (240 to 315 kg), they reported flows of total N and NAN through the rumen of 9.9 and 5.8 g/d (145 and 85 mg/kg BW^{0.75}) and flows through the abomasum of 17.0 and 13.4 g/d (248 and 195 mg/kg BW^{0.75}). In lambs (40 to 50 kg), respective flows of N and NAN from the rumen and abomasum were 103 and 76 and 244 and 181 mg/kg BW^{0.75}. In both steers and lambs, the contribution of the omasum and abomasum to the total endogenous N leaving the abomasum was greater than the contributions from the other sources.

A more physiologic approach for obtaining estimates of passage of endogenous N to the small intestine of cattle has been to measure flows of N fractions when diets considered free of rumen digestible protein are fed. In this case, flows of endogenous N are estimated as the difference between the sum of N intake and measured flows to the duodenum of microbial N and flows of total NAN. Hannah et al. (1991) and Lintzenich et al. (1995) fed dormant bluestem-range hay (2.3 and 2.8 percent CP, respectively) as the sole source of dietary energy and protein to Holstein steers (370 to 424 kg). Ad libitum intake of DM was 0.7 to 0.8 percent of BW (about 3.1 kg/d in both studies). Flows of endogenous N to the small intestine were calculated to be 278 (Hannah et al., 1991) and 279 mg/kg BW^{0.75} (Lintzenich et al., 1995). Hart and Leibholz (1990) fed variable amounts of alkali-treated wheat straw (1.7 to 4.1 kg/d) to 300 kg steers fitted with ruminal and abomasal (distal pyloric region) cannulas. The hay was demonstrated to be free of rumen digestible protein. The average flow of endogenous N to the abomasum was 325 mg/kg BW^{0.75}. The flow of endogenous N from the rumen to the omasum increased with increasing DMI, averaging 2.2 g/kg DMI (87 mg/kg BW^{0.75}), whereas the contribution of the omasum

to flow of endogenous N to the abomasum appeared unaffected by DMI, averaging 17.2 g N/d.

Brandt et al. (1980) used an alternative approach that allowed for the provision of N for ruminal microorganisms. Two lactating cows fitted with ruminal and duodenal cannulas were fed twelve daily meals of (kg/d) 4.86 cellulose, 0.48 straw, and 3.0 concentrate (corn starch, sugar, oil, and minerals). The basal diet was supplemented with constant ruminal infusions of ¹⁵N-enriched urea. From measured ¹⁵N surpluses in duodenal NAN, microbial N, and milk N they determined that 3.6 g of endogenous protein N passed to the duodenum of dairy cows for each kilogram of OM that passes to the small intestine. Assuming that dietary DM approximates 90 to 93 percent OM and that 60 to 65 percent of OM intake passes to the small intestine of dairy cows (Clark et al., 1992), then approximately 2.1 g of endogenous N passes to the small intestine for each kilogram of DM consumed (3.6 g × 0.915 × 0.625 = 2.1 g). The authors concluded that with normal diets, endogenous protein N may constitute 9 to 12 percent of NAN passing to the small intestine.

Vérité and Peyraud (1989) reported a regression equation that was developed to determine the contributions of microbial N, feed N, and endogenous N to passage of NAN to the small intestine. It was assumed in the regression model that flow of endogenous N to the small intestine is proportional to the intake of nondigestible OM (OM not digested in the entire digestive tract). Using a data set involving 405 measurements of NAN passage in sheep, growing cattle and cows, the resulting equation indicated that flow of endogenous N to the small intestine is equal to 5.3 g/kg of nondigestible OM intake, or approximately 1.7 g/kg DMI.

In summary, it is apparent that significant amounts of endogenous N may pass to the small intestine. The quantity

that passes to the duodenum in an animal of a given BW appears to be correlated closely to intake of indigestible OM. However, because OM digested in the rumen is not calculated in the model, for purpose of simplicity it was decided to predict passage of endogenous N to the duodenum from DMI. The equation selected for use in this publication is: endogenous N (g/d) = $1.9 \times \text{DMI (kg/d)}$. The value of 1.9 is less than the value of 2.1 reported by Brandt et al. (1980) and was selected for use in this model because it yields a mean bias closest to zero for predicting non-ammonia-non-microbial N in the model (see next section). The value of 1.9 also provides estimates of endogenous N that are consistent with the above cited data. For example, using a cow weighing 600 kg and consuming 25 kg of dry matter, the predicted flow of endogenous N is 47.5 g/d, or 392 mg/kg BW^{0.75}. The value of 392 mg/kg BW^{0.75} is 58 percent higher than the measured flow of 248 mg/kg BW^{0.75} in steers maintained by intragastric infusion and consuming no feed (Ørskov et al., 1986).

Evaluation of Model for Predicting Flows of N Fractions

The described approaches to predicting passage of MCP, RUP, and ECP to the small intestine were validated using 99 published studies that reported flows of N fractions [non-ammonia N (NAN), microbial N (MN), and non-ammonia-non-microbial N (NANMN)] to the small intestine (Table 5-8). Selected studies were limited to those in which duodenal N flow was partitioned into NAN, MN, and NANMN; data were not used if it was not explicitly clear that ammonia-N was measured and subtracted from total N for reporting flows of NAN. Of the 99 selected studies, 27 used growing cattle (106 treatment means) and 72 used lactating and non-lactating dairy cows (284 treatment means). The animals (155 to 785 kg BW) were fed a diversity of diets (e.g., 0 to 90% concentrate, mean = 50%; 8.0 to 24.8% CP, mean = 16.2%; and 7.2 to 12.8% RDP, mean 10.9%) at variable intakes of DM (0.95 to 4.40% of BW; mean = 2.86%). Although independently selected by a blind collaborator, 56 of the 72 studies involving cows in the 99-study data base used for evaluation were used for developing the equation for predicting passage of MCP. None of the growing cattle studies were used in developing the equation for predicting passage of MCP.

Figures 5-6, 5-7, and 5-8 are plots of predicted vs. measured flows and of residuals (predicted-measured) vs. measured flows for MN, NANMN (ruminally undegraded feed N + endogenous N), and NAN for cows. The plots for growing cattle showed the same tendencies as those for the cows so only the plots for cows are presented. On average, for all variables and for both growing cattle and cows, discrepancies were small between predicted and

measured flows. Mean biases of prediction for MN, NANMN, and NAN for growing cattle and cows were (g/d) -0.75, +0.44, -1.9 and +0.52, -0.12, +0.14, respectively. Mean biases of prediction for MN, NANMN, and NAN for the combined data set were (g/d) +0.18, -0.01, and -0.37. In 57 percent of the cases for growing cattle and 28 percent of the cases for cows (36 percent of the total cases), passage of microbial CP was restricted by the availability of RDP and therefore, predicted by RDP intake ($0.85 \times \text{RDP intake}$).

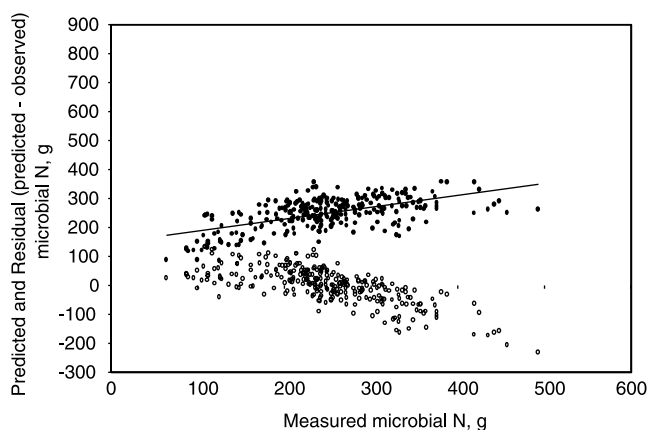
The degree of the negative slope-bias that is evident in the residual plots are of concern. However, some negative slope-bias was expected because of errors in measurement. A negative slope-bias was expected for NAN (Figure 5-8) because of errors associated with quantifying passage of digesta to the small intestine. Because measurements of digesta passage require the use of markers, flows can be under- or over-estimated to varying degrees. A greater negative slope-bias was expected for MN (Figure 5-6) and NANMN (Figure 5-7) because errors in measurement include errors in quantifying passage as well as estimating the content of MN in NAN. Primarily because of the error associated with the use of markers for estimating MN in NAN, estimates may be lower or higher than actual. To help determine if the negative slope-biases were attributable to the data used for evaluation, the model, or both, the residuals were regressed on some variables that were reported in most of the studies and considered to possibly influence the prediction accuracy of the model. These variables included BW, DMI (percent of BW and kg/d), concentrate intake (percent of DMI), diet CP (percent of DM), and CP intake. None of these factors contributed appreciably to the negative slope biases. Therefore, it was concluded that errors in the structure of the model are probably major contributors to the negative slope biases. The series of equations used for predicting flows of N fractions includes some nonlinear equations. Therefore, because of its nonlinear nature, the model is sensitive to generating bias predictions because of errors in model input (i.e., errors in measuring the independent variables).

Predicting Passage of Metabolizable Protein

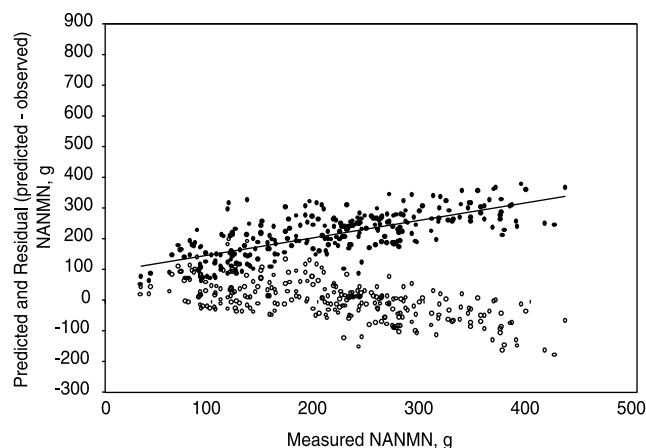
Microbial CP as provided by bacteria and protozoa is considered to contain 80 percent true protein; the remaining 20 percent of MCP is considered to be provided by nucleic acids (National Research Council, 1989). The true protein of MCP is assumed to be 80 percent digestible (National Research Council, 1989). Consequently, the conversion of MCP to MP is assumed to be 64 percent. Ruminally undegraded feed CP is assumed to be 100 percent true protein (National Research Council, 1989). As dis-

TABLE 5-8 Studies Used to Evaluate the Model Equations for Predicting Flows of MCP, RUP plus ECP, and NAN Flows to the Small Intestine

Aldrich et al. (1995)	Klusmeyer et al. (1990)	Robinson et al. (1985)
Aldrich et al. (1993a)	Köster et al. (1997)	Rode et al. (1985)
Aldrich et al. (1993b)	Kung et al. (1983)	Rooke et al. (1985)
Armentano et al. (1986)	Lardy et al. (1993)	Santos et al. (1984)
Beauchemin et al. (1999)	Lu et al. (1988)	Sarwar et al. (1991)
Bernard et al. (1988)	Lykos et al. (1997)	Schwab et al. (1992a)
Bohnert et al. (1999)	Lynch et al. (1991)	Schwab et al. (1992b)
Cameron et al. (1991)	Mabjeesh et al. (1996)	Song and Kennelly (1989)
Cecava et al. (1993)	Mansfield and Stern (1994)	Stern et al. (1983)
Cecava and Parker (1993)	McCarthy et al. (1989)	Stern et al. (1985)
Christensen et al. (1993a, b)	Merchen and Satter (1983)	Stokes et al. (1991b)
Christensen et al. (1996)	Milton et al. (1997)	Tesfa (1993)
Crocker et al. (1998)	Murphy et al. (1993)	Tice et al. (1993)
Cunningham et al. (1993)	Murphy et al. (1994)	van Vuuren et al. (1992)
Cunningham et al. (1994)	Narasimhalu et al. (1989)	van Vuuren et al. (1993)
Cunningham et al. (1996)	Ohajuruka et al. (1991)	Volden (1999)
Elizalde et al. (1999)	Oliveira et al. (1995)	Waltz et al. (1989)
Erasmus et al. (1992)	O'Mara et al. (1998)	Wessels et al. (1996)
Erasmus et al. (1994b)	O'Mara et al. (1997b)	Yang et al. (1997)
Espindola et al. (1997)	Overton et al. (1995)	Yang et al. (1999)
Feng et al. (1993)	Pantoja et al. (1995)	Yoon and Stern (1996)
Glenn et al. (1989)	Pantoja et al. (1994)	Yunker et al. (1998)
Goetsch et al. (1987)	Pena et al. (1986)	Zerbini et al. (1988)
Holden et al. (1994a)	Peyraud et al. (1997)	Zhu et al. (1997)
Holden et al. (1994b)	Pires et al. (1997)	Zinn (1988)
Johnson et al. (1998)	Poore et al. (1993)	Zinn (1993a)
Joy et al. (1997)	Prange et al. (1984)	Zinn (1993b)
Kalscheur et al. (1997a)	Putnam et al. (1997)	Zinn (1995)
Kalscheur et al. (1997b)	Ranggang et al. (1997)	Zinn et al. (1995)
Keery et al. (1993)	Rinne et al. (1997)	Zinn and Plascencia (1993)
Khorasani et al. (1996b)	Robinson (1997)	Zinn et al. (1994)
Klusmeyer et al. (1991a)	Robinson and Sniffen (1985)	Zinn and Shen (1998)
Klusmeyer et al. (1991b)	Robinson et al. (1994)	Zinn et al. (1996)

FIGURE 5-6 Plot of predicted vs. measured (filled circles) and residuals (predicted-measured; open circles) vs. measured flows of microbial N to the small intestine of dairy cows ($y = 0.4109x + 146.5$; $r^2 = 0.35$; mean bias = + 0.52; RMSPE = 63.1; $n = 284$).

cussed previously, estimates of intestinal digestibility have been assigned to the RUP fraction of each feedstuff; assigned values vary from 50 to 100 percent. Therefore, the contribution of RUP to MP is variable and dependent on feed type. Published data on the content and digestibil-

FIGURE 5-7 Plot of predicted vs. measured (filled circles) and residuals (predicted-measured; open circles) vs. measured flows of NANMN (rumen undegradable N plus endogenous N) to the small intestine of dairy cows ($y = 0.5701x + 91.193$; $r^2 = 0.51$; mean bias = -0.12; RMSPE = 63.1; $n = 275$).

ity of true protein in ECP is extremely limited. Ørskov et al. (1986) reported that NAN constituted 79 percent of total N in ruminal fluids and 74 percent of total N in abomasal fluids collected from 40-50 kg lambs nourished by N-free ruminal infusions of volatile fatty acids. Using a

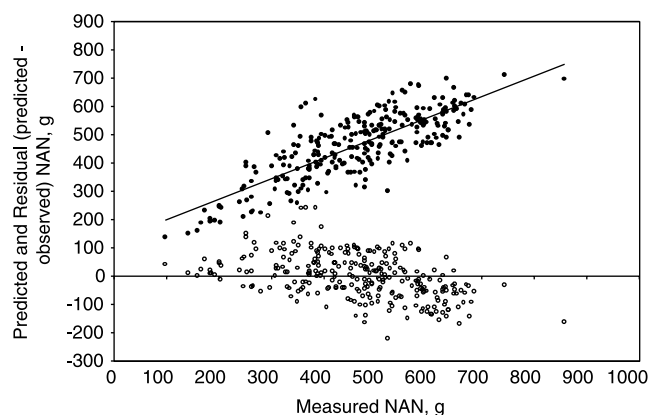


FIGURE 5-8 Plot of predicted vs. measured (filled circles) and residuals (predicted-measured; open circles) vs. measured flows of NAN (microbial N + rumen undegradable N + endogenous N) to the small intestine of dairy cows ($y = 0.7251x + 127.1$; $r^2 = 0.64$; mean bias = +0.14; RMSPE = 78.3; $n = 275$).

similar approach, Guilloteau (1986) found that 30 percent of abomasal endogenous N was AA-N. Based on these two experiments, the true protein content of ECP passing to the duodenum is assumed to be 50 percent. The true protein of ECP is assumed to be 80 percent digestible; consequently, the conversion of ECP to MP is assumed to be 40 percent.

METABOLIZABLE PROTEIN REQUIREMENTS

Previous National Research Council (1985, 1989) requirements for MP were based on the factorial method. The same approach is used in this edition. The protein requirement includes that needed for maintenance and production. The maintenance requirement consists of urinary endogenous N, scurf N (skin, skin secretions, and hair), and metabolic fecal N. The requirement for production includes the protein needed for the conceptus, growth, and lactation.

MP Requirements for Maintenance

Swanson (1977) derived the equation used to estimate the endogenous urinary protein requirement. The equation of Swanson ($UPN = 2.75 \times BW^{0.50}$) was in net protein units and was used as such in the previous *Nutrient Requirements of Dairy Cattle* publication (National Research Council, 1989). The protein system used in this version is based on MP. Assuming an efficiency of converting MP to net protein of 0.67 (National Research Council, 1989), the endogenous urinary protein requirement in MP units is $4.1 \times BW^{0.50}$.

The original equation of Swanson (1977) for predicting protein requirements for scurf protein also was in units of net protein ($SPN = 0.2 BW^{0.60}$) and used in the previous *Nutrient Requirements of Dairy Cattle* publication (National Research Council, 1989). Assuming an efficiency of converting MP to net protein of 0.67 (National Research Council, 1989), the scurf protein requirement in MP units is $0.3 \times BW^{0.60}$.

In the last edition (National Research Council, 1989), metabolic fecal protein (MFP) was calculated using an equation based on intake of indigestible DM (IDM) (i.e., $MFP, g/d = 90 \times IDM, kg/d$). Because of the errors associated with estimating the indigestibility of diets, the committee chose to calculate MFP directly from DM intake (DMI). Estimates of MFP have been made by two methods (Swanson, 1982). The first is by feeding diets of differing content of CP and regressing intake of digestible CP on intake of CP. The intercept is estimated MFP. Using this approach, Waldo and Glenn (1984) obtained a proportional intercept of 0.029 on the lactating dairy cow data of Conrad et al. (1960). Also using lactating cows, Boekholt (1976) obtained a proportional intercept of 0.033. Using sheep and cattle fed forage diets, Holter and Reid (1959) obtained an intercept of 0.034. The other approach for estimating MFP is to measure fecal N output when animals are fed low CP diets and subtract from fecal N an estimate of undigested feed N. Using this approach, Swanson (1977) estimated metabolic fecal N for ruminating cattle fed 70 natural and semi-synthetic low protein diets. By subtracting 10 percent of feed N from fecal N, Swanson (1977) obtained a mean estimate of metabolic fecal N of 4.7 g/kg DMI (29.4 g CP/kg of DMI). Based on the above data, the committee chose to calculate MFP (g/d) as: $MFP = 30 \times DMI (kg)$.

Metabolic fecal protein consists of bacteria and bacterial debris synthesized in the cecum and large intestine, keratinized cells, and a host of other compounds (Swanson, 1982). Using different solvents and centrifugation techniques, Mason (1979) reported that about 30 percent of the nonfeed portion of fecal N was soluble and about 70 percent was bacterial and endogenous debris. Quantitative data on the contribution of undigested bacterial CP synthesized in the rumen to metabolic fecal N are limited. In a series of experiments using cannulated lambs, Mason and White (1971) observed no degradation in the small intestine of the 2,6-diaminopimelic acid (DAPA)-containing fraction of bacterial cell-wall material. Based on differences in the quantities of DAPA passing through the terminal ileum and passing out of the rectum, the authors reported an 80 percent loss (apparent) of DAPA when the lambs were fed concentrate diets and a 30 percent loss when forage diets were fed. The true losses of the DAPA-containing material that originated in the rumen would be higher than the reported values to the extent that hindgut synthesis

of bacterial CP occurred, an event that is influenced by the availability of energy in the hindgut (Mason et al., 1981). Measurements of the amount of undigested ruminal bacterial CP that appears in the feces of dairy cattle fed a variety of diets are needed. Although uncertain of the amount of undigested ruminal bacterial CP that appears in the feces of dairy cattle, the subcommittee chose to assume that 50 percent of model estimated, intestinally indigestible MCP appears in the feces and that the other 50 percent is digested in the hindgut. Therefore, the equation for predicting the MP requirements for MFP (g/d) is: $MP = [(DMI \text{ (kg)} \times 30) - 0.50((\text{bacterial MP}/0.80) - \text{bacterial MP})]$.

In this edition, endogenous crude protein secretions are considered to contribute to MP supply. In view of the lack of published data, the efficiency of use of the absorbed MP for endogenous MP is assumed to be 0.67. Therefore the equation to calculate the MP requirement for endogenous MP is: $\text{endogenous MP}/0.67$.

In summary, the overall equation for predicting the MP requirement for maintenance (g/d) is: $MP = 4.1 \times BW^{0.50} \text{ (kg)} + 0.3 \times BW^{0.60} \text{ (kg)} + [(DMI \text{ (kg)} \times 30) - 0.50((\text{bacterial MP}/0.8) - \text{bacteria MP})] + \text{endogenous MP}/0.67$.

Protein Requirements for Pregnancy

Dry cows require nutrients for maintenance, growth of the conceptus, and perhaps growth of the dam. Estimating nutrient requirements for pregnancy by the factorial method requires knowledge of the rates of nutrient accretion in conceptus tissues (fetus, placenta, fetal fluids, and uterus) and the efficiency with which dietary nutrients are utilized for growth of the conceptus. Data are limited for dairy cattle.

This document differs from the last edition (National Research Council, 1989) for estimates of protein requirements for gestation during the last two months of pregnancy. Current estimates are from Bell et al. (1995). Other estimates are available, but they were obtained from beef cattle, dairy breeds other than Holsteins, or from research conducted more than 25 years ago. However, estimates from Bell et al. (1995) do not vary greatly from previous estimates and thus support the requirements published in the 1989 National Research Council report. Bell et al. (1995) measured rates of growth and conceptus chemical composition in multiparous Holstein cows that were serially slaughtered from 190 to 270 d of pregnancy. A quadratic regression equation best described protein accretion in the gravid uterus.

Estimates were derived from cows with a mean BW of 714 kg that carried a single fetus. Estimates of protein requirements to support pregnancy are solely a function of day of gestation and calf BW. The requirement for

metabolizable protein to meet the demands of pregnancy (MPPreg) was derived from the equation of Bell et al. (1995), which includes conceptus weight, calf birth weight and days of gestation as variables. The efficiency with which MP is used for pregnancy (EffMPPreg) is assumed to be 0.33. Because the experiments conducted by Bell included only animals more than 190 days pregnant and because the requirements for pregnancy are small before this time, pregnancy requirements are calculated only for animals more than 190 days pregnant. If the animal is between 190 and 279 days pregnant, the equation to compute the weight of the conceptus (CW) is:

$$CW = (18 + ((\text{DaysPreg} - 190) \times 0.665)) \times (CBW/45)$$

Where DaysPreg = days pregnant and CBW = calf birth weight.

The average daily gain due to pregnancy (ADGPreg) is: $ADGPreg = 665 \times (CBW/45)$.

The MPPreg is $MPPreg = (((0.69 \times \text{DaysPreg}) - 69.2) \times (CBW/45))/\text{EffMPPreg}$.

In the model, animals more than 279 days pregnant have the same requirements as animals that are 279 days pregnant.

Protein Requirement for Lactation

Protein required for lactation is based on the amount of protein secreted in milk. The equation for calculating protein in milk (kg/d) is $(YProtn) = \text{milk production, kg/d} \times (\text{milk true protein} / 100)$. The efficiency of use of MP for lactation is assumed to be 0.67. Use of this efficiency value in this edition's model resulted in MP balances of zero or less for 61 of the 206 diet treatments reported in the studies presented in Table 5-2. In all cases, cows were in early to mid lactation and averaged 30.9 kg/d of milk (range = 18.8 to 44.0). Crude protein, RDP, and RUP in diet DM averaged 16.1 percent (range = 13.8 to 20.8), 10.9 percent (range = 7.8 to 14.7), and 5.2 percent (range = 2.8 to 8.9). The equation to calculate MP requirement for lactation (MPLact) is (g/d) $MPLact = (YProtn/0.67) \times 1000$.

Protein Requirements for Growth

The protein requirements for heifers and steers are from the *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) (see growth section Chapter 11). The net protein requirement (NP, g/d) for growth is calculated using retained energy (RE), average daily weight gain (WG), and equivalent shrunk BW (EQSBW). The following equations are needed: if $WG = 0$ then $NPg = 0$ otherwise $NPg = WG \times (268 - (29.4 \times (RE / ADG)))$. If $(EQSBW < \text{or} = 478 \text{ kg})$ then efficiency of use of MP for growth $(\text{EffMP_NPg}) = (83.4 - (0.114 \times EQSBW)) /$

100 otherwise $\text{EffMP_NPg} = 0.28908$. Metabolizable protein for growth in g/d (MPGrowth) = $\text{NPg} / \text{EffMP_NPg}$.

AMINO ACIDS

Absorbed AA provided by ruminally synthesized MCP, RUP, and ECP are essential as the building blocks for the synthesis of tissue and milk proteins. Although to a lesser extent, absorbed AA are required also as precursors for the synthesis of other body metabolites. Amino acids other than leucine also serve as precursors for gluconeogenesis and all can be converted to fatty acids or serve as immediate sources of metabolic energy when oxidized to CO_2 . The metabolic fate of AA in ruminants has been reviewed (Lobley, 1992).

Amino acids in plant and animal proteins and those produced industrially in pure form for the feed industry by fermentative technology (lysine, threonine, and tryptophan) are of the L-form. In contrast, methionine produced by chemical synthesis is a DL-racemic mixture. Small amounts of D-AA exist in bacterial cell walls and in free form in a number of plants. Biologic use of absorbed D-AA requires conversion to the L-isomer, the efficiency of which is both AA and species dependent (Baker, 1994). The conversion of D-methionine to L-methionine has been of some concern in cattle nutrition because of the commercial availability of various types of ruminally protected DL-methionine. Titgemeyer and Merchen (1990a) noted a tendency for lower N retention when steers were infused abomasally with DL-methionine than with L-methionine. However, Campbell et al. (1996) concluded that D-methionine was used as effectively as L-methionine for N retention of growing cattle. Doyle (1981) and Reis et al. (1989) concluded that D-methionine was used as efficiently as L-methionine for wool growth.

Essential vs. Nonessential Amino Acids

Of the twenty primary AA that occur in proteins, ten are usually classified as being "essential" (or indispensable). These include arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val). Amino acids termed essential either cannot be synthesized by animal tissues or if they can (Arg and His), not at rates sufficient to meet requirements, particularly during the early stages of growth or for high levels of production. It is understood that when EAA are absorbed in the profile as required by the animal, the requirements for total EAA is reduced and their efficiency of use for protein synthesis is maximized (Heger and Frydrych, 1989). Amino acids classified as "nonessential" (or dispensable) are those which are readily synthesized from

metabolites of intermediary metabolism and amino groups from surplus AA. Unlike the EAA, there remains little evidence that the profile of absorbed nonessential AA (NEAA) is important for efficiency of use of absorbed AA for protein synthesis. If one or more of the NEAA are in short supply relative to metabolic need, most of the evidence indicates they can be synthesized in adequate amounts from one another or from one or more of the EAA that are absorbed in excess of need.

The classification of AA as being essential or nonessential originates from research with nonruminant animals. Research with dairy cattle is extremely limited. However, the early isotopic tracer studies of Black et al. (1957) and Downes (1961), using dairy cattle and sheep, indicated that the classification is similar to that of non-ruminants. Other studies in a more indirect way support that conclusion. For example, it was demonstrated that postruminal administration of mixtures of NEAA did not substitute for mixtures of EAA in supporting N retention of postweaned calves (Schwab et al., 1982) or milk protein production in lactating cows (Oldham et al., 1979; Schwab et al., 1976). Using the total intragastric nutrition technique, Fraser et al. (1991) observed that exclusion of NEAA from a supplemental mixture of EAA and NEAA decreased urinary N excretion without affecting productive N (milk N + retained N). Schwab et al. (1976) observed that increases in milk protein yields were generally of the same magnitude as for casein when only the 10 standard EAA were infused into the abomasum. Collectively, these observations indicate that when AA supplies approach requirements for total absorbable AA, requirements for total NEAA are met before the requirements for the most limiting of the EAA and that individual NEAA absorbed in amounts less than required for metabolic need can be synthesized in adequate amounts such that animal performance is not affected. These observations are consistent with those observed in *Nutrient Requirements of Swine* (National Research Council, 1998) and *Nutrient Requirements of Poultry* (National Research Council, 1994).

Although there is no evidence that NEAA as a group of AA become more limiting than EAA when dairy cattle are fed conventional diets, research is too limited to rule out the potential importance of selected NEAA to dairy cattle nutrition and production. For example, it is well-documented in nonruminants such as swine and poultry that the EAA, Met and Phe, are precursors to the synthesis of the NEAA, cysteine and tyrosine, respectively. Research indicates also that cysteine and its oxidation product cystine can satisfy approximately 50 percent of the need for total sulfur AA and that tyrosine can satisfy approximately 50 percent of the need for tyrosine + Phe (National Research Council, 1998; National Research Council, 1994). However, there are no reports involving dairy cattle as to the extent that cysteine/cystine and tyrosine can spare Met and

Phe in MP for maintenance and productive functions. Such information is ultimately needed to balance diets for AA and to know when cysteine/cystine or tyrosine in RUP can substitute for Met and Phe. A single study by Ahmed and Bergen (1983) indicated that as much as 58 percent of the total sulfur AA requirement of growing cattle can be met by cysteine and cystine. There are no reports that provide an example of the Met-sparing effect of cysteine/cystine in lactating dairy cows. Pruekvimolphon et al. (1997) concluded from an experiment with lactating dairy cows fed a Met-deficient diet that cystine in feather meal probably cannot substitute for Met in MP.

The percentage contributions of cysteine/cystine to total sulfur AA and of tyrosine to tyrosine + Phe of ruminal microorganisms and of feedstuffs are presented in Table 5-9. If cysteine/cystine can satisfy approximately 50 percent of the sulfur AA requirements and tyrosine can satisfy approximately 50 percent of the tyrosine + Phe requirements of dairy cattle, then it would appear there may often be an obligatory use of Met and Phe for cysteine and tyrosine synthesis. In cases where this exists, feedstuffs with higher concentrations of cysteine/cystine and tyrosine

in RUP would be important in reducing the need for Met and Phe in MP. An eventual understanding of the extent that cysteine/cystine can contribute to the requirements of total sulfur AA in MP is particularly important as Met has been identified as one of the most limiting EAA for growth and milk protein production. An apparent example of the Phe-sparing effect of tyrosine was provided by Rae and Ingalls (1984) who reported increased milk yields with supplemental tyrosine when cows were fed large amounts (17 percent of DM) of formaldehyde-treated canola meal. Substantial amounts of tyrosine have been shown to be destroyed or rendered unavailable by formaldehyde treatment (Rae et al., 1983; Sidhu and Ashes, 1977). The milk yield response of cows in the study by Rae and Ingalls (1984) may have resulted because of decreased bioavailability of tyrosine and an increased requirement for Phe to synthesize tyrosine.

Two NEAA that have received limited attention in regards to their importance to milk production in dairy cows are proline and glutamine. Bruckental et al. (1991) reported increased content and yield of fat in milk when proline was infused into the duodenum of early and midlac-

TABLE 5-9 Mean Percentage Contributions of Cysteine (and its oxidation product cystine) to Total Sulfur Amino Acids (methionine + cysteine + cystine) and of Tyrosine to Tyrosine + Phenylalanine in Ruminal Microbes and Feedstuffs

	Cysteine	Tyrosine		Cysteine	Tyrosine
<i>Ruminal microbes^a</i>			<i>Plant proteins^b</i>		
Bacteria	36	47	Brewer's grains, dry	52	40
Protozoa	40	46	Brewer's grains, wet	50	—
<i>Forages^b</i>			Canola meal	58	44
Alfalfa hay	48	41	Corn distillers grain w/sol.	51	38
Alfalfa silage	37	39	Corn gluten meal	43	46
Corn silage	47	35	Cottonseed meal	51	36
Grass hay	47	39	Fava beans	61	46
Grass pasture			Linseed meal	50	—
Grass silage	39	—	Lupin	65	53
Oat silage	28	—	Peas, field	60	42
Rye silage	36	—	Peanut meal	54	45
Sorghum silage	33	—	Rapeseed meal	55	44
Wheat silage	34	—	Safflower meal	53	42
<i>Grains and energy feeds^b</i>			Soybean meal	51	43
Barley	57	38	Sunflower meal	44	37
Corn	50	44	<i>Animal proteins^b</i>		
Corn gluten feed	57	45	Blood meal	52	31
Cottonseed	51	—	Feather meal	87	38
Oats	63	40	Fish meal, menhaden	24	45
Sorghum	51	42	Fish meal, anchovy	24	45
Triticale	58	38	Meat meal	44	39
Wheat	58	39	Meat and bone meal	42	40
<i>Fibrous byproduct feeds^b</i>			Skim milk powder	24	51
Beet pulp	47	57	Whey, wet	59	41
Citrus pulp	57	38			
Cottonseed hulls	47	—			
Rice bran	52	42			
Soybean hulls	60	—			
Wheat bran	57	42			

^aValues were calculated from mean AA concentrations as reported by Martin et al. (1996) and Storm and Ørskov (1983).

^bContributions of cysteine to total sulfur AA were calculated from AA concentrations presented in Tables 15-2a,b. Contributions of tyrosine to tyrosine + phenylalanine were calculated largely from AA concentrations presented in the Degussa book (Fickler et al., 1996); the remaining values were calculated from data presented in *Nutrient Requirements of Swine* (National Research Council, 1998).

tation cows. Proline infusion increased content and yield of protein in milk during midlactation but not in early lactation. In the same study, it was observed that proline infusion reduced mammary gland uptake of Arg by 40 to 50 percent. Glutamine has been hypothesized to be one of the first-limiting AA for milk protein synthesis in cows during early lactation (Meijer et al., 1993, 1995). The reasons for glutamine being suggested to be deficient were low concentrations of free glutamine in plasma of cows during early lactation and increased metabolic requirements during periods of energy deficiency. However, there are no reported studies in which intestinal supplies of glutamine were increased in cows during early lactation and lactational responses measured. Increasing duodenal supplies during late lactation did not increase content or yield of protein in milk (Meijer and van der Koelen, 1994). Proline and glutamine (including its intermediate precursor glutamic acid) are similar in that: (1) concentrations of both are considerably higher in milk casein (11.6 and 22.3 percent, respectively) than in the true protein fraction of either ruminal bacteria (3.5 and 12.6 percent, respectively) or of most feedstuffs (Fickler et al., 1996; Storm and Ørskov, 1983), (2) extraction by the lactating mammary gland is considerably less than the quantities secreted in milk protein (Clark, 1975; Clark et al., 1978; Illg et al., 1987), and (3) both can be synthesized in the mammary gland from Arg, an EAA, and ornithine (Clark et al., 1975; Mephram and Linzell, 1967; Mezl and Knox, 1977). Glutamine has received widespread attention in humans because of its numerous physiologic roles and its increased requirements during stress and illness. The additional quantities of glutamine required for stress and mild illness can be met by adaptive mechanisms for biosynthesis and utilization (Neu et al., 1996). However, during serious or long illness, glutamine producing tissues are unable to meet increased needs and thus, glutamine becomes conditionally essential (Young and El-Khoury, 1995). Currently, there are no reports of glutamine becoming a conditionally EAA for dairy cattle. However, such might be expected, particularly in young calves and early postpartum cows, when nutritional status is compromised for extended periods of time because of disease and metabolic disorders.

Limiting Essential Amino Acids

As noted in the previous section, research indicates that the dairy animal's requirement for total NEAA for growth and milk protein production are met before the requirement for at least the most limiting of the EAA. If this is true, then it follows that the efficiency of use of MP for protein synthesis will be determined by how well the profile of EAA in MP matches the profile required by the animal and by the amount of total EAA in MP. This logic has led to an interest in identifying the EAA that are most limiting

when dairy cattle are fed diets that differ in ingredient composition. Knowledge of how the sequence of AA limitation is influenced by diet composition is useful for selecting feed protein supplements that will improve the profile of AA in MP. Also, knowledge of the first limiting EAA when a diet of known composition is fed is requisite information for initial studies to determine AA requirements.

Lysine and Met have been identified most frequently as first-limiting EAA in MP of dairy cattle. The most direct evidence of their limitation has been observed by infusing individual AA or combinations of EAA into the abomasum or duodenum and measuring effects on N retention and milk protein production. Feeding ruminally inert supplements of ruminally protected Met (RPMet) and ruminally protected Lys (RPLys) and measuring effects on weight gains of growing cattle and milk protein production of lactating cows have confirmed and extended the results of infusion studies. Use of the reflex closure of the reticular groove also has provided a means of delivering AA to the small intestine of weaned calves (Abe et al., 1997, 1998).

Use of the above approaches indicate that the sequence of Lys and Met limitation is determined by their relative concentrations in RUP. For example, Lys was identified as first limiting for young post-weaned calves (Abe et al., 1997), growing cattle (Abe et al., 1997; Burris et al., 1976; Hill et al., 1980), and lactating cows (King et al., 1991; Polan et al., 1991; Schwab et al., 1992a) when corn and feeds of corn origin provided most or all of dietary RUP. In contrast, Met was identified as first-limiting for young post-weaned calves (Donahue et al., 1985; Schwab et al., 1982), growing cattle (Hopkins et al., 1999; Klemesrud and Klopfenstein, 1994; Lusby, 1994; Robert et al., 1999) and lactating cows (e.g., Armentano et al., 1997; Rulquin and Delaby, 1997; Robert et al., 1994; Schingoethe et al., 1988) when smaller amounts of corn were fed, when high forage diets were fed, or when most of the supplemental RUP was provided by soybean products, animal-derived proteins, or a combination of the two. Relative to concentrations in ruminal bacteria, feeds of corn origin are low in Lys and similar in Met whereas soybean products and most animal-derived proteins are similar in Lys and low in Met (Table 5-10). Lysine and Met were identified as co-limiting when lactating cows were fed diets without (Schwab et al., 1976) or with minimal protein supplementation (Rulquin, 1987).

That Lys and Met are often the first two limiting EAA for both growth and milk protein production may be expected. First, Met was identified as first limiting (Richardson and Hatfield, 1978; Titgemeyer and Merchen, 1990b) and Lys was identified as second limiting (Richardson and Hatfield, 1978) in MCP for N retention of growing cattle. Second, most feedstuffs have lower amounts of Lys and Met, particularly of Lys, in total EAA than in MCP (Table 5-10). And last, contributions of Lys and Met to total EAA in body lean tissue and milk are similar (Table 5-10).

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TABLE 5-10 A Comparison of the EAA Profiles of Body Tissue and Milk With That of Ruminal Bacteria and Protozoa and Common Feeds

Item	Arg	His	Ile	Leu	Lys (% of total EAA)	Met	Phe	Thr	Trp	Val	EAA (%CP)
<i>Animal products</i>											
Lean tissue ^a	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1	—
Milk ^b	7.2	5.5	11.4	19.5	16.0	5.5	10.0	8.9	3.0	13.0	—
<i>Rumen microbes</i>											
Bacteria ^c	10.2	4.0	11.5	16.3	15.8	5.2	10.2	11.7	2.7	12.5	—
Bacteria ^d	10.6	4.3	11.6	15.5	17.3	4.9	10.0	11.0	2.6	12.2	—
Protozoa ^e	9.3	3.6	12.7	15.8	20.6	4.2	10.7	10.5	2.8	9.7	—
<i>Forages^{f,g}</i>											
Legume (alfalfa) hay	12.5	4.7	10.3	17.9	12.4	3.8	11.6	10.6	3.6	12.7	41.2
Legume (alfalfa) silage	10.9	4.7	11.1	17.9	12.1	3.8	11.7	10.7	2.7	14.1	35.6
Corn silage, normal	6.2	5.7	10.6	27.2	7.9	4.8	12.1	10.1	1.4	14.1	31.6
Grass hay	11.7	4.9	10.0	18.8	10.5	3.9	11.8	10.9	3.7	13.6	33.1
Grass silage	9.4	5.1	10.9	18.8	10.1	3.7	13.4	10.2	3.3	15.0	32.6
<i>Grains^f</i>											
Barley	13.4	6.1	9.2	18.5	9.6	4.5	13.5	9.1	3.1	13.0	37.7
Corn, grain, cracked	11.5	7.8	8.2	27.9	7.1	5.3	11.5	8.8	1.8	10.0	40.1
Corn gluten feed	10.9	8.3	8.8	25.4	7.7	4.5	10.4	9.8	1.6	12.6	35.4
Oats	16.6	5.9	9.1	17.7	10.1	4.2	12.5	8.4	2.9	12.6	41.2
Sorghum	9.4	5.7	9.3	31.9	5.4	4.2	12.3	7.8	2.5	11.6	42.8
Wheat	13.6	7.1	9.6	19.3	8.1	4.6	13.3	8.4	3.5	12.3	34.4
<i>Plant proteins^f</i>											
Brewers grains, dry	14.7	5.1	9.8	20.0	10.4	4.3	11.7	9.1	2.5	12.1	39.2
Canola meal	16.5	6.6	9.0	15.9	13.2	4.4	9.5	10.4	3.4	11.1	42.6
Corn DDG w/sol.	10.7	6.6	9.8	25.4	5.9	4.8	12.9	9.1	2.3	12.4	37.8
Corn gluten meal	7.1	4.7	9.1	37.2	3.7	5.2	14.1	7.5	1.2	10.3	45.2
Cottonseed meal	26.0	6.6	7.3	13.8	9.7	3.7	12.5	7.6	2.8	10.0	42.6
Linseed meal	20.9	4.8	11.0	14.5	8.7	4.2	11.1	8.9	3.7	12.3	42.2
Peanut meal	27.6	6.0	8.1	15.9	8.3	2.9	12.1	6.7	2.4	9.8	40.1
Safflower meal	22.4	6.5	7.3	16.7	8.1	3.7	11.7	7.1	3.6	12.9	39.0
Soybean meal	16.2	6.1	10.1	17.2	13.9	3.2	11.6	8.7	2.8	10.2	45.3
Sunflower meal	20.8	6.2	9.9	15.2	8.0	5.6	11.0	8.7	2.9	11.7	42.2
<i>Animal proteins^f</i>											
Blood meal, ring dried	7.8	11.3	2.2	22.7	15.9	2.1	12.1	7.7	2.8	15.4	56.4
Feather meal	16.2	2.7	11.4	19.9	6.0	1.8	11.6	11.1	1.7	17.6	42.7
Fish meal, menhaden	13.1	6.4	9.2	16.2	17.2	6.3	9.0	9.4	2.4	10.8	44.5
Meat and bone meal	19.5	5.3	7.7	17.2	14.5	3.9	9.4	9.1	1.6	11.8	35.7
Whey, dry	5.0	4.5	12.1	21.2	17.6	3.3	7.0	14.1	3.5	11.7	42.2

^aFrom Ainslie et al. (1993); average values of empty, whole body carcasses as reported in 3 studies.

^bEach value is an average of 3 observations from Jacobson et al. (1970), McCance and Widdowson (1978), and Waghorn and Baldwin (1984).

^cFrom Clark et al. (1992); average values from 61 dietary treatments.

^dFrom Storm and Ørskov (1983); average values from 62 literature reports.

^eFrom Storm and Ørskov (1983); average values from 15 literature reports.

^fCalculated from values presented in this edition of *Nutrient Requirements of Dairy Cattle* feed table.

^gLegume and grass hays and silages are mid-maturity.

Responses of growing cattle to improved supplies of Lys and Met in MP include variable increases in BW gains and feed efficiency (Hopkins et al., 1999; Robert et al., 1999; Veira et al., 1991) and variable decreases in urinary N excretion (Abe et al., 1997, 1998; Campbell et al., 1996, 1997; Donahue et al., 1985; Schwab et al., 1982). Production responses of lactating dairy cows to increased supplies of Lys and Met in MP include variable increases in content and yield of protein in milk, milk yield, and feed intake. The nature of production responses of lactating cows to increased postruminal supplies of Lys and Met have been reviewed (Rulquin and Vérité, 1993; Schwab 1995b, 1996a; Garthwaite et al., 1998). Collectively, these reviews and other more recent studies (Piepenbrink et al., 1999; Nocek

et al., 1999; Sniffen et al., 1999a,b; Freeden et al., 1999; Rode et al., 1999; Wu et al., 1999; Nichols et al., 1998; Rulquin and Delaby, 1997) indicate: (1) that content of protein in milk is more responsive than milk yield to supplemental Lys and Met, particularly in post-peak lactation cows, (2) that increases in milk protein percentage are independent of milk yield, (3) that casein is the most influenced milk protein fraction, (4) that increases in milk protein production to increased supplies of either Lys or Met in MP are the most predictable when the resulting predicted supply of the other AA in MP is near or at estimated requirements (Rulquin et al., 1993; Schwab, 1996a; Sloan et al., 1998), (5) that milk yield responses to Lys and Met are more common in cows during early lactation than in

mid or late lactation cows, and (6) production responses to increased supplies of Lys and Met in MP typically are greater when CP in diet DM approximates normal levels (14 to 18 percent) than when it is lower or higher. That milk protein percentage is more sensitive than milk yield to improved concentrations of Lys and Met in MP of post-peak lactation cows was demonstrated by Chapoutot et al. (1992). The authors used a multiple switch-back experiment to determine individual responses of 40 post-peak lactation cows to ruminally protected Lys and Met. The RPAA blend was fed in amounts to provide 23 g/d of digestible Lys and 7 g/d of digestible Met. They observed that 37 cows responded with greater content of milk protein, 31 responded with greater protein yield, and 16 responded with more milk.

In addition to the effects on milk protein production, there are reports also of increased percentages of fat in milk with increased amounts of Met or Met plus Lys in MP. These increases in milk fat have been observed in postruminal infusion studies (Socha et al., 1994b) and when Met (Brunschwig and Augéard, 1994; Brunschwig et al., 1995; Yang et al., 1986) or Met and Lys (Bremmer et al., 1997; Canale et al., 1990; Rogers et al., 1987; Xu et al., 1998) were supplied in ruminally protected forms. The increases in milk fat generally have been observed in association with increases in milk protein but increases also have been observed without increases in milk protein (Varvikko et al., 1999). Increases in percentages of fat in milk with improved Met and Lys nutrition also have not been predictable. For example, the infusion of graded amounts of Met (0, 3.5, 7.0, 10.5, and 16.0 g/d) into the duodenum of post-peak lactation cows fed a corn-based diet supplemented with soybean products and blood meal increased percentages in milk of both fat (3.73, 3.86, 3.78, 3.91, and 4.15) and true protein (3.00, 3.07, 3.09, 3.13, and 3.15) (Socha et al., 1994b). However, when the same cows fed the same feedstuffs were infused with similar amounts of Met during peak lactation (Socha et al., 1994c) or mid lactation (Socha et al., 1994a), percentages of fat in milk did not change but protein in milk increased.

It is not clear why increased amounts of Met and Lys in MP may sometimes increase fat content of milk. One reason may involve a possible effect of Met on *de novo* synthesis of short- and medium-chain fatty acids in the mammary gland. This was suggested by Pisulewski et al. (1996) who demonstrated that the infusion of Met into the duodenum of early lactation cows increased proportions of short- and medium-chain fatty acids and decreased proportions of long-chain fatty acids in milk fat. Christensen et al. (1994) reported a similar trend in the fatty acid composition of milk when lactating cows were fed ruminally protected Met and Lys. However, others did not observe an effect of increased postruminal supplies of Met on fatty acid composition of milk (Casper et al., 1987; Chow et al.,

1990; Karunanandaa et al., 1994; Kowalski et al., 1999; Rulquin and Delaby, 1997; Varvikko et al., 1999). Another reason may relate to the role of AA in the intestinal and hepatic synthesis of chylomicrons and very low density lipoproteins (VLDL). Required substrates for the synthesis of chylomicrons and VLDL, in addition to the presence of the long-chain fatty acids that stimulate their formation, include apolipoproteins and phospholipids (Bauchart et al., 1996). The synthesis of apolipoproteins requires AA. The synthesis of phosphatidylcholine (lecithin), the most abundant phospholipid, requires choline. It has been demonstrated that a portion of the dairy cows' requirement for Met is as a methyl donor for choline synthesis (Sharma and Erdman, 1988) and that in some studies (Sharma and Erdman, 1988, 1989; Erdman, 1994), but not in others (Erdman and Sharma, 1991; Grummer et al., 1987), choline can be a limiting nutrient for milk fat synthesis. That Met and Lys may sometimes be limiting for the synthesis of chylomicrons or VLDL such that the availability of long-chain fatty acids for milk fat synthesis is reduced has not been demonstrated. However, there is limited evidence that formation or secretion of these lipoproteins can be enhanced with improved Met and Lys nutrition (Auboirn et al., 1995; Durand et al., 1992). Decreases in plasma nonesterified fatty acids concentrations in preruminant calves (Auboirn et al., 1995; Chilliard et al., 1994) and lactating cows (Pisulewski et al., 1996; Rulquin and Delaby, 1997) with increased amounts of Met in MP have been reported. However, decreases in plasma nonesterified fatty acids concentrations are generally considered to reflect reduced mobilization of fatty acids from body reserves rather than increased utilization.

Attempts to identify EAA that may become limiting after Lys and Met in dairy cattle are limited. Using the total intragastric nutrition technique, Fraser et al. (1991) concluded that His was limiting after Met and Lys for lactating cows when casein was the infused protein. Similar conclusions could not be drawn from the abomasal infusion experiments of Schwab et al. (1976) and Rulquin (1987) when lactating cows were fed diets of conventional ingredients. Rulquin (1987) concluded that Thr was not limiting after Lys and Met. Schwab et al. (1976) concluded from five infusion experiments that the sequence of limiting EAA after Lys and Met for lactating cows will be determined by the ingredient composition of the diet. Amino acid extraction efficiencies, transfer efficiencies, and ratios of uptake to output have been used in many studies to evaluate the order of limiting AA. Nichols et al. (1998) and Piepenbrink et al. (1999) concluded that AA extraction efficiency is the most accurate of the three methods for estimating the sequence of AA limitation because no errors from estimates of blood flow are involved. Use of this method identified Phe and Ile as most frequently limiting after Lys and Met (Nichols et al., 1998; Piepenbrink et al.,

1998; Liu et al., 2000) when corn-based diets are supplemented with common protein supplements such as soybean meal, corn distillers dried grains, canola meal, or a mixture of canola meal, corn gluten meal, blood meal, and fish meal.

Although research is limited, there is little direct evidence to indicate that other EAA might be more limiting than either Lys or Met. Two exceptions may be Arg and His. Abomasal infusion of Arg (13.7 g/d) increased N retention of 159-kg Holstein steers fed direct-cut vegetative wheat silage (12.3 percent CP) as the sole feed. In contrast, abomasal (178 g/d) and intravenous (112 g/d) infusions of Arg did not affect milk production or milk composition when post-peak lactating Holstein cows (544 kg) were fed a 15.3 percent CP diet of alfalfa-grass silage, corn silage, corn, and soybean meal (Vicini et al., 1988). Vanhatalo et al. (1999) concluded that His was the first-limiting EAA when post-peak lactating Finnish Ayrshire cows were fed a grass silage-based diet without feeds of corn origin and without protein supplementation. The diet contained 56 percent grass silage ensiled with an acid-based additive, 18 percent barley, 18 percent oats, 6.7 percent beet pulp, and 1.3 percent minerals and vitamins. The abomasal infusion of 6.5 g/d His increased yields of milk (23.6 vs. 22.9 kg/d) and milk protein (721 vs. 695 g/d) but not milk protein content. The infusions of either 6.0 g/d of Met or 19.0 g/d of Lys or both in combination with 6.5 g/d of His did not further increase milk protein production. Factors that probably contributed to His being first limiting in the study by Vanhatalo et al. (1999) are: (1) the low content of RUP in dietary DM, (2) the low content of His in microbial protein as compared to feed proteins (Table 5-10), and (3) the low content of His in barley and oats as compared to corn (Table 5-10). Mackle et al. (1999) found no response in milk yield or milk composition when Holstein cows in early lactation fed a 16.2 percent CP diet (based on alfalfa hay, corn, and soybean products) were abomasally infused with branched-chain AA (55.5, 39.0, and 55.5 g/d of Leu, Ile, and Val, respectively). Hopkins et al. (1994) provided daily intraperitoneal infusions of branched-chain AA plus Arg (46.1, 31.4, 38.3, and 25.0 g/d of Leu, Ile, Val, and Arg, respectively) over a 2-h period each day to Holstein cows in early lactation fed 13.6 percent CP diets that contained 15.0 or 22.4 percent ADF, respectively. The infusion of AA did not increase the content or yield of protein in milk but it did appear to attenuate the decreases in content and yield of fat in milk, when cows were fed the low fiber diet. Analysis of milk fat for fatty acids indicated that the infused AA may have increased *de novo* synthesis of C₄ to C₁₆ fatty acids, particularly the C₁₆ fatty acids. It is well-documented that Arg and the branched-chain AA are taken up by the mammary gland well in excess of their direct output in milk protein (Clark et al., 1978; Nichols et al., 1998; Piepenbrink et al., 1999) and that they can be con-

verted to NEAA or utilized as energy sources in the mammary gland (Mephram, 1982; Wohlt et al., 1977).

Predicting Passage to the Small Intestine

As reviewed in the previous section, the efficiency of use of MP by dairy cattle is influenced by its content of EAA. To advance AA nutrition research (e.g., to define the ideal content of EAA in MP) and to implement the results of such research (e.g., to select protein and AA supplements to optimize the balance of EAA in MP) models are needed that predict accurately the EAA composition of duodenal protein. In recognition of this need, it was the goal of the subcommittee to extend the use of the MP system developed for this revision of *Nutrient Requirements of Dairy Cattle* to one that would predict directly the EAA composition of duodenal protein. The EAA content of MP and flow to the duodenum of the individual digestible EAA could be calculated from knowledge of: (1) the predicted EAA composition of duodenal protein; (2) the predicted contribution of each protein fraction (microbial protein, the RUP fraction of each feedstuff, and endogenous protein) to the total flow of each EAA; (3) the digestibility coefficients assigned to microbial protein, the RUP fraction of each feedstuff, and endogenous protein; and (4) the predicted flows of MP.

The subcommittee considered both factorial and multivariate regression approaches. Prediction models based on the factorial method require the assignment of AA values to model-predicted supplies of ruminally synthesized microbial protein, ruminally undegraded feed proteins, and if predicted, endogenous protein. The challenge associated with such an approach is to have the predicted flows of protein fractions and their assigned AA values be accurate. Indeed, it can be assumed that there are errors in predicting flows of protein fractions as well as in assigning AA values to each fraction. To the extent that this occurs, then at each step in the factorial process, errors of prediction are aggregated, and depending on the number of steps involved, the aggregated error can be quite large. The net result of such errors are biases of prediction of mean values.

Two examples of published factorial approaches for predicting AA passage to the small intestine are the AA submodel of the Cornell Net Carbohydrate and Protein System (CNCPS) (O'Connor et al., 1993) and the AA submodel developed by Rulquin et al. (1998). The CNCPS AA submodel, adopted in conjunction with the CNCPS model for Level II of the *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) model, was developed to predict directly the absolute flows of each of the EAA. The AA submodel of Rulquin et al. (1998), which uses the PDI system (INRA, 1989) to predict flows of protein fractions, was developed to predict directly the content of AA in duodenal protein and not the absolute flows of the

individual AA. This approach provided for a true integration of the AA submodel with the protein model. The *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) and Rulquin et al. (1998) models differ in the AA values assigned to microbial protein and RUP. In the *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) model, predicted flows of microbial protein are partitioned into cell wall and non-cell wall fractions and estimated EAA compositions of each (O'Connor et al., 1993) are assigned. The EAA values assigned to the predicted digestible RUP fractions of feedstuffs are those of the insoluble protein fraction of feedstuffs and not of total CP (O'Connor et al., 1993). In the model of Rulquin et al. (1998), the average AA composition of liquid-associated bacteria from 66 publications are assigned to microbial protein. The AA profile of the RUP fraction of feedstuffs is assumed to be the same as in the original feedstuff. The two submodels also differ in that endogenous protein is considered in the model of Rulquin et al. (1998) but not in the *Nutrient Requirements of Beef Cattle* (National Research Council, 1996) model.

Both models were tested against published AA flow data and reasonable results were obtained. However, in both cases, the evaluation studies indicated biases of prediction for individual AA. Based on slopes of regression lines that related observed flows obtained from 200 diets (as reported in 12 lactating cow studies and 9 nonlactating cow studies) to predicted flows, O'Connor et al. (1993) observed that the CNCPS model over-predicted flows of Thr and Leu and under-predicted flows of Arg. Rulquin et al. (1998) tested their model against abomasal and duodenal digesta AA compositions measured in 133 dairy cow diets and 49 growing cattle diets. Mean percentage differences between predicted and measured concentrations (g/100 g AA) were: Arg (+5.6%), His (+0.9%), Ile (−1.5%), Leu (−5.8%), Lys (−4.7%), Met (+12.3%), Thr (−0.2%), Phe (+0.4%), and Val (+0.8%). As a result of these biases, the authors adjusted the initial model by covariance (i.e., regression) analysis. This improved the accuracy of prediction. In summary, if the two described models were perfect both in structure (i.e., all of the contributing variables were included) and parameters (i.e., assigned constants were correct), and measured profiles of AA in duodenal digesta protein used for evaluation were without systematic errors, then a comparison of predicted values with measured values would have revealed no biases of prediction of mean values.

In contrast to the described factorial models in which both the structure and the parameters were determined on theoretic grounds, the multivariate regression or semi-factorial approach allows for some of the parameters to be determined by regression. This allows the model (i.e., equations) to adapt to the measured data, and allows for at least partial correction of the errors of the mechanistically

determined variables. The result is that semi-mechanistic models generally are better at predicting (forecasting) than full mechanistic models when forecasting is within the inference range of the model. Because of the potential for increased accuracy of prediction, and because the approach eliminated the need to assign AA values to ruminally synthesized microbial protein and endogenous protein (AA values had to be assigned only to feedstuffs), the semi-mechanistic method was the method of choice by the subcommittee for predicting the content of EAA in total EAA of duodenal protein. This approach required the development of an equation for each of the EAA and one for predicting flows of total EAA.

The approach used for developing the AA submodel was as follows. A data set of observed abomasal and duodenal AA flows was compiled from 57 published studies involving 199 treatment means (Table 5-11). The data set included 155 treatment means from cows (lactating and dry) and 44 treatment means from growing cattle (dairy and beef). Only one experiment reported flows of Trp; thus, no equation could be developed for predicting the content of Trp in total EAA of duodenal protein. For data to be included in the final data set, the following requirements had to be met: (1) DMI was reported or could be calculated from the information given, (2) ingredient composition of diets was reported, (3) feedstuffs used in the experiments were represented in the feed library of the model for N fractions, K_d , and AA composition, and (4) flows (g/d) to the duodenum of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val were reported. An exception was made in regard to requirement # 3 in that N fractions and K_d for barley straw were used for oat straw, but the AA composition of oat straw was used. The first three requirements were necessary because the information is model-required data. For experiments that employed a factorial arrangement of treatments and reported main effect means only, data were used only if one of the main effects was not related to diet (e.g., for an experiment with main effects of protein source and feeding frequency, data for the main effect of protein source was used). Body weights of animals had to be estimated for 15 of the 57 published studies; in all cases, these 15 studies involved cows. Body weights were estimated from reported information on breed, stage of lactation, and BW reported by the same authors in other papers.

The 199 treatment means for duodenal flows of each EAA in the final data set represented 199 unique and diverse diets fed to cattle ranging in BW from 191 to 717 kg. Intake of DM ranged from 3.6 to 26.7 kg/d. Feedstuffs, their frequency of use, and the means and ranges of their contribution to diet DM are summarized in Table 5-12. Diets varied in percent concentrate (0 to 86%, mean = 46%), dietary CP (8.5 to 29.6%, mean = 16.2%), dietary RDP (4.6 to 18.2, mean = 10.7%), and dietary RUP (2.2 to 11.9%, mean = 5.5%). The descriptive statistics of the

TABLE 5-11 Experiments Used to Develop Equations for Predicting Amino Acid Passage to the Small Intestine

Aldrich et al. (1995)	Klusmeyer et al. (1991b)	Robinson (1997)
Aldrich et al. (1993a)	Klusmeyer et al. (1990)	Robinson et al. (1991a)
Aldrich et al. (1993b)	Lardy et al. (1993)	Robinson et al. (1994)
Armentano et al. (1986)	Lynch et al. (1991)	Santos et al. (1984)
Bernard et al. (1988)	Mabjeesh et al. (1996)	Schwab et al. (1992a)
Bohnert et al. (1999)	Mansfield and Stern (1994)	Schwab et al. (1992b)
Cameron et al. (1991)	McCarthy et al. (1989)	Stern et al. (1983)
Cecava et al. (1993)	McNiven et al. (1995)	Stern et al. (1985)
Cecava and Parker (1993)	Merchen and Satter (1983)	Titgemeyer et al. (1988)
Christensen et al. (1993a, b)	Murphy et al. (1993)	van Vuuren et al. (1992)
Christensen et al. (1996)	Narasimhalu et al. (1989)	van Vuuren et al. (1993)
Cunningham et al. (1993)	O'Mara et al. (1998)	Volden (1999)
Cunningham et al. (1994)	O'Mara et al. (1997b)	Waltz et al. (1989)
Cunningham et al. (1996)	Overton et al. (1995)	Wessels et al. (1996)
Erasmus et al. (1992)	Palmquist et al. (1993)	Zerbini et al. (1988)
Erasmus et al. (1994b)	Pena et al. (1986)	Zinn (1988)
Holden et al. (1994b)	Pisulewski et al. (1996)	Zinn (1993b)
Keery et al. (1993)	Prange et al. (1984)	Zinn and Shen (1998)
Klusmeyer et al. (1991a)	Putnam et al. (1997)	

TABLE 5-12 Feedstuffs and the Extent of Their Use in the 199 Diets in the Data Set Used to Develop Equations to Predict the Content of Individual EAA in Total EAA of Duodenal Protein

Feedstuff	N ^a	Contribution to dietary DM (%)		Feedstuff	N ^a	Contributions to dietary DM (%)	
		Mean	Range			Mean	Range
<i>Forages</i>				<i>Protein supplements</i>			
Corn silage	108	35	8–80	Alfalfa meal	5	9	5–10
Grass, fresh	10	87	56–100	Blood meal	22	4	0.6–10
Grass, hay	26	21	5–100	Brewers grains, dry	2	34	25–44
Grass, silage	17	58	38–100	Brewers grains, wet	1	32	—
Grass-legume, silage	18	19	11–26	Canola meal	10	12	4–20
Legume, fresh	5	86	65–100	Casein	4	3	2–4
Legume, hay	61	17	5–65	Corn distillers grains	14	8	4–28
Legume, silage	37	33	8–65	Corn gluten meal	17	6	1–19
Oat, silage	10	18	9–30	Feather meal	6	4	0.3–10
Oat, straw	13	6	3–95	Feather meal with viscera	3	4	2–6
Sorghum, sudan hay	7	11	10–12	Fish meal, anchovy	1	5	—
Sorghum, sudan, silage	6	68	66–70	Fish meal, menhaden	23	5	2–13
Wheat, silage	8	33	23–45	Meat meal	5	2	0.3–9
Wheat, straw	1	25	—	Rapeseed meal	7	6	1–19
<i>Energy feeds</i>				Soybean meal, expeller	6	8	4–15
Barley, grain	24	26	4–46	Soybean meal, heated	3	11	5–15
Barley, grain, heated	1	46	—	Soybean meal, nonenz browned	2	17	16–17
Barley, grain, steam-rolled	12	36	12–50	Soybean meal, solvent	78	9	0.3–20
Corn, grain	119	24	1–49	Sunflower meal	2	12	10–13
Corn, grain and cob	6	40	37–42	Urea	66	0.5	0.1–2.0
Corn, grain, high moisture	19	25	2–32	<i>Energy and protein feeds</i>			
Corn, grain, steam-flaked	7	51	16–65	Cottonseed, whole, extruded	1	42	—
Corn, hominy	1	22	—	Cottonseed, whole, heated	1	43	—
Corn, starch	19	5	0.3–17	Cottonseed, whole, raw	1	41	—
Fat	33	3	0.2–6	Soybean seed, raw	5	12	6–20
Molasses	75	4	0.5–13	Soybean seed, roasted	5	17	16–19
Oats, grain	5	21	17–25	<i>Byproduct feeds</i>			
Sorghum, grain	1	10	—	Beet pulp	7	18	9–36
Sugar/dextrose	2	3	—	Corn gluten feed	9	14	6–32
Wheat, grain	5	23	5–29	Soy hulls	21	15	0.3–36
Wheat, grain, steam. flaked	2	51	50–52	Tapioca	4	7	2–20
				Wheat middlings	16	8	0.2–34

^aNumber of diets in which the feedstuff was an ingredient.

animal, diet, and EAA flow data used in the development of the equations are presented in Table 5-13. All of the required animal and diet data for the 199 diets were entered into this edition's model for predicted intakes of RUP and RDP and for predicted duodenal flows of MCP, RUP, and endogenous CP. The CP content of feedstuffs was obtained from the experiment if reported; otherwise, model default values (± 1.0 SD) were used.

The following approach was used to identify the independent variables and a model structure that would most accurately predict the content of each EAA (except Trp) in total EAA of duodenal protein and flows of individual EAA to the small intestine. The first step involved calculating the content of each EAA in total EAA of the RUP fraction of each diet in the data set. The three equations used for this purpose are presented; Lys is used as the example EAA.

$$\text{RUP}_{\text{Lys}} = \sum_f (\text{DMI}_f \times \text{CP}_f \times \text{RUP}_f \times \text{Lys}_f \times 0.001) \quad (5-3)$$

where:

- RUP_{Lys} = amount of Lys supplied by total diet RUP, g
- DMI_f = intake of DM of each feedstuff contributing RUP, kg
- CP_f = crude protein content of each feedstuff contributing RUP, g/100 g DM
- RUP_f = ruminally undegraded protein content of each feedstuff contributing RUP, g/100 g CP
- Lys_f = lysine content of each feedstuff contributing RUP, g/100 g CP

$$\text{RUPEAA} = \text{RUP}_{\text{Arg}} + \text{RUP}_{\text{His}} + \text{RUP}_{\text{Ile}} +$$

$$\text{RUP}_{\text{Leu}} + \text{RUP}_{\text{Lys}} + \text{RUP}_{\text{Met}} + \text{RUP}_{\text{Phe}} + \text{RUP}_{\text{Thr}} + \text{RUP}_{\text{Trp}} + \text{RUP}_{\text{Val}} \quad (5-4)$$

where:

RUPEAA = amount of essential AA supplied by RUP, g

$$\text{RUP}_{\text{LysPctRUPEAA}} = 100 \times (\text{RUP}_{\text{Lys}}/\text{RUPEAA}) \quad (5-5)$$

where:

RUP_{LysPctRUPEAA} = Lys as percentage of essential AA in RUP, each g/100 g essential AA.

The content of each EAA in total EAA of the RUP fraction of each diet was estimated in recognition of the belief that the resulting values would be significant predictors of the contributions that each EAA makes to total EAA in duodenal protein. Multivariate analysis of measurements of AA passage to the small intestine indicated that the concentrations of individual AA in RUP and the proportional contribution of RUP to total protein passing to the duodenum explained most of the variation in AA profiles of duodenal protein (Rulquin and Vérité, 1993). Dietary RUP and the percentage contributions of Lys and Met to total EAA in diet RUP also emerged as significant independent variables in regression equations developed for predicting concentrations of Lys and Met in total EAA of duodenal protein of lactating dairy cows (Schwab, 1996b; Socha, 1994).

The second step involved the identification of significant independent variables to develop equations to predict percentages of each EAA (excluding Trp) and total EAA in duodenal protein. Variables that were evaluated as potential significant predictors of the content of each EAA in total EAA (e.g., g/100 g total EAA) of duodenal protein were: "Trial," dietary CP and predicted dietary RUP as

TABLE 5-13 Descriptive Statistics of the Data Used for Developing Equations for Predicting Content of Individual EAA in Total EAA of Duodenal Protein and for Predicting Flows of Total EAA to the Small Intestine

Item	Mean	Median	Minimum	Maximum	SD
Animal characteristics					
DMI, kg/d	15.5	16.4	3.6	26.7	6.4
BW, kg	515.2	568.0	191.0	717.0	128.0
DMI, %BW	2.9	2.9	1.3	4.4	0.8
Diet characteristics, %DM					
CP	16.2	16.5	8.5	29.6	2.7
RUP ^a	5.5	5.3	2.2	11.9	1.6
Concentrate	46.3	50.0	0.0	85.7	18.0
AA in duodenal protein, %EAA					
Arg	10.4	10.3	7.1	16.1	1.2
His	5.0	4.9	3.1	9.2	0.8
Ile	10.8	10.9	6.4	14.5	1.4
Leu	20.2	20.4	9.6	28.5	2.5
Lys	14.4	14.7	9.7	18.0	1.4
Met	4.3	4.1	2.2	7.1	0.9
Phe	11.3	11.2	9.8	15.1	0.7
Thr	11.1	11.1	8.9	13.8	0.8
Val	12.5	12.6	9.0	15.7	1.2
EAA flow to duodenum, g/d	894.1	938.5	169.2	1970.0	463.7

^aPredicted by the model.

percentages of dietary DM, the percentage of each EAA in dietary RUP (e.g., RUPLys, g/100 g RUP), the percentage of each EAA in total EAA of dietary RUP (e.g., RUPLysPctRUPEAA, g/100 g), and the percentage of predicted RUP in predicted flows of total duodenal protein (predicted MCP + predicted RUP + predicted endogenous protein). The potential independent variables considered for predicting flows of total EAA to the duodenum were: "Trial," dietary CP and predicted dietary RUP as percentages of diet DM, the percentage of total EAA in dietary RUP, RUPEAA intake (g/d), predicted flows of endogenous protein (g/d), and model predicted MCP (g/d). Trial was included in all models as a class variable to account for variation caused by independent variables or factors that are not continuous (e.g., feeding frequency, sampling methods, microbial markers used, etc.) and for which their inclusion risks overparameterization of the model. Significant independent variables were identified by using the backward elimination procedure of multiple regression. Briefly, independent variables, their squared terms (except for "Trial"), and all possible two-way interactions (excluding interactions with "Trial") were entered into the model. The following algorithm was used to reduce the model to significant ($P < 0.05$) independent variables. First, non-significant ($P > 0.05$) interactions were removed sequentially from the model. Second, non-significant main effects were removed from the model if no interactions or squared term of the main effect was significant. Third, if variance inflation factors (VIF) were all less than 100 then the model was accepted. If a term had a VIF greater than 100, it was removed. If more than one had a VIF greater than 100, the term with the largest P value was removed. In that case, all steps were repeated until an accepted model was obtained at the third step. When an apparently acceptable model was generated, the Difference in Fits Statistic (DFFITS) was used as the basis for omitting outliers; absolute values of DFFITS ≥ 2 were omitted (Bowerman and O'Connell, 1990). The variables that emerged as significant predictors of the content of individual EAA in total EAA of duodenal protein were Trial, each EAA as a percentage of EAA in RUP, and RUP as a percentage of total duodenal protein.

The third step involved the use of PROC MIXED of SAS (a random effects model) to develop the final equations. This was done to yield more accurate parameter estimates and to increase the utility of the prediction equations for purpose of field application (i.e., Trial effects would be unknown). In brief, two random coefficient models for each EAA and for total EAA were fitted for the prediction equations generated by using PROC GLM. The first random coefficient model utilized unstructured covariance to test whether the intercept and slope within trials were significantly ($P < 0.05$) correlated, which was not the case for any of the equations. The second random

coefficients model, which models a different variance component for each random effect (the default structure), then was used to generate the final prediction equations.

Arginine

$$Y = 7.31 + 0.251X_1 \text{ (RMSE} = 0.278\text{)}$$

where:

Y = Arg, % of EAA in duodenal protein

X_1 = Arg, % of EAA in RUP

Histidine

$$Y = 2.07 + 0.393X_1 + 0.0122X_2 \text{ (RMSE} = 0.156\text{)}$$

where:

Y = His, % of EAA in duodenal protein

X_1 = His, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Isoleucine

$$Y = 7.59 + 0.391X_1 - 0.0123X_2 \text{ (RMSE} = 0.174\text{)}$$

where:

Y = Ile, % of EAA in duodenal protein

X_1 = Ile, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Leucine

$$Y = 8.53 + 0.410X_1 + 0.0746X_2 \text{ (RMSE} = 0.541\text{)}$$

where:

Y = Leu, % of EAA in duodenal protein

X_1 = Leu, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Lysine

$$Y = 13.66 + 0.3276X_1 - 0.07497X_2 \text{ (RMSE} = 0.400\text{)}$$

where:

Y = Lys, % of EAA in duodenal protein

X_1 = Lys, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Methionine

$$Y = 2.90 + 0.391X_1 - 0.00742X_2 \text{ (RMSE} = 0.168\text{)}$$

where:

Y = Met, % of EAA in duodenal protein

X_1 = Met, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Phenylalanine

$$Y = 7.32 + 0.244X_1 + 0.0290X_2 \text{ (RMSE} = 0.194\text{)}$$

where:

Y = Phe, % of EAA in duodenal protein

X_1 = Phe, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Threonine

$$Y = 7.55 + 0.450X_1 - 0.0212X_2 \text{ (RMSE} = 0.167\text{)}$$

where:

Y = Thr, % of EAA in duodenal protein

X_1 = Thr, % of EAA in RUP

X_2 = RUP, % of duodenal protein (MCP + RUP + endogenous CP)

Valine

$$Y = 8.68 + 0.314X_1 \text{ (RMSE} = 0.216\text{)}$$

where:

Y = Val, % of EAA in duodenal protein

X_1 = Val, % of EAA in RUP

Total essential amino acids

$$Y = 30.9 + 0.863X_1 + 0.433X_2 \text{ (RMSE} = 58.8\text{)}$$

where:

Y = EAA in duodenal protein, g

X_1 = EAA supplied by RUP, g

X_2 = MCP, g

The model predicts flows (g/d) of individual EAA to the small intestine by multiplying predicted concentrations of each EAA in duodenal total EAA by predicted flows of total EAA. Plots of predicted vs. measured values and of residuals (predicted – measured) vs. measured values for Lys, Met, and total EAA are presented in Figures 5-9 through 5-11.

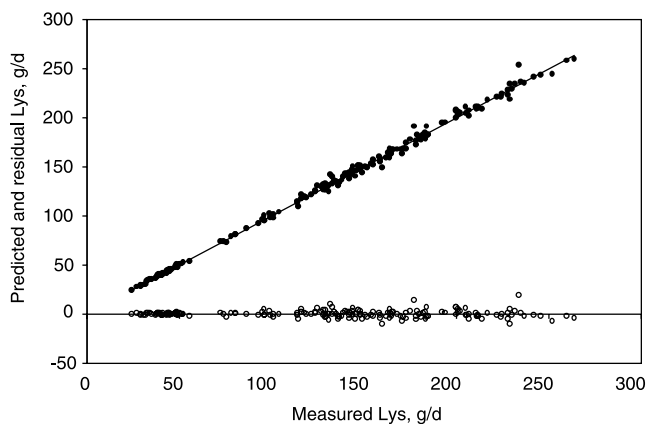


FIGURE 5-9 Plot of predicted vs. measured (filled circles) and residuals (predicted – measured; open circles) vs. measured (Lys, g/d) (from predicted Lys, percent of EAA and predicted EAA, g/d) (mean bias = 2.4×10^{-2} ; RMSPE = 3.5; n = 186).

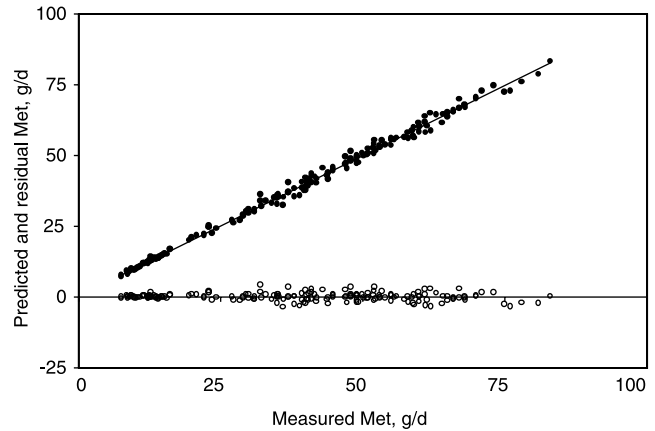


FIGURE 5-10 Plot of predicted vs. measured (filled circles) and residuals (predicted – measured; open circles) vs. measured Met, g/d (from predicted Met, percent of EAA and predicted EAA, g/d) (mean bias = 2.2×10^{-3} ; RMSPE = 1.3; n = 182).

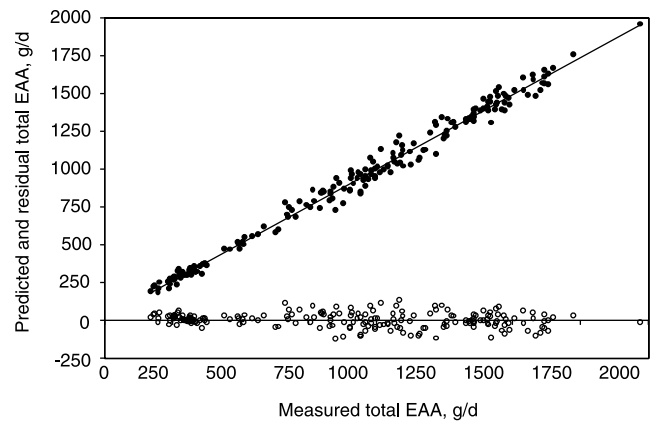


FIGURE 5-11 Plot of predicted vs. measured (filled circles) and residuals (predicted – measured; open circles) vs. measured flow of total EAA (mean bias = 3.06×10^{-5} ; RMSPE = 47.8; n = 196).

The subcommittee also evaluated the use of a semi-mechanistic approach to predict directly the “flows” of individual EAA to the duodenum. Using the same data base, the theoretically based model structure for each EAA was $Y = \beta_0 + \beta_1X_1 + \beta_2X_2$ where: Y = flow to duodenum(g), β_0 = parameter estimate for contribution of endogenous protein (g), β_1 = parameter estimate of the fractional contribution of RUP to flows from RUP, X_1 = model predicted flow of the EAA (g), β_2 = parameter estimate of the fractional content of the EAA in MCP, and X_2 = model predicted flow of MCP (g). The parameter estimates that resulted appeared reasonable, indicating that the model does an adequate job of predicting flows of MCP and RUP and that the content of EAA in MCP is similar to mean values reported in the literature (e.g., Clark et al., 1992). A comparison of the root mean square prediction errors (RMSPE) obtained from two sets of resid-

ual plots (“g/d” and “% of total EAA”) for each of the two approaches is presented in Table 5-14.

The residual plots indicated that the equations that predict percentages directly predict more accurately both the “percentages” of individual EAA in duodenal total EAA and “flows” (g/d) of individual EAA. The lower RMSPE for predicting “percentages” and “flows” when percentages are predicted directly (and flows are calculated) result partially because errors of prediction are “condensed” into two variables (i.e., the prediction of the percentage and prediction of total EAA, from which the product yields prediction of flow). In contrast, prediction errors of all nine EAA are aggregated into total EAA and subsequently into the calculation of percentages for the more theoretically based model. Thus, the equations that predict directly the percentages of each EAA in total EAA of duodenal protein were accepted for use in this publication.

Knowledge of predicted flows of digestible EAA and the EAA content of MP is more important than knowing the predicted flows of total EAA and the EAA content of total duodenal protein. This is because the AA in undigested protein are not absorbed and do not contribute to meeting the AA requirements of the animal. The EAA composition of MP will generally be different from that of total duodenal protein. This is because of differences among feedstuffs in both the digestibility and the EAA composition of their RUP fractions, differences in the proportional contributions that microbial protein and RUP make to total EAA passage, and mean differences in the digestibility of microbial protein and total dietary RUP. Because undigested AA do not contribute to meeting the AA requirements of the animal, and because the AA composition of MP is likely to differ from the AA composition of total duodenal protein, it is desirable also to express EAA requirements in terms of digestible (i.e., metabolizable) requirements rather than on the basis of total flows. In recognition of the need for research aimed at defining AA requirements

and the need for models designed to predict as accurately as possible passage of digestible EAA to the small intestine, the model was extended to predict flows of digestible EAA and the EAA composition of MP. The following 9 equations are used; again, Lys is used as the example EAA.

$$\text{RUP}_{\text{Lys}} = \sum_f (\text{DMI}_f \times \text{CP}_f \times \text{RUP}_f \times \text{Lys}_f \times 0.01) \quad (5-6)$$

where:

- RUP_{Lys} = amount of Lys supplied by total diet RUP, g
- DMI_f = intake of DM of each feedstuff contributing RUP, kg
- CP_f = crude protein content of each feedstuff contributing RUP, g/100 g DM
- RUP_f = ruminally undegraded protein content of each feedstuff contributing RUP, g/100 g CP
- Lys_f = lysine content of each feedstuff contributing RUP, g/100 g CP

The preceding equation is used to calculate for each feedstuff, and subsequently the diet, the amount of Lys supplied by RUP. Equation 5-6 is extended in the following manner to calculate the amount of digestible Lys supplied by RUP, which weights feedstuffs appropriately for differences of digestibility of RUP and concentration of Lys among feeds.

$$\text{dRUP}_{\text{Lys}} = \sum_f (\text{DMI}_f \times \text{CP}_f \times \text{RUP}_f \times \text{RUP}_{\text{digestibility}_f} \times \text{Lys}_f \times 0.001) \quad (5-7)$$

where:

- dRUP_{Lys} = amount of digestible Lys supplied by total diet RUP, g
- DMI_f = intake of DM of each feedstuff contributing RUP, kg
- CP_f = crude protein content of each feedstuff contributing RUP, g/100 g DM
- RUP_f = ruminally undegraded protein content of each feedstuff contributing RUP, g/100 g CP

TABLE 5-14 Comparison of Root Mean Square Prediction Errors (RMSPE) Obtained from Plots of Residuals (predicted-measured vs. measured) for Equations That Predicted Directly the Flow of Each EAA With Those Accepted for Use in the Model That Predict Directly the Percentage of Each EAA in Total EAA of Duodenal Protein

Amino acid	Flow		Percentage	
	RMSPE from plots for %	RMSPE from plots for g/d ^a	RMSPE from plots for %	RMSPE from plots for g/d ^b
Arg	0.46	6.1	0.24	2.8
His	0.26	3.0	0.13	1.3
Ile	0.34	4.4	0.14	1.3
Leu	0.51	9.4	0.45	4.8
Lys	0.45	7.0	0.33	3.5
Met	0.22	2.7	0.14	1.3
Phe	0.28	5.9	0.16	1.5
Thr	0.25	5.6	0.14	1.5
Val	0.22	5.4	0.17	1.7
Total EAA		40.6		47.8

^aPercentages of each EAA in duodenal total EAA were calculated from predicted flows of individual EAA.

^bFlows of each EAA to the duodenum were calculated from predicted flows of total EAA and predicted percentages of each EAA in duodenal total EAA.

$RUP_{digestibility_i}$ = digestibility coefficient of ruminally undegraded protein for each feedstuff contributing RUP, g/100 g RUP
 Lys_i = lysine content of each feedstuff contributing RUP, g/100 g CP

The preceding two equations then are combined to yield the calculation of digestible RUPLys as a percentage of total RUPLys for the diet.

$$PctdRUPLys = 100 \times (dRUPLys/RUPLys) \quad (5-8)$$

where:

$PctdRUPLys$ = digestibility coefficient for Lys supplied by RUP, g/100 g
 $dRUPLys$ = amount of digestible Lys supplied by total diet RUP, g
 $RUPLys$ = amount of Lys supplied by total diet RUP, g

In order to calculate the supply of total digestible Lys, two "pools" must be considered. The first pool is the amount supplied by RUP. The equation for predicting total EAA has associated with it a coefficient of 0.863 for RUPEAA, which indicates that the total EAA supplied by RUP (thus, individual AA supplied by RUP) is "discounted" by 13.8 percent (i.e., $100 - 86.3$). Theoretically, the total flow (g/d) of Lys from RUP can be calculated.

$$TotalRUPLysFlow = 0.863 \times RUPLys \quad (5-9)$$

where:

$TotalRUPLysFlow$ = adjusted total supply of Lys from RUP, g
 $RUPLys$ = amount of Lys supplied by total diet RUP, g

The second "pool" is the amount of Lys supplied from MCP and endogenous CP, and is calculated by difference from total Lys flow and the supply of Lys from RUP as calculated in Equation 5-9.

$$TotalMCPEndoLysFlow = LysFlow - TotalRUPLysFlow \quad (5-10)$$

where:

$TotalMCPEndoLysFlow$ = supply of Lys from MCP and endogenous CP, g
 $LysFlow$ = total amount of Lys in duodenal protein, g
 $TotalRUPLysFlow$ = adjusted total supply of Lys from RUP, g

The amount of digestible Lys supplied by each of the two pools and total digestible Lys is calculated as follows:

$$dTotalRUPLys = TotalRUPLysFlow \times PctdRUPLys \times 0.01 \quad (5-11)$$

where:

$dTotalRUPLys$ = supply of digestible Lys from RUP, g
 $TotalRUPLysFlow$ = adjusted total supply of Lys from RUP, g

$PctdRUPLys$ = digestibility coefficient for Lys supplied from RUP (i.e., Equation 5-8), g/100g

$$dTotalMCPEndoLys = 0.80 \times TotalMCPEndoLysFlow \quad (5-12)$$

where:

$dTotalMCPEndoLysFlow$ = supply of Lys from MCP and endogenous CP, g

$$TotalDigestibleLys = \text{Equation 5-11} + \text{Equation 5-12} \quad (5-13)$$

The final step is to calculate digestible Lys as percentage of MP.

$$dLysPctMP = 100 \times (TotalDigestibleLys / (MPBact + MPFeed + MPEndo)) \quad (5-14)$$

where:

$dLysPctMP$ = digestible Lys as percentage of MP, %
 $TotalDigestibleLys$ = total amount of digestible Lys (i.e., Equation 5-13), g
 $MPBact$ = model predicted MP from MCP, g
 $MPFeed$ = model predicted MP from RUP, g
 $MPEndo$ = model predicted MP from endogenous CP, g

Requirements for Lysine and Methionine in Metabolizable Protein for Lactating Cows

The AA requirements of dairy cattle are not known with much certainty. Attempts have been made to quantify AA requirements of cattle using the factorial approach (Oldham, 1981; O'Connor et al., 1993). The factorial method is a mathematic approach of calculating requirements from a segmentation of the requirements into individual and independent components, and from knowledge of pool sizes and the rates by which nutrients move through digestive and metabolic pools. More specifically, calculating requirements for absorbed AA using this approach requires at a minimum a knowledge of: (1) net protein requirements for maintenance, growth, pregnancy, and lactation, (2) AA composition of products, and (3) efficiencies of use of absorbed AA for maintenance and product formation. The Cornell Net Carbohydrate and Protein System for evaluating cattle diets and the associated AA submodel (O'Connor et al., 1993) is the most tested of the AA factorial models published to date in the United States. It was the opinion of the subcommittee, however, that current knowledge is too limited, both for model construction and model evaluation, to put forth a model that quantifies AA requirements for dairy cattle. Indeed, there have been few direct attempts to quantify AA requirements of dairy cattle (Campbell et al., 1997; Fenderson and Bergen, 1975; Titgemeyer et al., 1988; Williams and Smith, 1974). This is due largely to the technical difficulties involved in provid-

ing graded amounts of a limiting AA to sites of absorption in ruminants at various production levels, while simultaneously measuring AA flows to the small intestine and weight gains or milk production.

An alternate and more direct approach to defining AA requirements is to use the dose-response approach to estimate required AA concentrations in MP for maximal use of MP for protein synthesis. Thus far, the most progress has been made for Lys and Met in lactating cows. Two dose-response approaches have been used. The first is the “direct” dose-response approach, whereby postruminal supplies of Lys (Rulquin et al., 1990; Schwab et al., 1992b) or Met (Pisulewski et al., 1996; Socha et al., 1994a,b,c) were increased in graded fashion via intestinal infusion and production responses and AA flows to the small intestine were measured. A constant amount of supplemental Met was provided in each of the Lys experiments and a constant amount of supplemental Lys was provided in each of the Met experiments to reduce the possibility that they would limit responses. This approach indicated that for cows fed corn-based diets, Lys must contribute about 7.0 percent and Met about 2.5 percent of total AA in duodenal digesta for maximum content and yield of protein in milk.

The second method for estimating the optimum amounts of Lys and Met in MP for lactating cows is an “indirect” dose-response approach. This approach was used by Rulquin et al. (1993) and involved five steps: (1) predicting concentrations of digestible Lys and Met in protein truly digested in the small intestine (PDI) for control and treatment groups in experiments in which postruminal supplies of Lys, Met, or both were increased (either by intestinal infusion or by feeding in ruminally protected form) and production responses were measured, (2) identifying “fixed” concentrations of Lys and Met in PDI that were intermediate to the lowest and highest values in the greatest number of Lys experiments and Met experiments, respectively, (3) calculating by linear regression a “reference production value” for each production parameter in each Lys experiment that corresponded to the “fixed” level of Lys in PDI and in each Met experiment that corresponded to the “fixed” level of Met in PDI, (4) calculating “production responses” (plus and minus values) for control and treatment groups relative to the “reference production values,” and (5) regressing the production responses on the predicted concentrations of Lys and Met in PDI. Experiments involving ruminally protected Lys or Met were limited to those in which data on ruminal stability and postruminal release of Lys and Met had been obtained in the author’s laboratory.

Using the described approach, Rulquin et al. (1993) obtained curvilinear (monomolecular) dose-response relationships for content and yield of milk protein to increasing concentrations of Lys in PDI. The authors reported that concentrations of Met in PDI had no apparent effect on

milk protein responses to Lys in PDI. In contrast, concentrations of Lys lower than 6.5 percent of PDI limited responses to increases in Met. Thus, curvilinear dose-response relationships for content and yield of milk protein to increasing concentrations of Met in PDI were obtained from the data for Lys concentrations greater than 6.5 percent of PDI. Assuming that Lys and Met requirements were met when protein yield responses were slightly below the maximum attainable values (as determined from the derived exponential equations), the authors concluded that the requirements for Lys and Met in PDI are the amounts that would result in the production of 16 g less milk protein (i.e., 0.5 kg milk containing 3.2 percent true protein) than the maximum attainable values. Using the derived equations, the calculated requirements for Lys and Met in PDI were 7.3 percent and 2.5 percent, respectively.

The “indirect” dose-response approach described by Rulquin et al. (1993) was used in this revision to determine the requirements for Lys and Met in MP for lactating cows. A unique and practical feature of this approach is that the requirement values are estimated using the same model as that used to estimate the contributions of feedstuffs to AA passage to the small intestine. Experiments were identified in which Lys (18 experiments; 63 treatments) or Met (27 experiments; 87 treatments) was infused continuously into the abomasum or duodenum or fed in ruminally protected form (Table 5-15). Experiments were not considered if diet or feed intake information was insufficient for model input, or if Lys and Met were supplemented together and there was no corresponding control where one of the two AA was supplemented at the same concentration. Of the 36 different experiments that were identified (9 experiments involved the administration of one or more quantities of both Lys and Met), 24 were Latin squares and of these 18 were infusion experiments. Experiments in which ruminally protected products were fed were restricted to those that had data for viability reported in peer-reviewed literature and estimates of ruminal escape were 80 percent or higher. Experiments involving rumina-

TABLE 5-15 Studies Used to Determine the Dose-Response Relationships for Lysine and Methionine in Metabolizable Protein

Armentano et al. (1997)	Rogers et al. (1987)
Casper et al. (1987)	Rulquin and Delaby (1997)
Casper and Schingoethe (1988)	Rulquin and Delaby (1994)
Guinard and Rulquin (1994)	Rulquin et al. (1994)
Illg et al. (1987)	Schingoethe et al. (1988)
King et al. (1991)	Schwab et al. (1976)
Munneke et al. (1991)	Schwab et al. (1992a)
Papas et al. (1984a)	Schwab et al. (1992b)
Papas et al. (1984b)	Socha (1994)
Piepenbrink et al. (1999)	Socha et al. (1994a)
Pisulewski et al. (1996)	Socha et al. (1994b)
Polan et al. (1991)	Yang et al. (1986)

lly protected products with published estimates of ruminal escape less than 80 percent were not used because of the concern that ruminally released Met may affect ruminal fermentation and AA passage to the small intestine. All experiments utilized Holstein cows. All but 2 experiments involved early and mid lactation cows. Ten experiments involved both multiparous and primiparous cows and 26 experiments involved only multiparous cows. Cows produced an average of 31.5 kg milk in the Lys experiments (range = 20.7 to 46.3 kg) and an average of 33.7 kg milk in the Met experiments (range = 20.9 to 43.1 kg).

To calculate concentrations of Lys and Met in MP, all cow and diet data were entered into the model. Published nutrient composition of the individual ingredients was used when available; otherwise, model default values were used. When nutrient composition of ingredients was not published but nutrient composition of the total diet was included, nutrient composition of individual ingredients (usually only the forages) was changed so that the composition of the diet was the same as the published composition. In all cases, model default values were used for the AA composition of feeds. Contributions of supplemental Lys and Met to predicted flows of digestible Lys and Met originating from the basal diet were estimated as follows: (1) the intestinal availability of infused Lys and Met was considered to be 100 percent, (2) ruminally protected sources of Lys and Met containing polymers in the surface coating (see next section, "Ruminally Protected Amino Acids") were considered to have a ruminal escape of 90 percent and an intestinal digestibility coefficient of 90 percent (Rogers et al., 1987; Schwab, 1995a) so 81 percent (0.90×0.90) of the fed amounts of Lys and Met was considered digestible, and (3) the ruminally protected Met product, Ketionin (Rumen Kjemi; Oslo, Norway), was considered to have a ruminal escape of 80 percent and an intestinal digestibility of 75 percent (Schwab, 1995a; Yang et al., 1986) so 60 percent of the fed amounts of Met was considered digestible.

Predicted concentrations of Lys in MP varied between 4.33 percent and 9.83 percent and for Met between 1.70 percent and 3.36 percent. The "fixed" concentration of Lys in MP that was selected (6.67 percent) to calculate the required "reference production values" was intermediate to the lowest and highest concentrations in 16 of the 18 Lys experiments. This eliminated the experiments of Polan et al. (1991) (6 treatments with predicted concentrations of Lys in MP between 4.32 percent and 5.87 percent) and Rogers et al. (1987) (4 treatments with predicted concentrations of Lys in MP between 6.76 and 7.55 percent). The "fixed" concentration of Met in MP (2.06 percent) that was selected was intermediate to the lowest and highest concentrations in all of the 27 Met experiments. The "reference production values" for each experiment and the "production responses" (plus and minus values) for each pro-

duction parameter for each treatment were calculated as described above. The final database contained 53 observations for Lys and 87 observations for Met.

As observed by Rulquin et al. (1993), changes in milk yield, milk fat content, and milk fat yield to changes in concentrations of Lys and Met in MP were small and inconsistent. These observations were expected (see section, "Limiting Essential Amino Acids"). Therefore, no attempt was made to use these production measurements as response criteria for establishing requirements for Lys and Met in MP.

Four statistical models were used to describe the relationships between increasing concentrations of Lys and Met in MP and milk protein content and yield responses. These were: (1) a straightforward quadratic model (SAS, GLM procedure), (2) a negative exponential curve model (SAS, NLIN procedure), (3) a segmented quadratic model with a plateau (SAS, NLIN procedure), and (4) a rectilinear model (referred to in the literature as a linear abrupt threshold and plateau model, essentially consisting of a straight line followed by a plateau) (SAS, NLIN procedure). Analyses involving all models indicated that low concentrations of Met in MP limited responses to increasing concentrations of Lys in MP and that low concentrations of Lys in MP limited responses to increasing concentrations of Met in MP. The final regression analysis for Lys was limited to data where Met was 1.95 percent or more of MP ($n = 41$ of 53) and for Met it was limited to data where Lys was 6.50 percent or more of MP ($n = 48$ of 87). Using these restricted databases, the rectilinear model was either equal to or superior to the other models for describing protein content and protein yield responses to increasing amounts of both Lys and Met in MP. Based on these findings, the rectilinear model was accepted as the final model. An advantage of the rectilinear model is that the breakpoint in the nutrient dose-response line provides an objective, mathematically determined estimate of nutrient requirements. However, a requirement predicted by this type of break-point analysis is usually lower than that predicted by a curvilinear model because of the implicit smoothness constraint of curvilinear models. The appropriateness of different models for defining AA requirements have been discussed (Baker, 1986; Fuller and Garthwaite, 1993; Owens and Pettigrew, 1989).

The plots of predicted concentrations of Lys and Met in MP and the corresponding responses for milk protein content for all data are presented in Figure 5-12; the equivalent plots for milk protein yield are in Figure 5-13. The rectilinear dose-response relationships for the restricted databases are in the same figures. There are several noteworthy observations. First, the breakpoint estimates for the required concentrations of Lys and Met in MP for maximal yield of milk protein (7.08 percent and 2.35 percent, respectively; Figure 5-13) are similar to those

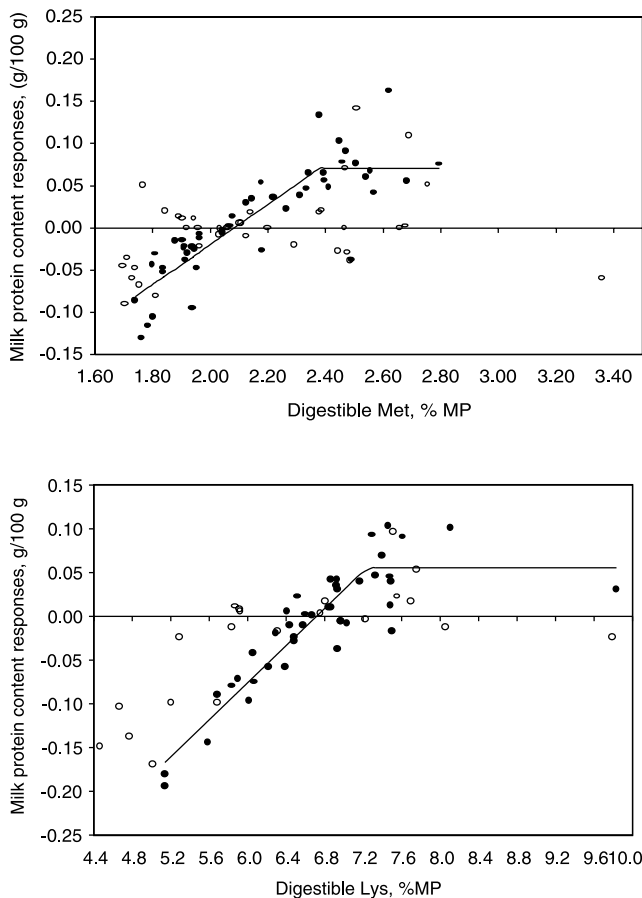


FIGURE 5-12 Milk protein content responses as a function of digestible Lys and Met concentrations in MP. Regression analysis for Lys was limited to data where Met was 1.95 percent or more of MP (filled circles) [$y = -0.712 + 0.106x$ for the linear part of the model and $y = -0.712 + 0.106 \times 7.24$ for the plateau (SE = 0.12 for x value of breakpoint); $r^2 = 0.85$; SE = 0.029; $n = 41$]. Regression analysis for Met was limited to data where Lys was 6.50 percent or more of MP (filled circles) [$y = -0.496 + 0.238x$ for the linear part of the model and $y = -0.496 + 0.238 \times 2.38$ for the plateau (SE = 0.07 for x value of breakpoint); $r^2 = 0.76$; SE = 0.033; $n = 48$]. The “trial” effect was not significant and therefore, not included in the model.

required for maximal content of milk protein (7.24 percent and 2.38 percent; Figure 5-12). For both AA, the nutrient-response relationships were determined more accurately for protein content than for protein yield.

Based on these results, it is concluded that optimal use of MP for the combined functions of maintenance and milk protein production requires concentrations of Lys and Met in MP (as determined by this edition’s model) that approximate 7.2 percent and 2.4 percent, respectively. Second, the resultant requirement values are strikingly similar to the values of 7.3 percent and 2.5 percent proposed by Rulquin et al. (1993). As noted previously, the requirements proposed by Rulquin et al. (1993) were calculated

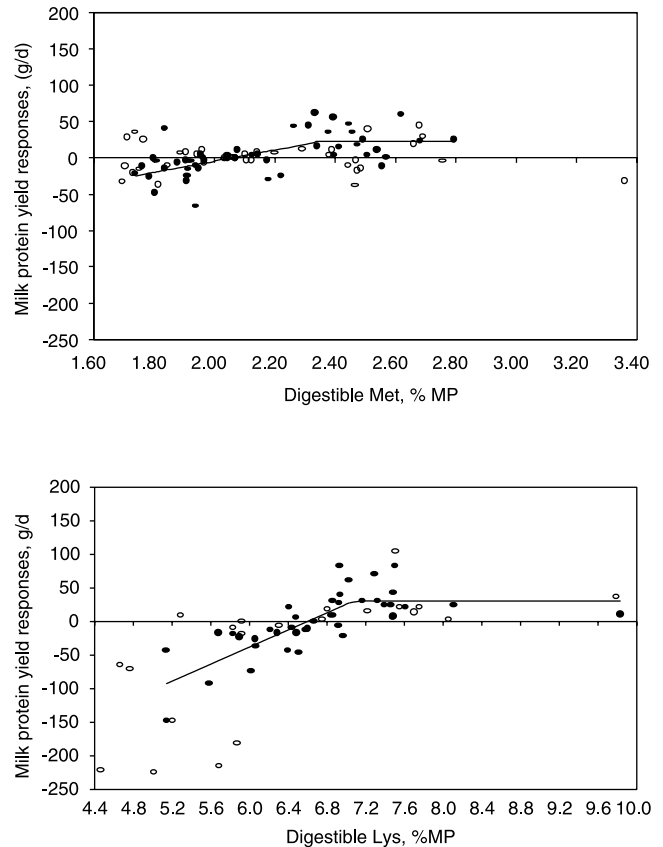


FIGURE 5-13 Milk protein yield responses as a function of digestible Lys and Met concentrations in MP. Regression analysis for Lys was limited to data where Met was 1.95 percent or more of MP (filled circles) [$y = -419.6 + 63.62x$ for the linear part of the model and $y = -419.6 + 63.62 \times 7.08$ for the plateau (SE = 0.18 for x value of breakpoint); $r^2 = 0.62$; SE = 27.9; $n = 41$]. Regression analysis for Met was limited to data where Lys was 6.50 percent or more of MP (filled circles) [$y = -159.1 + 77.30x$ for the linear part of the model and $y = -159.1 + 77.30 \times 2.35$ for the plateau (SE = 0.13 for x value of breakpoint); $r^2 = 0.40$; SE = 21.8; $n = 48$]. The “trial” effect was not significant and therefore, not included in the model.

to be somewhat less than required for maximum response as determined using an exponential representation of milk protein yield responses. Third, the observed optimum concentrations of Lys and Met in MP for the combined functions of maintenance and milk protein production (7.3 percent and 2.4 percent) are within their reported concentrations in milk protein (7.1 to 8.2 percent and 2.4 to 2.7 percent, respectively) (Rulquin et al., 1993; Waghorn and Baldwin, 1984). This observation may be considered as providing evidence of the reasonableness of the observed requirements. And last, an examination of Figures 16-4 and 16-5 indicates that implementation of diet formulation strategies that increase Lys and Met in MP to concentra-

tions that approach or meet the requirement levels can result in more actual milk than MP allowable milk. Indeed, achieving the optimum concentrations of the most limiting AA in MP is the first step in balancing diets for AA. The subcommittee encourages more research aimed at determining the ideal profile of EAA in MP of growing cattle and lactating cows. The results of such efforts are needed to combine protein supplements and ruminally protected AA in ways to meet AA requirements of dairy cattle with minimal MP, and thus, minimal RUP.

Ruminally Protected Amino Acids

As discussed, Lys and Met are two of the most limiting AA for protein synthesis in dairy cattle fed corn-based diets. A challenge in diet formulation, particularly for animals requiring higher RUP diets, is to achieve the desired concentrations of both Lys and Met in MP by relying solely on feed protein supplements. Supplements of crystalline Lys and Met have not been considered efficacious because of rapid deamination in the rumen (Chalupa, 1976; Onodera, 1993). Thus, a considerable effort has been made to develop technologies for supplying Met and Lys in forms that would allow them to escape ruminal degradation without compromising substantially their digestibility in the small intestine. The physical-chemical properties of Lys are such that application of most technologies are currently limited to Met.

The methods that have been evaluated for protecting free AA from ruminal degradation have been reviewed (Loerch and Oke, 1989; Schwab, 1995a). Technologically, the approaches in current use fall into one of three categories: (1) surface coating with a fatty acid/pH-sensitive polymer mixture, (2) surface coating or matrices involving fat or saturated fatty acids and minerals, and (3) liquid sources of Met hydroxy analog (DL-2-hydroxy-4-methylthiobutanoic acid; HMB).

Technology # 1 provides for a postruminal delivery system that is independent of digestive enzyme function and dependent on the differences in pH between the rumen and abomasum. The resulting ruminally inert products have an apparent high coefficient of rumen protection (Mbanzamihigo et al., 1997; Robert and Williams, 1997; Schwab, 1995a) and possess high intestinal release coefficients of the coated AA (Robert and Williams, 1997). This technology appears to be the most effective in increasing Met in MP as evidenced by the largest increases in blood Met concentrations (Blum et al., 1999; Robert et al., 1997).

Several variations of technology # 2 have been evaluated (Loerch and Oke, 1989; Schwab, 1995a). The physical-chemical properties of Lys are such that this technology has generally been limited to Met. The technology relies in identifying a combination of process and materials that provides a coating or matrix that gives a reasonable degree of protection against ruminal degradation, provided by the

relatively inert characteristics of saturated fat in the rumen, while providing also for a reasonable degree of intestinal release. The apparent bioavailability of Met (ruminal escape $\times$ intestinal release) from RPMet products using this approach is less than RPMet products utilizing technology # 1 (Bach and Stern, 2000; Berthiaume et al., 2000; Blum et al., 1999; Mbanzamihigo et al., 1997; Overton et al., 1996).

Technology # 3 (i.e., liquid HMB) is currently being evaluated as an alternative to coated or encapsulated forms of Met. The Ca salt of HMB, commonly known as Met hydroxy analog, has been studied extensively as a supplement for increasing milk and milk fat production (Loerch and Oke, 1989). The Ca salt of HMB is no longer manufactured but liquid HMB is available and is used in the poultry and swine industry as a substitute for Met. It is well documented in nonruminants that following absorption, HMB is first converted to the α -keto analog of Met and then transaminated to L-Met (Baker, 1994). The combined efficiencies of absorption and conversion rates to Met in nonruminants is still being questioned. Baker (1994) summarized the efficiency estimates for dietary HMB and concluded that appropriate "Met bioavailability" values (molar basis) for rats, chickens, and pigs were 70, 80, and 100 percent, respectively. Comparable "Met bioavailability" data (ruminal escape $\times$ intestinal absorption $\times$ conversion to Met) is not available for ruminants. However, studies indicate that HMB is more resistant to ruminal degradation than free Met (Belasco, 1972, 1980; Patterson and Kung, 1988), that it can be absorbed across the ruminal and omasal epithelium (McCollum et al., 2000), and that ruminants possess the enzymes involved in the conversion of HMB to Met (Belasco, 1972, 1980; Papas et al., 1994). The study of Koenig et al. (1999) is the only reported attempt to quantify ruminal escape and intestinal absorption of liquid HMB in dairy cattle. In this study, a 90-g pulse-dose of HMB was given to lactating dairy cows fed a diet containing 30 g/d HMB. Based on fractional rate constants for ruminal and duodenal disappearance of HMB and passage of liquid, the workers reported that 50 percent of the HMB escaped ruminal degradation. However, the extent to which dietary HMB substitutes for absorbed Met for protein synthesis remains questionable because of observed minimal effects on blood Met concentrations (Johnson et al., 1999; Robert et al., 1997) and milk protein concentrations (Johnson et al., 1999; Rode et al., 1998).

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