

Nitrogen Metabolism in the Rumen*

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ABSTRACT

Protein metabolism in the rumen is the result of metabolic activity of ruminal microorganisms. The structure of the protein is a key factor in determining its susceptibility to microbial proteases and, thus, its degradability. Ruminal protein degradation is affected by pH and the predominant species of microbial population. Ruminal proteolytic activity decreases as pH decreases with high-forage dairy cattle-type rations, but not in high-concentrate beef-type rations. Accumulation of amino acid (AA) N after feeding suggests that AA uptake by rumen microorganisms could be the limiting factor of protein degradation in the rumen. In addition, there are several AA, such as Phe, Leu, and Ile, that are synthesized by rumen microorganisms with greater difficulty than other AA. The most common assessment of efficiency of microbial protein synthesis (EMPS) is determination of grams of microbial N per unit of rumen available energy, typically expressed as true organic matter or carbohydrates fermented. However, EMPS is unable to estimate the efficiency at which bacteria capture available N in the rumen. An alternative and complementary measure of microbial protein synthesis is the efficiency of N use (ENU). In contrast to EMPS, ENU is a good measurement for describing efficiency of N capture by ruminal microbes. Using EMPS and ENU, it was concluded that optimum bacterial growth in the rumen occurs when EMPS is 29 g of bacterial N/kg of fermented organic matter, and ENU is 69%, implying that bacteria would require about $1.31 \times$ rumen-available N per unit of bacterial N. Because the distribution of N within bacterial cells changes with rate of fermentation, AA N, rather than total bacterial N should be used to express microbial protein synthesis. (**Key words:** rumen, nitrogen, metabolism, microbial)

Abbreviation key: CHO = carbohydrate, EMPS = efficiency of microbial protein synthesis, ENU = efficiency of N utilization, FOM = fermented OM, TDN = total digestible nutrients.

INTRODUCTION

Nutritional models for feeding protein to dairy cattle have evolved from basic CP (NRC, 1978; ARC, 1980) to more complex systems based on rumen-degradable and undegradable protein (INRA, 1988; NRC, 1985, 1989; AFRC, 1992; NRC, 2001). The basic structure of all of the models is similar with N inputs provided by dietary, recycled, and endogenous N. Dietary protein is divided into rumen-degradable and undegradable protein with RDP composed of nonprotein and true protein N. True protein is degraded to peptides and AA and eventually deaminated into ammonia N or incorporated into microbial protein. Nonprotein N is composed of N present in DNA, RNA, ammonia, AA, and small peptides with the N from peptides, AA, and ammonia being used for microbial growth. Rumen output consists of ammonia N, undegraded protein (dietary or endogenous), and microbial protein. When dietary RDP is in excess of the amount required by ruminal microorganisms, the protein is degraded to ammonia N, absorbed, metabolized to urea in the liver, and lost in the urine. Under typical dairy cattle feeding conditions, manipulation of rumen protein degradation or the efficiency of N use (ENU) in the rumen is the most effective strategy to reduce N losses (Tamminga, 1996). Losses of N may be reduced by decreasing protein degradation in the rumen and(or) increasing N use by ruminal microorganisms.

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 (Storm and Ørskov, 1983). The total amount of microbial protein flowing to the small intestine depends on nutrient availability and efficiency of use of these nutrients by ruminal bacteria. Therefore, N metabolism in the rumen can be divided into 2 distinct events: pro-

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tein degradation, which provides N sources for bacteria, and microbial protein synthesis.

There have been several comprehensive reviews on N metabolism in the rumen (Clark et al., 1992; Stern et al., 1994; Jouany, 1996; Firkins et al., 1998; Dewhurst et al., 2000). This paper will review new developments in understanding protein metabolism in the rumen, with emphasis on protein degradation, microbial protein synthesis, and efficiency of microbial protein synthesis with special focus on issues that have not been sufficiently emphasized in previous reviews. For example, most studies have focused on ammonia concentration in the rumen despite the fact that peptides and AA are similar in concentration to ammonia. Also, current feeding systems ignore aspects affecting protein degradation such as pH and nutrient interactions, and consider microbial protein content to be constant, independent of growing conditions.

RUMINAL DEGRADATION OF PROTEIN

The first step of 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 those 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 AA. 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. Peptides can be further degraded by peptidases into AA, and the latter can be incorporated into microbial protein or further deaminated to VFA, CO₂, and ammonia (Tamminga, 1979). The fate of absorbed peptides and AA once inside the microbial cell will depend on the availability of energy [carbohydrates (CHO)]. 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 will be fermented into VFA (Figure 1). Some ruminal bacteria lack mechanisms of AA transport from the cytoplasm to the extracellular environment, and AA absorbed in excess must be ex-

creted from the cytoplasm as ammonia (Tamminga, 1979).

Most studies evaluating protein degradation in the rumen have been conducted using the *in situ* technique, which only measures protein degradation, but not the use of peptides and AA by ruminal bacteria. Nugent and Mangan (1981) observed that peptides and AA did not accumulate after feeding proteins and suggested that proteolysis was the rate-limiting step and, therefore, key in controlling protein degradation. However, Broderick et al. (1991) demonstrated that rapidly degraded proteins may result in the accumulation of peptides and AA within the first 2 h after feeding, suggesting that rates of peptidolysis and deamination play an important role in the control of protein degradation. Recently, Cardozo et al. (2004) found, in continuous culture fermenters receiving a typical dairy ration, that the concentration of peptides, AA, and ammonia were within the same range for up to 8 h after feeding. They reported an accumulation of AA N at 2 and 4 h after feeding (Figure 2), suggesting that AA uptake could be the limiting factor of protein degradation in the rumen. Therefore, manipulation of protein degradation can be achieved not only by modulation of proteolysis, but also through changes in peptidolysis and deamination. For example, monensin reduced ammonia N concentration through the inhibition of the hyper-ammonia-producing bacteria, a small group of ruminal bacteria that are responsible for the production of most of the ammonia (Chen and Russell, 1989). Ferme et al. (2004) also reported that the inhibition of major ammonia-producing bacteria (such as *Prevotella ruminantium* and *Prevotella bryantii*) resulted in a reduction in ammonia N concentration in continuous culture fermenters of ruminal microbes. Continuous culture fermenters have low numbers of protozoa; however, *in vivo*, protozoa play a major role in protein degradation. The most important aspect of protozoa is their ability to engulf large molecules, protein, CHO, or even ruminal bacteria (Van Soest, 1994). In addition, protozoa play a role in regulating bacterial N turnover in the rumen, and they supply soluble protein to sustain microbial growth. Because protozoa are not able to use ammonia N (Onodera et al., 1977), a fraction of previously engulfed insoluble protein is later returned to the rumen fluid in the form of soluble protein (Dijkstra, 1994). This is one of the main reasons why defaunation decreases ammonia N concentration in the rumen (Eugene et al., 2004).

Factors Affecting Ruminal Protein Degradation

The most important factors affecting microbial protein degradation include the type of protein, interactions with other nutrients (mainly CHO within the

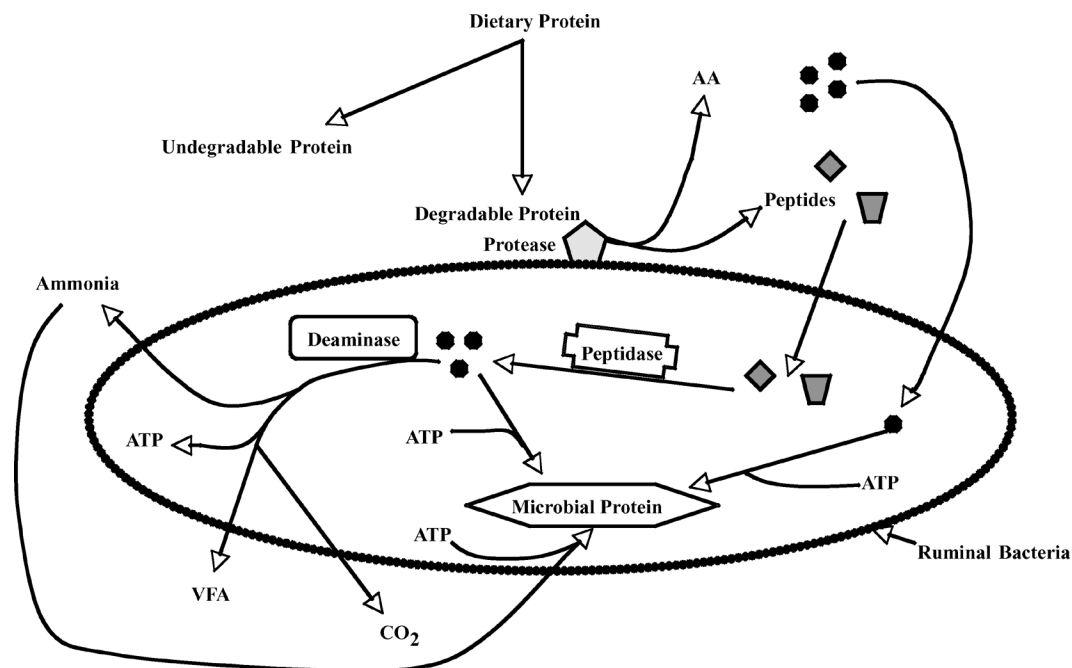


Figure 1. Schematic representation of protein degradation and fate of end products in the rumen.

same feedstuff and within the rumen contents), and the predominant microbial population (dependent on the type of ration, ruminal passage rate, and ruminal pH).

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, but 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 (tertiary and quaternary structure) 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 soybean meal 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).

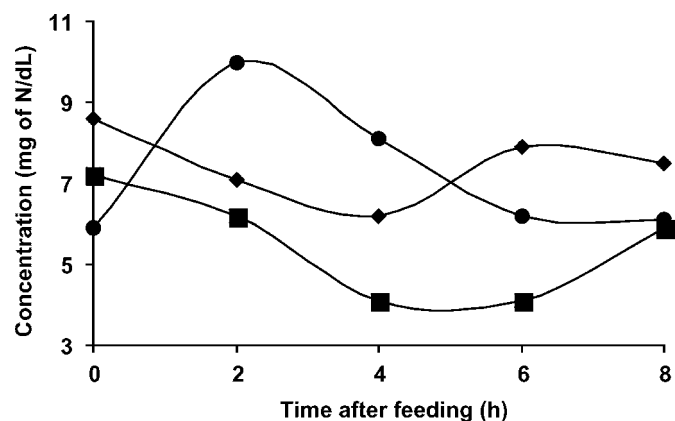


Figure 2. Concentrations of AA peptides, and ammonia in continuous culture fermenters fed a 60:40 forage:concentrate ration every 8 h (adapted from Cardozo et al., 2004). Peptide N = \blacklozenge , AA N = \bullet , and ammonia N = \blacksquare .

Ruminal dilution rate. Protein degradation is inversely related to passage rate through the rumen (Ørskov and McDonald, 1979). The NRC (2001) devel-

oped 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 of DM of a 70:30 forage-to-concentrate ration would increase from 0.049 to 0.057/h for wet forages, from 0.040 to 0.046/h for dry forages, and from 0.056 to 0.068/h for concentrates if the same cow would consume 26 kg of 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 undegraded dietary protein 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 Kopečný and Wallace (1982); however, protein degradation is reduced at the lower end of the ruminal pH environment. Cardozo et al. (2000, 2002) conducted 2 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 soybean meal and heat-processed soybean meal 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, despite the fact that pH was >6.0 in both types of animals, illustrating that the reduction of protein degradation is not only due 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 popu-

lation. 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 Cartailier, 1988; Tománková and Kopečný, 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 *Streptomyces 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 nonproteolytic 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 1). 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 caused by interactions among nutrients. It could be hypothesized that reduction in cellulolytic 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.

MICROBIAL PROTEIN SYNTHESIS

The rumen is a complex environment inhabited by different microbial species, each of them with different nutrient requirements and metabolisms. 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.

Table 1. Effect of pH on bacterial population and digestion of CP and NDF in continuous culture fermenters (adapted from Endres and Stern, 1993).

Item	pH	Protein supplement ¹			
		SSS	SSN	SNN	NNN
Bacteria, cells $\times 10^8$ /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					
CP 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

¹Approximately 50% of dietary CP was in the form of soybean meal (SBM). SSS = 100% SBM, SSN = 67% SBM and 33% nonenzymatically browned SBM (NSBM), SNN = 33% SBM and 67% NSBM, and NNN = 100% NSBM.

²pH effect ($P < 0.01$).

The role of protozoa on rumen metabolism has been reviewed elsewhere (Jouany, 1996; Williams and Coleman, 1997) and will not be discussed in depth in this review. Protozoa can account for about 40% of the rumen microbial biomass (Russell and Rychlik, 2001) and have a direct involvement in digestion of protein and CHO. Protozoa are capable of degrading fibrous and nonfibrous CHO (NFC) (Williams and Withers, 1991), and bacteria are their main supply of protein. The contribution of protozoa to the supply of protein to the small intestine is limited, approximately 11% of total CP flow (Shabi et al., 2000) because they are selectively retained in the rumen. The true contribution of protozoa to animal performance is not clear, and there is no consensus on the value of protozoa to ruminants. Defaunation usually results in decreased protein degradation and concentrations of peptides and AA in the rumen (Ivan et al., 1991). Also, under conditions of low ruminal pH, protozoal numbers would diminish and so would concentrations of peptides and AA in the rumen. 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, inducing low ruminal pH.

Bacteria can use CHO 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 CHO as an energy source for the synthesis of peptide bonds. Readily fermentable CHO, such as starch or sugars, are more effective than other CHO sources, such as cellulose, in promoting microbial growth (Stern and Hoover, 1979). Sev-

eral 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 CHO decreased ammonia N concentrations because of improved N uptake by ruminal microbes. However, the optimum ratio of NFC to ammonia N has not yet been determined. Hoover and Stokes (1991) suggested that in pH-controlled continuous culture fermenters, maximum microbial growth is attained with a 2:1 NFC:RDP ratio. Although this ratio is not feasible under practical conditions, it illustrates the importance of supplying adequate amounts of available N when energy is not limiting. 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 CHO fermentation, large quantities of N can be lost as ammonia, and, conversely, when the rate of CHO fermentation exceeds protein degradation rate, microbial protein synthesis can decrease (Nocek and Russell, 1988). However, the effects of a more synchronized supply of CHO and N to ruminal microorganisms reported in the literature are not consistent. Some in vivo studies (Casper and Schingoethe, 1989; Herrera-Saldana et al., 1990; Matras et al., 1991) indicated a positive response in animal performance with better synchronization of ruminally available CHO and N, whereas some batch culture studies (Henning et al., 1991; Newbold and Rust, 1992) showed no improvement. Interpretation of these types of studies is difficult because rate of energy and(or) protein availability is often confounded with the total amount of energy and(or) protein availability, and ingredients are also different among treatments within a study.

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 the stabilization of microbial growth even when N supply is not well synchronized.

Based on the preferential use of energy by ruminal bacteria, they are classified as cellulolytic and amylolytic. Russell et al. (1992) proposed a simplified model to describe energy and protein requirements of microbial subpopulations. Microbes that degrade structural CHO (cellulolytic) have low maintenance requirements, grow slowly, and use ammonia N as their main N source, whereas microorganisms that degrade nonstructural CHO (amylolytic) have higher maintenance requirements, grow rapidly, and use ammonia, peptides, and 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 microbial 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 (Bryant, 1973).

Russell et al. (1983) reported that microorganisms that ferment NFC derived up to 66% of their protein from peptides or AA, and the rest of the protein was derived from ammonia N. Those researchers claimed

that this proportion was not influenced by rate of microbial growth and that, in the absence of CHO, all peptide N would be converted to ammonia. However, the optimum concentration of peptides in the rumen needed to maximize microbial protein synthesis has not been determined (NRC, 1996). Assuming that bacteria transform available peptides into microbial protein with an efficiency of 80% (Russell et al., 1983) and that NFC-fermenting bacteria may use up to 66% of the available N in the form of peptides, it could be concluded that to maximize microbial protein synthesis, bacteria would need 1.2 g of peptide N/kg of OM fermented in the rumen. Atasoglu et al. (1999) reported that the proportion of bacterial N derived from ammonia decreased as the ratio of ammonia N:total available N decreased. Other studies by Siddons et al. (1985) and Firkins et al. (1987) also reported a negative relationship between ammonia N concentration and the percentage of microbial protein derived from NPN. These observations suggest that ammonia N accumulation in the rumen is the result of preferential use of peptides or AA by microbes, either as a source of N or as a source of energy. Therefore, the proportion of bacterial N derived from ammonia N is not a fixed value, and the proposed value of 1.2 g of peptide N/kg of OM fermented may not apply to all rations.

Recently, Atasoglu et al. (2004) studied the fate of N and carbons from AA in ruminal mixed microorganisms. Results 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 confirmed the theory that ruminal bacteria have difficulty synthesizing Phe, Leu, and Ile (Allison, 1965; Oltjen et al., 1971; Amin and Onodera, 1997) and proposed that Lys is a potential AA limiting growth of rumen bacteria. Therefore, ensuring generous supplies of specific AA might result in greater microbial growth.

In addition to adequate supplies of CHO and N sources, as well as other nutritional factors, such as sulfur supply, other non-nutritional factors, such as ruminal pH and dilution rate, also play an important role in microbial protein synthesis. Under practical conditions, efficiency of microbial protein synthesis (EMPS) remains relatively constant within a wide range of 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 following the model $Y_{ij} = B_0 + B_1X_{ij} + S_i + b_iX_{ij} + e_{ij}$, where $B_0 + B_1X_{ij}$ is the fixed effect part of the model, $S_i + b_iX_{ij} + e_{ij}$ is the random effect part of the model, Y

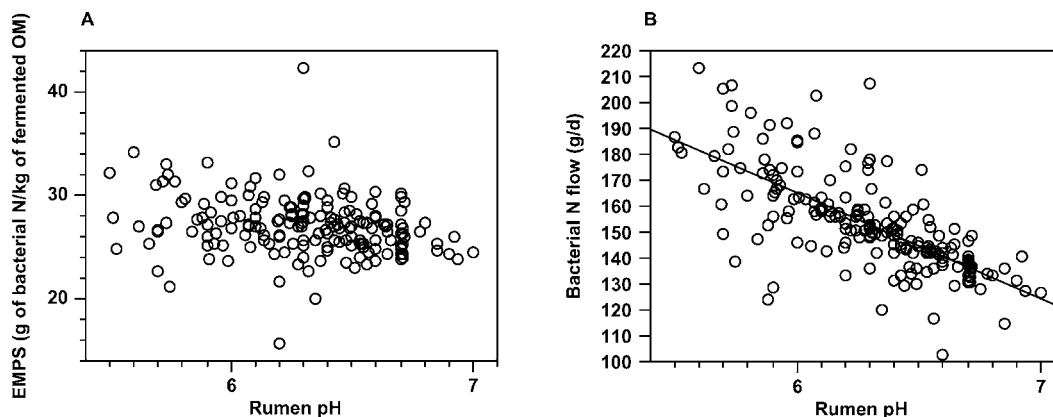


Figure 3. Relationship between rumen pH and efficiency of microbial protein synthesis (EMPS; A) or total bacterial N flow (B), both adjusted for the average study effect. Rumen pH and EMPS had no relationship (A). Bacterial N flow and rumen pH had a significant relationship (B): $Y = 410 - 40.767\text{pH}$; $R^2 = 0.50$; $\text{RMSE} = 13.49$; $P < 0.001$.

represents the dependent variable, X represents the independent variable, and S represents the study. Figure 3A 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 bacterial N flow is negatively related to pH (Figure 3B). 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 (low pH).

Changes in dilution rate of liquid and solids fractions of the ruminal content can also exert an important effect on ruminal fermentation and microbial growth (Isaacson et al., 1975; Russell et al., 1992). Solids and liquid dilution rates depend on various factors including level of intake (Merchen et al., 1986; Faichney, 1993), proportion of forage in the ration (Rode and Satter, 1988), and particle size of the ration (Uden, 1988; Woodford and Murphy, 1988). 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). However, as dilution rates increase, ruminal degradation of OM and energy availability for microbial growth decrease, reducing the expected flow of bacterial N. Meng et al. (1999) reported that, in single-flow continuous cultures, when dilution rate increased from 0.025 to 0.20/h, EMPS increased 2.2-fold, whereas microbial N flow increased only 1.5-

fold, likely because of a reduction of OM truly digested, which decreased from 62.5 to 44.0%.

The increase in microbial protein synthesis and EMPS that is obtained with high dilution rates has been attributed to the 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. 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; Firkins et al., 1992; Hoover and Miller, 1992). *In vivo* results have been discordant with *in vitro* observations, with increases in ruminal liquid dilution rate resulting in higher (Zinn and Owens, 1983; Robinson and Sniffen, 1985), similar (Chamberlain and Thomas, 1979; Merchen et al., 1986; Firkins et al., 1987), or even lower (Sniffen and Robinson, 1987; Owen and Goetsch, 1986) EMPS. The apparent inconsistency between *in vivo* and *in vitro* experiments may be attributed to problems associated with modifying rate of passage *in vivo*, without causing simultaneous changes in other variables, resulting in confounding effects.

EMPS

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 most common assessment of efficiency of microbial growth is the determination of grams of microbial N/unit of rumen-available energy, usually expressed as true OM or CHO fermented. This expression

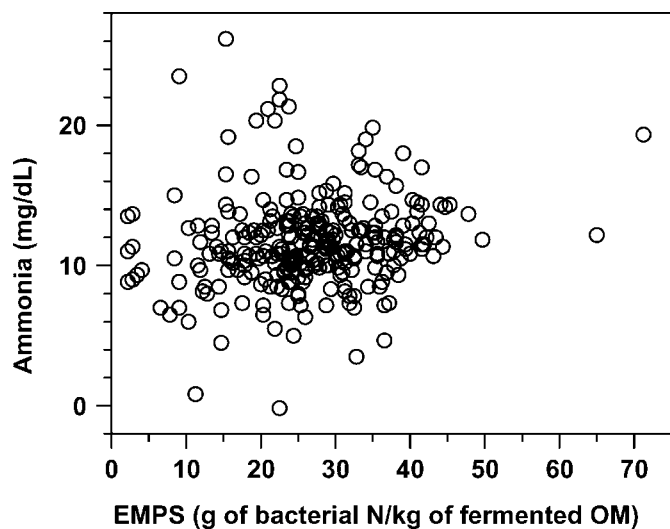


Figure 4. Relationship between efficiency of microbial protein synthesis (EMPS) and ruminal ammonia N concentrations (adjusted for the average study effect). The graph was constructed using literature data ($n = 285$). The independent variable, EMPS, was not correlated with ammonia N concentrations.

of efficiency is justified because it is believed that energy is the most limiting factor in microbial growth. Thus, maximizing the amount of microbial protein per unit of fermentable matter should result in maximum microbial growth. Although, EMPS is affected by similar factors to that required for total microbial protein synthesis, these 2 measurements do not always follow the same trend. Efficiency of microbial protein synthesis is a useful indication of how much energy is directed toward N deposition in microbes; however, EMPS is unable to predict how much available N is actually being used by microbes. In fact, mixed model regression analyses conducted as described previously in this review show that EMPS is insensitive to ruminal ammonia N concentrations (Figure 4), illustrating the inability of EMPS to estimate the efficiency at which bacteria capture available N in the rumen. The NRC (2001) described a negative relationship between ruminal N balance and EMPS, suggesting that with an abundance of N, EMPS is lower than when available N is limiting bacterial growth. This relationship is difficult to justify and has limitations. Ruminal N balance is calculated as RDP supply minus RDP requirements, with the latter calculated as a function of total digestible nutrients (TDN) supply from the ration and assuming a constant efficiency of adjusted TDN use for microbial protein synthesis ($0.131 \times \text{adjusted TDN}$). However, the negative relationship between EMPS and N balance described by NRC (2001) could be a reflection of the reduction in EMPS as TDN availability increases. The supply of TDN that would sustain microbial growth in the

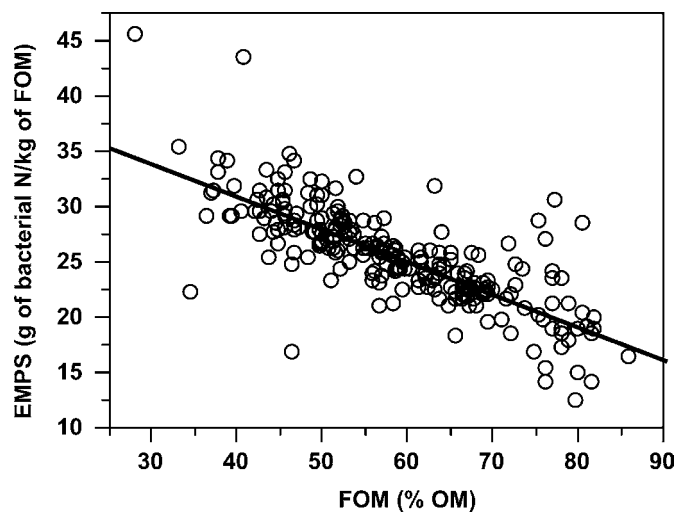


Figure 5. Relationship between fermented OM (FOM; % of OM intake) and efficiency of microbial protein synthesis (EMPS) adjusted for the average study effect. $Y = 42.9 - 0.295\text{FOM} (\% \text{ of OM})$; $R^2 = 0.61$; $\text{RMSE} = 2.83$; $P < 0.001$; $n = 239$.

rumen is dependent on the degradation of OM. In fact, the analysis of literature data using mixed model regression conducted as described previously demonstrate (Figure 5) that EMPS is negatively correlated with fermented OM (%), indicating that the relationship between EMPS and N balance suggested by NRC (2001) might be a result of the negative relationship between TDN (used for the calculation of N balance) and EMPS. Furthermore, the regression line obtained has an identical slope (-0.30) to that described in the NRC (2001) for the relationship between ruminal N balance and EMPS. This relationship could also be partially driven by total DMI. However, performing multiple regression analysis as described previously, including DMI in the model, resulted in a coefficient for fermented OM (FOM) (%) of -0.27 , but the term DMI was not significant in the model.

Several authors introduced alternative measures for ENU in the rumen. For example, Griswold et al. (2003) used the proportion of N intake converted into microbial N, and Bach et al. (1999) proposed to express ENU by ruminal microbes as

$$\text{ENU} = \frac{\text{grams of bacterial N}}{\text{grams of available N}} \times 100.$$

Available N represents N that could potentially be used by rumen bacteria, comprised of rumen-degradable protein plus endogenous protein. In continuous culture fermenters, available N can be determined easily as dietary N intake + N infused through artificial saliva – undegraded N. In vivo determination of avail-

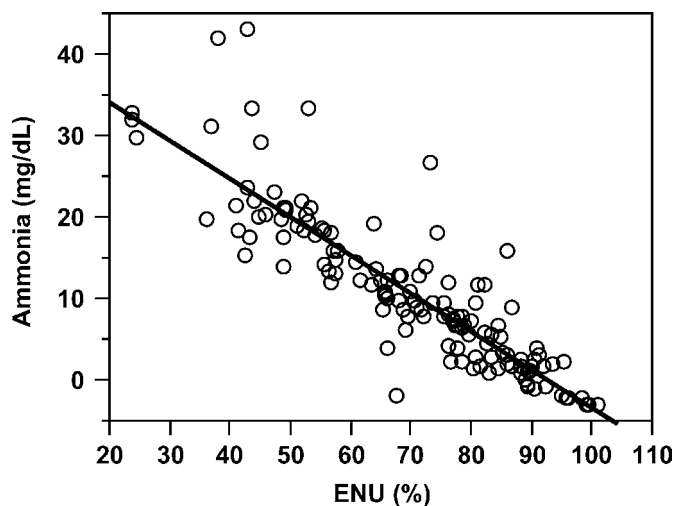


Figure 6. Relationship between efficiency of N utilization (ENU) and ammonia concentration in continuous culture (adjusted for the average study effect). $Y = 43.6 - 0.469\text{ENU}$; $R^2 = 0.78$; $\text{RMSE} = 4.53$; $P < 0.001$; $n = 136$.

able N is more complicated because of N that is recycled from urea and ammonia flows to the rumen via saliva and the rumen wall, which needs to be estimated; however, these values are seldom reported in the literature. Data obtained only from continuous culture studies and adjusted for the effect of study (Figure 6) as described previously show that as ENU increases, ammonia N accumulation in the fermenters decreases ($R^2 = 0.78$; $P < 0.001$), supporting the ability of ENU to effectively describe efficiency of N capture by rumen microbes.

As a result of this discussion, EMPS and ENU appear to be complementary measurements of efficiency of microbial nutrient use; EMPS is a reliable indicator of energy use, and ENU is a reliable indicator of N use. The plot of EMPS vs. ENU (Figure 7) demonstrates a quadratic relationship with an optimum efficiency of growth obtained with an EMPS of 29 g of microbial N/kg of FOM and an ENU of 69% of microbial N/100 g of rumen-available N. This implies that bacteria will be able to capture 69% of available N and will produce 29 g of microbial N/kg of OM fermented under optimal conditions (maximum efficiency of energy use and maximum N capture). Therefore, a reasonable goal would be to ensure the supply of 42 (29/0.69) g of rumen-available N/kg of OM truly fermented in the rumen. The NRC (2001) assumes a fixed ENU from RDP by rumen bacteria of 85%, which implies a need for rumen degradable N of $1.18 \times \text{RDP} \times \text{microbial N}$. The proposed relationship for analyses presented herein would be $1.31 \times \text{rumen-available N} \times \text{microbial N}$. These 2 values are very similar, because the greater need for N in the analysis presented herein is due to the fact that

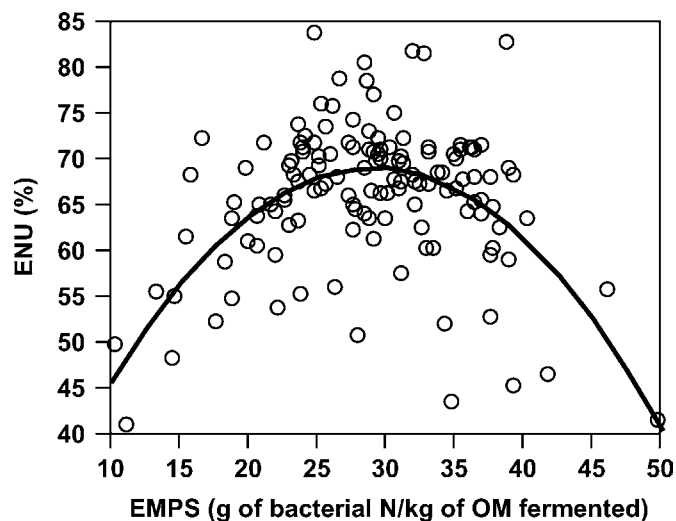


Figure 7. Relationship between efficiency of microbial protein synthesis (EMPS) and efficiency of N utilization (ENU) in continuous culture fermenters (adjusted for the average study effect). The graph was constructed using literature data ($n = 136$) and mixed model regression analysis following the model $Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + S_i + b_1X_{ij} + b_2X_{ij}^2 + e_{ij}$, where $B_0 + B_1X_{ij} + B_2X_{ij}^2$ is the fixed effect part of the model and $S_i + b_1X_{ij} + b_2X_{ij}^2 + e_{ij}$ is the random effect part of the model. Y represents ENU, X represents EMPS, and S represents the study. $Y = 15.31 + 3.724\text{EMPS} - 0.0643\text{EMPS}^2$; $R^2 = 0.33$; $\text{RMSE} = 6.54$; $P < 0.001$. The coefficient of EMPS^2 was significantly ($P < 0.001$) different from zero.

available N accounts for N recycled, whereas RDP does not. Assuming that urea N transfer from plasma to the rumen is between 8 and 10% (NRC, 2001), the 1.18 factor for RDP is converted into a 1.30 factor for available N in the rumen. These observations demonstrate that under optimal conditions, the NRC (2001) estimate is correct, but use of the NRC (2001) estimate will be incorrect when EMPS and ENU deviate from 29 g of bacterial N/kg of FOM and 69% ENU, respectively. For example, when EMPS is 40 g of bacterial N/kg of FOM, efficiency of use of rumen available N would be around 60% (Figure 7), and thus the need of available N to sustain bacterial growth would increase to $1.4 \times \text{rumen-available N} \times \text{microbial N}$. However, within the range of EMPS between 25 and 35 g of bacterial N/kg of FOM, the NRC (2001) estimate seems adequate.

It is important to note that using EMPS or ENU has limitations associated with the measurement of grams of microbial N rather than grams of AA N in bacterial cells. The NRC (2001) assumes that 80% of microbial N is in the form of AA; however, the physiological state (size and macromolecular composition) of cellular growth is determined mainly by the rate at which nutrients become available rather than the specific composition of the medium. Cellular components such as total mass, protein, RNA, and DNA are exponential func-

Table 2. Effects of type of energy supplement on rumen microbial composition (adapted from Bach et al., 1999).

Item	Ration ¹			
	Pasture only	PBP	PC	PSH
Bacterial glycogen content, % of OM	1.4	4.8	6.4	2.0
Bacterial N content, % of OM	7.1	5.6	4.1	5.9

¹PBP = Pasture with beet pulp; PC = pasture with corn; and PSH = pasture with soybean hulls.

tions of growth rate. With more rapid growth, a larger number of dividing cells contains more cellular components and lower cell membrane and cell wall (Schaechter, 1976); thus, the proportions of the various components differ with different growth rates. For example, RNA increases much faster than cell mass, protein increases somewhat slower, and DNA much slower (Bremer and Dennis, 1987). Thus, the relative composition of bacteria changes with growth rate (Shahab et al., 2004). In addition, changes in microbial composition depend on energy and N availability. Under CHO starvation, bacteria store a labile reserve of N in the form of glutamine (Siddons and Paradine, 1983). Similarly, in the presence of a N deficiency with respect to CHO availability, bacteria tend to use CHO to synthesize slimes, capsules, and other extracellular and intracellular α -dextran-glucose polymers (McAllan and Smith, 1976; Wanner and Egli, 1990). Several researchers (Cecava et al., 1988; Olubobokun et al., 1988) reported lower N concentrations in ruminal bacteria from cows receiving rapidly fermentable rations compared with cows receiving a more slowly fermentable ration. Similarly, Bach et al. (1999) reported that increasing supply of available CHO to a basal pasture ration resulted in increasing glycogen contents in microbial cells (Table 2). These observations suggest that availability of N has major consequences on bacterial chemical composition and may be used to estimate quality of CHO and N synchronization in the rumen; these observations also imply that using AA N rather than bacterial N in the numerator would be a more precise expression of EMPS or ENU.

CONCLUSIONS

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. Total microbial N flow is negatively correlated with ruminal pH, but there is no relationship between ruminal pH and EMPS. In general, in vitro studies with pure or mixed cultures of ruminal bacteria indicate a greater synthe-

sis and EMPS with increases in liquid and solids dilution rates. Traditional measurement of EMPS is a valid indicator of how bacteria use available energy, but should be complemented with a measure of efficiency that bacteria capture available N in the rumen. Efficiency of N use by rumen bacteria varies depending on the efficiency of energy use. Therefore, it is suggested that the use of a fixed value based on available energy to estimate requirements of rumen-degradable protein may be inaccurate. Also, because the distribution of N within bacterial cells changes with rate of fermentation, AA N should be considered as a measure of microbial protein synthesis rather than total bacterial N.

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