New Concepts in Protein Nutrition of Ruminants

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Summary

- Microbial protein synthesis in the rumen
  - the goal of the ruminant nutritionist seems to be to maximize EMPS, however, the real goal should be to maximize microbial CP flow
  - Total microbial N flow is negatively correlated with ruminal pH but there is no relationship between ruminal pH and efficiency of microbial protein synthesis
- Microbial protein degradation in the rumen
  - protein degradation in the rumen is the result of microbial activity and depends on protein type, ruminal dilution rate, ruminal pH, substrate being fermented and predominant species of rumen flora
  - after maximizing microbial protein synthesis, it is clear that substantial quantities of rumen undegradable protein (RUP) from protein supplements must be incorporated into the diet of high producing ruminants
- Protein digestion in the small intestine
  - considerable variation among and within feedstuffs exists in intestinal digestion of protein
  - cost of intestinally absorbable dietary protein or absorbable amino acids can be used as a guideline to select protein supplements for high producing ruminant animals

Introduction

Amino acids are supplied to the duodenum of ruminants by microbial protein synthesized in the rumen, undegraded dietary protein and endogenous protein. Microbial protein usually accounts for a substantial portion of the total amino acids entering the small intestine. Microbial protein is a high quality protein for the animal that is highly digestible in the small intestine. Because microbial growth rates affect amino acid supply to the ruminant animal, it is important to maximize microbial protein synthesis in the rumen. Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein. The total amount of microbial protein flowing to the small intestine depends on nutrient availability and efficiency of utilization of these nutrients by ruminal bacteria.

Ruminal degradation of protein from dietary feed ingredients is one of the most important factors influencing intestinal amino acid supply to ruminants. Proteolysis determines the availability of ammonia nitrogen, amino acids, peptides and branched-chain volatile fatty acids, which influence microbial growth rates in the rumen. Rate and extent of ruminal proteolysis not only affect microbial protein synthesis but also the quantity and quality of undegraded dietary protein that reach the duodenum. Although microbial protein alone may be adequate for low producing ruminants, it can be

inadequate for supporting higher levels of growth, wool or milk production. As animal production increases, additional protein must be provided from dietary protein that leaves the rumen undegraded to meet the animal's protein requirement. The use of high rumen undegradable proteins (RUP) in diets fed to ruminants with high protein requirements can improve the amino acid supply to the animal provided that enough degradable protein is included in the diet to maximize microbial protein.

When formulating diets for ruminants, various criteria can be used to select the protein supplement including palatability, ruminal protein degradability, protein quality, intestinal absorption of amino acids, cost per unit of protein, availability and consistency of product, and impact on animal performance. Nutritional models for feeding protein to dairy cattle in the USA have evolved from basic crude protein (NRC, 1978) to more complex systems based on rumen degradable protein (RDP), RUP and intestinal digestion of RUP (NRC, 2001). The NRC (1989) recognized that intestinal digestion of protein supplements differs; however, empirical data were lacking and as a result, a constant value of 80% was used for all feeds. The main problem was the lack of reliable techniques for estimating intestinal digestion of proteins. With improved techniques, the NRC (2001) has assigned estimates of intestinal digestion to the RUP fraction of each feedstuff. These estimates were obtained from literature using the mobile bag technique and the three-step in situ/in vitro procedure of Calsamiglia and Stern (1995). Values used in the French Protein System were adopted for feeds with limited or no data.

Feeding proteins to ruminants that are resistant to microbial degradation in the rumen can provide a practical way to increase dietary protein and alter the amino acid profile of the protein reaching the small intestine for digestion and absorption. However, the effects of feeding high RUP on intestinal amino acid supply and animal performance have been inconsistent. Lack of response has been attributed to various factors including depression in ruminal microbial protein synthesis and factors related to quality of the dietary protein such as inadequate or overprotection of protein, reduced intestinal availability of amino acid or inherent amino acid limitations of the dietary protein. A considerable amount of variation among and within feeds in ruminal degradation and intestinal digestion of protein has been reported (Calsamiglia and Stern, 1995; Howie et al., 1996; Yoon et al., 1996). It is important that this variation be considered when determining the value of feeds as sources of protein for the ruminant animal.

Ruminal Nitrogen Metabolism

Ruminal Microbial Protein Synthesis The ultimate goal of proper rumen nutrition is to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves the supply of AA to the small intestine, but also decreases N losses. The rumen is a complex environment inhabited by different microbial species, with each species having different nutrient requirements and metabolism. Therefore, considering the nutrient requirements of ruminal microorganisms is crucial to understanding N metabolism in the rumen as well as the factors that may modify it (Bach et al., 2005).

Factors that affect microbial protein synthesis. Bacteria can utilize carbohydrates and proteins as energy sources. Carbohydrates are the main energy source for bacteria, although they can also be used as carbon skeletons for protein synthesis in combination with ammonia. Ruminal microbial protein synthesis depends on supply of adequate amounts and type of carbohydrate as an energy source for the synthesis of

peptide bonds. Readily fermentable carbohydrates such as starch or sugars are more effective than other sources such as cellulose in promoting microbial growth (Stern and Hoover, 1979). Several in vitro (Stern et al., 1978; Henning et al., 1991) and in vivo (Casper and Schingoethe, 1989; Cameron et al., 1991) studies demonstrated that infusions of increasing amounts of readily fermentable carbohydrate decreased ammonia-N concentrations due to improved N uptake by ruminal microbes. However, the optimum ratio of non-fibrous carbohydrates to ammonia-N has not yet been determined. In addition to the importance of the amounts of nutrient supply, the synchrony at which nutrients become available is also important. When rate of protein degradation exceeds the rate of carbohydrate fermentation, large quantities of N can be lost as ammonia, and conversely when the rate of carbohydrate fermentation exceeds protein degradation rate, microbial protein synthesis can decrease (Nocek and Russell, 1988). Whereas the concept of synchronous protein and energy supply has a solid theoretical basis, it is likely that in the complex ecosystem of mixed ruminal microorganisms when nutrient supply is synchronized for a specific subpopulation, it might not be synchronized for other populations. Therefore, average microbial efficiency remains fairly stable. Also, recycled N to the rumen may contribute to stabilize microbial growth even when N supply is not well synchronized (Bach et al., 2005).

Ruminal bacteria are classified as cellulolytic and amylolytic based on their preferential use of energy. Russell et al. (1992) proposed a simplified model to describe energy and protein requirements of microbial subpopulations. Microbes that degrade structural carbohydrates (cellulolytic) have low maintenance requirements, grow slowly, and use ammonia-N as their main N source, whereas microorganisms that degrade non-structural carbohydrates (amylolytic) have higher maintenance requirements, grow rapidly, and use ammonia, peptides, and amino acids (AA) as N sources (Russell et al., 1992). However, bacterial growth has been shown to increase with addition of AA and (or) peptides in cellulolytic and amylolytic bacteria (Maeng and Baldwin, 1976; Argyle and Baldwin, 1989; Kernick, 1991). Similarly, fiber digestion was reported to increase with the supply of AA (Griswold et al., 1996; Carro and Miller, 1999) and peptides (Cruz Soto et al., 1994) to pure cellulolytic bacteria. Atasoglu et al. (2001) demonstrated with pure cultures of cellulolytic bacteria, that the incorporation of ammonia-N into microbial cell-N decreased as the proportion of AA increased in the medium, suggesting that cellulolytic bacteria would use AA if available. Similar findings were reported with increasing concentrations of peptides, although Atasoglu et al. (2001) reported a greater preference of cellulolytic bacteria for incorporating AA-N compared with peptide-N into their cell-N. However, at typical ruminal peptide and AA concentrations, about 80% of the cell-N is derived from ammonia-N. Addition of branched-chain AA that will ferment to branched-chain VFA, and addition of peptides to ruminal fluid has increased fiber digestion, microbial protein production and growth efficiencies (Russell and Sniffen, 1984; Thomsen, 1985). The increase in microbial growth observed with addition of AA and (or) peptides, may be due to direct incorporation of AA into microbial protein and (or) to increased availability of carbon skeletons from AA deamination, which can be used for energy production or as carbon skeletons for new microbial AA.

Atasoglu et al. (2004) studied the fate of N and carbons from AA in ruminal mixed microorganisms and showed that several AA were synthesized by rumen microorganisms with greater difficulty than others. In general, it is believed that rumen microbes do not have an absolute requirement for any AA; however, Atasoglu et al. (2004) suggested that some AA may be limiting growth. They also proposed that lysine is a potential AA

limiting growth of rumen bacteria. Therefore, ensuring generous supplies of specific AA might result in greater microbial growth. Demeyer and Fievez (2004) suggested that low concentrations of peptides and AA could potentially limit microbial growth when feeding rations rich in starch with fine particle size, by inducing a low ruminal pH.

In addition to supply of carbohydrate and N sources, there are other nutritional factors such as sulfur supply, and non-nutritional factors such as ruminal pH and dilution rate that play important roles in microbial protein synthesis. Under practical conditions, efficiency of microbial protein synthesis (EMPS) remains relatively constant within a wide range of ruminal pH. To assess the potential effect of ruminal pH on EMPS, a meta-analysis as described by St-Pierre (2001) was conducted with literature providing in vivo data (n = 187) using a mixed model regression analysis. Figure 1a shows the results of this meta-analysis with the observations adjusted for the average study effect, and illustrates no relationship between ruminal pH and EMPS. These observations agree with in vitro studies (Hoover and Miller, 1992; de Veth and Kolver, 2001a,b; Calsamiglia et al., 2002). In contrast, total microbial N flow is negatively related to pH (Figure 1b). Low ruminal pH is the result of fermentation of large amounts of available OM. When the quantity of OM fermented increases, microbial protein synthesis also increases (Hoover and Stokes, 1991). As a result, the negative relationship between pH and bacterial N flow is a consequence of the increased supply of energy with highly fermentable rations resulting in lower ruminal pH.

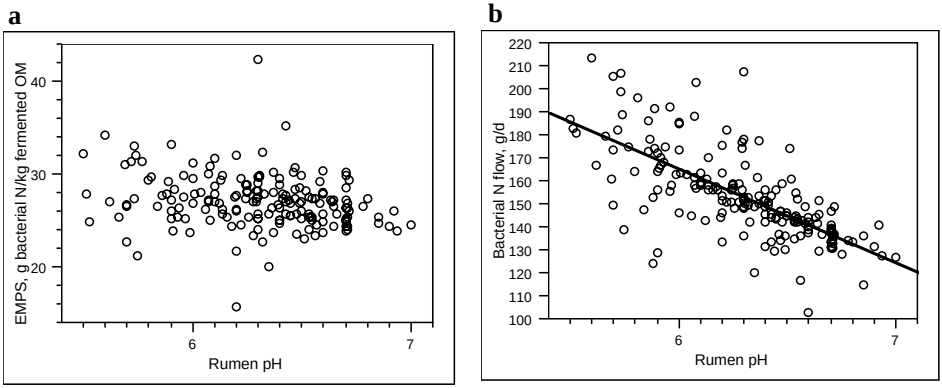

Figure 1. Relationship between rumen pH, and efficiency of microbial protein synthesis **(a)** or total bacterial N flow **(b)**, both adjusted for average study effect. Rumen pH and EMPS had no relationship (A). Bacterial N flow and rumen pH had a significant relationship (B): Y = 410 – 40.767pH; R^2 = 0.50, RMSE=13.49, $P < 0.001$ (adapted from Bach et al., 2005).

Changes in ruminal liquid and solids dilution rates can also impact ruminal fermentation and microbial growth (Isaacson et al., 1975; Russell et al., 1992). Ruminal dilution rates depend on various factors including level of intake, proportion of forage in the ration and particle size of the ration. In general, in vitro studies with pure or mixed cultures of rumen bacteria indicate a greater synthesis and EMPS with increases in liquid dilution rate (Isaacson et al., 1975), solids dilution rate (Hoover et al., 1982; Schadt et al., 1999) or both (Crawford et al., 1980; Shriver et al., 1986). The increase in microbial protein synthesis and EMPS that is obtained with high dilution rates has been attributed to selection of microbial species with greater rates of growth, a higher proportion of the microbial population in the exponential phase of growth, and a dilution of the maintenance requirements of microbes (Bach et al., 2005). In addition, high dilution rates

are associated with shorter retention times in the rumen, which reduce bacterial lysis, and bacterial predation by protozoa (Stern and Hoover, 1979; Hoover and Miller, 1992; Firkins et al., 1992).

Importance of microbial protein to ruminants. The theoretical contribution of microbial protein to the total protein requirement of the lactating dairy cow, calculated at three efficiencies of microbial protein synthesis, is presented in Table 1. Contribution of microbial protein to total protein requirement was determined using NRC values (2001) for a 680-kg lactating dairy cow producing 25, 35, or 45 kg/d of 4% FCM. At these three milk yields, microbial protein would contribute 51, 49 and 48%, respectively, of the total protein required by the cow when microbial synthesis in the rumen is 30 g of N/kg of organic matter truly digested (OMTD). When milk yield is 45 kg/d, the contribution of microbial protein to total protein required by the cow would increase from 32 to 63% as efficiency of microbial protein synthesis increases from 20 to 40 g of N/kg of OMTD. Stern and Hoover (1979) reviewed the literature and reported that approximately 30 g of N were synthesized per kilogram of OMD in the rumen; values ranged from 10 to 50 g. Efficiencies of microbial protein synthesis used in Table 1 fall into the range of reported values; therefore, calculated contributions of microbial protein clearly depicted the importance of optimizing microbial protein synthesis in the rumen of high producing dairy cows. In addition, these calculations demonstrate that, as milk yield increases, a substantial quantity of RUP from protein supplements must leave the rumen to meet the protein requirement of the cow. Contribution of microbial protein to growing beef cattle calculated at three efficiencies of microbial protein synthesis is presented in Table 2. Contribution of microbial protein to total protein requirement was determined using NRC values (1996) for a 250 kg growing beef animal with average daily gains (ADG) of 300, 1200 and 1700 g/day. At these three ADG, microbial protein would contribute 95, 57 and 40%, respectively, of the total protein required by the animal when microbial synthesis in the rumen is 25 g of N/kg of organic matter truly digested (OMTD). Lower efficiencies of microbial protein synthesis, ranging from 20 to 30 g N/kg OMTD, were used because beef cattle are generally less efficient, most probably due to a high population of amylolytic bacteria. Microbial protein contribution to total protein requirement demonstrated a more dramatic difference in beef cattle compared with dairy cattle. This response is probably due to the lack of increase in dry matter intake in beef cattle as ADG increases. However, in both situations, it is clear that substantial quantities of RUP from protein supplements must be incorporated into the diet of high producing ruminants.

Table 1. Contribution of microbial protein to total protein requirement of lactating dairy cattle.[a]

Microbial synthesis, g of N/kg of OM truly digested [b]	Contribution of microbial protein when daily milk production (kg) equals:		
	25 (55 lb)	35 (77 lb)	45 (100 lb)
	---------- % ----------		
20	34	33	32
30	51	49	48
40	68	65	63

[a] Requirements determined using NRC (2001).
[b] Assumed that 55% of OM intake is truly digested in the rumen.

Table 2. Contribution of microbial protein to total protein requirement of beef cattle (250 kg).[a]

Microbial synthesis, g of N/kg of OM truly digested [b]	Contribution of microbial protein when average daily gain (g/d) equals:		
	300 (0.64 lb)	1200 (2.7 lb)	1700 (3.75 lb)
	---------- % ----------		
20	76	46	30
25	95	57	40
30	114	69	48

[a] Requirements determined using NRC (1996).
[b] Assumed that 55% of OM intake is truly digested in the rumen.

Ruminal Protein Degradation Dietary protein degradation in the rumen involves attachment of bacteria to feed particles, followed by activity of cell-bound microbial proteases (Brock et al., 1982). Approximately 70 to 80% of ruminal microorganisms attach to undigested feed particles in the rumen (Craig et al., 1987) and 30 to 50% of the attached microbes have proteolytic activity (Prins et al., 1983). A large number of different microbial species form a consortium that attaches to a feed particle, acting symbiotically to degrade and ferment nutrients, including protein. Products resulting from this process are peptides and amino acids. Because the number of different bonds within a single protein is large, the synergistic action of different proteases is necessary for complete protein degradation (Wallace et al., 1997). The rate and extent at which protein degradation occurs will depend on proteolytic activity of the ruminal microflora and the type of protein (susceptibility and accessibility of peptide bonds).

Peptides and AA resulting from the extracellular rumen proteolytic activity are transported inside microbial cells (Figure 2). Peptides can be degraded further by peptidases into AA and the latter can be incorporated into microbial protein or further deaminated to VFA, CO_2 and ammonia (Tamminga, 1979). The fate of absorbed peptides and AA once inside the microbial cell will depend on the availability of energy in the form of carbohydrates. If energy is available, AA will be transaminated or used directly for microbial protein synthesis. However, if energy is limiting, AA will be deaminated and their carbon skeleton fermented into VFA. Some ruminal bacteria lack mechanisms of AA transport from the cytoplasm to the extra-cellular environment and AA absorbed in excess must be excreted from the cytoplasm as ammonia (Tamminga, 1979).

Estimates of rumen undegradable protein. Under ideal circumstances, the ruminant nutritionist would prefer to use "book values" for RUP of various protein supplements, such as those found in the NRC (2001) publication (Figure 3). However, ruminal protein degradation is a complex process influenced by various factors such as solubility, protein structure, microbial proteolytic activity, microbial access to the protein and ruminal retention time of dietary protein. There is an inherent variability in reported values for RUP of feeds that is related to conditions under which experiments are conducted; however, part of the variation can also be attributed to differences in quality of protein supplements.

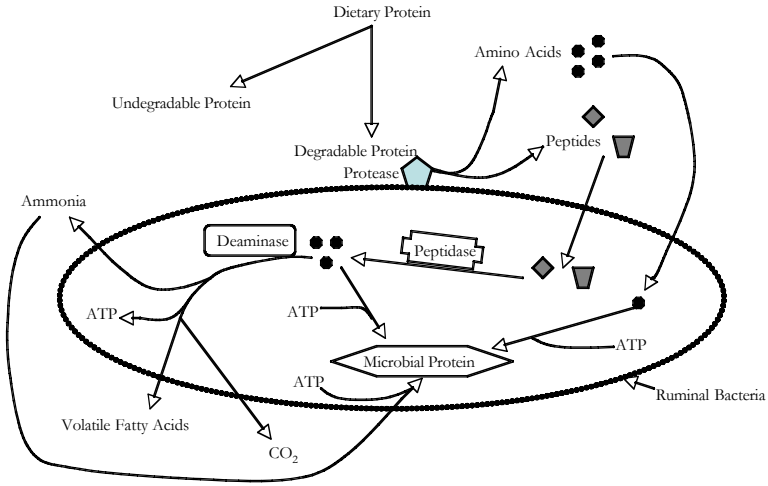

Figure 2. Proteolysis in the rumen and fate of fermentation end-products (adapted from Bach et al., 2005).

Rumen undegradable protein values for various protein supplements estimated at the University of Minnesota using the in situ Dacron polyester bag technique are shown in Table 3. Average RUP content of feeds in Table 3 is fairly similar to NRC (2001) values, mainly because similar techniques were used to estimate RUP. The large standard deviation and wide range for RUP values shown in Table 3 indicate a problem with quality control. Variation in RUP content within protein supplements can be attributed to processing method and/or raw material used. The RUP value can be altered by various factors including time before processing, humidity, temperature, heating time, etc. Determination of optimal processing conditions and routine control of these conditions can reduce variation and improve quality of protein supplements.

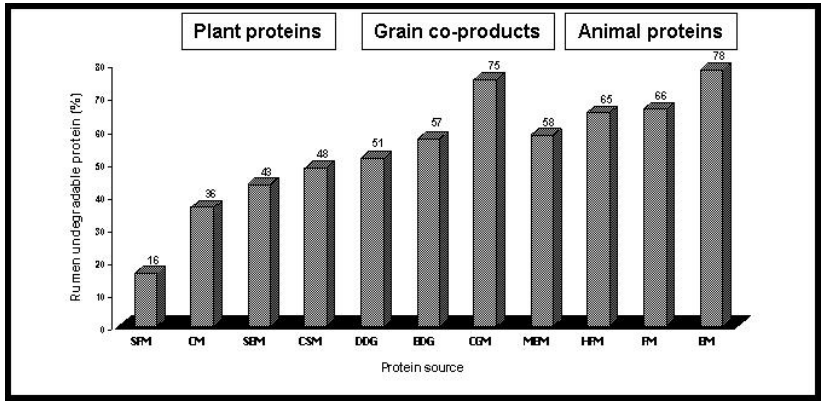

Figure 3. Rumen undegradable protein (%) of various protein sources including: sunflower meal (SFM), canola meal (CM), soybean meal (SBM), cottonseed meal (CSM), distillers dried grains with solubles (DDG), brewers dried grains (BDG), corn gluten meal (CGM), meat and bone meal (MBM), hydrolyzed feather meal (HFM), fish meal (FM) and blood meal (BM) adapted from NRC (2001).

Factors that affect ruminal protein degradation. Bach et al. (2005) indicated that the most important factors affecting microbial protein degradation in the rumen include the type of protein, interactions with other nutrients (mainly carbohydrate within the same feedstuff and within the rumen contents) and the predominant microbial population (dependent on the type of ration, ruminal passage rate and ruminal pH).

Table 3. Rumen undegradable protein (RUP), intestinal CP digestion (ID), and intestinally absorbable dietary protein (IADP) of various protein supplements [a].

Protein source	n	RUP (% of CP) Avg ± SD (range)	ID (% of RUP) Avg ± SD (range)	IADP[b] Avg ± SD (range)
Plant proteins				
Cottonseed meal, solvent	1	46	71	33
Cottonseed meal, mechanical	1	55	80	43
Soybean meal	5	25 ± 3 (22-29)	90 ± 4 (86-93)	22 ± 2 (20-25)
Soybean meal, expeller	6	47 ± 6 (38-53)	93 ± 7 (83-100)	44 ± 3 (38-53)
Soybean meal, non-enzymatically browned	6	66 ± 8 (57-77)	88 ± 4 (82-92)	58 ± 7 (49-67)
Grain by-products				
Brewers grains, dried	5	57 ± 5 (50-63)	77 ± 2 (73-79)	44 ± 5 (37-49)
Corn gluten meal	2	83 ± 2 (82-85)	89 ± 4 (86-91)	74 ± 5[c] (70-77)
Distillers grains, dried	5	56 ± 8 (47-64)	81 ± 5 (72-85)	46 ± 8 (36-53)
Animal proteins				
Blood meal, batch-dried	12	88 ± 6 (78-98)	63 ± 17 (29-86)	55 ± 14 (25-75)
Blood meal, ring-dried	10	83 ± 4 (76-89)	81 ± 6 (72-90)	67 ± 7 (58-76)
Feather meal, hydrolyzed	12	76 ± 11 (50-88)	67 ± 6 (58-75)	51 ± 9 (36-64)
Fish meal, Menhaden	13	65 ± 4 (59-73)	80 ± 5 (73-88)	52 ± 4 (43-57)
Meat and bone meal	11	59 ± 13 (40-88)	55 ± 10 (41-70)	33 ± 10 (21-56)

[a] Adapted from Stern et al. (1997).
[b] IADP (% of CP) = RUP (% of CP) x ID (% of RUP).
[c] Using in vivo estimate of RUP of 57% (Stern et al., 1983b), IADP = 51%.

Type of protein. Solubility of proteins is a key factor determining their susceptibility to microbial proteases and thus their degradability. For example, prolamins and glutelins are insoluble and slowly degraded, while globulins are soluble and highly degradable in the rumen (Romagnolo et al., 1994). However, the structure of the protein

is also important. Some albumins are soluble but contain disulfide bonds, making them slowly degradable in the rumen, illustrating that factors other than solubility affect rumen degradability of proteins. The presence of bonds within and between protein chains play an important role in determining protein degradation. For example, the acidic subunit glycinin (with strong disulfide bonds), the basic glycinin, and several Leu- containing peptides in the N-terminal group in SBM are fairly resistant to degradation (Schwingel and Bates, 1996). In addition, specific peptide bonds are more resistant to ruminal degradation than others. For example, dipeptides formed of Lys-Pro are hydrolyzed in the rumen 5-fold slower than the dipeptide Lys-Ala and dipeptides formed of Pro-Met are degraded 2.5-fold slower than dipeptides formed of Met-Ala (Yang and Russell, 1992). It has also been suggested that peptidases and deaminases may be regulated by end-product inhibition processes. Velle et al. (1997) infused increasing quantities (75, 150, 300, and 600 mmol) of different AA in the rumen, and found that AA degradation decreased as the quantity infused increased. Degradation of Met and His was specifically affected, which is consistent with observations by Volden et al. (1998) and Bach and Stern (1999).

Ruminal dilution rate. Protein degradation is inversely related to passage rate through the rumen (Ørskov and McDonald, 1979). The NRC (2001) developed equations of passage rate for wet and dry forages and concentrates based on DMI, fiber content, and forage to concentrate ratio of the diet. According to NRC (2001), digesta passage rate of a cow consuming 18 kg DM of a 70:30 forage to concentrate ration would increase from 0.049 to 0.057 h^{-1} for wet forages, from 0.040 to 0.046 h^{-1} for dry forages, and from 0.056 to 0.068 h^{-1} for concentrates if the same cow would consume 26 kg DM of a 40:60 forage to concentrate ration. With a standard ryegrass silage, alfalfa hay and soybean meal, this increase in passage rate would result in a reduction in protein degradation of 1.2, 2.1 and 3.5 percentage units, respectively. These changes are small, and represent only a modest increase in the flow of RUP supply to the small intestine.

Ruminal pH and substrate. The optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0 according to Kopecny and Wallace (1982); however, protein degradation is reduced at the lower end of the ruminal pH environment. Cardozo et al. (2000; 2002) conducted two dual flow continuous culture fermentation studies comparing high forage vs high concentrate rations at pH ranging from 4.9 to 7.0, and demonstrated that protein degradation was reduced as pH decreased with both types of rations. Although amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Siddons and Paradine, 1981; Wallace et al., 1997), protein degradation in the studies of Cardozo et al. (2000, 2002) was consistently lower when high-concentrate rations provided substrate to microbes, regardless of pH. In addition, Lana et al. (1998) reported that a decrease in ruminal pH from 6.5 to 5.7, reduced ruminal ammonia concentration only when bacteria were obtained from cattle fed a 100% forage ration, whereas bacteria from cattle fed 90% concentrate had lower ammonia-N concentration regardless of pH. These results indicate that protein degradation is affected by pH and type of ration, which may dictate the predominant type of microbial population present in the rumen. Devant et al. (2001) incubated SBM and heat-processed SBM in the rumen of dairy cattle fed a 60:40 forage to concentrate ration or in the rumen of beef cattle fed a 10:90 forage to concentrate ration using the in situ technique. Results demonstrated that protein degradation was lower with the beef-type ration, in spite of the fact that pH was above 6.0 in both types of animals, illustrating that the reduction of protein degradation is not due only to a pH effect, but is also related to type of substrate being fermented, or the predominant microbial population induced by a particular ration.

Nutrient interactions. The combined effect of pH and substrate on ruminal protein degradation may be explained by the resulting predominant microbial population. It is obvious that protein degradation occurs by the action of proteolytic enzymes, but there is evidence that supports the importance of other enzymatic activities on the degradation of protein. Assoumani et al. (1992) demonstrated that starch interferes with protein degradation. They noted that the addition of amylase increased total ruminal protein degradation of cereal grains between 6 and 20 percentage units. Positive effects of amylases on protein degradation were also reported by others (Aufrère and Cartailler, 1988; Tománková and Kopecny, 1995). Debroas and Blanchart (1993) found that NDF-bound protein was degraded by proteolytic bacteria only after microbial depolymerization of cellulose began. Kohn and Allen (1995) also reported an increase in protein degradation from 42.4 to 53.1% when cellulases were added to an in vitro proteolytic digestion. Similar results were obtained by Abdelgadir et al. (1996) when forages were pretreated with cellulase before undergoing an in vitro digestion with *S. griseus* protease. Many plant proteins are trapped in a fiber matrix that needs to be degraded before proteases can gain access to proteins for degradation. Therefore, it appears that protein degradation in the rumen requires the presence of several proteolytic and non-proteolytic enzymes, and the combination of several microbial and enzymatic activities are required for maximum protein degradation. This fact is clearly illustrated in a study by Endres and Stern (1993) who observed a reduction in CP and NDF digestion when pH decreased from 6.3 to 5.9. Proteolytic bacteria counts were not affected by pH, but cellulolytic bacteria counts were reduced by about 50% (Table 4). It is likely that with a high-concentrate ration, even if pH is high, starch-degrading bacteria predominate and fiber digestion is limited by the reduced number of cellulolytic bacteria, reducing the degradation of protein (Mould and Ørskov, 1983). Therefore, the effect of pH and (or) the substrate being fermented may affect the predominant microbial population and modify protein degradation due to interactions among nutrients. It could be hypothesized that reduction in cellullolytic bacteria as a consequence of low pH, leads to a reduction in fiber degradation, reducing access of proteolytic bacteria to proteins, indirectly diminishing protein degradation.

Table 4. Effect of pH on bacterial populations in continuous culture fermenters

		Protein supplement[1]			
Item	pH	SSS	SSN	SNN	NNN
Bacteria, cells x 10^8/ml					
Amylolytic	6.3	6.2	9.3	8.1	6.5
	5.9	17.8	6.9	9.8	8.3
Proteolytic	6.3	31.6	20.4	14.1	17.0
	5.9	12.9	20.4	12.3	14.4
Cellulolytic[2]	6.3	1.6	1.2	1.5	1.4
	5.9	0.4	0.6	0.7	0.6
Digestion, % of intake					
Crude protein[2]	6.3	52.7	50.3	47.2	44.7
	5.9	39.5	38.9	38.1	41.9
NDF[2]	6.3	35.6	37.3	44.6	41.4
	5.9	29.5	31.8	29.9	38.3

[1] Adapted from Endres and Stern (1993).
[2] Approximately 50% of dietary CP was in form of soybean meal (SBM). SSS: SBM 100%; SSN: 67% SBM and 33% nonezymatically browned SBM (NSBM); SNN: 33% SBM and 67% NSBM; NNN: 100% NSBM.
[3] pH effect ($P < 0.01$).

Intestinal Protein Digestion

Methods for Measuring Intestinal Protein Digestion The total amount of protein available for absorption from the small intestine depends on the flow of microbial and dietary protein to the duodenum and their respective intestinal digestibilities. Digestion of protein that leaves the rumen starts in the abomasum with acid-pepsin digestion and is completed in the small intestine with pancreatic and intestinal proteases. The NRC (1989) recognized that intestinal digestion of protein supplements may differ; however, empirical data were lacking, and as a result, a constant value of 80% was used for all feeds. The main problem was the lack of reliable techniques for estimating intestinal digestion of proteins (Stern et al., 1997). In vivo estimation of intestinal protein digestion involves expensive and labor-intensive experiments and requires the use of surgically prepared animals. Apparent digestion of protein is calculated as the disappearance of CP or amino acids between the duodenum and ileum, which is subject to considerable error associated with digesta sampling, use of digesta flow rate markers, and inherent animal variation. Therefore, several alternative procedures for estimation of intestinal protein digestion in ruminants have been developed. An in situ mobile bag technique was devised to determine intestinal protein digestion in ruminants (Hvelplund, 1985). A summary of intestinal protein digestion data adapted from Stern and Bach (1996) for various feed ingredients derived from 21 references using the mobile bag technique is presented in Table 5. Mean values ranged from 50.0 to 98.2% for lupine seeds and wheat flour middling, respectively.

Table 5. Intestinal digestion of crude protein (percentage) in ruminants using the mobile bag technique. [a]

Feed	n	Mean	SE
Plant Proteins			
Brewers Grains	1	76.6	
Canola seed, whole	3	84.6	13.3
Canola seed, whole, heated	4	75.4	5.7
Canola meal	3	75.0	5.9
Canola meal, heated	3	80.9	2.2
Coconut, cake	5	89.6	1.6
Coconut, expeller	1	93.8	
Coconut, mechanically extracted	2	88.3	0.01
Cotton seed	2	58.5	8.2
Cotton seed, meal	5	92.9	2.0
Corn gluten, meal	4	96.2	1.9
Corn gluten, feed	1	80.9	
Corn, distiller	2	79.4	15.6
Groundnut meal	1	94.1	
Horse beans, whole	2	88.3	2.5
Horse beans, whole extruded	1	90.7	
Linseed meal	4	85.3	2.4
Lupine seed	1	50.0	
Lupine seed, extruded	2	92.4	2.3
Palm kernel	7	87.8	1.7
Peanut meal	1	93.2	
Peanut, mechanically extruded	1	95.9	
Peas	3	87.9	6.5
Peas, extruded	1	98.0	
Rapeseed meal	12	70.9	4.7
Rapeseed meal, heated	2	78.1	5.1

Table 5.
(Continued)

Feed	n	Mean	SE
Plant Proteins (continued)			
Soybean Hulls	1	72.4	
Sesame meal	2	97.3	0.2
Soybean meal	19	96.4	4.7
Soybean meal, heated	1	96.9	
Soybean meal, formaldehyde	3	66.3	15.4
Soybeans, extruded	2	96.0	4.0
Soybeans, flaked	1	87.9	
Sunflower, meal	4	87.8	3.1
Wheat bran	1	70.9	
Wheat flour middling	1	98.2	
Wheat middling	1	78.2	
Animal Proteins			
Blood meal	3	77.3	10.7
Feather meal, hydrolyzed	1	68.3	
Fish Meal	11	83.9	7.4
Meat and bone meal	7	67.4	6.5
Forages			
Alfalfa	2	75.7	5.2
Alfalfa, hay	4	72.5	2.7
Alfalfa, silage	1	80.6	
Alfalfa, dehydrated	3	71.9	4.9
Barley, whole crop	1	88.5	
Barley, silage, whole crop	2	78.0	9.7
Barley, straw	2	61.7	7.4
Beet top silage	1	70.0	
Clover, red	1	86.0	
Corn Silage	2	67.9	19.6
Grass	5	74.3	3.0
Grass silage	3	87.1	2.9
Grass haylage	1	55.4	
Grass hay	2	82.6	3.7
Horse bean whole crop silage	1	68.3	
Pea, straw	2	73.5	13.9
Peas hole crop silage	1	61.9	
Peas, whole crop	1	94.6	
Wheat straw	1	61.5	
Grains			
Barley	5	92.3	1.3
Corn	5	90.7	1.3
Oats	3	76.9	6.0
Rye	3	88.9	2.7
Sorghum	1	80.5	
Wheat	4	90.8	2.4

[a] Adapted from Stern and Bach (1996).

A three-step procedure that combines in situ and in vitro methods was developed to estimate intestinal digestion of proteins in ruminants (Calsamiglia and Stern, 1995) with some values shown in Table 3. Mean values ranged from 55 to 93% for meat and bone meal and expeller processed soybean meal, respectively. Using these improved techniques, the NRC (2001) has assigned estimates of intestinal digestion to the RUP fraction of each feedstuff. More recently, McNiven et al. (2002) modified the three-step

procedure by replacing the in situ step with an in vitro method involving a pre-incubation with a protease enzyme followed by incubations in pepsin and pancreatin. They concluded that their method could predict intestinal protein digestion of heat damaged soybeans. However, recent evaluation of this modified procedure (Stern et. al., *unpublished data*) demonstrated that estimates were not accurate among a wide variety of feedstuffs and further research is warranted to evaluate the modified procedure.

Three-step in situ/in vitro procedure. The three-step procedure of Calsamiglia and Stern (1995) was developed to: 1) closely simulate physiological conditions of ruminants, including potential effects of ruminal fermentation; 2) be rapid, reliable, and inexpensive; 3) be applicable to a wide variety of protein supplements; and 4) accurately reflect differences in protein digestion. Dacron bags containing feed samples were suspended in the rumen for 16 h. Residue was incubated for 1 h in a 0.1 *N* HCl solution containing 1 g/L of pepsin. After incubation, pH was neutralized with 1 *N* NaOH and a pH 7.8 phosphate buffer containing 3 g/L of pancreatin was added to the solution followed by incubation at 38C. After a 24-h incubation, a 100% (wt/vol) trichloroacetic acid solution was added to precipitate undigested proteins. Preincubation of samples in the rumen did not affect pepsin-pancreatin digestion of residual CP in soybean meal, corn gluten meal, and blood meal, but decreased pepsin-pancreatin digestion of residual CP in hydrolyzed feather meal, fish meal, and meat and bone meal (80 vs 70, 88 vs 81, and 82 vs 56%, respectively, for nonruminal vs ruminal preincubation). Pepsin digestion before pancreatin digestion increased CP digestion of all proteins tested by a mean of 23 percentage units. The pancreatin digestion step was validated using 34 duodenal samples from which small intestinal CP digestion was determined in vivo. The regression equation of in vivo estimates on pancreatin digestion had a coefficient of determination of 0.91. Intestinal CP digestion of solvent-extracted soybean meal and non-enzymatically browned soybean meal was similar within method using either the in situ mobile bag technique or the three-step procedure (Mansfield and Stern, *unpublished data*). However, there was a substantial difference between the two procedures for estimating intestinal digestion. Estimates differed by almost 22 percentage units, 99.4 vs 77.8% between the in situ mobile bag technique and the three-step procedure, respectively, with almost complete disappearance of CP from the mobile-bags. Differences between methods may be a result of protein digestion that occurs in the large intestine. Hvelplund (1985) indicated that 50% of soybean meal protein that left the ileum was digested in the large intestine and observed a significant site of collection (ileum vs feces) x feed (soybean meal vs canola meal) interaction using the in situ mobile bag technique .

Application of the three-step procedure. Estimates of intestinal protein digestion of different protein supplements using the three-step procedure (Table 3) indicate that large variation exists among and within protein supplements. Low intestinal protein digestion was found for meat and bone meal (55%), batch-dried blood meal (63%), and hydrolyzed feather meal (67%), whereas all remaining protein supplements averaged greater than 70%. To evaluate the effects of different processing procedures on intestinal protein digestion of various animal proteins, Howie et al. (1996) obtained seven samples each of several animal byproducts. Estimates of intestinal digestion of RUP, determined using the three-step procedure, ranged from 40.9 to 70.1%($\surd$ = 56.0% ± 4.0), 59.2 to 75.2% ($\surd$ = 65.3% ± 2.1), 72.0 to 90.3% ($\surd$ = 79.6% ± 2.5) and 28.8 to 79.2% ($\surd$ = 61.4% ± 6.8) for meat and bone meal, hydrolyzed feather meal, ring-dried blood meal, and batch-dried blood meal, respectively. Yoon et al. (1996) used 18 Menhaden fish

meal samples from various processing plants to evaluate the effects of processing on intestinal digestion of protein and noted values ranging from 72.8 to 86.4% ($\surd$ = 77.7% ± 3.5). These results demonstrate that considerable variation exists in intestinal digestion of protein among and within different protein sources, and this variation can possibly be influenced by source of raw material, quality of raw material (storage time and temperature), drying conditions, and various other factors. Recently, Stern et al. (2005) used the in situ Dacron polyester bag technique and the three-step procedure to evaluate various processing methods of soybean meal. Rumen undegradable protein (Figure 4a) ranged from 23.2 to 68.3% for solvent-extracted soybean meal and non-enzymatically browned soybean meal, respectively. Intestinal CP digestion (Figure 4b) ranged from 57.7 % to 83.8% for non-enzymatically browned soybean meal and mechanical extracted soybean meal with fresh soygums, respectively. Ruiz Moreno and Stern (2005, *unpublished data*) used the three-step procedure to evaluate consistency in processing procedures. Intestinal protein digestion of three carloads for each of four ruminal protected soybean products [solvent extracted SBM (SE) heat treated (SOLH); SE SBM nonenzymatically browned (SOLNEB); mechanical-extracted (ME) SBM #1 with fresh soy gums (MEC1G); and ME SBM #2 (MEC2)] and three carloads of three sources of distillers dried grains with solubles (DDG-A, DDG-B and DDG-C) were evaluated. Means and standard deviation (sd) for SOLH, SOLNEB, MEC1G and MEC2 were 70.8, ± 2.1; 68.2, ± 1.0; 83.0, ± 1.55; and 81.5, ± 2.5, respectively (Figure 5a). Variation in processing of each protected soybean product was not great, however the mean intestinal protein digestion was fairly large ranging from 68.2 to 83.0% among the four soybean products. Means and standard deviations for DDG-A, DDG-B and DDG-C were 71.9, ± 3.2; 72.0, ± 0.5; and 80.6, ± 4.2, respectively (Figure 5b). Variation in processing was low for DDG-B (range of 71.7 to 72.6%), but there was a fairly large variation in processing of DDG-A (range of 69.6 to 75.5%), and DDG-C (range of 77.2 to 72.6%). Another noteworthy point is that the mean intestinal protein digestion of the three distillers dried grains products ranged from 71.9 to 80.6%. From these types of observations, it appears that the three-step procedure can be a useful method of evaluating quality control of protien within and among processing procedures.

Intestinally Absorbable Dietary Protein

Importance of estimating intestinally absorbable dietary protein. Intestinally absorbable dietary protein (IADP) is defined as the amount of protein from a specific feed that is available for absorption in the small intestine and is calculated as RUP multiplied by intestinal protein digestion. The IADP value provides an index of the quality of protein supplements as sources of RUP for ruminants. Because there is a large amount of variation associated with measurements of ruminal degradability and intestinal digestibility among and within protein supplements, and there seems to be no correlation between RUP and intestinal protein digestibility (Yoon et al., 1996), the use of published values for RUP and intestinal digestion to calculate IADP is not adequate. Therefore, only data from experiments where RUP and intestinal protein digestion were measured simultaneously were included in Table 3. Results indicate that corn gluten meal (74%) and ring-dried blood meal (67%) provided the largest amount of IADP, followed by non-enzymatically browned soybean meal, batch-dried blood meal, Menhaden fish meal and hydrolyzed feather meal (51 to 58%). Because corn gluten meal tends to stick together when wet, exposure of the surface area in Dacron polyester bags decreases as previously described (Stern et al., 1983a). Using an RUP value of 57%, determined in vivo by Stern et al. (1983b), instead of 83%, as determined in situ, and assuming that intestinal

digestion is 89%, the IADP for corn gluten meal would be approximately 51%. Using IADP to evaluate the various processing methods of soybean meal (Figure 4c), it should be noted that non-enzymatically browned soybean meal had an IADP of 39.4%, similar to mechanical extracted soybean meal with fresh soygums at 41.3%, emphasizing the importance of accounting for both RUP and intestinal digestion of protein (Stern et al., 2005).

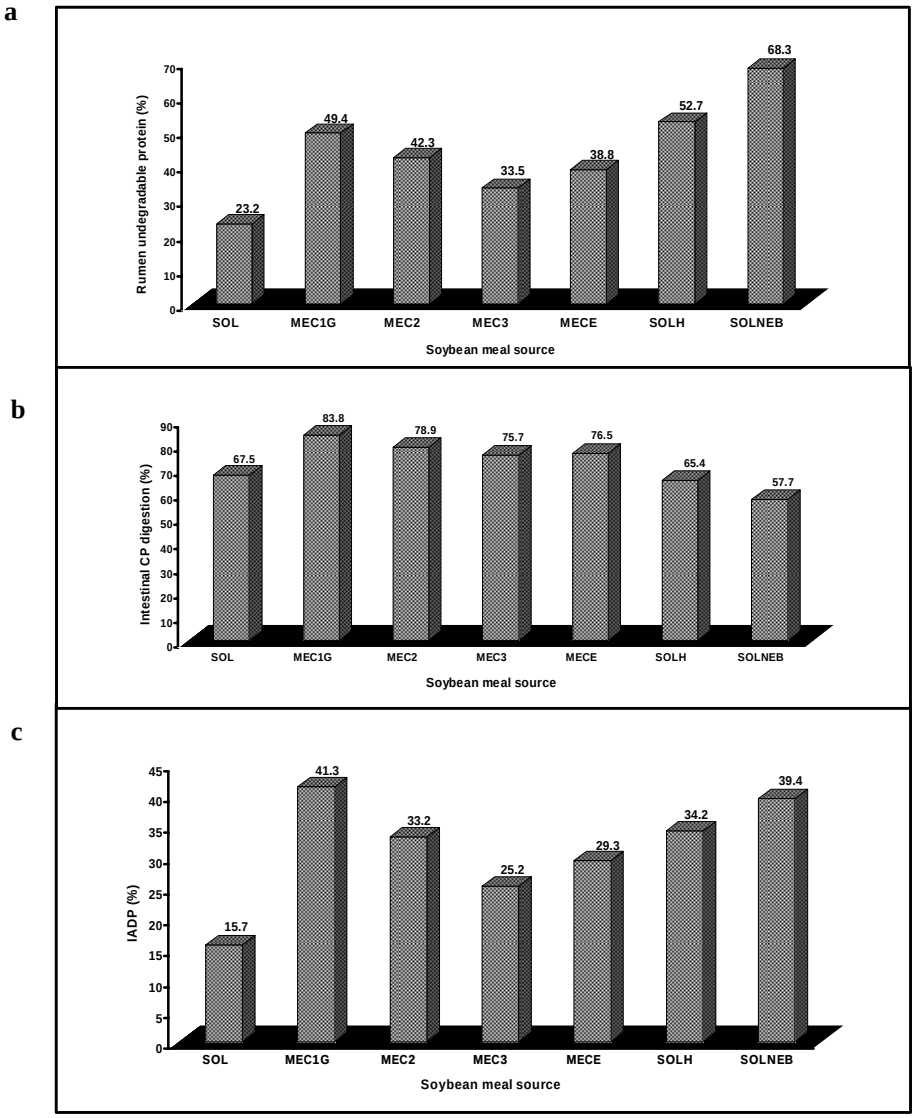

Figure 4. Rumen undegradable protein **(a)**, intestinal CP digestion **(b)** and intestinally absorbable dietary protein **(c)** of solvent-extracted (SE) SBM (SOL), mechanical-extracted (ME) SBM #1 with fresh soy gums (MEC1G), ME SBM #2 (MEC2), ME SBM #3 (MEC3), ME SBM extruded (MECE), SE SBM heat treated (SOLH), SE SBM nonenzymatically browned (SOLNEB) adapted from Stern et al. (2005).

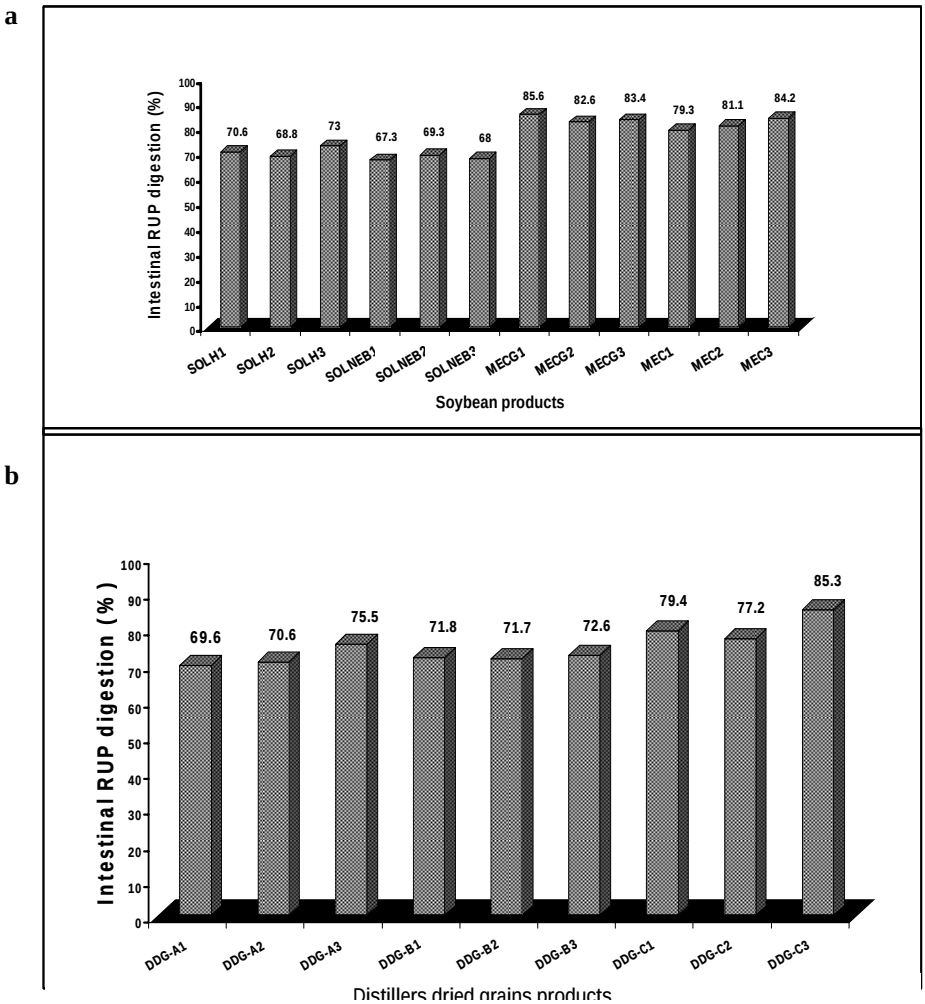

Figure 5. Intestinal protein digestion of three different carloads of (**a**) four different processed soybean products, SE SBM heat treated (SOLH); SE SBM nonenzymatically browned (SOLNEB); mechanical-extracted (ME) SBM #1 with fresh soy gums (MEC1G); and ME SBM #2 (MEC2); and (**b**) distillers dried grains with solubles from three different manufacturers (DDG-A; DDG-B; DDG-C).

Economical considerations. Determination of IADP and analysis of the amino acid profile of protein supplements make it possible to calculate the cost per kg of IADP or intestinally absorbable individual amino acids provided by each supplement. Table 6 was derived using data from Table 3, standard amino acid composition of feeds and market prices of feeds adapted from Feedstuffs. Results were calculated assuming that individual amino acids had similar ruminal degradability and intestinal digestibility as the crude protein of the original feed. In terms of IADP ($/kg), HFM (0.43) and CGM (0.54) were the cheapest sources and SBM the most expensive source (1.89) of IADP. Ring-dried and batch-dried BM were the cheapest ($/100 g) sources of lysine and CGM was

the cheapest source of methionine. However, it should be emphasized that there is potentially large differences associated with the cost of each protein due to variation in quality within supplements. The highest variation in cost of IADP ($/kg) was for batch-dried BM (0.73 to 2.20) and MBM (0.64 to 2.07). Although batch-dried BM on average was the second cheapest source of lysine, it is important to emphasize that it ranged from 0.86 to 2.58 for absorbable lysine ($/100 g) due to inconsistency in processing. Selection of the best protein supplement is highly dependent on the quality of the protein. In the future, cost per kg of IADP or absorbable amino acids can be used as a guideline for selecting the appropriate protein supplement to be used in diets fed to ruminants as market prices of feedstuffs fluctuate.

Table 6. Cost ($/kg) of intestinally absorbable dietary protein (IADP), absorbable lysine (A-Lys) and absorbable methionine (A-Met) supplied by various protein supplements.

Protein supplement	IADP[a] $/kg (Range)	A-Lys[b] $/100 g (Range)	A-Met[c] $/100 g (Range)
Feather meal, hydrolyzed	0.43 (0.30-0.66)	1.57 (1.10-2.44)	7.10 (4.98-1.01)
Corn gluten meal	0.54 (0.51-0.56)	3.42 (3.24-3.56)	2.24 (2.12-2.34)
Soybean meal, non-enzymatically browned	0.80 (0.70-0.95)	1.36 (1.18-1.61)	6.17 (5.35-7.31)
Blood meal, ring-dried	0.81 (0.72-0.95)	0.96 (0.85-1.11)	7.15 (6.34-8.31)
Brewers grains, dried	0.87 (0.78-1.03)	2.90 (2.60-3.45)	4.35 (3.91-5.17)
Soybean meal, expeller	0.95 (0.82-1.14)	1.61 (1.38-1.93)	7.32 (6.27-8.75)
Cottonseed meal, mechanical	0.99	2.43	5.89
Distillers grains, dried	1.01 (0.80-1.41)	4.04 (3.21-5.63)	7.21 (5.73-10.05)
Blood meal, batch-dried	1.01 (0.73-2.20)	1.18 (0.86-2.58)	8.82 (6.42-19.27)
Meat and bone meal	1.19 (0.64-2.07)	2.44 (1.31-4.24)	9.39 (5.02-16.28)
Cottonseed meal, solvent	1.19	2.92	7.08
Fish meal, Menhaden	1.36 (1.25-1.66)	1.81 (1.66-2.19)	4.93 (4.51-5.98)
Soybean meal	1.89 (1.67-2.09)	3.20 (2.83-3.54)	14.54 (12.85-16.07)

Conclusions

This review has demonstrated that 1) total microbial N flow is negatively correlated with ruminal pH but there is no relationship between ruminal pH and efficiency of microbial protein synthesis; 2) in vitro studies with pure or mixed cultures of ruminal bacteria generally indicate a greater synthesis and efficiency of microbial protein synthesis with increases in liquid and solids dilution rates; 3) after maximizing microbial protein synthesis, it is clear that substantial quantities of RUP from protein supplements must be incorporated into the diet of high producing ruminants; 4)protein degradation in the rumen is the result of microbial activity and depends on protein type,

ruminal dilution rate, ruminal pH, substrate being fermented and predominant species of rumen flora; 5) considerable variation among and within feedstuffs exists in ruminal degradation and intestinal digestion of protein; and 6) cost of intestinally absorbable dietary protein or absorbable amino acids can be used as a guideline to select protein supplements for high producing ruminant animals.

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