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Review Article

Effects of Source and Concentrations of Nitrogen and Carbohydrate on Ruminal Microbial Protein Synthesis

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Abstract: The objective of this review was to discuss microbial protein synthesis and the effects of sources and concentrations of nitrogen and carbohydrate on microbial protein synthesis. Even though ammonia-N is a satisfactory source of nitrogen for the growth of the majority of rumen microbes, substitution of intact protein for urea usually stimulates ruminal microbial protein synthesis. While protein sources high in degradable intake protein (DIP), such as soybean meal, appear to have properties that optimize microbial protein synthesis, protein sources low in DIP, such as fish meal, appear to limit microbial protein synthesis apparantly because of the low ruminal digestibility of fish meal. Similarly, a mixture of structural and non-structural carbohydrate sources is necessary for maximal microbial protein synthesis in the rumen.

Key Words: Microbial Protein Synthesis, Protein, Carbohydrate

Azot ve Karbonditrat Kaynağı ve Miktarının Rumen Mikrobiyal Protein Sentezi Üzerine Etkisi

Özet: Bu derleme de, rasyondaki azot ve karbonhidrat kaynakları ile miktarlarının mikrobiyel protein sentezi üzerine etkileri incelenmiştir. Amonyak azotu rumen mikroorganizmalarının çoğunluğu için yeterli azot kaynağı oluşturmasına rağmen, rasyonlarda üre yerine gerçek proteinlerin kullanıldığı durumlarda, mikrobiyel protein sentezinin genel olarak stimüle edildiği bildirilmektedir. Soya küspesi gibi rumende yıkımlanabilir protein (DIP) bakımından zengin yemler mikrobiyel protein sentezini optimize ederken, balık unu gibi DIP yönünden fakir yemler düşük rumen yıkılımından dolayı, mikrobiyel protein sentezini sınırlandırmaktadır. Benzer şekilde, maksimum mikrobiyel protein sentezi için yapısal ve yapısal olmayan karbonhidratların oluşturduğu bir karışıma ihtiyaç duyulmaktadır.

Anahtar Sözcükler: Mikrobiyel Protein Sentezi, Protein, Karbonhidrat

Introduction

Microbial protein synthesis is important in ruminants because microbial protein synthesized in the rumen provides from 50% to nearly all amino acids required for beef cattle depending on the undegraded crude protein concentration of the diet (1). Synthesis of microbial protein and growth of ruminal microbes depend on adequate energy (ATP), resulting from fermentation of organic matter in the rumen, and N resulting from degradation of non-protein and protein nitrogen sources (2). Other nutrients such as sulfur, phosphorus, and other minerals and vitamins are also required for microbial protein synthesis (3). Not only the quantity of nutrients, but also simultaneous ruminal degradation and utilization of dietary carbohydrates and protein are necessary for optimal microbial growth and protein synthesis (4). The Figure shows the various nutrients required for microbial growth (5).

Dietary crude protein (CP) consumed by ruminants can be divided into ruminally degradable (RDP) and undegradable (UDP) (6). As the RDP fraction of dietary CP enters the rumen, it is extensively degraded into peptides and, subsequently, amino acids and ammonia (7). Ruminal ammonia-N is the major nitrogen source used for protein synthesis by ruminal bacterial species. However, in addition to ruminal ammonia-N, many of the bacterial and protozoan species also require either preformed amino acids or peptides for optimal growth (8). Many studies have indicated that the addition of amino acids and/or peptides in the rumen environment has significantly increased microbial growth (9-12).

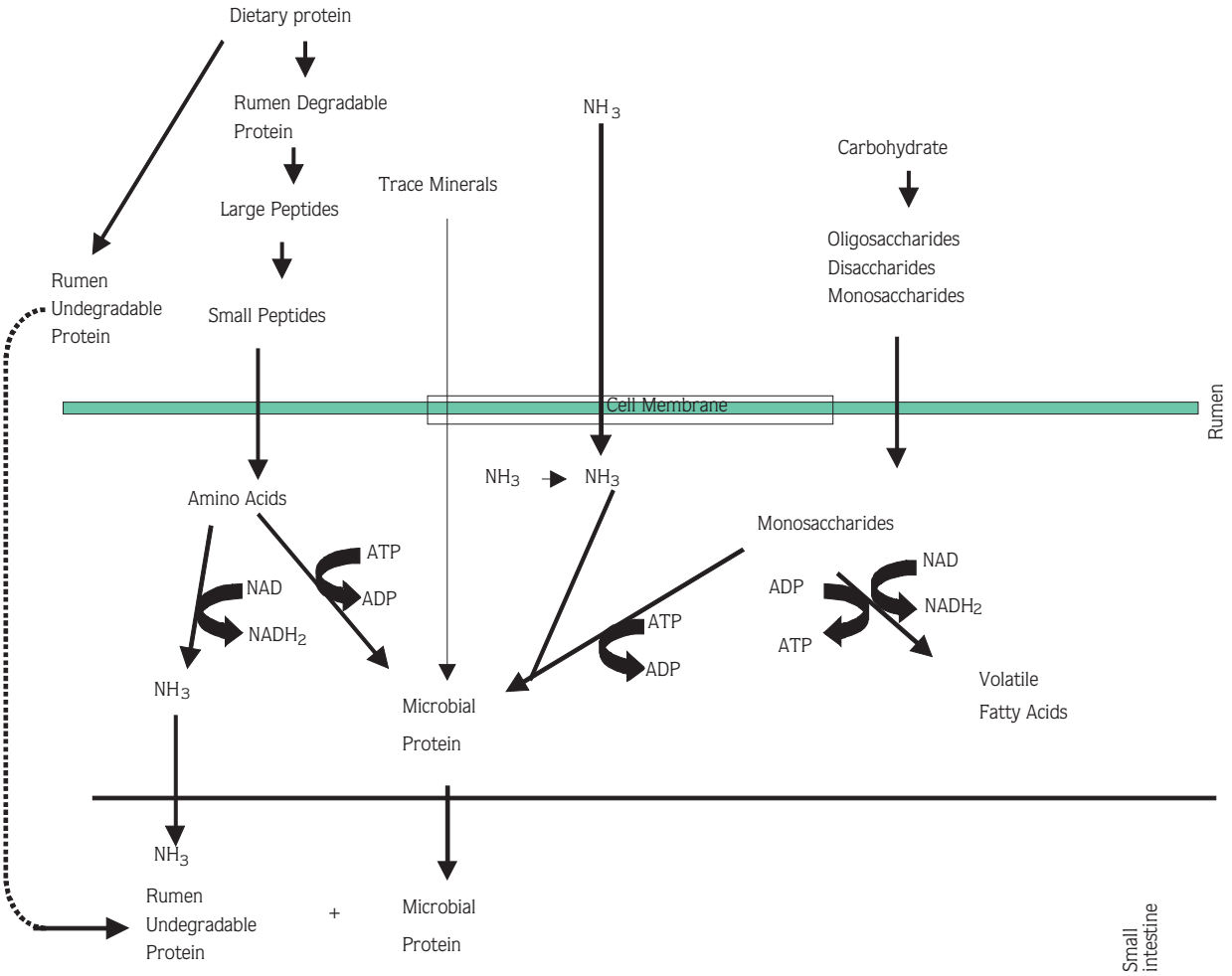

Figure: Nitrogen metabolism and microbial protein synthesis in ruminant animals. Adapted from Nocek et al. (5).

The energy requirement of microbes is provided by fermentation of the carbon skeleton of amino acids or carbohydrates by microbial enzymes. The ATP generated via protein fermentation is not adequate for optimal microbial growth (5,13). Therefore, most of the energy required for optimal microbial growth is provided by fermentation of carbohydrates in the rumen (11,13).

Factors Affecting Microbial Protein Synthesis and Efficiency

Numerous studies have been conducted to determine microbial protein synthesis in the rumen under various conditions (2,6,10,14- 21).

The efficiency of microbial protein synthesis varies significantly among studies. Some of these variations were attributed to the techniques used in these experiments. But there are other factors that caused differences in microbial protein synthesis among the studies (22). These factors include nitrogen concentrations, nitrogen sources, rates of nitrogen and carbohydrate degradation, carbohydrate sources, the ratio of forage to concentrate in the diets, dry matter intake, synchronization of nitrogen and simultaneous release of energy. Other factors such as rates of solid and liquid passage and dietary sulfur concentrations must also be considered (23,24)

The Effects of Nitrogen on Microbial Protein Synthesis and Efficiency

Concentrations. Ruminal microbial protein synthesis requires an adequate supply of nitrogen to achieve maximum efficiency (25). When the nitrogen

concentration is insufficient for optimal growth, uncoupled fermentation may occur, resulting in fermentation without efficient ATP use (26). In order to obtain maximum microbial growth, energy and nitrogen availability must be balanced (27).

Several researchers have observed that increasing CP concentrations in basal diets increased microbial protein synthesis. An infusion of urea at 22 g/d into the rumen of sheep fed a ryegrass hay and rolled maize diet containing 7.3% CP improved microbial protein synthesis (28). Galgal et al. (29) also reported that copra expeller pellet supplementation at 0.5 or 1.0% of body weight of low quality pangola grass hay increased total N intake. Efficiencies of microbial protein synthesis were 15.7, 21.3, and 26.0 g of N/kg true organic matter digested in the rumen of sheep fed the control diet or diets with 0.5% and 1.0% copra expeller pellet supplementation, respectively. Cows fed whole barley supplemented with urea at 0.14 kg/d or soybean meal at 2.4 kg/d had a higher fiber digestibility and a greater efficiency of microbial protein synthesis than with unsupplemented control diet (30).

Studies from the literature have indicated that concentrations of 11 to 13% CP in diets were adequate to obtain optimal microbial protein synthesis (9,31). When sheep were fed semi-purified, protein-free diets containing urea as the N source, the efficiency of microbial protein synthesis was improved when the urea concentration was increased from 0.95 to 1.82%. No further increases in microbial growth were observed when the N level was increased to 3.29%. It was concluded from this study that microbial protein synthesis was depressed when dietary CP level was below 11% (9). Satter and Roffler (31) reported that two percentage units of higher dietary nitrogen concentrations were required for maximal microbial growth in cattle compared to sheep. Therefore, it has been suggested that microbial growth will peak when dairy diets contain approximately 12 to 13% CP.

The optimal concentrations of ruminal ammonia-N required to maximize microbial protein synthesis are controversial, but 5 mg/dL of ammonia-N maximized microbial protein synthesis in vitro (15). The microbial growth was limited at ruminal ammonia concentrations closer to 2 mg ammonia-N/dL; however, excessive levels of ammonia-N up to 80 mg/dL did not increase microbial growth. Under in vitro conditions, ammonia-N started to accumulate when dietary nitrogen concentrations equivalent to 110-140 g CP/kg diet (DM basis) were added to the fermenters (15). Even under favorable conditions for microbial growth, the capture of ammonia-N for microbial protein synthesis was far from complete (16). Even though 5 mg ammonia-N/dL was found to be adequate for optimal microbial protein synthesis in the in vitro study, it is logical to say that more than 5 mg ammonia-N/dL may need to be supplied for maximal in vivo microbial growth because while in vitro conditions are usually static, in vivo conditions are usually dynamic.

After the optimal level of CP has been reached in the diets, further increases in CP concentrations will not improve microbial protein synthesis, but may increase total amino acids to the small intestine because of escape protein passage (32). Krop et al. (33) observed that increasing the urea level in low quality forage diets did not affect the microbial yield above a dietary CP level of 11.2%. The effect of increasing the urea level in the diets from 8.5 to 11.2% CP was associated with increasing ruminal ammonia-N concentrations from 3.7 to 22.2 mg/dL. Stern et al. (32) fed cows diets consisting of 50% concentrate, 35% corn silage, and 15% alfalfa hay that contained 13.1, 16.4, 20.4 or 22.9% CP by feeding increasing amounts of corn gluten meal. Increased amounts of CP increased the concentrations of ruminal ammonia from 9.6 to 14.4 mg/dL, but did not change the amounts of total bacterial amino acid entering the small intestine. Therefore, additional dietary CP increased the supply of total amino acids to the small intestine even though microbial protein synthesis was not altered.

Source. As previously discussed, most ruminal bacterial species use ammonia-N as the N source for growth. Therefore, microbial growth can occur in the rumen of animals fed semi-purified diets containing urea as the only N source (34). In order to obtain maximal efficiency of microbial protein synthesis in the rumen, however, preformed amino acids and peptides are also needed (35).

Similar to other systems describing ruminal protein metabolism, the metabolizable protein (MP) system attempts to describe the degree to which dietary protein is degraded in the rumen (RDP) and the amount that leaves the rumen as undegradable protein (UDP), which passes to the small intestine for digestion (1). The RDP fraction is further categorized as either quickly degradable protein (QDP) or slowly degradable protein

(SDP) (6). The amount and rate of RDP degradation are critical for microbial growth in the rumen because this fraction provides the N necessary for microbial growth. Microbial growth may be limited by the amount of available N in the rumen, even though 11 to 13% CP is provided in the diet if the RDP fractions of these diets are low (21). In the United Kingdom's MP system, QDP is assumed to be incorporated into ruminal bacterial protein with an efficiency of 80%. When energy is not limiting to rumen microbes, the SDP fraction is considered to be utilized within the rumen with an apparent efficiency of 100% (6).

Microbial protein synthesis responds better with some N sources than others. Klusmeyer et al. (36) found that daily microbial N flows into the duodenum were numerically greater in cattle fed diets supplemented with soybean meal compared to corn gluten meal in isonitrogeneous diets. The difference in microbial protein synthesis was apparently caused by a limitation of ruminal ammonia-N in the corn gluten meal diets. Ruminal ammonia-N concentrations were 2.5 and 1.9 mg/dL for corn gluten meal diets containing 14.5 and 11% CP compared to 10.5 and 5.4 mg/dL for SBM diets. Protein sources such as blood meal or corn gluten meal, which are high in undegradable intake protein (UIP), tended to have lower microbial efficiency compared to protein sources like sunflower meal, which had a high degradable intake protein (DIP) content (37). Similarly, Waltz et al. (38) found significantly higher efficiency of microbial protein synthesis for cattle diets with high RDP than for those fed diets of low RDP. Cows fed whole crop barley with no supplement or supplemented with urea, soybean meal or a mixture of urea and soybean meal consumed 1324, 1688, 1793, and 2157 g/d digestible CP, respectively. Supplementation with the mixture of urea and soybean meal resulted in the highest fiber digestibility, and greater efficiency of microbial protein synthesis than the unsupplemented diet. Because the ruminal ammonia-N concentration of cattle fed the unsupplemented diets was 6 mg/dL, the reduction in the efficiency of microbial growth in the low protein diet may have resulted from a lack of RDP (30). The data suggested that supplemental energy may be synchronized with ammonia and peptide production from forage protein degradation, which in turn may have resulted in greater microbial growth. Feeding high-quality forages or soybean meal, containing proteins that are rapidly degraded in the rumen, provides adequate N for the utilization of rumen microbes; however, a shortage of degradable N may also limit microbial protein synthesis in the rumen, especially when diets containing high concentrations of UIP are fed to animals (39).

Numerous studies have been conducted to evaluate the effects of amino acids and peptides as non-ammonia-N sources on microbial growth (9,11,35). In an in vitro study, Jones et al. (35) found that production of microbial CP and DM digestion were affected quadratically by peptide addition, with the highest values for each variable occurring in a diet containing 10% peptides. It was suggested that in diets containing high concentrations of nonstructural carbohydrates, excessive peptides could depress protein digestion and ammonia concentrations and, thereby, cause decreased OM digestibility and microbial protein synthesis. Similarly, Argyle and Baldwin (11) conducted an in vitro experiment to determine which amino acids limited growth of mixed ruminal bacteria. It was concluded that growth of mixed bacteria was a linear function of carbohydrate fermented, but was greatly stimulated by peptides and amino acids, which acted as multiplying factors for microbial growth. Moreover, bacterial affinity for peptides and amino acids was high enough that these organisms used these substrates very efficiently at the low levels normally found in the rumen. Stimulation of microbial protein synthesis was a general phenomenon that was more dependent upon how many different amino acids were available to bacteria in a given mixture than on specific growth limiting amino acids.

Hume et al. (9) fed sheep with diets containing nitrogen provided from urea, gelatin, casein and zein resulting in the synthesis of 17.1 19.8, 23.3 and 22.5 g microbial CP/100 g digestible organic matter, respectively. The lower microbial protein synthesis when urea was fed could have been caused by two reasons. First, urea is completely degraded in the rumen within two hours of feeding, resulting in peak ruminal ammonia-N concentrations at one to three hours after feeding. Although some ammonia-N was used for microbial growth, much of the ammonia is either absorbed or passed to the duodenum and lost in digestion (10). Second, NPN sources did not supply the amino acids and peptides required for optimal microbial growth (11).

Beever and Cottrill (6) indicated that optimal microbial growth occurs only if the supply of nitrogenous constituents for amino acids and protein synthesis is

adequate. Growth rate is generally limited from substrates supplying only ammonia. Both in vitro and in vivo experimentation have established the importance of performed AA if microbial yields are to be optimized. When isolated soy protein was added to a continuous culture system, less microbial growth was observed than when amino acids, peptides and urea were used as N sources (12). The reduced microbial yield when isolated soy protein was used may have resulted from the lower ruminal digestibility of soy protein than those of amino acids, peptides and urea, thereby yielding less ammonia-N for microbial growth.

The Effects of Carbohydrates on Microbial Protein Synthesis and Efficiency

Amount and Source of Carbohydrates. If N supply is not limiting, then the yield of microbial protein is considered to be energy dependent (6). Consideration of the nutrients required to support microbial growth indicates that the major source of energy used by ruminal microorganisms is the monosaccharides, which are derived from the ruminal catabolism of ingested soluble and structural carbohydrates (6). A fermentable carbohydrate that releases energy at the same rate as N is released from dietary N source is also required for the most efficient utilization of nutrients by rumen microbes (4).

Gomes et al. (40) found that supplementation of barley straw with 15.5 or 31.0% of a 1.2 to 1.0 mixture of corn and barley increased microbial protein synthesis from 12.8 to 14.1 and 17.5 g N/kg DOMI. This study implied that with low quality straw, supplementation of the diet with 31% concentrate increased both voluntary intake and microbial protein synthesis per unit of DOMI, apparently because of increased liquid and solid outflow rates when starch was included in the diet.

The above studies have shown that the addition of readily fermentable carbohydrates into the rumen increases microbial growth. However, increasing the energy level beyond an optimal level did not further increase microbial growth. McAllan et al. (41) found that cows fed two types of grass silage at two levels of concentrates had similar ammonia-N concentrations, but the efficiency of microbial protein synthesis was significantly higher in early cut grass silage than late cut grass silage. Because early cut grass silage had higher ruminal digestion coefficients for neutral detergent fiber (NDF) and acid detergent fiber (ADF), the greater microbial protein synthesis may have resulted from greater cell wall digestion in the rumen.

Data from the literature indicate that the type of nonstructural carbohydrate also influences microbial growth. Voigt et al. (42) found that steers fed barley, ground maize or fresh potatoes as supplements for diets containing grass meal and beet pulp had average efficiencies of microbial protein synthesis in the rumen of 16.4 g N/kg organic matter truly fermented in the rumen with a range of 10.6 to 21.4. Microbial efficiency was highest when the ratio of nitrogen-free extract to crude fiber in the diet was between 1.7 and 2.1 for barley, 1.8 for potatoes, and between 2.1 and 3.3 for maize as the starch source. The amino acid composition of the duodenal flow was also significantly influenced by the starch source. In another study, greater microbial protein passage was observed in cows fed barley compared to cows fed corn at similar starch intakes (43). This greater microbial protein synthesis was attributed to greater starch and OM digestion in the rumen for barley diets.

Starch is a more suitable energy source than glucose for maximum capture of ammonia-N for microbial synthesis (16). Replacing starch with glucose as the main energy source for bacteria in continuous culture fermentation caused a marked depression in the proportion of dietary urea-N captured for microbial growth. The growth of mixed ruminal bacteria is a linear function of the amounts of carbohydrate fermented in the rumen (11). However, Cecava et al. (18) did not see a significant difference between the different energy sources upon microbial growth. In that study, the difference in the energy levels (2.24 vs 2.92 Mcal metabolizable energy/kg DM) of the two diets may not have been sufficient to observe significant results.

Conclusion

Data reviewed from the literature indicated that calculating the protein requirement of ruminant based on dietary CP is not adequate. As indicated earlier, protein sources that are low in DIP may limit microbial protein synthesis when calculated to meet animal requirements based on dietary CP. In order to obtain maximal microbial protein synthesis, the nitrogen requirement of the rumen bacteria has to be met first. Even though microbial protein synthesis can occur in the rumen of animals fed semi-purified diets containing urea as the only N source,

nitrogen sources also must include amino acids and peptides in addition to NPN to maintain optimal microbial protein synthesis.

It seems that diets containing a mixture of structural and non-structural carbohydrate sources increase microbial protein synthesis and the efficiency of microbial protein synthesis because of an improved ruminal environment for more diverse ruminal bacteria species and increased amounts and type of substrates, available for microbial protein synthesis.

Therefore, if maximal microbial protein synthesis is aimed, diets must include amino acids and peptides in addition to NPN, and a mixture of structural and non-structural carbohydrate sources is necessary in order to obtain the maximal efficiency of microbial protein synthesis in the rumen.

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