5 Protein and Amino Acids

Dietary protein generally refers to crude protein (CP), which is defined for feedstuffs as the nitrogen (N) content × 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 microorganisms 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 (Kopecny 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, B_1, B_2, B_3, 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 acetic (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 B_1 is that percentage of total CP that is soluble in borate-phosphate buffer and precipitated with TCA. Fraction B_3 is calculated as the difference between the portions of total CP recovered with NDF (i.e., NDIN) and ADF (i.e., fraction C). Fraction B_2 is the remaining CP and is calculated as total CP minus the sum of fractions A, B_1, B_3, and C. Reported ranges for the fractional rates of degradation for the three B fractions are: B_1 (120–400 %/h), B_2 (3–16 %/h), and B_3 (0.06–0.55 %/h). The RDP and RUP values (percent of CP) for a feedstuff using this model are computed using the equations

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

and

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

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

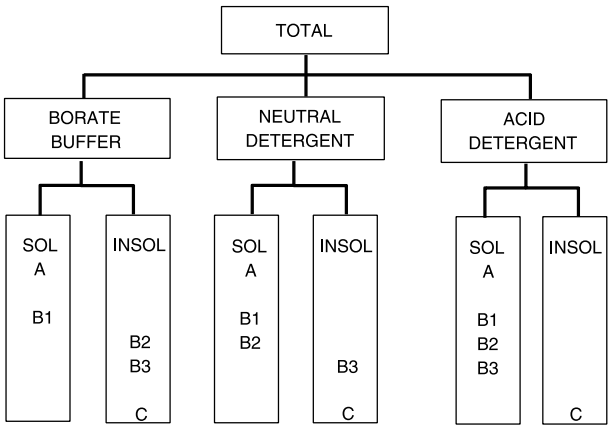


FIGURE 5-1 Analyses of crude protein fractions using borate-phosphate buffer and acid detergent and neutral detergent solutions (Roe et al., 1990; Sniffen et al, 1992).

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 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 degradabilites 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 B_1 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, rapeseed 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 N^{15} 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 N^{15} 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 C^{14}-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)
Blauwiekel and Kincaid (1986)	Hongerholt and Muller (1998)	Polan et al. (1997)
Blauwiekel 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: $2.3 - 0.1 \times CP$. 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) $= 17.7 \times DMI + 55.6 \times CP - 1.26 \times CP^2 + 31.8$ ($r^2 = 0.19$) 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 $55.63 - 2.52 \times CP$ 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:

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

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)
Blauwiekel 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)		

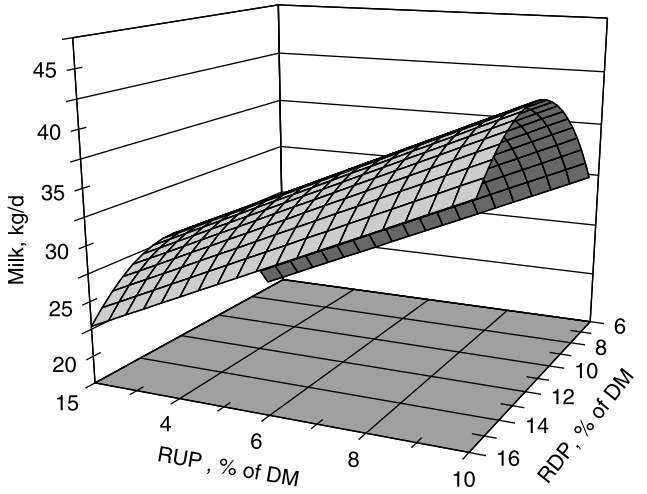


FIGURE 5-2 Response surface for data set described in "Animal Responses to CP, RDP, and RUP" section. Maximum milk yield occurred at 12.2 percent RDP (percent of diet DM). Dry matter intake was held constant at 20.6 kg/day.

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 × 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 (DMI = 14.4 + 0.58 × 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:

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

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 hypophysealpituitary-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 Ruminal 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 asynchronized (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 $(^{15}NH_4)_2SO_4$. 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

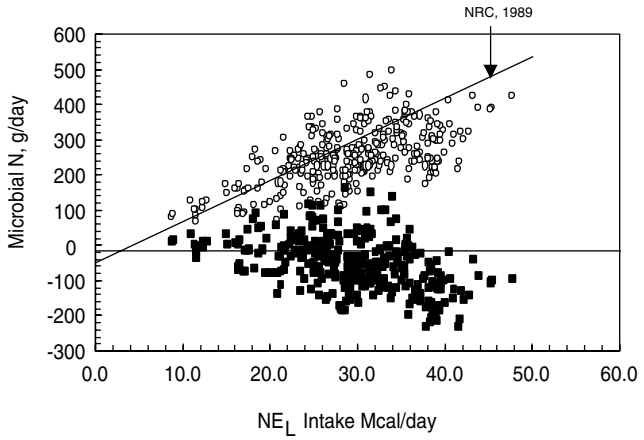


FIGURE 5-3 Plot of observed (open circles) and residuals (squares) for measured microbial N flow (g/day) versus estimated NE_L intake in lactating and dry dairy cows. The National Research Council, 1989, line is the predicted line where microbial N = $-30.93 + 11.45\ NE_L$. At high levels of NE_L intake, 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)	Klusmeyer et al. (1991b)	Robinson and Sniffen (1985)
Arieli et al. (1993)	Klusmeyer et al. (1990)	Robinson et al. (1991a)
Armentano et al. (1986)	Kung et al. (1983)	Robinson et al. (1997)
Benchaar et al. (1994a)	Lu et al. (1988)	Robinson et al. (1994)
Benchaar et al. (1991)	Lykos et al. (1997)	Robinson et al. (1985)
Benchaar 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)
Klusmeyer et al. (1991a)	Putnam et al. (1997)	Zhu et al. (1997)

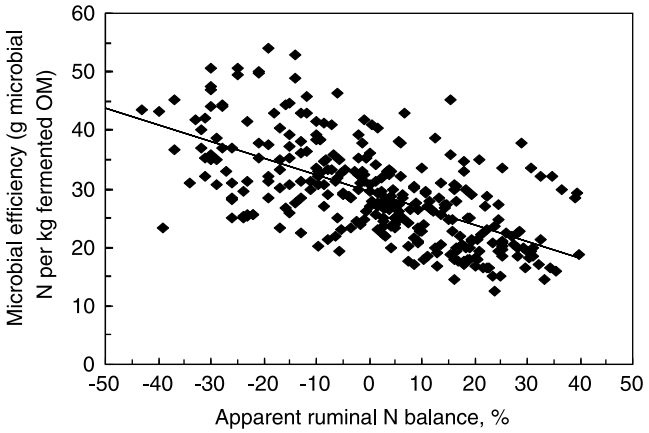


FIGURE 5-4 Relationship between measured efficiency of microbial protein synthesis (g microbial N/kg rumen fermented OM) and apparent ruminal N balance (microbial efficiency = 29.74 − 0.30 apparent ruminal N digestibility percent, $r^2 = 0.41$, $P < 0.001$, Sy = 6.49, n = 306).

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 × 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) × 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 nonfatty 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 (g microbial N/kg of TTDOM = 32.78 − 8.29 RDN:MN, r^2 = 0.35, P <0.001, Sy = 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.

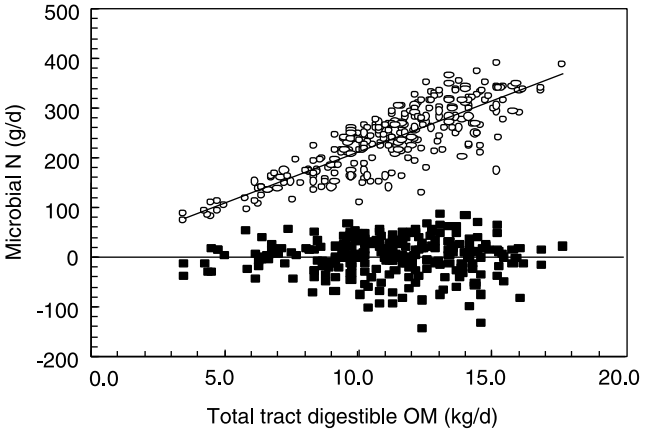


FIGURE 5-5 Plot of adjusted (open circles) and residuals (squares) for measured microbial N (g/d) versus measured total tract digestible OM (kg/d). (Microbial N = 21.03 total tract digestible OM. r^2 = 0.69, P < 0.001, Sy = 38.1, n = 266).

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 × 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 × MCP yield. Therefore, yield of MCP is calculated as 0.130 × TDN (discounted TDN, see Chapter 2) when RDP intake exceeds 1.18 × MCP yield. When RDP intake is less than 1.18 × 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 estimated 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:

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

where:

RDP = RDP of the feedstuff, percentage of CP
A = Fraction A, percentage of CP
B = Fraction B, percentage of CP
Kd = rate of degradation of the B fraction, %/h
Kp = rate of passage from the rumen, %/h

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

where:

RUP = RUP of the feedstuff, percentage of CP
B = Fraction B, percentage of CP
Kd = rate of degradation of the B fraction, %/h
Kp = rate of passage from the rumen, %/h
C = Fraction C, percentage of CP

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

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 Kp 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 [g/kg DM = 0.90 (RUP N-ADIN)/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
Diet	
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 *ad libitum* DMI
Evaluated feedstuff	
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)
Bag	
Material	Polyester
Pore size	40 to 60 μ
Incubation procedure	
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
Microbial correction	Required
Mathematic model	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 nonusable 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 l 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) trichloracetic 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 Jarrige (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 nonlactating, pregnant Holstein cows (650 and 700 kg) of 8.3 g/d or 51 mg/kg $BW^{0.75}$; for two steers (307 and 405 kg), the flows were 5.1 g/d or 58.2 mg/kg $BW^{0.75}$. 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)	Kopecný 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 ^{15}N-enriched urea. From measured ^{15}N 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 ($3.6 \text{ g} \times 0.915 \times 0.625 = 2.1$ 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 × 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 $BW^{.75}$ 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 × 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)	Younker 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)	Rangngang 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)

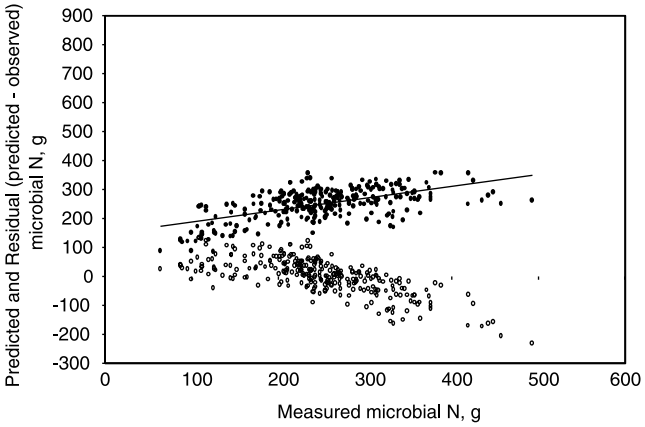


FIGURE 5-6 Plot of predicted vs. measured (filled circles) and residuals (predicted-measured; open circles) vs. measured flows of microbial N to the small intestine of dairy cows (y = 0.4109x + 146.5; r^2 = 0.35; mean bias = + 0.52; RMSPE = 63.1; n = 284).

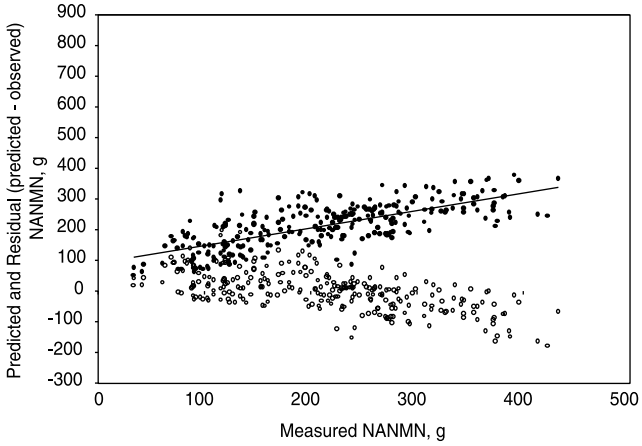


FIGURE 5-7 Plot of predicted vs. measured (filled circles) and residuals (predicted-measured; open circles) vs. measured flows of NANMN (rumen undegradable N plus endogenous N) to the small intestine of dairy cows (y = 0.5701x + 91.193; r^2 = 0.51; mean bias = −0.12; RMSPE = 63.1; n = 275).

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 digestibility 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

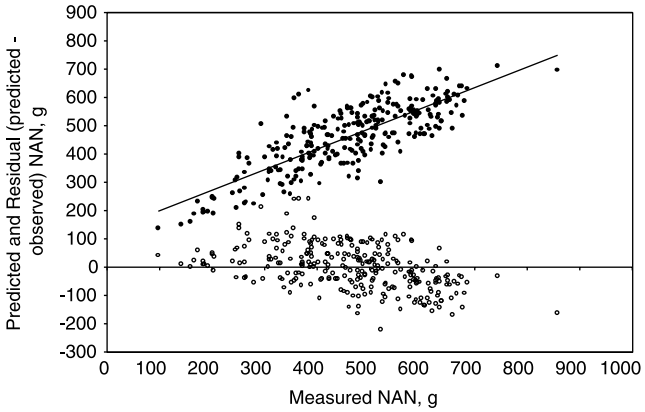


FIGURE 5-8 Plot of predicted vs. measured (filled circles) and residuals (predicted-measured; open circles) vs. measured flows of NAN (microbial N + rumen undegradable N + endogenous N) to the small intestine of dairy cows (y = 0.7251x + 127.1; r^2 = 0.64; mean bias = +0.14; RMSPE = 78.3; n = 275).

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 × $BW^{0.50}$) 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 × $BW^{0.50}$.

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 × $BW^{0.60}$.

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 × 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 × 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 (kg) × 30) − 0.50((bacterial MP/0.80)-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: endogenous MP/0.67.

In summary, the overall equation for predicting the MP requirement for maintenance (g/d) is: MP = $4.1 \times BW^{0.50}$ (kg) + $0.3 \times BW^{0.60}$ (kg) + [(DMI (kg) × 30) − 0.50((bacterial MP/0.8)-bacteria MP)] + 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 + ((DaysPreg − 190) × 0.665)) × (CBW/45)

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

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

The MPPreg is MPPreg = (((0.69 × DaysPreg) − 69.2) × (CBW/45))/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) = milk production, kg/d × (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) × 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 × (268–(29.4 × (RE / ADG))). If (EQSBW < or = 478 kg) then efficiency of use of MP for growth (EffMP_NPg) = (83.4 − (0.114 × EQSBW)) /

100 otherwise EffMP_NPg = 0.28908. Metabolizable protein for growth in g/d (MPGrowth) = NPg / 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. Pruekvimolphan 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
Ruminal microbes[a]			*Plant proteins*[b]		
Bacteria	36	47	Brewer's grains, dry	52	40
Protozoa	40	46	Brewer's grains, wet	50	—
Forages[b]			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
Grains and energy feeds[b]			Soybean meal	51	43
Barley	57	38	Sunflower meal	44	37
Corn	50	44	*Animal proteins*[b]		
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
Fibrous byproduct feeds[b]			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			

[a]Values were calculated from mean AA concentrations as reported by Martin et al. (1996) and Storm and Ørskov (1983).

[b]Contributions 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; Mepham 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).

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	Met	Phe	Thr	Trp	Val	EAA (%CP)
	(% of total EAA)										
Animal products											
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	—
Rumen microbes											
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	—
Forages[f,g]											
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
Grains[f]											
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
Plant proteins[f]											
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
Animal proteins[f]											
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

[a] From Ainslie et al. (1993); average values of empty, whole body carcasses as reported in 3 studies.
[b] Each value is an average of 3 observations from Jacobson et al. (1970), McCance and Widdowson (1978), and Waghorn and Baldwin (1984).
[c] From Clark et al. (1992); average values from 61 dietary treatments.
[d] From Storm and Ørskov (1983); average values from 62 literature reports.
[e] From Storm and Ørskov (1983); average values from 15 literature reports.
[f] Calculated from values presented in this edition of *Nutrient Requirements of Dairy Cattle* feed table.
[g] Legume 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 Augeard, 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 (Auboiron et al., 1995; Durand et al., 1992). Decreases in plasma nonesterified fatty acids concentrations in preruminant calves (Auboiron 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 Aryshire 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 C_4 to C_{16} fatty acids, particularly the C_{16} 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 converted to NEAA or utilized as energy sources in the mammary gland (Mepham, 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

		Contribution to dietary DM (%)				Contributions to dietary DM (%)	
Feedstuff	N[a]	Mean	Range	Feedstuff	N[a]	Mean	Range
Forages				*Protein supplements*			
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
Energy feeds				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	*Energy and protein feeds*			
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	*Byproduct feeds*			
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

[a]Number 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.

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

where:

RUPLys = 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

$$\text{RUPEAA} = \text{RUPArg} + \text{RUPHis} + \text{RUPIle} + \text{RUPLeu} + \text{RUPLys} + \text{RUPMet} + \text{RUPPhe} + \text{RUPThr} + \text{RUPTrp} + \text{RUPVal} \quad (5\text{-}4)$$

where:

RUPEAA = amount of essential AA supplied by RUP, g

$$\text{RUPLysPctRUPEAA} = 100 \times (\text{RUPLys}/\text{RUPEAA}) \quad (5\text{-}5)$$

where:

RUPLysPctRUPEAA = 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

[a] Predicted 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

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

where:
Y = Arg, % of EAA in duodenal protein
X_1 = Arg, % of EAA in RUP

Histidine

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

where:
- Y = Val, % of EAA in duodenal protein
- X_1 = Val, % of EAA in RUP

Total essential amino acids

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

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.

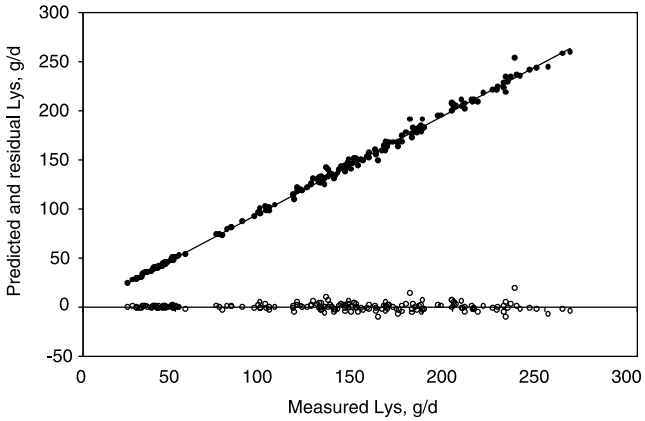


FIGURE 5-9 Plot of predicted vs. measured (filled circles) and residuals (predicted − measured; open circles) vs. measured (Lys, g/d) (from predicted Lys, percent of EAA and predicted EAA, g/d) (mean bias = 2.4×10^{-2}; RMSPE = 3.5; n = 186).

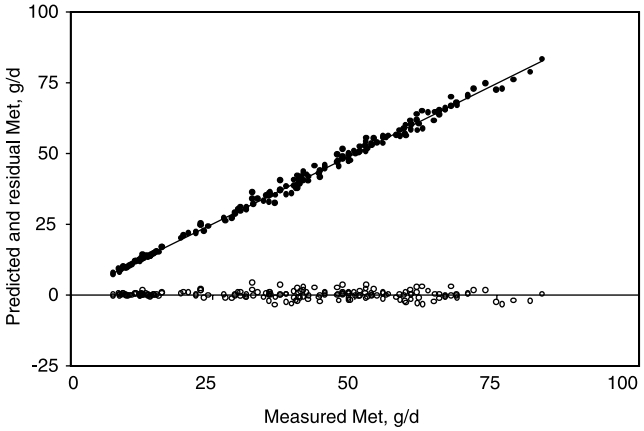


FIGURE 5-10 Plot of predicted vs. measured (filled circles) and residuals (predicted − measured; open circles) vs. measured Met, g/d (from predicted Met, percent of EAA and predicted EAA, g/d) (mean bias = 2.2×10^{-3}; RMSPE = 1.3; n = 182).

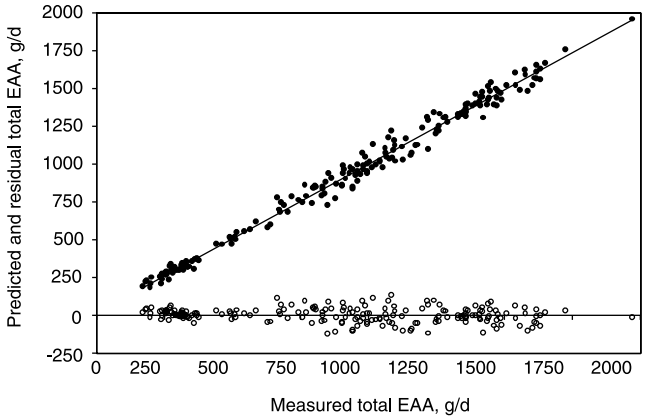


FIGURE 5-11 Plot of predicted vs. measured (filled circles) and residuals (predicted − measured; open circles) vs. measured flow of total EAA (mean bias = 3.06×10^{-5}; RMSPE = 47.8; n = 196).

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.

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

where:

- RUPLys = 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 preceeding 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.

$$\text{dRUPLys} = \Sigma_f (\text{DMI}_f \times \text{CP}_f \times \text{RUP}_f \times \text{RUPdigestibility}_f \times \text{Lys}_f \times 0.001) \quad (5\text{-}7)$$

where:

- dRUPLys = 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

	Flow		Percentage	
Amino acid	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

[a] Percentages of each EAA in duodenal total EAA were calculated from predicted flows of individual EAA.
[b] Flows of each EAA to the duodenum were calculated from predicted flows of total EAA and predicted percentages of each EAA in duodenal total EAA.

$RUPdigestibility_f$ = digestibility coefficient of ruminally undegraded protein for each feedstuff contributing RUP, g/100 g RUP
Lys_f = lysine content of each feedstuff contributing RUP, g/100 g CP

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

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

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.

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

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.

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

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:

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

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

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

where:

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

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

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

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

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).

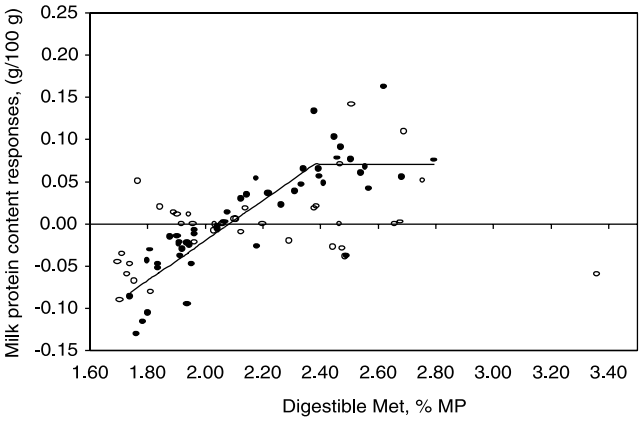
To calculate concentrations of Lys and Met in MP, all cow and diet data were entered into the model. Published nutrient composition of the individual ingredients was used when available; otherwise, model default values were used. When nutrient composition of ingredients was not published but nutrient 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.

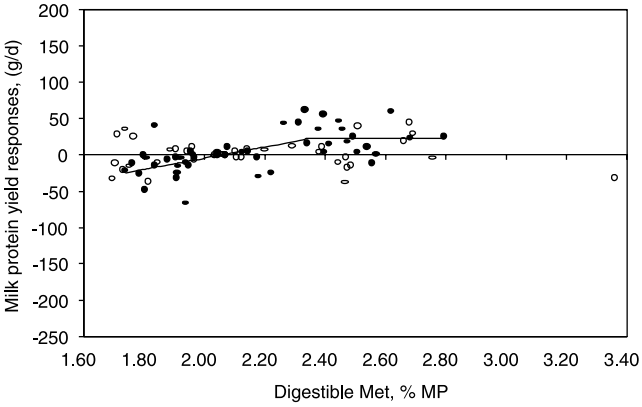
Predicted concentrations of Lys in MP varied between 4.33 percent and 9.83 percent and for Met between 1.70 percent and 3.36 percent. The "fixed" concentration of Lys in MP that was selected (6.67 percent) to calculate the required "reference production values" was intermediate to the lowest and highest 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 production parameter for each treatment were calculated as described above. The final database contained 53 observations for Lys and 87 observations for Met.

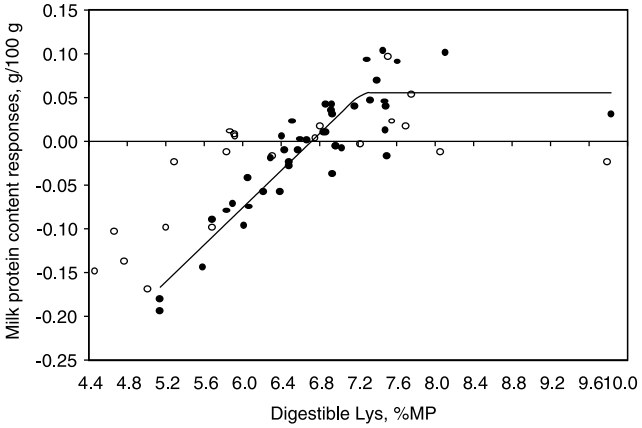
As observed by Rulquin et al. (1993), changes in milk yield, milk fat content, and milk fat yield to changes in concentrations of Lys and Met in MP were small and inconsistent. These observations were expected (see section, "Limiting Essential Amino Acids"). Therefore, no attempt was made to use these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







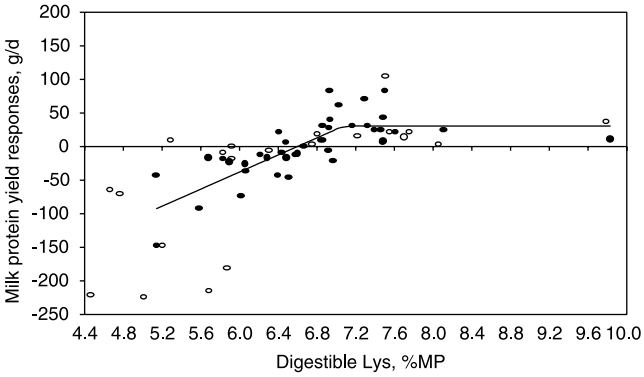


FIGURE 5-12 Milk protein content responses as a function of digestible Lys and Met concentrations in MP. Regression analysis for Lys was limited to data where Met was 1.95 percent or more of MP (filled circles) [y = −0.712 + 0.106x for the linear part of the model and y = −0.712 + 0.106 × 7.24 for the 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 × 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.

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 × 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 × 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.

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 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).

REFERENCES

Abe, M., T. Iriki, M. Funaba, and S. Onda. 1998. Limiting amino acids for a corn and soybean meal diet in weaned calves less than three months of age. J. Anim. Sci. 76:628–636.

Abe, M., T. Iriki, and M. Funaba. 1997. Lysine deficiency in postweaned calves fed corn and corn gluten meal diets. J. Anim. Sci. 75:1974–1982.

Agricultural and Food Research Council (AFRC). 1984. The Nutrient Requirements of Ruminant Livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Slough, England.

Agricultural and Food Research Council (AFRC). 1992. Nutritive requirements of ruminant animals: Protein. Nutr. Abstr. Rev. (Ser. B) 62:787–835.

Aharoni, Y., A. Arieli, and H. Tagari. 1993. Lactational response of dairy cows to change of degradability of dietary protein and organic matter. J. Dairy Sci. 76:3514–3522.

Ahmed, B. M., and W. G. Bergen. 1983. Metionine-cyst(e)ine relationship in steers. J. Anim. Sci. 57(Suppl.1):110. (Abstr.)

Ainslie, S. J., D. G. Fox, T. C. Perry, D. J. Ketchen, and M. C. Barry. 1993. Predicting amino acid adequacy of diets fed to Holstein steers. J. Anim. Sci. 71:1312–1319.

Aitchison, T. E., D. R. Mertens, A. D. McGilliard, and N. L. Jacobson. 1976. Effect of nitrogen solubility on nitrogen utilization in lactating dairy cattle. J. Dairy Sci. 59:2056–2062.

Akayezu, J. M., W. P. Hansen, D. E. Otterby, B. A. Crooker, and G. D. Marx. 1997. Yield response of lactating Holstein dairy cows to dietary fish meal or meat and bone meal. J. Dairy Sci. 80:2950–2963.

Aldrich, J. M., L. A. Holden, L. D. Muller, and G. A. Varga. 1996. Rumen availabilities of nonstructural carbohydrate and protein estimated from in situ incubation of ingredients versus diets. Anim. Feed Sci. Technol. 63:257–271.

Aldrich, C. G. N. R. Merchen, D. R. Nelson, and J. A. Barmore. 1995. The effects of roasting temperature applied to whole soybeans on site of digestion by steers: II. Protein and amino acid digestion. J. Anim. Sci. 73:2131–2140.

Aldrich, J. M., L. D. Muller, and G. A. Varga. 1993a. Effect of somatatropin administration and duodenal infusion of methionine and lysine on lactational performance and nutrient flow to the small intestine. Br. J. Nutr. 69:49–58.

Aldrich, J. M., L. D. Muller, G. A. Varga, and L. C. Griel, Jr. 1993b. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. J. Dairy Sci. 76:1091–1105.

Alexandrov, A. N. 1998. Effect of ruminal exposure and subsequent microbial contamination on dry matter and protein degradability of various feedstuffs. Anim. Feed Sci. Technol. 71:99–107.

Anderson, G. W., and B. A. Barton. 1988. Reproductive efficiency: potential nutrition-management interactions. Page 107 *in* Proc. Winter Dairy Management Schools. Cornell University, Ithaca, NY.

Annexstad, R. J., M. D. Stern, D. E. Otterby, J. G. Linn, and W. P. Hansen. 1987. Extruded soybeans and corn gluten meal as supplemental protein sources for lactating dairy cattle. J. Dairy Sci. 70:814–822.

Annison, E. F. 1956. Nitrogen metabolism in the sheep. Protein digestion in the rumen. Biochem. J. 64:705–714.

Antoniewicz, A. M., J. Kowalczyk, J. Kanski, Z. Gorska-Matusiak, and M. Nalepka. 1995. Rumen degradability of crude protein of dried grass and lucerne forage measured by in sacco incubation and predicted by near infrared spectroscopy. Anim. Feed Sci. Technol. 54:203–216.

Antoniewicz, A. M., A. M. van Vuuren, C. J. van der Koelen, and I. Kosmala. 1992. Intestinal digestibility of rumen undegraded protein of formaldehyde-treated feedstuffs measured by mobile bag and in vitro technique. Anim. Feed Sci. Technol. 39:111–124.

Argyle, J. L., and R. L. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial growth yields. J. Dairy Sci. 72:2017–2027.

Arieli, A., and G. Adin. 1994. Effect of wheat silage maturity on digestion and milk yield in dairy cows. J. Dairy Sci. 77:237–243.

Arieli, A., I. Bruckental, O. Kedar, and D. Sklan. 1995. In sacco disappearance of starch nitrogen and fat in processed grains. Anim. Feed Sci. Technol. 51:287–295.

Arieli, A., S. Mabjeesh, H. Tagari, I. Bruckental, and S. Zamwell. 1993. Evaluation of protein flow to the duodenum in dairy cattle by in sacco method. Livest. Prod. Sci. 35: 283–292.

Arieli, A., A. Ben-Moshe, S. Zamwel, and H. Tagari. 1989. In situ evaluation of the ruminal and intestinal digestibility of heat-treated whole cottonseeds. J. Dairy Sci. 72:1228–1233.

Armentano, L. E., S. J. Bertics, and G. A. Ducharme. 1997. Response of lactating cows to methionine or methionine plus lysine added to high protein diets based on alfalfa and heated soybeans. J. Dairy Sci., 80:1194–1199.

Armentano, L. E., S. J. Bertics, and J. Riesterer. 1993. Lack of response to addition of degradable protein to a low protein diet fed to midlactation dairy cows. J. Dairy Sci. 76:3755–3762.

Armentano, L. E., T. A. Herrington, C. E. Polan, A. J. Moe, J. H. Herbein, and P. Umstadt. 1986. Ruminal degradation of dried brewers grains, wet brewers grains, and soybean meal. J. Dairy Sci. 69:2124–2133.

Armentano, L. E., T. A. Herrington, and C. E. Polan. 1983. Ruminal degradation of dried brewers grains, wet brewers grains, and soybean meal in situ and in vitro. J. Dairy Sci. 66(Suppl. 1):171(Abstr.).

Armstead, I. P., and J. R Ling. 1993. Variations in the uptake and metabolism of peptides and amino acids by mixed ruminal bacteria in vitro. Appl. Environ. Microbiol 59:3360–3366.

Atwal, A. S., S. Mahadevan, M. S. Wolynetz, and Y. Yu. 1995. Increased milk production of cows in early lactation fed chemically treated soybean meal. J. Dairy Sc. 78:595–603.

Auboiron, S., D. Durand, J. C. Robert, M. J. Chapman, and D. Bauchart. 1995. Effect of dietary fat and L-methionine on the hepatic metabolism of very-low density lipoproteins in the preruminant calf, *Bos* spp. Reprod. Nutr. Dev. 35:167–168.

Bach, A., and M. D. Stern. 2000. Measuring resistance to ruminal degradation and bioavailability of ruminally protected methionine. Anim. Feed Sci. Technol. 84:23–32.

Bach, A., M. D. Stern, N. R. Merchen, and J. K. Drackley. 1998. Evaluation of selected mathematical approaches to the kinetics of protein degradation in situ. J. Anim. Sci. 76:2885–2893.

Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. J. Nutr. 116:2339–2349.

Baker, D. H. 1994. Utilization of precursors for L-amino acids. Page 37–47 in Amino Acids in Farm Animal Nutrition. J.P.F. D'Mello, ed. Cab International.

Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci. 78:2424–2434.

Balde, A. T., J. H. Vandersall, R. A. Erdman, J. B. Reeves, III, and B. P. Glenn. 1993. Effect of stage of maturity of alfalfa and orchardgrass on in situ dry matter and crude protein degradability and amino acid composition. Anim. Feed Sci. Technol. 44:29–43.

Barney, N. C. Personal communication.

Barney, D. J., D. G. Grieve, G. K. Macleod, and L. G. Young. 1981. Response of cows to dietary crude protein during midlactation. J. Dairy Sci. 64:655–661.

Barton, B. A. 1996a. Effects of dietary crude protein, breed, parity, and health status on the fertility of dairy cows. J. Dairy Sci. 70:2225–2236.

Barton, B. A. 1996b. Determining if reproduction is affected by a nutrient imbalance. Pages 17–32 *in* Proc. Tri-State Dairy Nutrition Conf.

Batajoo, K. K., and R. D. Shaver. 1998. In situ dry matter, crude protein, and starch degradabilities of selected grains and by-products. Anim. Feed Sci. Technol. 71:165–176.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. J. Dairy Sci. 76:3864–3881.

Bauchart, D., D. Gruffat, and D. Durand. 1996. Lipid absorption and hepatic metabolism in ruminants. Proc. Nutr. Soc. 55:39–47.

Beam, S. W., and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Repro. 56:133–142.

Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 1999. Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. J. Dairy Sci. 82:378–390.

Beauchemin, K. A., L. M. Rode, and M. V. Eliason. 1997. Chewing activities and milk production of dairy cows fed alfalfa as hay, silage, or dried cubes of hay or silage. J. Dairy Sci. 80:324–333.

Beckers, Y., A. Théwis, and B. Madoux. 1996. Intestinal digestibility of rumen undegraded N of concentrates measured by the mobile nylon bag technique. Anim. Feed Sci. Technol. 61:305–323.

Beckers, Y., A. Théwis, B. Madoux, and E. François. 1995. Studies on the in situ nitrogen degradability corrected for bacterial contamination of concentrate feeds in steers. J. Anim. Sci. 73:220–227.

Beever, D. E., H. R. Losada, D. L. Gale, M. C. Spooner, and M. S. Dhanoa. 1987. The use of monensin or formaldehyde to control the digestion of nitrogenous constituents of perennial ryegrass (*Lolium perenne* cv. Melle) and white clover (*Trifolium repens* cv. Blanca) in the rumen of cattle. Br. J. Nutr. 57:57–67.

Beever, D. E., M. S. Dhanoa, H. R. Losada, R. T. Evans, S. B. Cammell, and J. France. 1986. Br. J. Nutr. 56:439–454.

Beever, D. E., D. J. Thomson, and S. B. Cammell. 1976. The digestion of frozen and dried grass by sheep. J. Agric. Sci., Camb. 86:443–452.

Belasco, I. J. 1972. Stability of methionine hydroxy analog in rumen fluid and its conversion in vitro to methionine by calf liver and kidney. J. Dairy Sci. 55:353–357.

Belasco, I. J. 1980. Fate of carbon-14 labeled methionine hydroxy analog and methionine in the lactating dairy cow. J. Dairy Sci. 63:775–784.

Bell, A. W., R. Sleptis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78:1954–1961.

Benchaar, C., C. Bayourthe, R. Moncoulon, and M. Vernay. 1991. Ruminal digestion and intestinal absorption of lupine proteins extruded in the lactating cow. Reprod. Nutr. Dev. 316:655–665.

Benchaar, C., M. Vernay, C. Bayourthe, and R. Moncoulon. 1994a. Effects of extrusion of whole horse beans on protein digestion and amino acid absorption in dairy cows. J. Dairy Sci. 77:1360–1371.

Benchaar, C., R. Moncoulon, C. Bayourthe, and M. Vernay. 1994b. Effects of a supply of raw or extruded white lupin seeds on protein digestion and amino acid absorption in dairy cows. J. Anim. Sci. 72:492–501.

Bendall, J. R. 1964. Meat proteins. Page 225 in Symposium on Foods: Proteins and Their Reactions. H. W. Schultz (ed.). AVI Publication Co., Inc., Westport, CT.

Ben Salem, H., R. Krzeminski, A. Ferlay, and M. Doreau. 1993. Effect of lipid supply on in vivo digestion in cows: comparison of hay and corn silage diets. Can. J. Anim. Sci. 73:547–557.

Bernard, J. K., H. E. Amos, M. A. Froetschel, and J. J. Evans. 1988. Influence of supplemental energy and protein on protein synthesis and crude protein reaching the abomasum. J. Dairy Sci. 71:2658–2669.

Berthiaume, R., H. Lapierre, M. Stevenson, N. Cote, and B. W. McBride. 2000. Comparison of the in situ and in vivo intestinal disappearance of ruminally protected methionine. J. Dairy Sci. 83:(In press).

Bertrand, J. A., F. E. Pardue, and T. C. Jenkins. 1998. Effect of ruminally protected amino acids on milk yield and composition of Jersey cows fed whole cottonseed. J. Dairy Sci. 81:2215–2220.

Berzaghi, P., G. Cozzi, and I. Andrighetto. 1997. The use of near infrared analysis for in situ studies. J. Dairy Sci. 80:3263–3270.

Black, A. L., M. Kleiber, A. H. Smith, and D. N. Stewart. 1957. Acetate as a precursor of amino acids of casein in the intact dairy cow. Biochem. Biophys. Acta. 23:54.

Blauwiekel, R., and R. L. Kincaid. 1986. Effect of crude protein and solubility on performance and blood constituents of dairy cows. J. Dairy Sci. 69:2091–2098.

Blauwiekel, R., S. Xu, J. H. Harrison, K. A. Loney, R. E. Riley, and M. C. Calhoun. 1997. Effect of whole cottonseed, gossypol, and ruminally protected lysine supplementation on milk yield and composition. J. Dairy Sci. 80:1358–1365.

Blauwiekel, R., W. H. Hoover, S. D. Slider, and T. K. Miller. 1990. Effects of fish meal protein supplementation on milk yield and composition and blood constituents of dairy cows. J. Dairy Sci. 73:3217–3221.

Blethen, D. B., J. E. Wohlt, D. K. Jasaitis, and J. L. Evans. 1990. Feed protein fractions: relationship to nitrogen solubility and degradability. J. Dairy Sci. 73:1544–1551.

Blum, J. W., R. M. Bruckmaier, and F. Jans. 1999. Rumen-protected methionine fed to dairy cows: bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J. Dairy Sci. 82:1991–1998.

Boehme, W. R. 1982. Protein products of the rendering industry. Page 173 *in* CRC Handbook of Processing and Utilization in Agriculture. Vol 1. Animal Products. I. A. Wolff (ed.). CRC Press, Inc., Boca Raton, FL.

Boekholt, H. A. 1976. Nitrogen metabolism of the lactating cow and the role of gluconeogenesis from amino acids. Meded. Landbouwhogesch. Wageningen 76:10.

Bohnert, D. W., B. T. Larson, M. L. Bauer, A. F. Branco, K. R. McLeod, D. L. Harmon, and G. E. Mitchell, Jr. 1999. Nutritional evaluation of poultry by-product meal as a protein source for ruminants: Small intestinal amino acid flow and disappearance in steers. J. Anim. Sci. 77:1000–1007.

Bohnert, D. W., B. T. Larson, M. L. Bauer, A. F. Branco, K. R. McLeod, D. L. Harmon, and G. E. Mitchell, Jr. 1998. Nutritional evaluation of poultry by-product meal as a protein source fro ruminants: effects on performance and nutrient flow and disappearance in steers. J. Anim. Sci. 76:2474–2484.

Boila, R.J., and J. R. Ingalls. 1995. Prediction of rumen undegradable amino acids that are digested post-ruminally. Can. J. Anim. Sci. 75: 583–592.

Boila, R. J., and J. R. Ingalls. 1994. The post-ruminal digestion of dry matter, nitrogen and amino acids in wheat-based distillers dried grains and canola meal. Anim. Feed. Sci. Technol. 49:173–188.

Boila, R. J., and J. R. Ingalls. 1992. In situ rumen digestion and escape of dry matter, nitrogen and amino acids in canola meal. Can. J. Anim. Sci. 72:891–901.

Bowerman, B. L., and R. T. O'Connell. 1990. Linear statistical models: An applied approach. 2nd ed. PWS-Kent Publishing Co., Boston, MA.

Bowman, J. M., D. G. Grieve, J. G. Buchanan-Smith, and G. K. Macleod. 1988. Response of dairy cows in early lactation to sodium hydroxide-treated soybean meal. J. Dairy Sci. 71:982–989.

Brandt, M., K. Rohr, and P. Lezien. 1980. Bestimmung des endogenen Protein-N im Duodenalchymus von Milchkhen mit Hilfe von ^{15}N. J. Anim. Physiol. Anim. Nutr. 44:26.

Bremmer, D. R., T. R. Overton, and J. H Clark. 1997. Production and composition of milk from Jersey cows administered bovine somatotropin and fed ruminally protected amino acids. J. Dairy Sci. 80:1374–1380.

Broderick, G. A. 1992. Relative value of fish meal versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. J. Dairy Sci. 75:174–183.

Broderick, G. A. 1998. Can cell-free enzymes replace rumen microorganisms to model energy and protein supply? Pages 99-114 in In vitro Techniques for Measuring Nutrient Supply to Ruminants. E. R. Deaville, E. Owens, A. T. Adesogan, C. Rymer, J. A. Huntington, and T. L. J. Lawrence (eds.) Occasional Publication No. 22, British Society of Animal Sciences, Edinburgh.

Broderick, G. A., R. J. Wallace, and E. R. Ørskov. 1991. Control of rate and extent of protein degradation. Pages 541–592 in Physiological Aspects of Digestion and Metabolism in Ruminants. T. Tsuda, Y. Sasaki, and R. Kawashima (eds.) Academic Press, Orlando, FL.

Broderick, G. A., and R. J. Wallace. 1988. Effects of dietary nitrogen source on concentrations of ammonia, free amino acids and fluorescamine-reactive peptides in the sheep rumen. J. Anim. Sci., 66: 2233–2238.

Broderick, G. A., D. B. Ricker, and L. S. Driver. 1990. Expeller soybean meal and corn by-products versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. J. Dairy Sci. 73:453–462.

Brown, W. F., and F. M. Pate. 1997. Cottonseed meal or feather meal supplementation of ammoniated tropical grass hay for yearling cattle. J. Anim. Sci. 75:1666–1673.

Bruckental, I., D. Dori, M. Kaim, H. Lehrer, and Y. Folman. 1989. Effects of source and level of protein on milk yield and reproductive performance of high-producing primiparous and multiparous dairy cows. Anim. Prod. 48:319–329.

Bruckental, I., I. Ascarelli, B. Yosif, and E. Alumot. 1991. Effect of duodenal proline infusion on milk production and composition in dairy cows. Anim. Prod. 53:299–303.

Brunschwig, P., and P. Augeard. 1994. Acide aminé protégé: effet sur la production et la composition du lait des vaches sur régime ensilage de maïs. Journées de Recherches sur l'alimentation et la nutrition des Herbivores 16-17 INRA Theix.

Brunschwig, P., P. Augeard, B. K. Sloan, and K. Tanan. 1995. Feeding of protected methionine from 10d pre-calving and at the beginning of lactation to dairy cows fed a maize silage based ration. Recontres Recherches Ruminants 2:249.

Burris, W. R., J. A. Boling, N. W. Bradley, and A. W. Young. 1976. Abomasal lysine infusion in steers fed a urea supplemented diet. J. Anim. Sci. 42:699–705.

Burroughs, W.A., A.H. Trenkle, and R.L. Vetter. 1974. A system of protein evaluation for cattle and sheep involving metabolizable protein (amino acids) and urea fermentation potential of feedstuffs. Vet Med. Small Anim. Clin. 69:713–722.

Butler, W. R. 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81:2533–2539.

Butler, W. R., J. J. Calaman, and S. W. Beam. 1995. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J. Anim. Sci. 74:858–865.

Calsamiglia, S., and M. D. Stern. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73:1459–1465.

Calsamiglia, S., G. Caja, M. D. Stern, and B. A. Crooker. 1995a. Effects of ruminal versus duodenal dosing of fish meal on ruminal fermentation and milk composition. J. Dairy Sci. 78:1999–2007.

Calsamiglia, S., M. D. Stern, and J. L. Firkins. 1995b. Effects of protein source on nitrogen metabolism in continuous culture and intestinal digestion in vitro. J. Anim. Sci. 73:1819–1827.

Cameron, M. R., T. H. Klusmeyer, G. L. Lynch, J. H. Clark, and D. R, Nelson. 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J. Dairy Sci. 74:1321–1336.

Campbell, C. G., E. C. Titgemeyer, and G. St-Jean. 1997. Sulfur amino acid utilization by growing steers. J. Anim. Sci. 75:230–238.

Campbell, C. G., E. C. Titgemeyer, and G. St-Jean. 1996. Efficiency of D- vs L-methionine utilization by growing steers. J. Anim. Sci. 74:2482–2487.

Canale, C. J., L. D. Muller, H. A. McCahon, T. J. Whitsel, G. A. Varga, and M. J. Lormore. 1990. Dietary fat and ruminally protected amino acids for high producing dairy cows. J. Dairy Sci. 73:135–141.

Canfield, R. W., C. J. Sniffen, and W. R. Butler. 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73:2342–2349.

Carey, D. A., J. S. Caton, and M. Biondini. 1993. Influence of energy source on forage intake, digestibility, in situ forage degradation, and ruminal fermentation in beef steers fed medium-quality brome hay. J. Anim. Sci. 71:2260–2269.

Carroll, D. J., B. A. Barton, G. W. Anderson, and R. D. Smith. 1988. Influence of protein intake and feeding strategy on reproductive performance of dairy cows. J. Dairy Sci. 71:3470–3481.

Carroll, D. J., G. W. Anderson, and B. A. Barton. 1987. The influence of level of crude protein on the reproductive performance of the early lactation cow. J. Dairy Sci. 70 (Suppl. 1):207. (Abstr.)

Casper, D. P., and D. J. Schingoethe. 1988. Protected methionine supplementation to a barley-based diet for cows during early lactation. J. Dairy Sci. 71:164–172.

Casper, D. P., D. J. Schingoethe, and W. A. Eisenbeisz. 1990. Response of early lactation dairy cows fed diets varying in source of nonstructural carbohydrate and crude protein. J. Dairy Sci. 73:1039–1050.

Casper, D.P., H. A. Maiga, M. J. Brouk, and D. J. Schingoethe. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J. Dairy Sci. 82:1779–1790.

Casper, D. P., D. J. Schingoethe, C.-M. J. Yang, and C. R. Mueller. 1987. Protected methionine supplementation with extruded blend of soybeans and soybean meal for dairy cows. J. Dairy Sci. 70:321–330.

Caton, J. S., V. I. Burke, V. L. Anderson, L. A. Burgwald, P. L. Norton, and K. C. Olson. 1994. Influence of crambe meal as a protein source on intake, site of digestion, ruminal fermentation, and microbial efficiency in beef steers fed grass hay. J. Anim. Sci. 72:3238–3245.

Cecava, M. J. Personal communication.

Cecava, M. J., and J. E. Parker. 1993. Intestinal supply of amino acids in steers fed ruminally degradable and undegradable crude protein sources alone and in combination. J. Anim. Sci. 71:1596–1605.

Cecava, M. J., D. L. Hancock, and J. E. Parker. 1993. Effects of zinc-treated soybean meal on ruminal fermentation and intestinal amino acid flows in steers fed corn silage-based diets. J. Anim. Sci. 71:3423–3431.

Cecava, M. J., N. R. Merchen, L. L. Berger, R. I. Mackie, and G. C. Fahey, Jr. 1991. Effects of dietary energy level and protein source on nutrient digestion and ruminal nitrogen metabolism in sheep. J. Anim. Sci. 69:2230–2243.

Chalupa, W. 1976. Degradation of amino acids by mixed rumen microbial population. J. Anim. Sci. 43:829–834.

Chalupa, W., and J. E. Chandler. 1975. Methionine and lysine nutrition of growing cattle. J. Anim. Sci. 394. (Abstr.)

Chalupa, W., C. J. Sniffen, D. G. Fox and P. J. Van Soest. 1991. Model generated protein degradation nutritional information. Proc. Cornell Nutr. Conf., p. 44, Ithaca, NY.

Chan, S. C., J. T. Huber, C. B. Theurer, Z. Wu, K. H. Chen, and J. M. Simas. 1997. Effects of supplemental fat and protein source on ruminal fermentation and nutrient flow to the duodenum in dairy cows. J. Dairy Sci. 80:152–159.

Chapoutot, P., P. Schmidely, D. Sauvant, J. C. Robert, and B. Sloan. 1992. Influence of a ruminally protected blend of methionine and lysine (ML) on the dairy cow nutrition and production. J. Dairy Sci. 75(Suppl. 1):199. (Abstr.)

Chen, G., and J. B. Russell. 1988. Fermentation of peptides and amino acids by a monensin-sensitive ruminal peptostreptococcus. Appl. Environ. Microbiol. 54:2742–2749.

Chen, G., and J. B. Russell. 1989. More monensin-sensitive, ammonia-producing bacteria from the rumen. Appl. Environ. Microbiol. 55:1052–1057.

Chen, G., C. J. Sniffen, and J. B. Russell. 1987. Concentration and estimated flow of peptides from the rumen of dairy cattle: Effects of protein solubility and feeding frequency. J. Dairy Sci. 70:983–992.

Chen, K. H., J. T. Huber, C. B. Theurer, D. V. Armstrong, R. C. Wanderley, J. M. Simas, S. C. Chan, and J. L. Sullivan. 1993. Effect of protein quality and evaporative cooling on lactational performance of Holstein cows in hot weather. J. Dairy Sci. 76:819–825.

Cherney, D. J. R., J. J. Volenec, and J. H. Cherney. 1992. Protein solubility and degradation in vitro as influenced by buffer and maturity of alfalfa. Anim. Feed Sci. Technol. 37:9–20.

Chikunya, S., C. J. Newbold, L. Rode, X. B. Chen, and R. J. Wallace. 1996. The influence of non protein nitrogen, preformed amino acids and protein on microbial activity in the rumen of sheep receiving diets containing rapidly and slowly degraded fiber sources. Anim. Feed Sci. Technol. 63:333–340.

Chilliard, Y., C. Audigier, D. Durand, S. Auboiron, and D. Bauchart. 1994. Effects of portal infusions of methionine on plasma concentrations and estimated hepatic balances in underfed preruminant calves. Ann. Zootech. 43:299.

Chow, J. M., E. J. DePeters, and R. L. Baldwin. 1990. Effect of rumen-protected methionine and lysine on casein in milk when diets high in fat or concentrate are fed. J. Dairy Sci. 73:1051–1061.

Christensen, R. A., T. R. Overton, J. H. Clark, J. K. Drackley, D. R. Nelson, and S. A. Blum. 1996. Effects of dietary fat with or without nicotinic acid on nutrient flow to the duodenum of dairy cows. J. Dairy Sci. 79:1410–1424.

Christensen, R. A., M. R. Cameron, J. H. Clark, J. K. Drackley, J. M. Lynch, and D. M. Barbano. 1994. Effects of amount of protein and ruminally protected amino acids in the diet of dairy cows fed supplemental fat. J. Dairy Sci. 77:1618–1629.

Christensen, R. A., G. L. Lynch, J. H. Clark, and Y. Yu. 1993a. Influence of amount and degradability of protein on production of milk and milk components by lactating Holstein cows. J. Dairy Sci. 76:3490–3496.

Christensen, R. A., M. R. Cameron, T. H. Klusmeyer, J. P. Elliott, J. H. Clark, D. R. Nelson, and Y. Yu. 1993b. Influence of amount and degradability of dietary protein on nitrogen utilization by dairy cows. J. Dairy Sci. 76:3497–3513.

Clark, J. H. 1975. Lactational responses to postruminal administration of protein and amino acids. J. Dairy Sci. 58:1178–1197.

Clark, J. H., H. R. Spires, and C. L Davis. 1978. Uptake and metabolism of nitrogenous components by the lactating mammary gland. Fed. Proc. 37:1233–1238.

Clark, J. H., R. G. Derrig, C. L. Davis, and H. R. Spires. 1975. Metabolism of arginine and ornithine in the cow and rabbit mammary tissue. J. Dairy Sci. 58:1808–1813.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304–2323.

Cleale, R. M., IV, T. J. Klopfenstein, R. A. Britton, L. D. Satterlee, and S. R. Lowry. 1987. Induced non-enzymatic browning of soybean meal. III. Digestibility and efficiency of protein utilization by ruminants of soybean meal treated with xylose or glucose. J. Anim. Sci. 65:1327–1335.

Coblentz, W. K., I.E.O. Abdelgadir, R. C. Cochran, J. O. Fritz, W. H. Fick, K. C. Olson, and J. E. Turner. 1999. Degradability of forage proteins by in situ and in vitro enzymatic methods. J. Dairy Sci. 82:343–354.

Coblentz, W. K., J. O. Fritz, W. H. Fick, R. C. Cochran, and J. E. Shirley. 1998. In situ dry matter, nitrogen, and fiber degradation of alfalfa, red clover, and Eastern gamagrass at four maturities. J. Dairy Sci. 81:150–161.

Coblentz, W. K., J. O. Fritz, R. C. Cochran, W. L. Rooney, and K. K. Bolsen. 1997. Protein degradation in response to spontaneous heating in alfalfa hay by in situ and ficin methods. J. Dairy Sci. 80:700–713.

Cody, R. F., J. J. Murphy, and D. J. Morgan. 1990. Effect of supplementary crude protein level and degradability in grass silage-based diets on performance of dairy cows, and digestibility and abomasal nitrogen flow in sheep. Anim. Prod. 51:235–244.

Coleman, G. S. 1985. Possible causes of the high death rate of ciliate protozoa in the rumen. J. Agric. Sci., Camb. 105:39–43.

Conrad, H. R., J. W. Hibbs, and A. D. Pratt. 1960. Nitrogen metabolism in dairy cattle. I. Efficiency of nitrogen utilization by lactating cows fed various forges. Ohio Agric. Exp. Stn. Res. Bull. No. 861. Wooster, Ohio State University.

Cooper, P. B., and J. R. Ling. 1985. The uptake of peptides and amino acids by rumen bacteria. Proc. Nutr. Soc. 44:144A.

Cotta, M. A., and R. B. Hespell. 1984. Protein and amino acid metabolism of rumen bacteria. Pages 122–136 *in* Control of Digestion and Metabolism in Ruminants. L. P. Milligan, W. L. Grovum, and A. Dobson (ed.). Prentice-Hall Englewood Cliffs, NJ.

Cotta, M. A., and J. B. Russell. 1982. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. J. Dairy Sci. 65:226–234.

Cozzi, G., and C. E. Polan. 1994. Corn gluten meal or dried brewers grains as partial replacement for soybean meal in the diet of Holstein cows. J. Dairy Sci. 77:825–834.

Cozzi, G., I. Andrighetto, P. Berzaghi, and C. E. Polan. 1995. In situ ruminal disappearance of essential amino acids in protein feedstuffs. J. Dairy Sci. 78:161–171.

Cozzi, G., G. Bittante, and C. E. Polan. 1993. Comparison of fibrous materials as modifiers of in situ ruminal degradation of corn gluten meal. J. Dairy Sci. 76:1106–1113.

Crawford, R. J., W. H. Hoover, C. J. Sniffen, and B. A. Crooker. 1978. Degradation of feedstuff nitrogen in the rumen *vs* nitrogen solubility in three solvents. J. Anim. Sci. 46:1768–1775.

Crawley, D. D., and L. H. Kilmer. 1995. Effects of level and source of rumen degradable protein fed prepartum on postpartum performance of dairy cows. J. Dairy Sci. 78 (Suppl. 1):266. (Abstr.)

Crish, E. M., J. E. Wohlt, and J. L. Evans. 1986. Insoluble nitrogen for milk production in Holstein cows via increases in voluntary intake and nitrogen utilization. J. Dairy Sci. 69:1576–1586.

Crocker, L. M., E. J. DePeters, J. G. Fadel, H. Perez-Monti, S. J. Taylor, J. A. Wyckoff, and R. A. Zinn. 1998. Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. J. Dairy Sci. 81:2394–2407.

Crooker, B. A., C. J. Sniffen, W. H. Hoover, and L. L. Johnson. 1978. Solvents for soluble nitrogen measurements in feedstuffs. J. Dairy Sci. 61:437–447.

Cros, P., M. Vernay, C. Bayourthe, and R. Moncoulon. 1992a. Influence of extrusion on ruminal and intestinal disappearance of amino acids in whole horsebean. Can. J. Anim. Sci. 72: 359–366.

Cros, P., R. Moncoulon, C. Bayourthe, and M. Vernay. 1992b. Effect of extrusion on ruminal and intestinal disappearance of amino acids in white lupin seed. Can. J. Anim. Sci. 72: 89–96.

Cruz Soto, R., S. A. Muhammad, C. J. Newbold., C. S. Stewart, and R. J. Wallace. 1994. Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay and on the growth of rumen bacteria in vitro. Anim. Feed. Sci. Technol. 49:151–161.

Cunningham, K. D., M. J. Cecava, and T. R. Johnson. 1993. Nutrient digestion, nitrogen, and amino acid flows in lactating cows fed soybean hulls in place of forage or concentrate. J. Dairy Sci. 76:3523–3535.

Cunningham, K. D., M. J. Cecava, and T. R. Johnson. 1994. Flows of nitrogen and amino acids in dairy cows fed diets containing supplemental feather meal and blood meal. J. Dairy Sci. 77:3666–3675.

Cunningham, K. D., M. J. Cecava, T. R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620–630.

Cushnahan, A., C. S. Mayne, and E. F. Unsworth. 1995. Effects of ensilage of grass on performance and nutrient utilization by dairy cattle 2. Nutrient metabolism and rumen fermentation. Anim. Feed Sci. Technol. 60:347–359.

Dakowski, P., M. R. Weisbjerg, and T. Hvelplund. 1996. The effect of temperature during processing of rape seed meal on amino acid degradation in the rumen and digestion in the intestine. Anim. Feed. Sci. Technol. 58: 213–226.

Dale, N. 1996. Variation in feed ingredient quality: oilseed meals. Anim. Feed Sci. Technol. 59:129–135.

Dawson, L.E.R., and C. S. Mayne. 1998. The effect of silage fermentation characteristics on dry matter intake of steers. Anim. Sci. 66:105–113.

Dawson, L.E.R., and C. S. Mayne. 1997. The effect of infusion of putrescine and gamma amino butyric acid on the intake of steers offered grass silage containing three levels of lactic acid. Anim. Feed Sci. Technol. 66:15–29.

de Boer, G., J. J. Murphy, and J. J. Kennelly. 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977–982.

De Gracia, M., F. G. Owen, and S. R. Lowry. 1989. Corn gluten meal and blood meal mixture for dairy cows in midlactation. J. Dairy Sci. 72:3064–3069.

Deacon, M. A., G. deBoer, and J. J. Kennelly. 1988. Influence of Jet-Sploding and extrusion on ruminal and intestinal disappearance of canola soybeans. J. Dairy Sci. 71:745–753.

Denham, S. C., G. A. Morantes, D. B. Bates, and J. E. Moore. 1989. Comparison of two models used to estimate in situ nitrogen disappearance. J. Dairy Sci. 72:708–714.

DePeters, E. J., and D. L. Bath. 1986. Canola meal versus cottonseed meal as the protein supplement in dairy diets. J. Dairy Sci. 69:148–154.

DeVisser, H., A. Klop, C. J. van der Koelen, and A. M. van Vuuren. 1998. Starch supplementation of grass harvested at two stages of maturity prior to ensiling: intake, digestion, and degradability by dairy cows. J. Dairy Sci. 81:2221–2227.

Dewhurst, R. J., D. R. Davies, and R. J. Merry. 2000. Microbial protein supply from the rumen. Anim. Feed Sci. Technol. 85:1–21.

Dhiman, T. R., and L. D. Satter. 1993. Protein as the first-limiting nutrient for lactating dairy cows fed high proportions of good quality alfalfa silage. J. Dairy Sci. 76:1960–1971.

Dijkstra, J. 1994. Simulation of the dynamics of protozoa in the rumen. Br. J. Nutr. 72:679–699.

Donahue, P. B., C. G., J. D. Quigley, III, and W. E. Hylton. 1985. Methionine deficiency in early weaned dairy calves fed pelleted rations based on corn and alfalfa or corn and soybean proteins. J. Dairy Sci. 68:681–693.

Doreau, M., F. Legay, and D. Bauchart. 1991. Effect of source and level of supplemental fat on total and ruminal organic matter and nitrogen digestion in dairy cows. J. Dairy Sci. 74:2233–2242.

Downes, A. M. 1961. On the amino acids essential for the tissues of the sheep. Aust. J. Biol. Sci. 14:254.

Doyle, P. T. 1981. Sulfur and methionine metabolism in sheep. V. Utilization of methionine isomers. Aust. J. Biol. Sci. 34:47–59.

Durrand, D., Y. Chilliard, and D. Bauchart. 1992. Effects of lysine and methionine on in vivo hepatic secretion of VLDL in the high yielding dairy cow. J. Dairy Sci. 75 (Suppl. 1):279 (Abstr.).

Elizalde, J. C., N. R. Merchen, and D. B. Faulkner. 1999. Supplemental cracked corn for steers fed fresh alfalfa: II. Protein and amino acid digestion. J. Anim. Sci. 77:467–475.

Elrod, C. C., and W. R. Butler. 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J. Anim. Sci. 71:694–701.

England, M. L., G. A. Broderick, R. D. Shaver, and D. K. Combs. 1997. Comparison of in situ and in vitro techniques for measuring ruminal degradation of animal by-product proteins. J. Dairy Sci. 80:2925–2931.

Erasmus, L. J. 1993. Ruminal degradation of protein of various feedstuffs and its affect on post ruminal amino acid flow in high producing dairy cows. Ph. D. Diss. Univ. Pretoria, South Africa.

Erasmus, L. J., P. M. Botha, and H. H. Meissner. 1994a. Effect of protein source on ruminal fermentation and passage of amino acids to the small intestine of lactating cows. J. Dairy Sci. 77:3655–3665.

Erasmus, L. J., P. M. Botha, C. W. Cruywagen, and H. H. Meissner. 1994b. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feed stuffs. J. Dairy Sci. 77:541–551.

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056–3065.

Erdman, R. A. 1994. Production responses in field study herds fed rumen protected choline. J. Dairy Sci. 77(Suppl. 1):186. (Abstr.)

Erdman, R. A. 1995. Factors affecting microbial protein flow in dairy cows. Four-State Dairy Nutrition and Management Conference. LaCrosse, Wisconsin.

Erdman, R. A., and B. K. Sharma. 1991. Effect of dietary rumen-protected choline in lactating dairy cows. J. Dairy Sci. 74:1641–1647.

Erdman, R. A., and J. H. Vandersall. 1983. Effect of rumen protein degradability on milk yield of dairy cows in early lactation. J. Dairy Sci. 66:1873–1880.

Erdman, R. A., J. H. Vandersall, E. Russek-Cohen, and G. Switalski. 1987. Simultaneous measures of rates of ruminal digestion and passage of feeds for prediction of ruminal nitrogen and dry matter digestion in lactating dairy cows. J. Anim. Sci. 64:565–577.

Erdman, R. A., G. H. Proctor, and J. H. Vandersall. 1986. Effect of rumen ammonia concentration on in situ rate and extent of digestion of feedstuffs. J. Dairy Sci. 69:2312–2320.

Erickson, P. S., M. R. Murphy, and C. L. Davis. 1986. Malt sprouts as a source of supplemental protein for ruminants. J. Dairy Sci. 69:2959–2962.

Espindola, M. S., E. J. DePeters, J. G. Fadel, R. A. Zinn, and H. Perez-Monti. 1997. Effects on nutrient digestion of wheat processing and method of tallow addition to the diets of lactating dairy cows. J. Dairy Sci. 80:1160–1171.

Fairbairn, R., I. Alli, and B. E. Baker. 1988. Proteolysis associated with the ensiling of chopped alfalfa. J. Dairy Sci. 71:152–158.

Faldet, M. A., V. L. Voss, G. A. Broderick, and L. D. Satter. 1991. Chemical, in vitro, and in situ evaluation of heat treated soybean proteins. J. Dairy Sci. 74:2548–2554.

Fenderson, C. L., and W. G. Bergen. 1975. An assessment of essential amino acid requirements of growing steers. J. Anim. Sci. 41:1759–1766.

Feng, P., W. H. Hoover, T. K. Miller, and R. Blauwiekel. 1993. Interactions of fiber and nonstructural carbohydrates on lactation and ruminal function. J. Dairy Sci. 76:1324–1333.

Ferguson, J. D., and W. Chalupa. 1989. Impact of protein nutrition on reproduction in dairy cows. J. Dairy Sci. 72:746–766.

Ferguson, J. D., T. Blanchard, D. T. Galligan, D. C. Hoshall, and W. Chalupa. 1988. Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. J. Am. Vet. Assoc. 192:659–662.

Ferguson, K. A. 1975. The protection of dietary proteins and amino acids against microbial fermentation in the rumen. Pages 448–464 *in* Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (eds.). University of New England Publishing Unit, Armidale, NSW, Australia.

Ffoulkes, D., and R. A. Leng. 1988. Dynamics of protozoa in the rumen of cattle. Br. J. Nutr. 59:429–436.

Fickler, J., J. Fontaine, and W. Heimbeck. 1996. The Amino Acid Composition of Feedstuffs, Degussa Corporation, Ridgefield Park, NJ.

Firkins, J. L., M. S. Allen, B. S. Oldick, and N. R. St-Pierre. 1998. Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum. J. Dairy Sci. 81:3350–3369.

Fox, D. G., C. J. Sniffen, J. D. O'Conner, J. B. Russell, and P. J. Van Soest. 1990. The search: agriculture. Cornell Univ. Agric. Exp. Sta. No. 34., Ithaca.

Fraser, D. L., E. R. Ørskov, F. G. Whitelaw, and M. F. Franklin. 1991. Limiting amino acids in dairy cows given casein as the sole source of protein. Livestock Prod. Sci., 28:235–252.

Freeden, A. H., W. Chalupa, W. E. Julien, C. J. Sniffen, H. Sato, T. Fujieda, T. Ueda, and H. Suzuki, 1999. Effects of rumen-protected LYS and MET to periparturient cows on their productivity during 24 weeks post-partum. J. Dairy Sci. 82(Suppl. 1):121. (Abstr.)

Frydrych, Z. 1992. Intestinal digestibility of rumen undegraded protein of various feeds as estimated by the mobile bag technique. Anim. Feed Sci. Technol. 37:161–172.

Fuller, M. F., and P. Garthwaite. 1993. The form of response of body protein accretion to dietary amino acid supply. J. Nutr. 123:957–963.

Ganesh, D., and D. G. Grieve. 1990. Effect of roasting raw soybeans at three temperatures on in situ dry matter and nitrogen disappearance in dairy cows. J. Dairy Sci. 73:3222–3230.

Garcia, A. D., W. G. Olson, D. E. Otterby, J. G. Linn, and W. P. Hansen. 1989. Effects of temperature, moisture, and aeration on fermentation of alfalfa silage. J. Dairy Sci. 72:93–103.

Garcia-Bojalil, C. M., C. R. Staples, C. A. Risco, J. D. Savio, and W. W. Thatcher. 1998b. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: reproductive responses. J. Dairy Sci. 81:1385–1395.

Garcia-Bojalil, C. M., C. R. Staples, C. A. Risco, J. D. Savio, and W. W. Thatcher. 1998a. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: productive responses. J. Dairy Sci. 81:1374–1384.

Garthwaite, B. D., C. G. Schwab, and B. K. Sloan. 1998. Amino acid nutrition of the transition and early lactation cow. Proc. Cornell Nutr. Conf. pp. 38–50, Ithaca, NY.

Givens, D. I., E. R. Deaville, and A. R. Moss. 1997. The effect of fertilizer nitrogen on the solubility and rumen degradability of dry matter and nitrogen in wheat grain. Anim. Feed Sci. Technol. 66:247–256.

Glenn, B. P., G. A. Varga, G. B. Huntington, and D. R. Waldo. 1989. Duodenal nutrient flow and digestibility in Holstein steers fed formaldehyde- and formic acid-treated alfalfa or orchardgrass silage at two intakes. J. Anim. Sci. 67:513–528.

Goelema, J. O., A. Smits, L. M. Vaessen, and A. Wemmers. 1999. Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. Anim. Feed Sci. Technol. 78:109–126.

Goelema, J. O., M. A. M. Spreeuwenberg, G. Hof, A. F. B. van der Poel, and S. Tamminga. 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. Anim. Feed Sci. Technol. 76:35–50.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis. (Apparatus, reagents, procedures and some applications). Agric. Handbook No. 379.

Goering, H. K., C. H. Gordon, R. W. Hemken, D. R. Waldo, P. J. van Soest, and L. W. Smith. 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. J. Dairy Sci. 55:1275–1280.

Goetsch, A. L., F. N. Owens, M. A. Funk, and B. E. Doran. 1987. Effects of whole or ground corn with different forms of hay in 85 percent concentrate diets on digestion and passage rate in beef heifers. Anim. Feed Sci. Technol. 18:151–164.

Gordon, F. J., and A. C. Peoples. 1986. The utilization of wilted and unwilted silages by lactating cows and the influence of changes in the protein and energy concentration of the supplement offered. Anim. Prod. 43:355–366.

Grant, R. J., and S. G. Haddad. 1998. Effect of a mixture of feather and blood meals on lactational performance of dairy cows. J. Dairy Sci. 81:1358–1363.

Grings, E. E., R. E. Roffler, and D. P. Deitelhoff. 1992a. Evaluation of corn and barley as energy sources for cows in early lactation fed alfalfa-based diets. J. Dairy Sci. 75:193–200.

Grings, E. E., R. E. Roffler, and D. P. Deitelhoff. 1992b. Responses of dairy cows to additions of distillers dried grains with solubles in alfalfa-based diets. J. Dairy Sci. 75:1946–1953.

Grings, E. E., R. E. Roffler, and D. P. Deitelhoff. 1991. Response of dairy cows in early lactation to additions of cottonseed meal in alfalfa-based diets. J. Dairy Sci. 74:2580–2587.

Griswold, K. E., W. H. Hoover, T. K. Miller, and W. V. Thayne. 1996. Effect of form of nitrogen on growth of ruminal microbes in continuous culture. J. Anim. Sci. 74:483–491.

Grum, D. E., W. L. Shockey, and W. P. Weiss. 1991. Electrophoretic examination of alfalfa silage proteins. J. Dairy Sci. 74:146–154.

Grummer, R. C. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244–3257.

Grummer, R. R., K. Slark, S. J. Bertics, M. L. Luck, and J. A. Barmore. 1996. Soybeans versus animal sources of rumen-undegradable protein and fat for early lactation dairy cows. J. Dairy Sci. 79:1809–1816.

Grummer, R. R., L. E. Armentano, and M. S. Marcus. 1987. Lactation response to short-term abomasal infusion of choline, inositol, and soy lecithin. J. Dairy Sci. 70:2518–2524.

Guilloteau, P. 1986. Digestion des proteines chez le jeune ruminant. These de doctorates-sciences, specialite sciences naturelles. Universite de Paris VI. 246 p.

Guinard, J., and H. Rulquin. 1994. Effects of graded amounts of duodenal infusions of lysine on the mammary uptake of major milk precursors in dairy cows. J. Dairy Sci. 77:3565–3576.

Ha, J. K., and J. J. Kennelly. 1984. Effect of protein on nutrient digestion and milk production by Holstein cows. J. Dairy Sci. 67:2302–2307.

Hadwell, D. L., and J. L. Sommerfeldt. 1988. Chickpeas as a protein and energy supplement for high producing dairy cows. J. Dairy Sci. 71:762–772.

Hannah, S. M., R. C. Cochran, E. S. Vanzant, and D. L. Harmon. 1991. Influence of protein supplementation on site and extent of digestion, forage intake, and nutrient flow characteristics in steers consuming dormant bluestem-range forage. J. Anim. Sci. 69:2624–2633.

Harris, B. Jr., D. E. Dorminey, W. A. Smith, H. H. Van Horn, and C. J. Wilcox. 1992. Effects of feather meal at two protein concentrations and yeast culture on production parameters in lactating dairy cows. J. Dairy Sci. 75:3524–3530.

Hart, F. J., and J. Leibholz. 1990. A note on the flow of endogenous protein to the omasum and abomasum of steers. Anim. Prod. 51:217–219.

Harty, S. R., J-M. Akayezu, J. G. Linn, and J. M. Cassady. 1998. Nutrient composition of distillers grains with added solubles. J. Dairy Sci. 81:1201. (Abst.)

Heger, J., and Z. Frydrych. 1989. Efficiency of utilization of amino acids. Pages 31–56 *in* Absorption and Utilization of Amino Acids, Vol. 1. M. Friedman, ed. CRC Press, Inc., Boca Raton, FL.

Henderson, S. J., H. E. Amos, and J. J. Evans. 1985. Influence of dietary protein concentration and degradability on milk production, composition, and ruminal protein metabolism. J. Dairy Sci. 68:2227–2237.

Henning, P. H., D. G. Steyn, and H. H. Meissner. 1993. Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. J. Anim. Sci. 71:2516–2528.

Henson, J. E., D. J. Schingoethe, and H. A. Maiga. 1997. Lactational evaluation of protein supplements of varying ruminal degradabilities. J. Dairy Sci. 80:385–392.

Herrera-Saldana, R., R. Gomez-Alarcaon, M. Tobrabi, and T. Huber. 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy Sci. 73:142–148.

Higginbotham, G. E., M. Torabi, and J. T. Huber. 1989. Influence of dietary protein concentration and degradability on performance of lactating cows during hot environmental temperatures. J. Dairy Sci. 72:2554–2564.

Hill, G. M., J. A. Boling, and N. W. Bradley. 1980. Postruminal lysine and methionine infusion in steers fed a urea-supplemented diet adequate in sulfur. J. Dairy Sci. 63:1242–1247.

Hindle, V. A., A. Steg, A. M. van Vuuren, and J. Vroons-de Bruin. 1995. Rumen degradation and post-ruminal digestion of palm kernel by-products in dairy cows. Anim. Feed Sci. Technol. 51:103–121.

Hino, T., and J. B. Russell. 1987. Relative contributions of ruminal bacteria and protozoa to the degradation of protein *in vitro*. J. Anim. Sci. 64:261–270.

Hoffman, P. C., and L. E. Armentano. 1988. Comparison of brewers wet and dried grains and soybean meal as supplements for dairy cattle. Nutr. Reports Int'l 38:655–663.

Hoffman, P. C., N. M. Brehm, L. M. Bauman, J. B. Peters, and D. J. Undersander. 1999. Prediction of laboratory and in situ protein fractions in legume and grass silages using near-infrared reflectance spectroscopy. J. Dairy Sci. 82:764–770.

Hoffman, P. C., S. J. Sievert, R. D. Shaver, D. A. Welch, and D. K. Combs. 1993. In situ dry matter, protein, and fiber degradation of perennial forages. J. Dairy Sci. 76:2632–2643.

Hoffman, P. C., R. R. Grummer, R. D. Shaver, G. A. Broderick, and T. R. Drendel. 1991. Feeding supplemental fat and undegraded intake protein to early lactation dairy cows. J. Dairy Sci. 74:3468–3474.

Holden, L. A., B. P. Glenn, R. A. Erdman, and W. E. Potts. 1994a. Effects of alfalfa and orchardgrass on digestion by dairy cows. J. Dairy Sci. 77:2580–2594.

Holden, L. A., L. D. Muller, G. A. Varga, and P. J. Hillard. 1994b. Ruminal digestion and duodenal nutrient flows in dairy cows consuming grass as pasture, hay, or silage. J. Dairy Sci. 77:3034–3042.

Holter, J. A., and J. T. Reid. 1959. Relationship between the concentrations of crude protein and apparently digestible protein in forages. J. Anim. Sci. 18:1339–1349.

Holter, J. B., H. H. Hayes, W. E. Urban Jr., S. Ramsey, and H. Rideout. 1992. Response of Holstein cows to corn gluten meal used to increase undegradable protein in early or later lactation. J. Dairy Sci. 75:1495–1506.

Hongerholt, D. D., and L. D. Muller. 1998. Supplementation of rumen undegradable protein to the diets of early lactation Holstein cows on grass pasture. J. Dairy Sci. 81:2204–2214.

Hoover, W. H., and S. R. Stokes. 1991. Balancing carbohydrates and protein for optimum rumen microbial yield. J. Dairy Sci. 74:3630–3644.

Hopkins, B. A., A. H. Rakes, T. E. Daniel, C. A. Zimmerman, and W. J. Croom, Jr. 1994. Effects of intraperitoneal L-leucine, L-isoleucine, L-valine, and L-arginine on milk fat depression in early lactation cows. J. Dairy Sci. 77:1084–1092.

Hopkins, D. I., W. E. Kunkle, A. C. Hammond, D. B. Bates, and B. A. Reiling. 1999. Effects of bypass methionine on the performance of growing cattle fed bermudagrass hay supplemented with molasses-based supplements. J. Anim. Sci. 71(Suppl.1):202. (Abstr.)

Howard, H. J., E. P. Aalseth, G. D. Adams, L. J. Bush, R. W. McNew, and L. J. Dawson. 1987. Influence of dietary protein on reproductive performance of dairy cows. J. Dairy Sci. 70:1563–1571.

Howie, S. A., S. Calsamiglia, and M. D. Stern. 1996. Variation in ruminal degredation and intestinal digestion of animal byproduct proteins. Anim. Feed. Sci. Technol. 63:1–7.

Hristov, A. 1998. Nitrogen fractions and in sacco dry matter and crude protein degradability of fresh and frozen alfalfa. Anim. Feed Sci. Technol. 71:351–355.

Hristov, A. N., and S. G. Sandev. 1998. Proteolysis and rumen degradability of protein in alfalfa preserved as silage, wilted silage, or hay. Anim. Feed Sci. Technol. 72:175–181.

Hristov, A. N., and G. A. Broderick. 1996. Synthesis of microbial protein in ruminally cannulated cows fed alfalfa silage, alfalfa hay, or corn silage. J. Dairy Sci. 79:1627–1637.

Hristov, A. N., T. A. McAllister, and K. J. Cheng. 1997. Effect of carbohydrate level and ammonia availability on utilization of alpha amino nitrogen by mixed ruminal microorganisms in vitro. Proc. Western Section, Amer. Soc. Anim. Sci. 48:186–189.

Hughes, A. D. 1970. The non-protein nitrogen composition of grass silages. II. The changes occurring during the storage of silage. J. Agr. Sci. 75:421–431.

Hurrell, R. F., and R. A. Finot. 1985. Effect of food processing on protein digestibility and amino acid availability. Pages 233–258 *in* Digestiblity and Amino Acid Availability in Cereals and Oilseeds. J. W. Finely and D.T. Hopkins (eds.). American Association of Cereal Chemists, St. Paul, MN.

Hussein, H. S., R. M. Jordan, and M. D. Stern. 1991. Ruminal protein metabolism and intestinal amino acid utilization as affected by dietary protein and carbohydrate sources in sheep. J. Anim. Sci. 69:2143–2151.

Huyler, M. T., R. L. Kincaid, and D. F. Dostal. 1999. Metabolic and yield responses of multiparous Holstein cows to prepartum rumen-undegradable protein. J. Dairy Sci. 82:527–536.

Hvelplund, T. 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep and by in sacco procedure. Acta. Agric. Scand. Suppl. 25:132–144.

Hvelplund, T., M. R. Weisbjerg, and L. S. Anderson. 1991. Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique. Acta. Agric. Scand. Sect. A, Anim. Sci. 42:34–39.

Hvelplund, T., F. D. DeB Hovell, E. R. Ørskov, and D. J. Kyle. 1994. True intestinal digestibility of protein estimated with sheep on intragastric infusion and with the mobile bag technique. Proc. Soc. Nutr. Physiol. 3:64.

Ibrahim, M.N.M., S. Tamminga, and G. Zemmelink. 1995. Degradation of tropical roughages and concentrate feeds in the rumen. Anim. Feed Sci. Technol. 54:81–92.

Illg, D. J., J. L. Sommerfeldt, and D. J. Schingoethe. 1987. Lactational and systemic responses to the supplementation of protected methionine in soybean meal diets. J. Dairy Sci. 70:620–629.

Institute National de la Recherche Agronomique. 1989. Ruminant Nutrition: Recommended Allowances and Feed Tables. R. Jarrige, ed. John Libbey Eurotext, Paris.

Jacobson, D. R., H. H. Van Horn, and C. J. Sniffen. 1970. Lactating ruminants. Fed. Proc. 29:39–40.

Janicki, F. J., and C. C. Stallings. 1988. Degradation of crude protein in forages determined by in vitro and in situ procedures. J. Dairy Sci. 71:2440–2448.

Jaquette, R. D., A. H. Rakes, and W. J. Croom, Jr. 1986. Effects of dietary protein on milk, rumen, and blood parameters in dairy cattle fed low fiber diets. J. Dairy Sci. 69:1026–1034.

Jaquette, R. D., A. H. Rakes, and W. J. Croom, Jr. 1987. Effect of amount and source of dietary nitrogen on milk fat depression in early lactation dairy cows. J. Dairy Sci. 70:1202–1210.

Jarosz, L., T. Hvelplund, M. R. Weisbjerg, and B. B. Jensen. 1994. True digestibility of protein in the small intestine and the hind gut of cows measured with the mobile bag technique using ^{15}N-labelled roughage. Acta Agric. Scand., Sect. A, Anim. Asci. 44:146–151.

Jarrige, R. 1989. Ruminant Nutrition: Recommended Allowances and Feed Tables. Institut National de la Recherche Agronomique, Libbey, Eurotext, Paris, France.

Johnson H. E., N. L. Whitehouse, B. D. Garthwaite, M. S. Piepenbrink, and C. G. Schwab. 1999. Supplementation of corn and barley-based diets of late gestation and early lactation cows with liquid methionine hydroxy analog (HMB). J. Dairy Sci. 82(Suppl. 1):65. (Abstr.)

Johnson, L. M., J. H. Harrison, and R. E. Riley. 1998. Estimation of the flow of microbial nitrogen to the duodenum using urinary uric acid or allantoin. J. Dairy Sci. 81:2408–2420.

Jones, D. F., W. H. Hoover, and T. K. Miller Webster. 1998. Effects of concentrations of peptides on microbial metabolism in continuous culture. J. Anim. Sci. 76:611–616.

Jones-Endsley, J. M., M. J. Cecava, and T. R. Johnson. 1997. Effects of dietary supplementation on nutrient digestion and the milk yield of intensively grazed lactating dairy cows. J. Dairy Sci. 80:3283–3292.

Jordan, E. R., and L. V. Swanson. 1979. Effect of crude protein on reproductive efficiency, serum total protein, and albumin in the high producing dairy cow. J. Dairy Sci. 62:58–63.

Jordan, E. R., T. E. Chapman, D. W. Holtan, and L. V. Swanson. 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing dairy cows. J. Dairy Sci. 66:1854–1862.

Jouany, J. P. 1996. Effect of rumen protozoa on nitrogen utilization by ruminants. J. Nutr. 126:1335S–1346S.

Jouany, J. P., and K. Ushida. 1999. The role of protozoa in feed digestion. Review. AJAS 12:113–128.

Joy, M. T., E. J. DePeters, J. G. Fadel, and R. A. Zinn. 1997. Effects of corn processing on the site and extent of digestion in lactating cows. J. Dairy Sci. 80:2087–2097.

Kaim, M., H. Neumark, Y. Folman, and W. Kaufmann. 1987. The effect of two concentrations of dietary protein and of formaldehyde-treated soya-bean meal on the performance of high-yielding dairy cows. Anim. Prod. 4:333–345.

Kaim, M., Y. Folman, H. Neumark, and W. Kaufmann. 1983. The effect of protein intake and lactation number on post-partum body weight loss and reproductive performance of dairy cows. Anim. Prod. 37:229–235.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997a. Effect of dietary forage concentration and buffer addition on duodenal flow of *Trans*-$C_{18:1}$ fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104–2114.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997b. Effect of fat source on duodenal flow of *Trans*-$C_{18:1}$ fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2115–2126.

Kalscheur, K. F., J. H. Vandersall, R. A. Erdman, R. A. Kohn, and E. Russek-Cohen. 1999a. Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. J. Dairy Sci. 82:545–554.

Kalscheur, K. F., R. A. Kohn, and B. P. Glenn. 1999b. Effect of increasing ruminally degraded protein on lactation performance of dairy cows. J. Dairy Sci. 82 (Suppl. 1):95. (Abstr.)

Karunanandaa, K., L. E. Goodling, G. A. Varga, L. D. Muller, W. W. McNeill, T. W. Casssidy, and T. Lykos. 1994. Supplemental dietary fat and ruminally protected amino acids for lactating Jersey cows. J. Dairy Sci. 77:3417–3425.

Kaufmann, W., and Lüpping, W. 1982. Protected proteins and protected amino acids for ruminants. In: Protein Contribution of Feedstuffs for Ruminants: Application to feed formulation (E.L Miller,., I.H. Pike, and A.J.H. Van Es, eds.) pp. 36–75. Butterworth Scientific, London, England.

Keady, T.W.J., and R.W.J. Steen. 1996. Effects of applying a bacterial inoculant to silage immediately before feeding on silage intake, digestibility, degradability and rumen volatile fatty acid concentrations in growing beef cattle. Grass Forage Sci. 51:155–162.

Keady, T.W.J., R.W.J. Steen, D. J. Kilpatrick, and C. S. Mayne. 1994. Effects of inoculant treatment on silage fermentation, digestibility and intake by growing cattle. Grass Forage Sci. 49:284–294.

Keery, C. M., H. E. Amos, and M. A. Froetschel. 1993. Effects of supplemental protein source on intraruminal fermentation, protein degradation, and amino acid absorption. J. Dairy Sci. 76:514–524.

Kendall, E. M., J. R. Ingalls, and R. J. Boila. 1991. Variability in the rumen degradability and postruminal digestion of the dry matter, nitrogen and amino acids of canola meal. Can. J. Anim. Sci. 71:739–754.

Kenelly, J. J., D. L. Dalton, and J. K. Ha. 1988. Digestion and utilization of high moisture barley by lactating dairy cows. J. Dairy Sci. 71:1259–1266.

Kerry, C. M., and H. E. Amos. 1993. Effects of source and level of undegraded intake protein on nutrient use and performance of early lactation cows. J. Dairy Sci. 76:499–513.

Khalili, H., P. O. Osuji, N. N. Umunna, and S. Crosse. 1994. The effects of forage type (maize-lablab or oat-vetch) and level of supplementation (wheat middlings) on food intake, diet apparent digestibility, purine excretion and milk production of crossbred (*Bos taurus* × *Bos indicus*) cows. Anim. Prod. 58:321–382.

Khalili, H., T. Varvikko, and S. Crosse. 1992. The effects of forage type and level of concentrate supplementation on food intake, diet digestibility and milk production of crossbred cows (*Bos taurus* × *Bos indicus*). Anim. Prod. 54:183–189.

Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 1996a. Forage source alters nutrient supply to the intestine without influencing milk yield. J. Dairy Sci. 79:862–872.

Khorasani, G. R., G. De Boer, and J. J. Kennelly. 1996b. Response of early lactation cows to ruminally undegradable protein in the diet. J. Dairy Sci. 79:446–453.

Khorasani, G. R., G. deBoer, B. Robinson, and J. J. Kennelly. 1994a. Influence of dietary protein and starch on production and metabolic responses of dairy cows. J. Dairy Sci. 77:813–824.

Khorasani, G. R., P. H. Robinson, and J. J. Kennelly. 1994b. Evaluation of solvent and expeller linseed meals as protein sources for dairy cattle. Can. J. Anim. Sci. 74:479–485.

Khorasani, G. R., P. H. Robinson, and J. J. Kennelly. 1993. Effects of canola meal treated with acetic acid on ruminal degradation and intestinal digestibility in lactating dairy cows. J. Dairy Sci. 76:1607–1616.

Khorasani, G. R., G. deBoer, P. H. Robinson, and J. J. Kennelly. 1992. Effect of canola fat on ruminal and total tract digestion, plasma hormones, and metabolites in lactating cows. J. Dairy Sci. 75:492–501.

Kibelolaud, A. R., M. Vernay, C. Bayourthe, and R. Moncoulon. 1993. Effect of extruding on ruminal disappearance and lower gastrointestinal tract digestion of white lupin seeds. Can. J. Anim. Sci. 73: 571–579.

Kim, Y. K., D. J. Schingoethe, D. P. Casper, and F. C. Ludens. 1991. Lactational response of dairy cows to increased dietary crude protein with added fat. J. Dairy Sci. 74:3891–3899.

King, K. J., J. T. Huber, M. Sadik, W. G. Bergen, A. L. Grant, and V. L. King. 1990. Influence of dietary protein sources on the amino acid profiles available for digestion and metabolism in lactating cows. J. Dairy Sci. 73:3208–3216.

King, K. J., W. G. Bergen, C. J. Sniffen, A. L. Grant, D. B. Grieve, V. L. King, and N. K. Ames. 1991. An assessment of absorbable lysine requirements in lactating cows. J. Dairy Sci. 74:2530–2539.

Kirkpatrick, B. K., and J. J. Kennelly. 1987. In situ degradability of protein and dry matter from single protein sources and from a total diet. J. Anim. Sci. 65:567–576.

Klemesrud, M. J., and T. J. Klopfenstein. 1994. Addition of ruminal escape methionine and lysine to meat and bone meal. J. Dairy Sci. 77(Suppl. 1):94. (Abstr.)

Klover, E., L. D. Muller, G. A. Varga, and T. J. Cassidy. 1998. Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. J. Dairy Sci. 81:2017–2028.

Klusmeyer, T. H., G. L. Lynch, J. H. Clark, and D. R. Nelson. 1991a. Effects of calcium salts of fatty acids and protein source on ruminal fermentation and nutrient flow to duodenum of cows. J. Dairy Sci. 74:2206–2219.

Klusmeyer, T. H., G. L. Lynch, J. H. Clark, and D. R. Nelson. 1991b. Effects of calcium salts of fatty acids and proportion of forage in diet on ruminal fermentation and nutrient flow to duodenum of cows. J. Dairy Sci. 74:2220–2232.

Klusmeyer, T. H., R. D. McCarthy, Jr., J. H. Clark, and D. R. Nelson. 1990. Effects of source and amount of protein on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 73:3526–3537.

Koenig, K. M., L. M. Rode, C. D. Knight, and P. R. McCullough. 1999. Ruminal escape, gastrointestinal absorption, and response of serum methionine to supplementation of liquid methionine hydroxy analog in dairy cows. J. Dairy Sci. 82:355–361.

Komaragiri, M. V. S., and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. J. Dairy Sci. 80:929–937.

Kopecný, J., and R. J. Wallace. 1982. Cellular location and some properties of proteolytic enzymes of rumen bacteria. Appl. Environ. Microbiol. 43:1026–1033.

Kopecný, J., O. Tománková, and P. Homolka. 1998. Comparison of protein digestibility of rumen undegraded protein estimated by an enzymatic and mobile bag method: feeds for ruminants and anaerobic fungus. Anim. Feed Sci. Technol. 71:109–116.

Köster, H. H., R. C. Cochran, E. C. Titgemeyer, E. S. Vanzant, T. G. Nagaraja, K. K. Kreikemeier, and G. St. Jean. 1997. Effect of increasing proportion of supplemental nitrogen from urea on intake and utilization of low-quality, tallgrass-prairie forage by beef steers. J. Anim. Sci. 75:1393–1399.

Kowalski, Z. M., P. M. Pisulewski, and M. Spanghero. 1999. Effects of calcium soaps of rapeseed fatty acids and protected methionine on milk yield and composition in dairy cows. J. Dairy Res. 66:475–487.

Kowalski, Z. M., A. Marszaek, and C. R. Mills. 1997. The use of Ca salts of rape seed fatty acids to protect protein against degradation in the rumen. Anim. Feed Sci. Technol. 65:265–274.

Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen, and P. J. Van Soest. 1982. Nitrogen fractions in selected feedstuffs. J. Dairy Sci., 65: 217–225.

Kung, L., JR., J. T. Huber, and L. D. Satter. 1983. Influence of nonprotein nitrogen and protein of low rumen degradability on nitrogen flow and utilization in lactating dairy cows. J. Dairy Sci. 66:1863–1872.

Lardy, G. P., G. E. Catlett, M. S. Kerley, and J. A. Paterson. 1993. Determination of the ruminal excape value and duodenal amino acid flow of rapeseed meal. J. Anim. Sci. 71:3096–3104.

Laycock, K. A., and E. L. Miller. 1981. Nitrogen solubility and protein degradability of commercially and laboratory prepared rapeseed and soya-bean meals. Proc. Nutr. Soc. 40:103A.

Lees, J. A., J. D. Oldham, W. Haresign, and P. C. Garnsworthy. 1990. The effects of patterns of rumen fermentation on the response by dairy cows to dietary protein concentration. Brit. J. Nutr. 63:177–186.

Lehman, K. B., E. K. Okine, G. W. Mathison, and J. Helm. 1995. In situ degradabilities of barley grain cultivars. Can. J. Anim. Sci. 75:485–487.

Leng, R. A., and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072–1089.

Leng, R. A., D. Dellow, and G. Waghorn. 1986. Dynamics of large ciliate protozoa in the rumen of cattle fed on diets of freshly cut grass. Br. J. Nutr. 56:455–462.

Leonard, M., and E. Block. 1988. Effect of ration protein content and solubility on milk production of primiparous Holstein heifers. J. Dairy Sci. 71:2709–2722.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358.

Lindberg, J. E. 1985. Estimation of rumen degradability of feed protein with the in sacco technique and various in vitro methods: A review. Acta Agric. Scand. Suppl. 25:64–97.

Ling, J. R., and I. P Armstead. 1995. The in vitro uptake and metabolism of peptides and amino acids by five species of rumen bacteria. J. Appl. Bacteriol. 78:116–124.

Lintzenich, B. A., E. S. Vanzant, R. C. Cochran, J. L. Beaty, R. T. Brandt, Jr., and G. St. Jean. 1995. Influence of processing supplemental alfalfa on intake and digestion of dormant bluestem-range forage by steers. J. Anim. Sci. 73:1187–1195.

Liu, C., D. J. Schingoethe, and G. A. Stegeman. 2000. Corn distillers grains verus a blend of protein supplements with or without ruminally protected amino acids for lactating cows. J. Dairy Sci. 83:(In press).

Liu, Y., L., A. Steg, and V. A. Hindle. 1994. Rumen degradation and intestinal digestion of crambe and other oilseed by-products in dairy cows. Anim. Feed Sci. Technol. 45:397–409.

Lobley, G. E. 1992. Control of the metabolic fate of amino acids in ruminants: A review. J. Anim. Sci. 70:3264–3275.

Loerch, S. G., and B. O. Oke. 1989. Rumen protected amino acids in ruminant nutrition. *In* Absorption and Utilization of Amino Acids. Vol. III (M. Friedman, ed.) pp. 187–200. CRC Press. Boca Raton, FL.

Lu, C. D., N. A. Jorgensen, and L. D. Satter. 1988. Site and extent of nutrient digestion in lactating dairy cows fed alfalfa protein concentrate or soybean meal. J. Dairy Sci. 71:697–704.

Lundquist, R. G., D. E. Otterby, and J. G. Linn. 1986. Influence of formaldehyde-treated soybean meal on milk production. J. Dairy Sci. 69:1337–1345.

Lusby, K. S. 1994. Performance of beef calves supplemented with protein or energy with or without Smartamine-M. Pages 173–178 *in* 1994 Oklahoma State University Animal Science Research Report.

Lykos, T., and G. A. Varga. 1995. Effects of processing method on degradation characteristics of protein and carbohydrate sources in situ. J. Dairy Sci. 78:1789–1801.

Lykos, T, G. A. Varga, and D. Casper. 1997. Varying degradation rates of total nonstructrual carbohydrates: Effects on ruminal fermentation, blood metabolites, and milk production and composition in high producing Holstein cows. J. Dairy Sci. 80:3341–3355.

Lynch, G. L., T. H. Klusmeyer, M. R. Cameron, J. H. Clark, and D. R. Nelson. 1991. Effects of somatatropin and duodenal infusion of amino acids on nutrient passage to duodenum and performance of dairy cows. J. Dairy Sci. 74:3117–3127.

Mabjeesh, S. J., A. Ariele, I. Bruckental, S. Zamwell, and H. Tagari. 1996. Effect of type of protein supplementation on duodenal amino acid flow and absorption in lactating dairy cows. J. Dairy Sci. 79:1792–1801.

Mabjeesh, S. J., A. Arieli, I. Bruckental, S. Zamwell, and H. Tagari. 1997. Effect of ruminal degradability of crude protein and nonstructural carbohydrates on the efficiency of bacterial crude protein synthesis and amino acid flow to the abomasum of dairy cows. J. Dairy Sci. 80:2939–2949.

Mackle, T. R., Dwyer, D. A., and D. E. Bauman. 1999. Effects of branched-chain amino acids and sodium caseinate on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:161–171.

Macleod, G. K., and L. W. Cahill. 1987. Canola meal as a protein supplement in corn based dairy rations. Guelph Dairy Research Report; June pp. 100–108.

Madsen, J. 1986. Influence of feeding level on digestion and protein passage to the duodenum in cows fed high concentrate diets. Acta Agric. Scand. 36:275–285.

Madsen, J., and T. Hvelplund. 1990. Protein degradation in the rumen. Pages 103–124 *in* A Study of the Quantitative Nitrogen Metabolism in the Gastro-intestinal Tract, and the Resultant New Protein Evaluation System for Ruminants. The AAT-BPV System. T. Hvelplund and J. Madsen, eds. Institute of Animal Science, The Royal Veterinary and Agricultural University, Copenhagen.

Madsen, J., T. Hvelplund, M. R. Weisbjerg, J. Bertilsson, I. Olsson, R. Spörndly, O. M. Harstad, H. Volden, M. Tuori, T. Varvikko, P. Huntanen, and B. L. Olafsson. 1995. The AAT/PBV protein evaluation system for ruminants. A revision. The Norwegian J. Agric. Sci., Suppl. No. 19:1–37 (ISSN 0802-1600).

Maeng, W. J., and R. L. Baldwin. 1976. Factors influencing rumen microbial growth rates and yields: Effects of urea and amino acids over time. J. Dairy Sci. 72:2002–2016.

Maeng, W. J., H. Park, and H. J. Kim. 1997. The role of carbohydrate supplementation in microbial protein synthesis in the rumen. *In* Rumen Microbes and Digestive Physiology in Ruminants (Onodera et al, eds.), pp. 107–119. Japan Sci. Soc. Press, Tokyo/S. Karger, Basel.

Mahadevan, S., J. D. Erfle, and F. D. Sauer. 1980. Degradation of soluble and insoluble proteins by *Bacteroides amylophilus* protease and by rumen microorganisms. J. Anim. Sci. 50:723–728.

Maiga, H. A., D. J. Schingoethe, and J. Ellison Henson. 1997. Ruminal degradation, amino acid composition, and intestinal digestibility of

the residual components of five protein supplements. J. Dairy Sci. 79:1647–1653.

Maiga, H. A., D. J. Schingoethe, and J. E. Henson. 1996. Ruminal degradation, amino acid composition, and intestinal digestibility of the residual components of five protein supplements. J. Dairy Sci. 79: 1647–1653.

Makoni, N. F., J. A. Shelford, and L. J. Fisher. 1991. The rate and extent of silage nitrogen degradation in the rumen as influenced by wilting and duration of regrowth. Can. J. Anim. Sci. 71:245–248.

Mangan, J. L. 1972. Quantitative studies on nitrogen metabolism in the bovine rumen. The rate of proteolysis of casein and ovalbumin and the release and metabolism of free amino acids. Br. J. Nutr. 27:261–283.

Mansfield, H. R., and M. D. Stern. 1994. Effects of soybean hulls and lignosulfonate-treated soybean meal on ruminal fermentation in lactating dairy cows. J. Dairy Sci. 77:1070–1083.

Manson, F. J., and J. D. Leaver. 1988. The influence of dietary protein intake and of hoof trimming on lameness in dairy cattle. Anim. Prod. 47:191–199.

Mantysaari, P. E., C. J. Sniffen, T. V. Muscato, J. M. Lynch, and D. M. Barbano. 1989. Performance of cows in early lactation fed isonitrogenous diets containing soybean meal or animal by-product meals. J. Dairy Sci. 72:2958–2967.

Manyuchi, B., T. Smith, and S. Mikayiri. 1992. The use of poultry litter in ruminant diets 1. Poultry litter and cotton seed meal as supplements for weaner steers grazing natural pasture during the dry season or sheep fed natural pasture hay in pens. Zimbabwe J. Agric. Res. 30:91–103.

Marshall, S. A., C. P. Campbell, and J. G. Buchanan-Smith. 1993. Proteolysis and rumen degradability of alfalfa silages preserved with a microbial inoculant, spent-sulphite liquor, formic acid, or formaldehyde. Can J. Anim. Sci. 73:559–570.

Martin. C., L. Bernard, and B. Michalet-Doreau. 1996. Influence of sampling time and diet on amino acid composition of protozoal and bacterial fractions from bovine ruminal contents. J. Anim. Sci. 74:1157–1163.

Masoero, F., L. Fiorentini, F. Rossi, and A. Piva. 1994. Determination of nitrogen intestinal digestibility in ruminants. Anim. Feed Sci. Technol. 48: 253–263.

Mason, V. C. 1979. The quantitative importance of bacterial residues in the non-dietary faecal nitrogen of sheep. 2. Estimates of bacterial nitrogen in faecal material from 47 digestibility trials. Z. Tierphysiol., Tierernährg. U. Futtermittelkde. 41:131–139.

Mason, V. C., and F. White. 1971. The digestion of bacterial mucopeptide constituents in the sheep. 1. The metabolism of 2,6-diaminopimelic acid. J. Agric. Sci., Camb. 77:91–98.

Mason, V. C., P. Kessank, J. C. Onontwu, and M. P. Narang. 1981. Z. Tierphysiol., Tierernährg. U. Futtermittelkde. 45:174–184.

Mathers, J.C., and E. L. Miller. 1981. Quantitative studies of food protein degradation and the energetic efficiency of microbial protein synthesis in the rumen of sheep given chopped lucerne and rolled barley. Br. J. Nutr. 45:587–604.

Mbanzamihigo, L., E. Vandycke, and D. I. Demeyer. 1997. Degradation of methionine by rumen contents in vitro and efficiency of its protection. Anim. Feed Sci. Tech. 67:339–347.

McCance, R. A., and E. Widdowson. 1978. *In*: The Composition of Foods, 4th ed. D.A.T. Southgate and A.A. Paul, eds. HMSO, London, Engl.

McCarthy, R. D. Jr., T. H. Klusmeyer, J. L. Vicini, J. H. Clark, and D. R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002–2016.

McCollum, M. Q., M. Vázquez-Añón, J. J. Dibner, and K. E. Webb, Jr. 2000. Absorption of 2-hydroxy-4-(methylthio)butanoic acid by isolated sheep ruminal and omasal epithelia. J. Anim. Sci. 78:1078–1083.

McCormick, M. E., D. D. French, T. F. Brown, G. J. Cuomo, A. M. Chapa, J. M. Fernandez, J. F. Beatty, and D. C. Blouini. 1999. Crude protein and rumen undegradable protein effects on reproduction and lactation performance of Holstein cows. J. Dairy Sci. 82:2697–2708.

McGuffey, R. K., H. B. Green, and R. P. Basson. 1990. Lactation response of dairy cows receiving bovine somatotropin and fed rations varying in crude protein and undegradable intake protein. J. Dairy Sci. 73:2437–2443.

McKinnon, J. J., A. F. Mustafa, and R.D.H. Cohen. 1995. Nutritional evaluation and processing of canola hulls for ruminants. Can. J. Anim. Sci. 75:231–237.

McNiven, M. A., M. R. Weisbjerg, and T. Hvelplund. 1995. Influence of roasting or sodium hydroxide treatment of barley on digestion in lactating cows. J. Dairy Sci. 78:1106–1115.

McNiven, M. A., R.M.G. Hamilton, P. H. Robinson, and J. W. deLeeuw. 1994. Effect of flame roasting on the nutritional quality of common cereal grains for non-ruminants and ruminants. Anim. Feed Sci. Technol. 47:31–40.

Meijer, G. A. L., and C. J. van der Koelen. 1994. Duodenal infusion of glutamine does not affect protein synthesis in late lactation dairy cows. J. Dairy Sci. 77(Suppl. 1):242. (Abstr.)

Meijer, G. A. L., J. van der Meulen, and A. M. van Vuuren. 1993. Glutamine is a potentially limiting amino acid for milk production in dairy cows: A hypothesis. Metabolism 42: 358–364.

Meijer, G. A. L., J. van der Meulen, J. G. M. Bakker, C. J. van der Koelen and A. M. van Vuuren. 1995. Free amino acids in plasma and muscle of high yielding dairy cows in early lactation. J. Dairy Sci. 78:1131–1141.

Mepham, T. B. 1971. Amino acid utilization by the lactating mammary gland. Page 297 *in* Lactation. (I. R. Falconer, ed.) Butterworths, London.

Mepham, T. B. 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65:287–298.

Mepham, T. B., and J. L. Linzell. 1967. Urea formation by the lactating goat mammary gland. Nature 214:507–518.

Merchen, N. R., and L. D. Satter. 1983. Changes in nitrogenous compounds and sites of digestion of alfalfa harvested at different moisture contents. J. Dairy Sci. 66:789–801.

Messman, M. A., W. P. Weiss, and M. E. Koch. 1994. Changes in total and individual proteins during drying, ensiling, and ruminal fermentation of forages. J. Dairy Sci. 77:492–500.

Mezl, V. A., and W. E. Knox. 1977. Metabolism of arginine in lactating rat mammary gland. Biochem. J. 164:105–113.

Mhgeni, D. M., T. Hvelplund, and M. R. Weisbjerg. 1994. Intestinal digestibility of rumen undegraded dietary protein from tropical roughages estimated by the mobile bag technique. Acta Agric. Scand., Sect. A, Anim. Sci. 44:230–235.

Michalet-Doreau, B., and P. Cerneau. 1991. Influence of foodstuff particle size on in situ degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35:69–81.

Michalet-Doreau, B., and P. Nozière. 1998. Validation of in situ nitrogen degradation measurements: comparative proteolytic activity of solid-adherent microorganisms isolated from rumen content and nylon bags containing various feeds. Anim. Feed Sci. Technol. 70:41–47.

Michalet-Doreau, B., and M. Y. Ould-Bah. 1992. *In vitro* and *in sacco* methods for the estimation of dietary nitrogen degradability in the rumen: a review. Anim. Feed Sci. and Technol. 40:57–86.

Milton, C. T., R. T. Brandt, Jr., and E. C. Titgemeyer. 1997. Urea in dry-rolled corn diets: Finishing steer performance, nutrient digestion, and microbial protein production. J. Anim. Sci. 75:1415–1424.

Mir, P. S., Z, Mir, and L. Townley-Smith. 1993. Comparison of the nutrient content and in situ degradability of fenugreek (*Trigonella foenum-graecum*) and alfalfa hays. Can J. Anim. Sci. 73:993–996.

Mir, Z., P. S. Mir, S. Bittman, and L. J. Fisher. 1992. Ruminal degradation characteristics of corn and corn-sunflower intercropped silages prepared at two stages of maturity. Can. J. Anim. Sci. 72:881–889.

Moller, P. D. 1985. Results of grass silage based rations on the nitrogen absorption in the gastro-intestinal tract of dairy cows applied in the Nordic protein evaluation system. Acta Agric. Scand. Suppl. 25:49–63.

Moshtaghi Nia, S. A., and J. R. Ingalls. 1992. Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. Can. J. Anim. Sci. 72: 83–88.

Moshtaghi Nia, S. A., and J. R. Ingalls. 1995. Evaluation of moist heat treatment of canola meal on digestion in the rumen, small intestine, large intestine, and total digestive tract of steers. Can. J. Anim. Sci. 75: 279–283.

Munneke, R. L., D. J. Schingoethe, and D. P. Casper. 1991. Lactational evaluation of ruminally protected methionine in diets containing extruded soybeans and urea. J. Dairy Sci. 74:227–233.

Mupeta, B., M. R. Weisbjerg, T. Hvelplund, and J. Madsen. 1997. Digestibility of amino acids in protein rich tropical feeds for ruminants estimated with the mobile bag technique. Anim. Feed Sci. Technol. 69: 271–280.

Murphy, J. J., and J. J. Kennelly. 1987. Effect of protein concentration and protein source on the degradability of dry matter and protein in situ. J. Dairy Sci. 70:1841–1849.

Murphy, T. A., S. C. Loerch, and B. A. Dehority. 1994. The influence of restricted feeding on site and extent of digestion and flow of nitrogenous compounds to the duodenum in steers. J. Anim. Sci. 72:2487–2496.

Murphy, M., H. Khalili, and P. Huhtanen. 1993. The substitution of barley by other carbohydrates in grass silage based diets to dairy cows. Anim. Feed Sci. Technol. 41:279–296.

Murphy, M., P. Uden, D. L. Palmquist, and H.Wiktorsson. 1987. Rumen and total diet digestibilities in lactating cows fed diets containing full-fat rapeseed. J. Dairy Sci. 70:1572–1582.

Mustafa, A. F., D. A. Christensen, and J. J. McKinnon. 1998. Effects of moist heat treatment on crude protein composition and degradability of field peas. Can. J. Anim. Sci. 78:453–456.

Mustafa, A. F., J. J. McKinnon, P. A. Thacker, and D. A. Christensen. 1997. Effect of borage meal on nutrient digestibility and performance of ruminants and pigs. Anim. Feed Sci. Technol. 64:273–285.

Mustafa, A. F., D. A. Christensen, and J. J. McKinnon. 1996. Chemical characterization and nutrient availability of high and low fiber canola meal. Can. J. Anim. Sci. 76:579–586.

Nakamura, T., T. J. Klopfenstein, and R. A. Britton. 1994a. Evaluation of acid detergent insoluble nitrogen as an indicator of protein quality in nonforage proteins. J. Anim. Sci. 72:1043–1048.

Nakamura, T., T. J. Klopfenstein, D. J. Gibb, and R. A. Britton. 1994b. Growth efficiency and digestibility of heated proteins fed to growing ruminants. J. Anim. Sci. 72:774–782.

Nakamura, T., T. J. Klopfenstein, F. G. Owen, R. A. Britton, R. J. Grant, and T. S. Winowiski. 1992. Nonenzymatically browned soybean meal for lactating dairy cows. J. Dairy Sci. 75:3519–3523.

Napoli, G. M., and F. J. Santini. 1989. The effect of a protein energy supplement on pasture protein and fibre digestion in the rumen of grazing steers. Anim. Feed Sci. Technol. 25:39–53.

Narasimhalu, P., E. Teller, M. Vanbelle, M. Foulon, and F. Dasnoy. 1989. Apparent digestibility of nitrogen in rumen and whole tract of Friesan cattle fed direct-cut and wilted grass silages. J. Dairy Sci. 72:2055–2061.

National Research Council. 1985. Ruminant Nitrogen Usage. Washington D.C.: National Academy Press.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle, 6th rev. ed. Washington, D.C.: National Academy Press.

National Research Council. 1994. Nutrient Requirements of Poultry, 9th rev. ed. Washington, D.C.: National Academy Press.

National Research Council. 1996. Nutrient Requirements of Beef Cattle, 7th rev. ed. Washington, D.C.: National Academy Press.

National Research Council. 1998. Nutrient Requirements of Swine, 10th rev. ed. Washington, D.C.: National Academy Press. 189 pp.

Negi, S. S., B. Singh, and H.P.S. Makkar. 1988. Rumen degradability of nitrogen in typical cultivated grasses and leguminous fodders. Anim. Feed Sci. Technol. 22:79–89.

Neu, J., V. Shenoy, and R. Chakrabarti. 1996. Glutamine nutrition and metabolism: Where do we go from here? FASEB J. 10:829–837.

Newbold, J. R., and S. R. Rust. 1992. Effect of asynchronous nitrogen and energy supply on growth of ruminal bacteria in batch culture. J. Anim. Sci. 70:538–546.

Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows. J. Dairy Sci. 81:482–491.

Nocek, J. E. 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. J. Dairy Sci. 71:2051–2069.

Nocek, J. E., and A. L. Grant. 1987. Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen contamination of haycrop forages preserved at different dry matter percentages. J. Anim. Sci. 64:552–564.

Nocek, J. E., and J. E. English. 1986. *In situ* degradation kinetics: evaluation of rate determinationprocedure. J. Dairy Sci., 69:77–87.

Nocek, J. E., G. D. Young, W. Chalupa, C. J. Sniffen, W. E. Julien, T. Ueda, T. Fujieda, I. Shinzato, H. Sato, and H. Suzuki. 1999. The effect of rumen-protected lysine on production performance of lactating dairy cows. J. Dairy Sci. 82(Suppl. 1):94. (Abstr.)

Nocek, J. E., K. A. Cummins, and C. E. Polan. 1979. Ruminal disappearance of crude protein and dry matter in feeds and combined effects in formulated diets. J. Dairy Sci. 62:1587–1598.

Noel, R. J. 2000. Official feed terms. Pages 187–200 In Association of American Feed Control Officials, Official Publication 2000.

Nolan, J. V. 1975. Quantitative models of nitrogen metabolism in sheep. In: Digestion and Metabolism in the Ruminant (McDonald, I. W. and A.C. I. Warner, eds.), pp 416–431. University of New England Publishing Unit, Armidale, Australia.

Nolan, J. V. 1993. Nitrogen kinetics. Pages 123–163 *in* Quantitative Aspects of Ruminant Digestion and Metabolism. J. M. Forbes and J. France (eds.). CAB International Wallingford, UK.

Nugent, J. H. A., and J. L. Mangan. 1978. Rumen proteolysis of fraction I leaf protein, casein, and bovine serum albumin. Proc. Nutr. Soc. 37:48A.

Nugent, J. H. A., and J. L. Mangan. 1981. Characteristics of the rumen proteolysis of Fraction 1 (18S) leaf protein from lucerne (*Medicago sativa L.*). Br. J. Nutr. 46:39–58.

Nugent, J. H. A., W. T. Jones, D. J. Jordan, and J. L. Mangan. 1983. Rates of proteolysis in the rumen of the soluble proteins casein, Fraction 1 (18S) leaf protein, bovine serum albumin, and bovine submaxillary mucoprotein. Br. J. Nutr. 50:357–368.

O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. J. Anim. Sci. 71: 1298–1311.

O'Mara, F. P., G. K. Stakelum, P. Dillon, J. J. Murphy, and M. Rath. 1997a. Rumen fermentation and nutrient flows for cows fed grass and grass supplemented with molassed beet pulp pellets. J. Dairy Sci. 80:2466–2474.

O'Mara, F. P., J. J. Murphy, and M. Rath. 1997b. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J. Anim. Sci. 75:1941–1949.

O'Mara, F. P., J. J. Murphy, and M. Rath. 1998. Effect of amount of dietary supplement and source of protein on milk production, ruminal fermentation, and nutrient flows in dairy cows. J. Dairy Sci. 81:2430–2439.

Ohajuruka, O. A., Z. Wu, and D. L. Plamquist. 1991. Ruminal metabolism, fiber, and protein digestion by lactating cows fed calcium soap or animal-vegetable fat. J. Dairy Sci. 74:2601–2609.

Oldham, J. D. 1981. Amino acid requirements for lactation in high-yielding dairy cows. In Recent Developments in Ruminant Nutrition. W. Haresign and D. J. A. Cole (eds.), pp 49–81. Butterworths, London.

Oldham, J. D., J. D. Sutton, and A. B. McAllan. 1979. Protein digestion and utilization by dairy cows. Ann. Rech. Vet. 10:290–293.

Oldick, B. S., and J. L. Firkins. 1996. Imbalanced, inadequate diets effect reproduction performance, bottom line. Feedstuffs. 51:12–14,25.

Oliveira, J. S., J. T. Huber, J. M. Simas, C. B. Theurer, and R. S. Swingle. 1995. Effect of sorghum grain processing on site and extent of digestion of starch in lactating dairy cows. J. Dairy Sci. 78:1318–1327.

Olson, K. C., J. S. Caton, D. R. Kirby, and P. L. Norton. 1994. Influence of yeast culture supplementation and advancing season on steers grazing mixed-grass prairie in the Northern Great Plains: I. Dietary composition, intake, and in situ nutrient disappearance. J. Anim. Sci. 72:2149–2157.

Onodera, R. 1993. Methionine and lysine metabolism in the rumen and the possible effects of their metabolites on the nutrition and physiology of ruminants. Amino Acids 5:217–232.

Opstvedt, J., R. Miller, R. W. Hardy, and J. Spinelli. 1984. Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gaifdneri*). J. Agric. Food Chem. 32:929–935.

Ørskov, E. R. 1982. Protein Nutrition in Ruminants. Academic Press Inc., San Diego, CA.

Ørskov, E. R., and I. McDonald. 1979. The estimate of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Camb.) 92:499–503.

Ørskov, E. R., N. A. MacLeod, and D. J. Kyle. 1986. Flow of nitrogen from the rumen and abomasum in cattle and sheep given protein-free nutrients by intragastric infusion. Br. J. Nutr. 56:241–248.

Orten, J. M., and O. W. Neuhaus. 1975. Human Biochemistry. Ninth Edition. C. V. Mosby Company, Saint Louis, MO.

Overton, T. R., D. W. LaCount, T. M. Cicela, and J. H. Clark. 1996. Evaluation of a ruminally protected methionine product for lactating dairy cows. J. Dairy Sci. 79:631–638.

Overton, T. R., M. R. Cameron, J. P. Elliott, J. H. Clark, and D. R. Nelson. 1995. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. J. Dairy Sci. 78:1981–1998.

Owen, F. G., and L. L. Larson. 1991. Corn distillers dried grains versus soybean meal in lactation diets. J. Dairy Sci. 74:972–979.

Owens, F. N., and A. L. Goetsch. 1986. Digesta passage and microbial protein synthesis. Pages 196–226 *in* Control of Digestion and Metabolism in Ruminants. L. P. Milligan, W. L. Grovum, and A. Dobson, ed. Prentice-Hall, Englewood Cliffs, NJ.

Owens, F. N., and J. E. Pettigrew. 1989. Subdividing amino acid requirements into portions for maintenance and growth. In Absorption and Utilization of Amino Acids. Vol. I (M. Friedman, ed.) pp. 15–30. CRC Press, Boca Raton, FL.

Palmquist, D. L., M. R. Weisbjerg, and T. Hvelplund. 1993. Ruminal, intestinal, and total digestibilities of nutrients in cows fed diets high in fat and undegradable protein. J. Dairy Sci. 76:1353–1364.

Palmquist, D. L., and W. P. Weiss. 1994. Blood and hydrolyzed feather meals as sources of undegradable protein in high fat diets for cows in early lactation. J. Dairy Sci. 77:1630–1643.

Palmquist, D. L., M. R. Weisbjerg, and T. Hvelplund. 1993. Ruminal, intestinal, and total digestibilities of nutrients in cows fed diets high in fat and undegradable protein. J. Dairy Sci. 76:1353–1364.

Pantoja, J., J. L. Firkins, and M. L. Eastridge. 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. J. Dairy Sci. 78:2247–2258.

Pantoja, J., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. J. Dairy Sci. 77:2341–2356.

Papas, A. M., C. J. Sniffen, and T. V. Muscato. 1984a. Effectiveness of rumen protected methionine for delivering methionine postruminally in dairy cows. J. Dairy Sci. 67:545–552.

Papas, A. M., J. L. Vicini, J. H. Clark, and S. Peirce-Sandner. 1984b. Effects of rumen-protected methionine on plasma free amino acids and production by dairy cows. J. Nutr. 114:2221–2227.

Papas, A., G. A .B. Hall, E. E. Hatfield, and F. N. Owens. 1974. Response of lambs to oral or abomasal supplementation of methionine hydroxy analog or methionine. J. Nutr. 104:653–659.

Paster, B. J., J. B. Russell, C. M. J., Yang J. M. Chow, C. R. Woese, and R. Tanner. 1993. Phylogeny of the ammonia-producing ruminal bacteria P*eptostreptococcus anaerobius, Clostridium sticklandii*, and *Clostridium aminophilum* sp. nov. Int. J. System. Bacteriol. 43:107–110.

Patterson, J. A., and L. Kung, Jr. 1988. Metabolism of DL-methionine and methionine analogs by rumen microorganisms. J. Dairy Sci. 71:3292–3301.

Pena, F., H. Tagari, and L. D. Satter. 1986. The effect of heat treatment of whole cottonseed on site and extent of protein digestion in dairy cows. J. Anim. Sci. 62:1423–1433.

Pereira, J. C., M. D. Carro, J. González, M. R. Alvir, and C. A. Rodríguez. 1998. Rumen degradability and intestinal digestibility of brewers grains as affected by origin and heat treatment and of barley rootlets. Anim. Feed Sci. Technol. 74:107–121.

Petit, H. V., and G. F. Tremblay. 1992. In situ degradability of fresh grass and grass conserved under different harvesting methods. J. Dairy Sci. 75:774–781.

Petit, H. V., P. Savoie, D. Tremblay, G. T. Dos Santos, and G. Butler. 1994. Intake, digestibility, and ruminal degradability of shredded hay. J. Dairy Sci. 77:3043–3050.

Peyraud, J. L., L. Astigarraga, and P. Faverdin. 1997. Digestion of fresh perennial ryegrass fertilized at two levels of nitrogen by lactating dairy cows. Anim. Feed Sci. Technol. 64:155–171.

Piepenbrink, M. S., and D. J. Schingoethe. 1998. Ruminal degradation, amino acid composition, and estimated intestinal digestibilities of four protein supplements. J. Dairy Sci. 81:454–461.

Piepenbrink, M. S., C. G. Schwab, B. K. Sloan, and N. L.Whitehouse. 1999. Importance of dietary concentrations of absorbable lysine on maximizing milk protein production of mid-lactation cows. J. Dairy Sci. 82(Suppl. 1):93. (Abstr.)

Piepenbrink, M. S., D. J. Schingoethe, M. J. Brouk, and G. A. Stegeman. 1998. Systems to evaluate the protein quality of diets fed to lactating cows. J. Dairy Sci. 81:1046–1061.

Pires, A. V., M. L. Eastridge, J. L. Firkins, and Y. C. Lin. 1997. Effects of heat treatment and physical processing of cottonseed on nutrient digestibility and production performance by lactating cows. J. Dairy Sci. 80:1685–1694.

Pisulewski, P. M., H. Rulquin, J. L. Peyraud, and R. Verite. 1996. Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. J. Dairy Sci. 79:1781–1791.

Polan, C. E., D. E. Stieve, and J. L. Garrett. 1998. Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia, or microbial inoculant. J. Dairy Sci. 81:765–776.

Polan, C. E., G. Cozzi, P. Berzaghi, and I. Andrighetto. 1997. A blend of animal and cereal protein or fish meal as partial replacement for soybean meal in the diets of lactating Holstein cows. J. Dairy Sci. 80:160–166.

Polan, C. E., K. A. Cummins, C. J. Sniffen, T. V. Muscato, J. L. Vicini, B.A. Crooker, J. H. Clark, D. G. Johnson, D. E. Otterby, B. Guillaume, L. D. Muller, G. A. Varga, R. A. Murray, and S. B. Peirce-Sandner. 1991. Responses of dairy cows to supplemental rumen-protected forms of methionine and lysine. J. Dairy Sci. 74:2997–3013.

Polan, C. E., T. A. Herrington, W. A. Wark, and L. E. Armentano. 1985. Milk production response to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal. J. Dairy Sci. 68:2016–2026.

Poore, M. H., J. A. Moore, T. P. Eck, R. S. Swingle, and C. B. Theurer. 1993. Effect of fiber source and ruminal starch degradability on site and extent of digestion in dairy cows. J. Dairy Sci. 76:2244–2253.

Powers, W. J., H. H. Van Horn, B. Harris, Jr., and C. J. Wilcox. 1995. Effects of variable sources of distillers dried grains plus solubles on milk yield and composition. J. Dairy Sci. 78:388–396.

Prakash, P., D. V. Reddy, R. Ramachandra Reddy, and N. Krishna. 1996. The catalytic effect of supplementation of protein meals on utilization of rice straw-poultry droppings-rice bran diet in buffaloes. Anim. Feed Sci. Technol. 63:229–245.

Prange, R. W., M. D. Stern, N. A. Jorgensen, and L. D. Satter. 1984. Site and extent of protein digestion in lactating cows fed alfalfa silage or baled alfalfa hay. J. Dairy Sci. 67:2308–2314.

Prestløkken, E. 1999. In situ ruminal degradation and intestinal digestibility of dry matter and protein in expanded feedstuffs. Anim. Feed Sci. Technol. 71:1–23.

Prigge, E. C., M. J. Baker, and G. A. Varga. 1984. Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers. J. Anim. Sci. 59:237–245.

Prins, R. A., J. C. Van Gestel, and G. H. M. Counotte. 1979. Degradation of amino acids and peptides by mixed rumen microorganisms. Z. Tierphysiol. Tierernahr. Futtermittelk. 42:333–339.

Pruekvimolphan S., R. R. Grummer, and S. J. Bertics. 1997. Effects of feather meal on lactation performance of dairy cows fed methionine deficient diets. J. Dairy Sci. 80(Suppl. 1):248. (Abstr.)

Punia, B. S., J. Leibholz, and G. J. Faichney. 1992. Rate of production of protozoa in the rumen and flow of protozoal nitrogen to the duodenum in sheep and cattle given a pelleted diet of lucerne hay and barley. J. Agric. Sci., Camb. 118:229–236.

Putnam, D. E., C. G. Schwab, M. T. Socha, N. L. Whitehouse, N. A. Kierstead, and B. D. Garthwaite. 1997. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci. 80:374–384.

Rae, R. C., and J. R. Ingalls. 1984. Lactational response of dairy cows to oral administration of L-tyrosine. J. Dairy Sci. 67:1430–1438.

Rae, R. C., and R. R. Smithard. 1985. Estimation of true nitrogen digestibility in cattle by a modified nylon bag technique. Proc. Nutr. Soc. 44:116A.

Rae, R. C., J. R. Ingalls, and J. A. McKirdy. 1983. Effect of dietary crude protein level and formaldehyde treated canola meal on milk production and nitrogen utilization during early lactation. Can. J. Anim. Sci. 63:905–915.

Ragland-Gray, K. K., H. E. Amos, M. A. McCann, C. C. Williams, J. L. Sartin, C. R. Barb, and F. M. Kautz. 1997. Nitrogen metabolism and hormonal responses of steers fed wheat silage and infused with amino acids or casein. J. Anim. Sci. 75:3038–3045.

Rangngang, M. B., M. L. Nelson, and S. M. Parish. 1997. Ruminal undegradability of blood meal and effects of blood meal on ruminal and postruminal digestion in steers consuming vegetative orchardgrass hay. J. Anim. Sci. 75:2788–2795.

Reis, P. J., D. A. Tunks, and L. F. Sharry. 1989. Incorporation of abomasal and intravenous doses of [^{35}S] cysteine and [^{35}S] methionine into wool. J. Agric. Sci. 112:313–319.

Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acid in growing cattle. J. Anim. Sci. 46:740–745.

Rinne, M., S. Jaakkola, and P. Huhtanen. 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. Anim. Feed Sci. Technol. 67:1–17.

Rioux, R., G. T. Dos Santos, H. V. Petit, and J. G. Proulx. 1995. Effect of cultivars on in vitro and ruminal degradation of the nitrogen fraction in birdsfoot trefoil silages. J. Dairy Sci. 78:1766–1773.

Robert, J. C., and P. E. V. Williams. 1997. Influence of forage type on the intestinal availability of methionine from a rumen protected form. J. Dairy Sci. 80(Suppl. 1):248. (Abstr.)

Robert, J. C., B. K. Sloan, and S. Bourdeau. 1994. The effects of supplementation of corn silage plus soybean meal diets with rumen protected methionine on the lactational performance of dairy cows in early lactation. J. Dairy Sci. 77(Suppl. 1):92. (Abstr.)

Robert, J. C., B. K. Sloan, N. Jouan, and J. Math. 1999. Influence of supplementation with protected methionine on the growth of heifers. J. Dairy Sci. 82(Suppl. 1):91. (Abstr.)

Robert, J. C., P. E. V. Williams, and B. Bouza. 1997. Influence of source of methionine and protection technology on the postruminal delivery and supply to the blood of dairy cows of an oral supply of methionine. J. Dairy Sci. 80(Suppl. 1):248. (Abstr.)

Robinson, P. H. 1997. Modifying duodenal flow of amino acids by manipulation of dietary protein sources. Can. J. Anim. Sci. 77:241–251.

Robinson, P. H., and J. J. Kennelly. 1988a. Ammonia or sulphur dioxide treatment of high-moisture barley on in situ rumen degradability and in situ whole-tract digestibility. Can. J. Anim. Sci. 68:779–786.

Robinson, P. H., and J. J. Kennelly. 1988b. Influence of intake of rumen undegradable protein on milk production of late lactation Holstein cows. J. Dairy Sci. 71:2135–2142.

Robinson, P. H., and J. J. Kennelly. 1988b. Influence of ammoniation of high moisture barley on its in situ rumen degradation and influence on rumen fermentation in dairy cows. Can. J. Anim. Sci. 68:839–851.

Robinson, P. H., and M. A. McNiven. 1994. Influence of flame roasting and feeding frequency of barley on performance of dairy cows. J. Dairy Sci. 77:3631–3643.

Robinson, P. H., and M. A. McNiven. 1993. Nutritive value of raw and roasted sweet white lupins (*Lupinus albus*) for lactating dairy cows. Anim. Feed Sci. Technol. 43:275–290.

Robinson, P. H., and R. E. McQueen. 1994. Influence of supplemental protein source and feeding frequency on rumen fermentation and performance in dairy cows. J. Dairy Sci. 77:1340–1353.

Robinson, P. H., and C. J. Sniffen. 1985. Forestomach and whole tract digestibility for lactating cows as influenced by feeding frequency. J. Dairy Sci. 68:857–867.

Robinson, P. H., M. Gill, and J. J. Kennelly. 1997. Influence of time of feeding a protein meal on ruminal fermentation and forestomach digestion in diary cows. J. Dairy Sci. 80:1366–1373.

Robinson, P. H., G. R. Khorasani, and J. J. Kennelly. 1994. Forestomach and whole tract digestion in lactating dairy cows fed canola meal treated with variable levels of acetic acid. J. Dairy Sci. 77:552–559.

Robinson, P. H., G. de Boer, and J. J. Kennelly. 1991a. Influence of source of rumen-degraded nitrogen and whole tract digestion, plasma hormone and metabolite concentrations as well as milk yield and composition in dairy cows. Can. J. Anim. Sci. 71:417–428.

Robinson, P. H., G. de Boer, and J. J. Kennelly. 1991b. Effect of bovine somatatropin and protein on rumen fermentation and forestomach and whole tract digestion in dairy cows. J. Dairy Sci. 74:3505–3517.

Robinson, P. H., R. E. McQueen, and P. L. Burgess. 1991c. Influence of rumen undegradable protein levels on feed intake and milk production of dairy cows. J. Dairy Sci. 74:1623–1631.

Robinson, P. H., C. J. Sniffen, and P. J. Van Soest. 1985. Influence of level of feed intake on digestion and bacterial yield in the forestomachs of dairy cattle. Can. J. Anim. Sci. 65:437–444.

Rode, L. M., and L. D. Satter. 1988. Effect of amount and length of alfalfa hay in diets containing barley or corn on site of digestion and rumen microbial protein synthesis in dairy cows. Can J. Anim. Sci. 68:445–454.

Rode, L. M., C. D. Knight, K. A. Andrews, and K. M. Koenig. 1998. Effects of pre- and post-partum Alimet® supplementation on milk production of dairy cows. J. Dairy Sci. 81(Suppl. 1):294. (Abstr.)

Rode, L. M., D. C. Weakley, and L. D. Satter. 1985. Effect of forage amount and particle size in diets of lactating dairy cows on site of digestion and microbial protein synthesis. Can. J. Anim. Sci. 65:101–111.

Rode, L., T. Fujieda, H. Sato, H. Suzuki, W. E. Julien, W. V. Chalupa, and C. J. Sniffen. 1999. Rumen-protected amino (RPAA) acid supplementation pre- and postpartum in commercial herds. J. Dairy Sci. 82(Suppl. 1):121. (Abstr.)

Rodwell, V. W. 1985. Proteins. *In*: D. W. Martin, Jr., P. A. Mayes, V. W. Rodwell, and D. K. Granner (Ed.) Harper's Review of Biochemistry, Twentieth Edition. pp. 32–40. Lange Medical Publications, Los Altos, CA.

Roe, M. B., C. J. Sniffen, and L. E. Chase. 1990. Techniques for measuring protein fractions in feedstuffs. Proc. Cornell Nutr. Conf., p. 81–88, Ithaca, NY.

Roffler, R. E., J. E. Wray, and L. D. Satter. 1986. Production responses in early lactation to additions of soybean meal to diets containing predominantly corn silage. J. Dairy Sci. 69:1055–1062.

Rogers, J. A., H. R. Conrad, B. A. Dehority, and J. A. Grubb. 1986. Microbial numbers, rumen fermentation, and nitrogen utilization of steers fed wet or dried brewers grains. J. Dairy Sci. 69:745–753.

Rogers, J. A., U. Krishnamoorthy, and C. J. Sniffen. 1987. Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. J. Dairy Sci. 70:789–798.

Romagnolo, D., C. E. Polan, and W. E. Barbeau. 1994. Electrophoretic analysis of ruminal degradability of corn proteins. J. Dairy Sci. 77:1093–1099.

Rooke, J. A. 1985. The nutritive values of feed proteins and feed protein residues resistant to degradation by rumen microorganisms. J. Sci. Food Agric. 36:629–637.

Rooke, J. A., H. A. Greife, and D. G. Armstrong. 1985. The digestion by cattle of silage-containing diets fed at two dry matter intakes. Br. J. Nutr. 53:691–708.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen, and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525–534.

Rulquin, H. 1987. Determination de certains acides amines limitants chez la vache laitiere par la methode administrations post-ruminales. Reprod. Nutr. Develop. 27(1B):299–300.

Rulquin, H., and L. Delaby. 1994. Lactational responses of dairy cows to graded amounts of rumen-protected methionine. J. Dairy Sci. 77(Suppl. 1):91 (Abstr.)

Rulquin, H., and L. Delaby. 1997. Effects of the energy balance of dairy cows on lactational responses to rumen-protected methionine. J. Dairy Sci. 80:2513–2522.

Rulquin, H., and R. Vérité. 1993. Amino acid nutrition of dairy cows: production effects and animal requirements. Pages 55–77 *in* Recent Advances in Animal Nutrition. P. C. Garnsworthy and D. J. A. Cole, eds. Nottingham University Press.

Rulquin, H., J. Guinard, and R. Vérité. 1998. Variation of amino acid content in the small intestine digesta of cattle: Development of a prediction model. Livestock Prod. Sci. 53:1–13.

Rulquin, H., C. Hurtaud, and L. Delaby. 1994. Effects of dietary protein level on lactational responses of dairy cows to rumen-protected methionine and lysine. Ann. Zootech. 43:245.

Rulquin, H., L. Le Henaff, and R. Vérité. 1990. Effects on milk protein yield of graded levels of lysine infused into the duodenum of dairy cows fed diets with two levels of protein. Reprod. Nutr. Dev. 30(Suppl. 2):238 (Abstr.).

Rulquin, H., P. M. Pisulewski, R. Vérité, and J. Guinard. 1993. Milk production and composition as a function of postruminal lysine and methionine supply: a nutrient-response approach. Livest. Prod. Sci., 37:69–90.

Rulquin, H., R. Vérité, J. Guinard, and P. M. Pisulewski. 1995. Dairy cows requirements for amino acids. Pages 143–160 *in* Animal Science Research and Development: Moving Toward a New Century. M. Ivan, ed., ISBN 0-662-23589-4, Centre for Food and Animal Research, Ottawa, Canada.

Russell, J. B., and C. J. Sniffen. 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. J. Dairy Sci. 67:987–994.

Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. J. Dairy Sci. 66:763–775.

Russell, J. B., H. J. Strobel, and G. Chen. 1988. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Appl. Environ. Microbiol. 54: 872–877.

Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70:3551–3561.

Salter, D. N., K. Daneshaver, and R. H. Smith. 1979. The origin of nitrogen incorporated into compounds in the rumen bacteria of steers given protein- and urea-containing diets. Br. J. Nutr. 41:197–209.

Santos, F. A. P., J. E. P. Santos, C. B. Theurer, and J. T. Huber. 1998a. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81:3182–3213.

Santos, F. A. P., J. T. Huber, C. B. Theurer, R. S. Swingle, J. M. Simas, K. H. Chen, and P. Yu. 1998b. Milk yield and composition of lactating cows fed steam-flaked sorghum and graded concentrations of ruminally degradable protein. J. Dairy Sci. 81:215–220.

Santos, K. A., M. D. Stern, and L. D. Satter. 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58:244–255.

Sarwar, M., J. L. Firkins, and M. L. Eastridge. 1991. Effect of replacing neutral detergent fiber of forage with soyhulls and corn gluten feed for dairy heifers. J. Dairy Sci. 74:1006–1017.

Satter, L. D. 1986. Protein supply from undegraded dietary protein. J. Dairy Sci. 69:2734–2749.

Sauvant D., and J. van Milgen. 1995. Dynamic aspects of carbohydrate and protein breakdown and the associated microbial matter synthesis. *In* Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction: Proceedings of the Eighth International Symposium on Ruminant Physiology. Ferdinand Enke Verlag, Stuttgart, Germany.

Schingoethe, D. J., D. P. Casper, C. Yang, D. J. Illg, J. L. Sommerfeldt, and C. R. Mueller. 1988. Lactational response to soybean meal, heated soybean meal, and extruded soybeans with ruminally protected methionine. J. Dairy Sci. 71:173–180.

Schroeder, G. E., L. J. Erasmus, and H. H. Meissner. 1996. Chemical and protein quality parameters of heat processed sunflower oilcake for dairy cattle. Anim. Feed Sci. Technol. 58:249–265.

Schwab, C. G. 1995a. Protected proteins and amino acids for ruminants. Pages 115–141 *in* R.J. Wallace and A. Chesson, eds. Biotechnology in Animal Feeds and Animal Feeding. V.C.H. Press, Weinheim, Germany.

Schwab, C. G. 1995b. Rumen-protected amino acids—their role in nutrition of high-producing ruminants. Pages 161–175 *in* M. Ivan, ed., Animal Science Research and Development: Moving Toward a New Century. ISBN 0-662-23589-4, Centre for Food and Animal Research, Ottawa, Canada.

Schwab, C. G. 1996a. Amino acid nutrition of the dairy cow: Current status. Proc. Cornell Nutr. Conf., p. 184–198, Ithaca, NY.

Schwab, C. G. 1996b. Rumen-protected amino acids for dairy cattle: Progress towards determining lysine and methionine requirements. Anim. Feed Sci. Technol. 59:87–101.

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M.M.A. Mesbah. 1992a. Amino acid limitation and flow to the duodenum at four stages

of lactation. I. Sequence of lysine and methionine limitation. J. Dairy Sci. 75:3486–3502.

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992b. Amino acid limitation and flow to duodenum at four stages of lactation. 2. Extent of lysine limitation. J. Dairy Sci. 75:3503–3518.

Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254–1270.

Schwab, C. G., S. J. Muise, W. E. Hylton, and J. J. Moore, III. 1982. Response to abomasal infusion of methionine of weaned dairy calves fed a complete pelleted starter ration based on by-product feeds. J. Dairy Sci. 65:1950–1961.

Scollan, N. D., H. J. Kim, W. J. Maeng, H. Park, M. A. Neville, R.T. Evans, and A. B. McAllan. 1996. The effect of cereal carbohydrate supplementation on nutrient flow to the duodenum and microbial protein synthesis in steers fed grass silage. *In* Proceedings of the XI the International Silage Conference (Jones, D. I. H. et al., eds.) pp. 228–229.

Sebek, L. B., and H. Everts. 1999. *In situ* degradation of dry matter and crude protein in ewes and dairy cows. Anim. Sci. 68:801–808.

Seymour, W. M., and C. E. Polan. 1986. Dietary energy regulation during gestation on subsequent lactational response to soybean meal or dried brewers grains. J. Dairy Sci. 69:2837–2845.

Seymour, W. M., C. E. Polan, and J. H. Herbein. 1992. In vivo degradation of protein in diets formulated for two degradabilities. J. Dairy Sci. 75:2447–2453.

Shabi, Z., A. Arieli, I. Bruckental, Y. Aharoni, S. Zamwel, A. Bor, and H. Tagari. 1998. Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dairy cows. J. Dairy Sci. 81:1991–2000.

Sharma, B. K., and R. A. Erdman. 1988. Abomasal infusion of choline and methionine with or without 2-amino-2-methyl-1-propanol for lactating dairy cows. J. Dairy Sci. 71:2406–2411.

Sharma, B. K., and R. A. Erdman. 1989. Effects of dietary and abomasally infused choline on milk production responses of lactating dairy cows. J. Nutr. 119:248–254.

Sicilano-Jones, J. L. Personal communication.

Siddons, R. C., and J. Paradine. 1983. Protein degradation in the rumen of sheep and cattle. J. Sci. Food and Agric. 34:701–708.

Sidhu, G. S., and J. R. Ashes. 1977. Amino acid availability from protected protein—a critical factor for response in milk production. Proc. Nutr. Soc. Aust. 2:81. (Abstr.)

Sievert, S. J., and R. D. Shaver. 1993. Effect of nonfiber carbohydrate level and *Aspergillus oryzae* fermentation extract on intake, digestion, and milk production in lactating dairy cows. J. Anim. Sci. 71:1032–1040.

Sinclair, L. A., P. C. Garnsworthy, J. R. Newbold, and P. J. Buttery. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. Camb. 120:251–263.

Sinclair, L. A., P. C. Garnsworthy, J. R. Newbold, and P. J. Buttery. 1995. Effects of synchronizing the rate of dietary energy and nitrogen release in diets with similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 124:463–472.

Singh, C. K., P. H. Robinson, and M. A. McNiven. 1995. Evaluation of raw and roasted lupin seeds as protein supplements for lactating cows. Anim. Feed Sci. Technol. 52:63–76.

Sloan, B. K., B. D. Garthwaite, and C. G. Schwab. 1998. Practical formulation of dairy cow diets for digestible amino acids to improve nitrogen efficiency and the bottom line. Proc. Cornell Nutr. Conf., p. 51–64, Ithaca, NY.

Sloan, B. K., P. Rowlinson, and D. G. Armstrong. 1988. The influence of a formulated excess of rumen degradable protein or undegradable protein on milk production in dairy cows in early lactation. Anim. Prod. 46:13–22.

Sniffen, C. J. 1974. Nitrogen utilization as related to solubility of NPN and protein in feeds. Proc. Cornell Nutr. Conf., p. 12–18, Ithaca, NY.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. carbohydrate and protein availability. J. Anim. Sci., 70:3562–3577.

Sniffen, C. J., C. S. Ballard, D. S. Tsang, W. Chalupa, W. E. Julien, B. Perkins, T. Fujieda, T. Ueda, and H. Suzuki. 1999a. Effect of rumen-protected amino acids (RPAA) on performance of lactating Holstein cows. J. Dairy Sci. 82(Suppl. 1):94. (Abstr.)

Sniffen, C. J., D. S. Tsang, C. S. Ballard, W. Chalupa, W. E. Julien, T. Fujieda, T. Ueda, H. Sato, and H. Suzuki. 1999b. Effect of rumen-protected lysine and methionine supplementation with different sources of metabolizable protein on milk production of high producing dairy cows. J. Dairy Sci. 82(Suppl. 1):94. (Abstr.)

Socha, M. T. 1994. Determining the methionine requirements of lactating dairy cows. Thesis. Ph. D. dissertation University of New Hampshire. 174 p.

Socha, M. T., C. G. Schwab, D. E. Putnam, N. A. Kierstead, N. L. Whitehouse, B. D. Garthwaite, and G. A. Ducharme. 1994a. Determining methionine requirements of dairy cows during midlactation by postruminally infusing incremental amounts of methionine. J. Dairy Sci. 77(Suppl. 1):93. (Abstr.)

Socha, M. T., C. G. Schwab, D. E. Putnam, N. A. Kierstead, N. L. Whitehouse, B. D. Garthwaite, and G. A. Ducharme. 1994c. Determining methionine requirements of dairy cows during peak lactation by postruminally infusing incremental amounts of methionine. J. Dairy Sci. 77(Suppl. 1):92. (Abstr.)

Socha, M. T., C. G. Schwab, D. E. Putnam, N. L. Whitehouse, N. A. Kierstead, B. D. Garthwaite, and G. A. Ducharme. 1994b. Determining methionine requirements of dairy cows during early lactation by postruminally infusing incremental amounts of methionine. J. Dairy Sci. 77(Suppl. 1):65. (Abstr.)

Song, M. K., and J. J. Kennelly. 1989. Effect of ammoniated barley silage on ruminal fermentation, nitrogen supply to the small intestine, ruminal and whole tract digestion, and milk production of Holstein cows. J. Dairy Sci. 72:2981–2990.

Spain, J. N., C. E. Polan, and B. A. Watkins. 1995. Evaluating effects of fish meal on milk fat yield of dairy cows. J. Dairy Sci. 78:1142–1153.

Spicer, L. A., C. B. Theurer, J. Sowe, and T. H. Noon. 1986. Ruminal and postruminal utilization of nitrogen and starch from sorghum grain, corn, and barley based diets by beef steers. J. Anim Sci. 65:521–529.

Stallings, C. C., Y. M. Acosta, and C. E. Polan. 1991. Predicting diet protein degradability from individual ingredient estimations in diets containing barley silages. J. Dairy Sci. 74:3486–3491.

Staples, C. R., W. W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73:938–947.

Stanford, K., T. A. McAllister, B. M. Lees, Z. J. Xu, and K. J. Cheng. 1996. Comparison of sweet white lupin seed, canola meal and soybean meal as protein supplements for lambs. Can. J. Anim. Sci. 76:215–219.

Steg, A., W. M. van Straalen, V. A. Hindle, W. A. Wesink, F. M. H. Dooper, and R. L. M. Schils. 1994. Rumen degradation and intestinal digestion of grass and clover at two maturity levels during the season in dairy cows. Grass Forage Sci. 49:378–390.

Stensig, T., and P. H. Robinson. 1997. Digestion and passage kinetics of forage fiber in dairy cows as affected by fiber-free concentrate in the diet. J. Dairy Sci. 80:1339–1352.

Stern, M. D., A. Bach, and S. Calsamiglia. 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim Sci. 75:2256–2276.

Stern, M. D., and L. D. Satter. 1984. Evaluation of nitrogen solubility and the dacron bag technique as methods for estimating protein degradation in the rumen. J. Anim. Sci. 58:714–724.

Stern, M. D., G. A. Varga, J. H. Clark, J. L. Firkins, J. T. Huber, and D. L. Palmquist. 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the rumen. J. Dairy Sci. 77:2762–2786.

Stern, M. D., K. A. Santos, and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating cattle fed heat-treated whole soybeans. J. Dairy Sci. 68:45–56.

Stern, M. D., L. M. Rode, R. W. Prange, R. H. Stauffacher, and L. D. Satter. 1983. Ruminal protein degradation of corn gluten meal in lactating dairy cattle fitted with duodenal T-type cannulae. J. Anim. Sci. 56:194–205.

Stern, M. D., W. H. Hoover, C. J. Sniffen, B. A. Crooker, and P. H. Knowlton. 1978. Effects of nonstructural carbohydrate, urea and soluble protein level on microbial protein synthesis in continuous culture of rumen contents. J. Anim. Sci. 47:944–951.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. P. Manski. 1991a. Impact of carbohydrate and protein levels on bacterial metabolism in continuous culture. J. Dairy Sci. 74:860–870.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. Blauweikel. 1991b. Ruminal digestion and microbial utilization of diets varying in type of carbohydrates and protein. J. Dairy Sci. 74:871–881.

Storm, E., and E. R. Ørskov. 1983. The nutritive value of rumen micro-organisms in ruminants. 1. Large scale isolation and chemical composition of rumen micro-organisms. Br. J. Nutr. 50:463–470.

Stutts, J. A., W. A. Nipper, R. W. Adkinson, J. E. Chandler, and A. S. Achacoso. 1988. Protein solubility, in vitro ammonia concentration, and in situ disappearance of extruded whole cottonseed and other protein sources. J. Dairy Sci. 71:3323–3333.

Subuh, A.M.H., T. G. Rowan, and T.L.J. Lawrence. 1994. Effect of heat or formaldehyde treatment and differences in basal diet on the rumen degradability of protein in soyabean meal and in rapeseed meals of different glucosinolate content. Anim. Feed Sci. Technol. 49:297–310.

Susmel, P., C. R. Mills, M. Colitti, and B. Stefanon. 1993. In vitro solubility and degradability of nitrogen in concentrate ruminant feeds. Anim. Feed Sci. Technol. 42:1–13.

Susmel, P., M. Spanghero, B. Stefanon, C. R. Mills, and C. Cargnelutti. 1991. Effect of NDF concentration and physical form of fescue hay on rumen degradability, intake, and rumen turn-over of cows. Anim. Prod. 53:305–313.

Susmel, P., B. Stefanon, C. R. Mills, and M. Spanghero. 1990. Rumen degradability of organic matter, nitrogen and fibre fractions in forages. Anim. Prod. 51:515–526.

Swanson, E. W. 1977. Factors for computing requirements of protein for maintenance of cattle. J. Dairy Sci. 60:1583–1593.

Swanson, E.W. 1982. Estimation of metabolic protein requirements to cover unavoidable losses of endogenous nitrogen in maintenance of cattle. Pp. 183–197 in Protein Requirement for Cattle: Symposium. F.N. Owens, ed. MP-109, Oklahoma State University, Stillwater.

Tamminga, S., R. Ketelaar, and A. M. van Vuuren. 1991. Degradation of nitrogenous compounds in conserved forages in the rumen of dairy cows. Grass Forage Sci. 46:427–435.

Tamminga, S., van der Koelen, C. J., and van Vuuren, A. M. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. Livestock Prod. Sci. 6:255–262.

Teller, E., M. Vanbelle, M. Foulon, G. Collignon, and B. Matatu. 1992. Nitrogen metabolism in rumen and whole digestive tract of lactating dairy cows fed grass silage. J. Dairy Sci. 75:1296–1304.

Tesfa, A. T. 1993. Effects of rape-seed oil supplementation on digestion, microbial protein synthesis and duodenal microbial amino acid composition in ruminants. Anim. Feed Sci. Technol. 41:313–328.

Tice, E. M., M. L. Eastridge, and J. L. Firkins. 1993. Raw soybeans and roasted soybeans of different particle sizes. 1. Digestibility and utilization by lactating cows. J. Dairy Sci. 76:224–235.

Titgemeyer, E. C., and N. R. Merchen. 1990a. Sulfur-containing amino acid requirements of rapidly growing steers. J. Anim. Sci. 68:2075–2083.

Titgemeyer, E. C., and N. R. Merchen. 1990b. The effect of abomasal methionine supplementation on nitrogen retention of growing steers postruminally infused with casein or nonsulfur-containing amino acids. J. Anim. Sci. 68:750–757.

Titgemeyer, E. C., N. R. Merchen, and L. L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci., 67:262–275.

Titgemeyer, E. C., N. R. Merchen, L. L. Berger, and L. E. Deetz. 1988. Estimates of lysine and methionine requirements of growing steers fed corn silage-based or corn-based diets. J. Dairy Sci. 71:421–434.

Todorov, N. A., and D. G. Griginov. 1991. Comparison of the infusion method, mobile bag technique and in vitro method for determination of the true protein digestibility in small intestine of cattle. 6th Int. Symp. Protein Metabolism and Nutrition Herning, Denmark, p. 80–82.

Uden, P., and P. J. Van Soest. 1984. Investigations of the *in situ* bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. J. Anim. Sci. 58:213–221.

Van Barneveld, R. J., E. S. Batterham, and B. W. Norton. 1994a. The effects of heat on amino acids for growing pigs. 1. A comparison of ileal and faecal digestibilities of amino acids in raw and heat-treated field peas (Pisum sativum cultivar Dundale). Br. J. Nutr. 72:243–256.

Van Barneveld, R. J., E. S. Batterham, and B. W. Norton. 1994b. The effects of heat on amino acids for growing pigs. 2. Utilization of ileal-digestible lysine from heat-treated field peas (Pisum sativum cultivar Dundale). Br. J. Nutr. 72:221–241.

Van Barneveld, R. J., E. S. Batterham, and B. W. Norton. 1994c. The effects of heat on amino acids for growing pigs. 3. The availability of lysine from heat-treated field peas (Pisum sativum cultivar Dundale) determined using the slope-ratio assay.. Br. J. Nutr. 72:257–275.

van der Aar, P. J., L. L. Berger, G. C. Fahey, Jr., and N. R. Merchen. 1984. Effects of alcohol treatment of soybean meal on ruminal escape of soybean meal protein. J. Anim. Sci. 59:483–489.

van der Koelan, C. J., P. W. Goedhart, A. M. van Vuuren, and G. Savoini. 1992. Sources of variation of the in situ nylon bag technique. Anim. Feed Sci. Technol. 38:35–42.

Van Gylswyk, N. O. 1990. Enumeration and presumptive identification of some functional groups of bacteria in the rumen of dairy cows fed silage-based diets. FEMS Microbiol. Ecol. 73:243–254.

Van Kessel, J. S., and J. B. Russell. 1997. The endogenous polysaccharide utilization rate of mixed ruminal bacteria and the effect of energy starvation on ruminal fermentation rates. J. Dairy Sci. 80:2442–2448.

Van Soest, P. J. 1994. Nutritional Ecology of The Ruminant. Second Edition. Cornell University Press, Ithaca, NY.

Van Soest, P. J., N. L. Conklin, and P. J. Horvath. 1987. Tannins in foods and feeds. Proc. Cornell Nutr. Conf. For Feed Manufactures, Ithaca, NY, Cornell University, Syracuse, NY, p. 115–122.

van Straalen, W. M., and G. Huisman. 1991. The digestibility of bypass crude protein from grass silage in the intestine of dairy cows measured by the mobile nylon bag technique. 6th Int. Symp. Protein Metabolism and Nutrition Herning, Denmark, p. 83–85.

van Straalen, W. M., F. M. H. Dooper, A. M. Antoniewicz, I. Kosmala, and A. M. van Vuuren. 1993. Intestinal digestibility in dairy cows of protein from grass and clover measured with mobile nylon bag and other methods. J. Dairy Sci. 76:2970–2981.

van Straalen, W. M., J. J. Odinga, and W. Mostert. 1997. Digestion of feed amino acids in the rumen and small intestine of dairy cows measured with nylon-bag techniques. Br. J. Nutr. 77:83–97.

van Vuuren, A. M., C. J. van der Koelen, and J. Vroons-de Bruin. 1993. Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows. J. Dairy Sci. 76:2692–2700.

van Vuuren, A. M., F. Krol-Kramer, R. A. van der Lee, and H. Corbijn. 1992. Protein digestion and intestinal amino acids in dairy cows fed fresh *Lolium perenne* with different nitrogen contents. J. Dairy Sci. 75:2215–2225.

van Vuuren, A. M., S. Tamminga, and R. S. Ketelaar. 1991. In sacco degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing cows. J. Agric. Sci. (Camb.) 116:429–436.

van Vuuren, A. M., K. Bergsma, F. Krol-Kramer, and J.A.C. van Beers. 1989. Effects of addition of cell wall degrading enzymes on the chemical composition and the in sacco degradation of grass silage. Grass Forage Sci. 44:223–230.

Valentine, S. C., and B. D. Bartsch. 1988. Degradation of dry matter, crude protein, fat, crude fibre and nitrogen-free-extract in milled barley and lupin grains incubated in nylon bags in the rumen of dairy cows. J. Agric. Sci. (Camb.) 110:395–398.

Vanhatalo, A., and E. Ketoja. 1995. The role of the large intestine in post-ruminal digestion of feeds as measured by the mobile-bag method in cattle. Br. J. Nutr. 73:491–505.

Vanhatalo, A., and T. Varvikko. 1995. Effect of rumen degradation on intestinal digestion of nitrogen of ^{15}N-labelled rapeseed meal and straw measured by the mobile-bag method in cows. J. Agric. Sci. (Camb.). 125:253–261.

Vanhatalo, A., I. Aronen, and T. Varvikko. 1995. Intestinal nitrogen digestibility of heat-moisture treated rapeseed meals as assessed by the mobile-bag method in cows. Anim. Feed Sci. Technol. 55:139–152.

Vanhatalo, A., P. Dakowski, and P. Huhtanen. 1996. Effects of stage of growth and duration of rumen incubation time on intestinal digestibility of rumen-undegradable nitrogen of grass by mobile-bag method in cows. Acta Agric. Scand., Sect. A, Anim. Sci. 46:1–10.

Vanhatalo, A., P. Huhtanen, V. Toivonen, and T. Varvikko. 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. J. Dairy Sci. 82:2674–2685.

Vanzant, E. S., R. C. Cochran, and E. C. Titgemeyer. 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717–2729.

Vanzant, E. S., R. C. Cochran, E. E. Titgemeyer, S. D. Stafford, K. C. Olson, D. E. Johnson, and G. St. Jean. 1996. In vivo and in situ measurements of forage protein degradation in beef cattle. J. Anim. Sci. 74:2773–2784.

Varvikko, T., A. Vanhatalo, T. Jalava, and P. Huhtanen. 1999. Lactation and metabolic responses to graded abomasal doses of methionine and lysine in cows fed grass silage diets. J. Dairy Sci. 82:2659–2673.

Varvikko, T., and A. Vanhatalo. 1992. Effect of supplementary energy and protein feeding on the true digestion of grass-silage organic matter, cell walls and nitrogen estimated by the combined synthetic-fiber-bag method in cows. Can. J. Anim. Sci. 72:671–678.

Vasquez-Anon, M., A. J. Heinrichs, J. M. Aldrich, and G. A. Varga. 1993. Effect of postweaning age on rate of protein disappearance in calves weaned at 5 weeks of age. J. Dairy Sci. 76:2749–2757.

Veira, D. M., J. R. Seoane, and J. G. Proulx. 1991. Utilization of grass silage by growing cattle: Effect of a supplement containing ruminally protected amino acids. J. Anim. Sci. 69:4703–4709.

Vérité, R., and J. L. Peyraud. 1989. Protein: the PDI system. In : Jarrige, R. (Ed.): Ruminant Nutrition: Recommended Allowances and Feed Tables. INRA. John Libbey, Paris. pp. 33–48.

Vicini, J. L., J. H. Clark, W. L. Hurley, and J. M. Bahr. 1988. Effects of abomasal or intravenous administration of arginine on milk production, milk composition, and concentrations of somatotropin and insulin in plasma of dairy cows. J. Dairy Sci. 71:658–665.

Vieira, R.A.M., J. Pereira, P.A.M. Malafaia, and A. C. de Queiroz. 1997. The influence of elephant-grass (*Pennisetum purpureum* Schum., Mineiro variety) growth on the nutrient kinetics in the rumen. Anim. Feed Sci. Technol. 67:151–161.

Vik-Mo, L. 1989. Degradability of forages in sacco. 2. Silages of grasses and red clover at two cutting times, with formic acid and without additive. Acta Agric. Scand. 39:53–64.

Virtanen, A. I. 1966. Milk production of cows on protein-free feed. Science. 153:1603–1614.

Voigt, J., B. Piatkowski, H. Englemann, and E. Rudolph. 1985. Measurement of the postruminal digestibility of crude protein by the bag technique in cows. Arch. Tierernaehr. 8:555–562.

Volden, H. 1999. Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. J. Anim. Sci. 77:1905–1918.

Volden, H., and O. M. Harstad. 1995. Effect of rumen incubation on the true digestibility of feed protein in the digestive tract determined by nylon bag techniques. Acta Agric. Scand., Sect. A, Anim. Sci. 45:106–115.

von Keyserlingk, G.E.M., and G. W. Mathison. 1993. The effect of ruminal escape protein and ambient temperature on the efficiency of utilization of metabolizable energy by lambs. J. Anim. Sci. 71:2206–2217.

von Keyserlingk, M.A.G., and G. W. Mathison. 1989. Use of the in situ technique and passage rate constants in predicting voluntary intake and apparent digestibility of forages by steers. Can. J. Anim. Sci. 69:973–987.

von Keyserlingk, M. A. G., J. A. Shelford, R. Purchala, M. L. Swift, and L. J. Fisher. 1998. In situ disappearance of amino acids from the grass silages in the rumen and intestine of cattle. J. Dairy Sci. 81:140–149.

von Keyserlingk, M. A. G., M. L. Swift, R. Puchala, and J. A. Shelford. 1996. Degradability characteristics of dry matter and crude protein of forages in ruminants. Anim. Feed Sci. Technol. 57:291–311.

Voss, V. L., D. Stehr, L. D. Satter, and G. A. Broderick. 1988. Feeding lactating dairy cows proteins resistant to ruminal degradation. J. Dairy Sci. 71:2428–2439.

Waghorn, G. C., and R. L. Baldwin. 1984. Model of metabolite flux within mammary gland of the lactating cow. J. Dairy Sci. 67:531–544.

Wagner, D. G., and J. K. Loosli. 1967. Studies on the energy requirements of high- producing dairy cows. Cornell Exp. Agric. Stn. Mem. No. 400. Ithaca, N.Y., Cornell University Press.

Waldo, D. R., and B. P. Glenn. 1984. Comparison of new protein systems for lactating dairy cows. J. Dairy Sci. 67:1115–1133.

Walhain, P., M. Foucart, and A. Théwis. 1992. Influence of extrusion on ruminal and intestinal disappearance in sacco of pea (*Psium sativum*) proteins and starch. Anim. Feed Sci. Technol. 38:43–55.

Wallace, R. J. 1983. Hydrolysis of ^{14}C-labelled proteins by rumen microorganisms and by proteolytic enzymes prepared from rumen bacteria. Br. J. Nutr. 50:345–355.

Wallace, R. J. 1985. Adsorption of soluble proteins to rumen bacteria and the role of adsorption in proteolysis. Br. J. Nutr. 53:399–408.

Wallace, R. J. 1994. Amino acid and protein synthesis, turnover, and breakdown by rumen microorganisms. In: Principles of Protein Metabolism in Ruminants (Asplund, J. M., ed.), pp 71–111, CRC Press, Boca Raton, Florida.

Wallace, R. J. 1996. Ruminal microbial metabolism of peptides and amino acids. J. Nutr. 126:1326S–1334S.

Wallace, R. J. 1997. Peptide metabolism and its efficiency in ruminant production. In Rumen Microbes and Digestive Physiology in Ruminants (Onodera, R. et al., eds.), pp 95–105. Japan Sci. Soc. Press, Tokyo/S Karger, Basel.

Wallace, R. J., and C. A. Munro. 1986. Influence of the rumen anaerobic fungus *Neocallimastix frontalis* on the proteolytic activity of a defined mixture of rumen bacteria growing on a solid substrate. Lett. Appl. Microbiol. 3:23–26.

Wallace, R. J., and K. N. Joblin. 1985. Proteolytic activity of a rumen anaerobic fungus. Fed. Eur. Microbiol. Soc. Microbial. Lett. 29:19.

Wallace, R. J., C, Atasoglu, and C. J. Newbold. 1999. Role of peptides in rumen microbial metabolism. Review. AJAS 12:139–147.

Waltz, D. M., and M. D. Stern. 1989. Evaluation of various methods for protecting soya-bean protein from degradation by rumen bacteria. Anim. Feed Sci. Technol. 25:111–122.

Waltz, D. M., M. D. Stern, and D. J. Illg. 1989. Effect of ruminal protein degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. J. Dairy Sci. 72:1509–1518.

Wanderly, R. C., G. A. Alhadhrami, M. Pessarkli, J. L. Aquino-Ramos, and J. T. Huber. 1999. An assessment of the microbial colonization of forage in the rumen of dairy cows and camels. Anim. Feed Sci. Technol. 76:207–218.

Wang, Y., T. A. McAllister, M. D. Pickard, Z. Xu, L. M. Rode, and K. - J. Cheng. 1999. Effect of micronizing full fat canola seed on amino acid disappearance in the gastrointestinal tract of dairy cows. J. Dairy Sci. 82:537–544.

Wang, Y., T. A. McAllister, D. R. Zobell, M. D. Pickard, L. M. Rode, S. Mir, and K. J. Cheng. 1997. The effect of micronization of full-fat canola seed on digestion in the rumen and total tract of dairy cows. Can. J. Anim. Sci. 77:431–440.

Waters, C. J., M. A. Kitcherside, and A. J. F. Webster. 1992. Problems associated with estimating the digestibility of undegraded dietary nitrogen from acid-detergent insoluble nitrogen. Anim. Feed Sci. Technol. 39:279–291.

Wattiaux, M. A., D. K. Combs, and R. D. Shaver. 1994. Lactational responses to ruminally undegradable protein by dairy cows fed diets based on alfalfa silage. J. Dairy Sci. 77:1604–1617.

Webb, K. E., J. C. Matthews, and D. B. DiRienzo. 1992. Peptide absorption: A review of current concepts and future perspectives. J. Anim. Sci. 70:3248–3257.

Webster, A. J. F. 1987. Metabolizable protein- the U.K. approach. *In*: G. Alderman and R. Jarrige (eds.), Feed Evaluation and Protein Requirement System for Ruminants. Commission of European Communities, EUR 10657 EN, Luxembourg, p. 47–54.

Webster, A. J. F., M. A. Kitcherside, J. R. Keirby, and P. A. Hall. 1984. Evaluation of protein feeds for dairy cows. Anim. Prod. 8:548.

Weigel, D. J., J. P. Elliott, and J. H. Clark. 1997. Effects of amount and ruminal degradability of protein on nutrient digestibility and production by cows fed tallow. J. Dairy Sci. 80:1150–1159.

Weisbjerg, M. R., C. F., Boersting, and T. Hvelplund. 1992. The influence of tallow on rumen metabolism, microbial biomass synthesis and fatty acid composition of bacteria and protozoa. Acta Agric. Scand. 42:138–147.

Weisbjerg, M. R., T. Hvelplund, S. Hellberg, S. Olsson, and S. Sanne. 1996. Effective rumen degradability and intestinal digestibility of individual amino acids in different concentrates determined in situ. Anim. Feed Sci. Technol. 62:179–188.

Weiss, W. P., D. O. Erickson, G. M. Erickson, and G. R. Fisher. 1989. Barley distillers grains as a protein supplement for dairy cows. J. Dairy Sci. 72:980–987.

Wen-Shyg, P. C., C. Kuen-Jaw, K. Kwen-Sheng, H. Jenn-Chung, and Y. Bi. 1995. Studies on the protein degradabilities of feedstuffs in Taiwan. Anim. Feed Sci. Technol. 55:215–226.

Wessels, R. H., E. C. Titgemeyer, C. K. Armendariz, and G. St. Jean. 1996. Lasalocid effects on ruminal degradation of protein and postruminal supply of amino acids in Holstein steers. J. Dairy Sci. 79:1802–1808.

Wheeler, J. G., H. E. Amos, M. A. Froetschel, J. C. Coomer, T. Maddox, and J. M. Fernandez. 1995. Responses of early lactation cows fed winter and summer annual forages and undegradable intake protein. J. Dairy Sci. 78:2767–2781.

Wilkerson, V. A., T. J. Klopfenstein, and W. W. Stroup. 1995. A collaborative study of in situ forage protein degradation. J. Anim. Sci. 73:583–588.

Williams, A. P., and R. H. Smith. 1974. Concentrations of amino acids and urea in the plasma of the ruminating calf and estimation of the amino acid requirements. Br. J. Nutr. 32:421–433.

Windschitl, P. M. 1991. Lactational performance of high producing dairy cows fed diets containing salmon meal and urea. J. Dairy Sci. 74:3475–3485.

Windschitl, P. M., and M. D. Stern. 1988. Evaluation of calcium lignosulfonate-treated soybean meal as a source of rumen protected protein for dairy cattle. J. Dairy Sci. 71:3310–3322.

Wohlt, J. E., J. H. Clark, R. G. Derrig, and C. L. Davis. 1977. Valine, leucine, and isoleucine metabolism by lactating bovine mammary tissue. J. Dairy Sci. 60:1875–1882.

Wohlt, J. E., S. L. Chmiel, P. K. Zajac, L. Backer, D. B. Blethen, and J. L. Evans. 1991. Dry matter intake, milk yield and composition, and nitrogen use in Holstein cows fed soybean, fish, or corn gluten meals. J. Dairy Sci. 74:1609–1622.

Wright, T. C. 1996. Effects of rumen-undegradable protein and feed intake on milk protein production in dairy cows. Thesis. University of Guelph. 92 p.

Wu, Z., and L. D. Satter. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. J. Dairy Sci. 83:1042–1051.

Wu, Z., C. Le Guilloux, and L. D. Satter. 1999. Supplementing rumen protected methionine to lactating cows fed different amounts of protein. J. Dairy Sci. 82(Suppl. 1):65. (Abstr.)

Wu, Z., R. J. Fisher, C. E. Polan, and C. G. Schwab. 1997. Lactational performance of cows fed low or high ruminally undegradable protein prepartum and supplemental methionine and lysine postpartum. J. Dairy Sci., 80:722–729.

Xu, S., J. H. Harrison, and R. E. Riley. 1996. Characteristics of nitrogen fractions and amino acids of feedstuffs common to the Pacific Northwest. Professional Anim. Scientist 12:223–237.

Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, H. Watanabe, T. Ueda, and H. Suzuki. 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. J. Dairy Sci. 81:1062–1077.

Yan, T., D. C. Patterson, F. J. Gordon, and D. J. Kilpatrick. 1998. Effects of bacterial inoculation of unwilted and wilted grass silages 1. Rumen microbial activity, silage nutrient degradability and digestibility. J. Agric. Sci. (Camb.) 131:103–112.

Yang, W. Z, K. A. Beauchemin, and L. M. Rode. 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci. 82:391–403.

Yang, W. Z., K. A. Beauchemin, K. M. Koenig, and L. M. Rode. 1997. Comparison of hull-less barley, barley, or corn for lactating cows: Effects on extent of digestion and milk production. J. Dairy Sci. 80:2475–2486.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 1996. Ruminal digestion kinetics of temper-rolled hulless barley. Can. J. Anim. Sci. 76:629–632.

Yang, C.-M.J., D. J. Schingoethe, and D. P. Casper. 1986. Protected methionine and heat-treated soybean meal for high producing dairy cows. J. Dairy Sci. 69:2348–2357.

Yong-Gang, L., A. Steg, and V. A. Hindle. 1994. Rumen degradation and intestinal digestion of crambe and other oilseed by-products in dairy cows. Anim. Feed Sci. Technol. 45:397–409.

Yoon, I. K., and M. D. Stern. 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. J. Dairy Sci. 79:411–417.

Yoon, I. K., K. J. Lindquist, D. D. Hongerholt, M. D. Stern, B. A. Crooker, and K. D. Short. 1996. Variation in menhaden fish meal characteristics and their effects on ruminal protein degradation as assessed by various techniques. Anim. Feed Sci. Technol. 60:13–27.

Young, V. R., and A. E. El-Khoury. 1995. The notion of the nutritional essentiality of amino acids revisited with a note of the indispensable

amino acid requirements in adults. *In* Amino Acid Metabolism and Therapy in Health and Nutritional Disease (L. Cynober, ed), pp. 191–233, CRC Press, Boca Raton, FL, USA.

Younker, R. S., S. D. Winland, J. L. Firkins, and B. L. Hull. 1998. Effects of replacing forage fiber or nonfiber carbohydrates with dried brewers grains. J. Dairy Sci. 81:2645–2656.

Yu, Y., and J. W. Thomas. 1976. Estimation of the extent of heat damage in alfalfa haylage by laboratory measurement. J. Anim. Sci. 42:766–774.

Zerbini, E., and C. E. Polan. 1985. Protein sources evaluated for ruminating Holstein calves. J. Dairy Sci. 68:1416–142

Zerbini, E., C. E. Polan, and J. H. Herbein. 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in Holstein cows during early and midlactation. J. Dairy Sci. 71:1248–1258.

Zhu, J. S., S. R. Stokes, and M. R. Murphy. 1997. Substitution of neutral detergent fiber from forage with neutral detergent fiber from by-products in the diets of lactating cows. J. Dairy Sci. 80:2901–2906.

Zimmerman, C. A., A. H. Rakes, R. D. Jaquette, B. A. Hopkins, and W. J. Croom, Jr. 1991. Effects of protein level and forage source on milk production and composition in early lactation dairy cows. J. Dairy Sci. 74:980–990.

Zimmerman, C. A., A. H. Rakes, T. E. Daniel, and B. A. Hopkins. 1992. Effect of total and rumen undegradable protein on the performance of cows fed low fiber diets. J. Dairy Sci. 75:1954–1964.

Zinn, R. A. 1993a. Characteristics of ruminal and total tract digestion of canola meal and soybean meal in a high-energy diet for feedlot cattle. J. Anim. Sci. 71:796–801.

Zinn, R. A. 1993b. Influence of processing on the comparative feeding value of barley for feedlot cattle. J. Anim. Sci. 71:3–10.

Zinn, R. A. 1995. Characteristics of digestion of linted and lint-free cottonseed in diets for feedlot cattle. J. Anim. Sci. 73:1246–1250.

Zinn, R. A., 1988. Crude protein and amino acid requirements of growing-finishing Holstein steers gaining 1.43 kilograms per day. J. Anim. Sci. 66:1755–1763.

Zinn, R. A., A. Plascencia, and R. Barajas. 1994. Interaction of forage level and monensin in diets for feedlot cattle on growth performance and digestive function. J. Anim. Sci. 72:2209–2215.

Zinn, R. A., and A. Plascencia. 1993. Interaction of whole cottonseed and supplemental fat on digestive function in cattle. J. Anim. Sci. 71:11–17.

Zinn, R. A., and Y. Shen. 1998. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. J. Anim. Sci. 76:1280–1289.

Zinn, R. A., C. F. Adam, and M. S. Tamayo. 1995. Interaction of feed intake level on comparative ruminal and total tract digestion of dry-rolled and steam-flaked corn. J. Anim. Sci. 73:1239–1245.

Zinn, R. A., Y. Shen, C. F. Adam, M. Tamayo, and J. Rosalez. 1996. Influence of dietary magnesium level on metabolic and growth-performance responses of feedlot cattle to laidlomycin propionate. J. Anim. Sci. 74:1462–1469.