Turkish Journal of Veterinary & Animal Sciences

Volume 25 | Number 5 Article 7

1-1-2001

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M. AKİF KARSLI

JAMES R. RUSSELL

Turk J Vet Anim Sci
25 (2001) 681-686

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Mehmet Akif KARSLI
Yüzüncü Yıl University, Faculty of Veterinary, Department of Animal Nutrition and Nutritional Diseases, Van - TURKEY
James R. RUSSELL
Department of Animal Science, Iowa State University, Ames, IA/ USA

Received: 22.02.2000

Abstract: The effects of some dietary factors, other than source and amount of N and carbohydrate, on the amount and efficiency of microbial protein synthesis are discussed in this review. Specifically, these factors include dry matter intake of animals, forage:concentrate ratio of diets, rate of N and carbohydrate degradation, synchronized release of N and energy from diets, rate of passage, and other factors, such as vitamins and minerals. It seemed that diets containing a mixture of forages and concentrates increase the efficiency of microbial protein synthesis because of an improved rumen environment for the growth of more diverse bacteria species.

Key Words: Microbial Protein Synthesis, Intake, Rates of Passages

Kimi Diyetsel Faktörlerin Rumen Mikrobiyal Protein Sentezi Üzerine Etkileri

Özet: Bu derlemede, N ve karbonhirat kaynağı ve miktarı dışında, mikrobiyel protein sentez miktarı ve etkinliğini etkileyen kimi diyetsel faktörler tartışılmıştır. Spesifik olarak bu faktörler, hayvanların yem tüketimi, rasyonların kaba:konsantre yem oranları, rasyon N ve karbonhidrat yıkılım hızı, rasyonların N ve enejilerinin mikrobiyel protein sentezi için senkronizasyon durumu, rumen içeriğinin rumeni terk etme hızı ve vitamin-mineral gibi diğer diyetsel faktörlerden oluşmaktadır. Mikrobiyel protein sentez etkinliğinin kaba-konsantre yem karışımı içeren rasyonları tüketen hayvanlarda, daha fazla bakteri türünün üreyebilmesi için uygun rumen ortamının sağlanmasına bağlı olarak arttığı yönünde bir izlenim ortaya çıkmaktadır.

Anahtar Sözcükler: Mikrobiyel Protein Sentezi, Yem Tüketimi, Rumen İçeriğinin Rumeni Terk Hızı

Efficiency and Variation in Efficiency of Microbial Protein Synthesis

Daily microbial protein synthesis is different from the efficiency of microbial protein synthesis. Daily microbial protein synthesis is the product of the efficiency of microbial protein synthesis (1), which usually is defined as grams of microbial crude protein (MCP)/ kilogram or 100 grams of organic matter (OM) digested in the rumen (1, 2).

Because a major energy source of OM is carbohydrate for microbial protein synthesis, some researchers have suggested that it would be more appropriate if the efficiency of microbial protein synthesis is expressed as a function of carbohydrate digested rather than OM digested in the rumen (3).

The efficiency of microbial protein synthesis greatly differs in animals fed different diets, even within similar diets. The average efficiency of microbial protein synthesis is 13.0, ranging from 7.5 to 24.3 for forage based diets (based on 34 studies); 17.6, ranging from 9.1 to 27.9 for forage-concentrate mix diets (based on 36 studies), and 13.2, ranging form 7.0 to 23.7 g MCP/ 100 g for concentrate diets (based on 14 studies) of OM truly digested in the rumen. Overall, the average efficiency of microbial protein synthesis is 14.8, ranging from 7.0 to 27.9 g MCP/100 g of OM truly digested in the rumen. The efficiency of microbial protein synthesis was predicted to be around 13 g MCP/100 g of total digestible nutrient (TDN) for beef cows (4, 5). Hoover and Stokes (1) proposed that sources of carbohydrates, such as different ratios of structural to nonstructural carbohydrates, would have little effects on the efficiency of microbial protein synthesis. On the other hand, the rate of digestion of carbohydrates would have greater impact on the efficiency of microbial protein synthesis. It

is well known that the rapid digestion of nonstructural carbohydrate results in reduced ruminal pH. The efficiency of microbial protein synthesis is reported to be low in animals fed high-concentrate diets because of reduced ruminal pH (5). The efficiency of microbial protein synthesis is also low in low-quality forages because of slow carbohydrate digestion, as well as the slow rate of particulate and liquid dilution turnover (5). In addition to slow carbohydrate degradation, in situ data showed that the ratio of degraded nitrogen (N) to OM in the rumen greatly varied in the rumen in times after feeding (6). The values (10 to 70 g N/kg of OM) were below and above the optimal value for microbial protein synthesis (30 to 40 g N/kg of OM), indicating periods of both severe undersupply and oversupply of N moieties in relation to energy availability, which may severely compromise optimal microbial metabolism and efficiency. The limitation associated with the efficiency of microbial protein synthesis with low- and high-fiber diets seems to be reduced when forage-concentrate mix diets were fed to animals (7). Forage supplies readily degradable protein and concentrate provides soluble carbohydrate at the initiation of feeding. This is reversed in later phases of feeding so that rumen microbes have enough substrate at all times (8).

In addition to N and carbohydrate, there are several factors which may influence the amount and efficiency of microbial protein synthesis, as discussed above. These factors include intake (9, 10), forage:concentrate ratio of a diet (11), rate of N and carbohydrate degradation (12), synchronization (13, 14), passage rates (15, 16), and other dietary factors.

Effects of Dry Matter Intake on Microbial Protein Synthesis and Efficiency

Data from the literature indicate that there is a strong positive correlation between DM intake (DMI) and microbial growth (7, 9, 10). Although increasing the level of intake decreased the percentage of organic matter digested in the rumen, the total amount of OM digested in the rumen increased. Therefore, more nutrients were supplied for microbial growth (9, 10). Djouvinov et al. (10) found that increasing DMI with the addition of straw to barley-based diets significantly increased microbial protein synthesis in the rumen in one experiment, but did not significantly change the efficiency of microbial protein synthesis. In a second experiment, the addition of polyvinylchloride (PVC) to provide ballast to increase dry matter intake of diets containing ground barley and dehydrated alfalfa increased DMI. Furthermore, the efficiency of microbial protein synthesis linearly increased with increasing levels of DMI (r^2=.99). The different effects of DMI on the efficiency of microbial growth between the two experiments was probably related to the extent of changes in DMI. Dry matter intake increased by 33 and 44% in the first and second experiments, resulting in 60% and 120% increases in solid turnover rates, respectively, increasing microbial protein synthesis by 19 to 36% and 34 to 42%, respectively.

Similarly, Gomes et al. (7) discovered that the supplementation of straw diets with starch linearly increased the amounts of OM digested and solid and liquid outflow rates. Therefore, increasing the level of starch linearly increased microbial yields, resulting in a strong correlation between the digestible organic matter intake (OMI) and the microbial protein synthesis (r^2=0.89). Clark et al. (9) also demonstrated that microbial protein synthesis was positively correlated with OMI (r^2=.69).

The increase in microbial protein synthesis with increased feed intake is probably the result of the increased passage rate. The increased passage of microbial protein to the small intestine occurred as a result of the increased passage of both fluids and solids with increased intake (7, 10). A higher dilution rate reduced the retention time of bacteria in the rumen and, therefore, reduced the maintenance energy requirement and increased the available energy for growth (17). The faster rate of growth coupled with the faster passage of microbes to the small intestine may reduce the recycling of energy and N within the rumen because of decreased cell lysis (9).

As intake of high-fiber diets increases, the neutrol detergent fiber (NDF) turnover rate increases while the ratio of bacterial OM to NDF in rumen ingesta is reduced. This suggests that as intake increases, there is a greater flow of particles from the rumen that are at an earlier stage of digestion with fewer attached microbes. Thus, microbial recycling is reduced concomitant with the increased rate of feed OM flow leading to increased microbial yield (18).

Effects of Forage: Concentrate Ratio of Diet on Microbial Protein Synthesis and Efficiency

As indicated earlier, the average efficiency of microbial protein synthesis was higher in forage-concentrate mix diets than for all-forage diets. Synthesis of microbial protein is improved by varying the source and degradability of energy incorporated into the diet (14). In contrast to results of Salter et al. (19), several studies have reported increased utilization of ruminal ammonia nitrogen for microbial protein synthesis when diets contained readily digestible carbohydrates rather than starch in high-fiber diets (20). The difference between these studies (19, 20) could be the varying carbohydrate and nitrogen sources in the diets. As proposed by Hoover and Stokes (1), the rate of carbohydrate digestion in diets and the synchronization of this rate with that of N release has an impact on microbial protein synthesis. Huber and Kung (21) reported that the major factor limiting the utilization of non-protein N (NPN) was a source of readily available energy. Microbial N synthesis was highest when highly ruminally available nonstructural carbohydrates were combined with highly ruminally available proteins, and lowest when highly ruminally available nonstructural carbohydrate were combined with poorly ruminally available protein. This situation would suggest that N utilization for forages having high readily degradable protein (RDP) will improve microbial growth when forages are supplemented with ruminally available nonstructural carbohydrates (21).

Czerkawski et al. (11) reported that sheep fed a diet composed of a mixture of hay and concentrate had greater microbial growth in the rumen compared to those fed concentrate and hay separately. The increase in microbial growth may have resulted from a better non-protein nitrogen to protein ratio in the mixed diet because the concentration of NPN is generally higher in forages than in concentrates. While forages may supply N as highly degradable protein or non-protein N, concentrates may slowly supply N mainly as peptides and/or amino acids needed for microbial protein synthesis (8). It could also be caused by better utilization of amino acids and peptides in the mixed diet.

The effect of readily fermentable carbohydrate supplementation on the efficiency of microbial protein synthesis is dependent on the level of supplementation. Efficiency tends to be increased when readily fermentable carbohydrate is supplemented at less than 30% of the total diet, but decreased when the supplementation level is greater than 70% (22). The decrease in efficiency of microbial protein passage to the small intestine when diets containing more than 70% concentrate are fed may occur because of a rapid rate of nonstructural carbohydrate degradation, resulting in an uncoupled fermentation (17). Uncoupled fermentation occurs because energy is released much faster than it is captured and utilized by the ruminal bacteria (9). Adding forage or structural carbohydrate to a diet that is high in concentrate may allow ruminal bacteria to utilize the energy for growth more efficiently as energy is released in a more uniform pattern throughout the day (8). Furthermore, as the proportion of forage increases in dietary dry matter, there is greater saliva flow, a higher ruminal pH, improved cation exchange capacity, improved hydration, improved mat formation, leading to decreased retention times, and greater microbial growth as microbial generation times are reduced (18).

Effects of Rate of N and Carbohydrate Degradation on Microbial Protein Synthesis and Efficiency

Although the crude protein content of many practical diets may be greater than the 11% CP required to support optimal microbial growth, the resistance of proteins to microbial degradation may limit microbial protein synthesis (2).

It seems that proteins which have lower rates of ruminal degradation tend to improve the efficiency of microbial protein synthesis, probably because of the better capture of released N by rumen microbes. Broderick (23) reported that heating legume forages increased microbial N flow to the duodenum from 11 to 15 g/kg OMD. Forage heating significantly decreased the rate of N disappearance from Dacron bags incubated in the rumen of steers (24, 25). Makkar et al. (12) indicated that the efficiency of microbial protein synthesis was greater in forages containing saponin and tannins, which reduce ruminal N degradability.

The readily degradable fraction of protein is higher in forages than in grains. Approximately 40% of protein in fresh alfalfa is soluble in the rumen environment (24). The solubility of the protein in corn grain was lower than in alfalfa (26). Therefore, while 2.0 g of available N per 100 g digestible organic matter has been reported to be required for optimal microbial growth for animals fed

forages, the level of degradable N in grains may limit microbial protein synthesis when supplemented at this level. Research indicates that more than 2.0 g of available N per 100 g digestible organic matter is required for optimal microbial growth in feedstuffs like grains which are resistant to ruminal microbial degradation (2).

The primary function of the microbial carbohydrate metabolism is to release the ATP required for microbial growth. Thus, patterns and rates of microbial nitrogen metabolism are dependent upon the rates of carbohydrate fermentation (1). Baldwin and Denham (8) divided carbohydrates into three groups according to their solubility in the rumen environment. These groups included: soluble carbohydrates, such as soluble sugars and organic acids; carbohydrates with intermediate solubility, such as starch and pectin; and insoluble carbohydrates, such as cellulose and hemicellulose. Fermentation rates of soluble sugars and starches are very high up to 2 h postfeeding, but decrease almost completely approximately 4 h postfeeding. Soluble sugars and starch provide higher levels of ATP than structural carbohydrate up to 4 h postfeeding, but they provide almost no ATP for microbial growth after 4 h postfeeding. Approximately 3 to 4 h postfeeding, cellulose and hemicellulose degradation start and continue for a long period (up to 96 h) postfeeding, providing ATP for later microbial growth (8). Therefore, feeding a mixture of forage and concentrate resulted in greater microbial protein synthesis compared to feeding only concentrate or forage (11).

Effects of Synchronization on Microbial Protein Synthesis and Efficiency

Matching the release of ammonia-N from dietary protein with the release of usable energy may improve N utilization (27). Sinclair et al. (14) found that wheat straw and barley diets containing rapeseed meal as a slow release N source, or urea as a rapid release N source, contained equal amounts of rumen degradable protein and OM truly degraded in the rumen. The efficiency of microbial protein synthesis, however, was 11 to 20% greater in sheep fed a diet supplemented with rapeseed meal than with urea. This increase in efficiency of microbial protein synthesis in sheep fed the rapeseed supplemented diet may have resulted from a lower rate of N and carbohydrate release and the better capture of these nutrients by rumen microbes. Similarly, synchronization for rapid fermentation with highly degradable starch and protein sources stimulated greater microbial protein flow to the duodenum when compared to diets with unsynchronized N and energy release (28). In contrast, the degree of energy and N synchronization, as controlled by intraruminal infusion, affected neither the duodenal microbial flow nor the efficiency of microbial protein synthesis in cattle (29). However, ammonia-N levels throughout a 24-hour period were high enough to support maximum microbial growth when urea was fed once a day (27).

Spreading the urea dosage throughout the day had no effect on N capture efficiency by ruminal bacteria when starch was used as the energy source (27). Salter et al. (19) also reported that spreading the dosages of urea and glucose to synchronize nitrogen and energy supplied to steers consuming a wheat straw-based diet had no effect on microbial N-capture efficiency. Henning et al. (29) concluded that merely improving the degree of synchronization between energy and N release rates in the rumen did not increase microbial yield. However, in their studies urea was used as the nitrogen source, and therefore, utilized amino acid and/or peptides might have limited ruminal microbial protein synthesis. In order to increase microbial yield, it seems that the manipulation of energy and N fermentation in the rumen should first be aimed at obtaining the most even ruminal energy supply pattern possible within a particular dietary regimen. The second goal is to supply the total daily amount of ruminally available N sufficient for use of the total amount of energy expected to be released in the rumen per day.

Effects of Passage Rate on Microbial Protein Synthesis and Efficiency

Microbes leave the rumen in either a liquid or solid phase of the digesta. Therefore, it is logical to assume that changes in the rate of solid or liquid passage would affect the amount of microbial protein flow to the duodenum. Cell yield efficiency increases as the dilution rate increases (2). The fundamental principle is that the mean age of the ruminal microbial population is decreased at higher dilution rates. At high ruminal dilution rates, values of 20-45 g cell/100 g carbohydrate fermentation have been reported (30). Theoretically, the ideal situation for each species of microbes to achieve

maximum yield, would be an outflow rate equal to the division time of that species. Such a condition would ensure that a minimum amount of energy is used to maintain the microbial population. However, this condition is impossible to achieve because different species divide at different rates in different nutritional environments (31).

It has been reported that at least 50% of the microbes leaving the rumen are associated with feed particles (18). However, this proportion could be diet dependent. While the majority of carbohydrates come from starch in high concentrate diets, fiber is the major source of carbohydrate in high forage diets. Therefore, while the rate of liquid passage may play an important role in high concentrate diets, the rate of solid passage could be more important in high forage diets. Cole et al. (15) reported that the efficiency of microbial protein synthesis was linearly (r^2=.85) correlated with the liquid dilution rate in high concentrate diets, but Rode et al. (16) found that efficiency was linearly (r^2=.77) correlated with particulate turnover rate with high fiber diets.

Other Dietary Factors Affecting Microbial Protein Synthesis and Efficiency

In addition to N and carbohydrate supply, microbial yield is affected by the concentrations of trace minerals and vitamins (18). Dietary sulfur concentration has been found to affect microbial growth (18). The amount of sulfur required by rumen microorganisms for synthesis of methionine and cysteine ranges from .11 to .20% of the total diet, depending on the status of the cattle (32). Limited intake of sulfur may restrict microbial protein synthesis when large amounts of non-protein nitrogen are fed to ruminant animals, such as urea (18). Phosphorus is another mineral required for the synthesis of ATP and protein by rumen microbes (2). Microbial protein synthesis can be limited by an insufficient supply of P for microbial growth (2).

Summary

Dietary CP in ruminant diets serves as a source of metabolizable protein to the ruminant by providing both ruminal degraded protein for microbial protein synthesis and ruminal undegraded protein.

Microbial protein synthesis is dependent upon suitable N and carbohydrate sources. Even though trace minerals and vitamins are adequate for maximal microbial protein synthesis in many feeding conditions, microbial protein synthesis could be limited by inadequate trace minerals and vitamins, in some cases. Data reviewed from the literature indicated that calculating the protein requirement of ruminant animals based on dietary CP is not adequate. As indicated earlier, protein sources, which are low in degradable intake protein (DIP) may limit the 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. Nitrogen sources also must include amino acids and peptides in addition to NPN.

Diets containing a mixture of forages and concentrates increase microbial protein synthesis because of improved synchronization of nutrient release, an improved ruminal environment for more diverse ruminal bacteria species, increased amounts and types of substrates, increased intake and, subsequently, increased rates of solid and liquid passage.

Although the majority of differences in the efficiency of microbial protein synthesis have been from the diets used, some of the differences have, unfortunately, been caused by the techniques used and assumptions made. Many different markers have been used to determine the microbial N flow to the duodenum. Different estimates among the markers have been shown. Even though none of the markers are perfect, the use of bacterial purines has been recommended by Stern et al. (33). Caution should also be taken when we make assumptions about the composition of rumen microbes because the composition of microbes may vary greatly (34).

References

1. Hoover, W. H. and S. R. Stokes.: Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci. 1991; 74: 3630-3645.

2. Stern, M. D. and W. H. Hoover.: Methods for determining and factors affecting rumen microbial protein synthesis: A review. J. Anim. Sci. 1979; 49: 1590-1602.

3. Nocek, J. E. and J. B. Russell.: Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial protein synthesis and milk production. J. Dairy Sci. 1988; 71: 2070-2107.

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

5. NRC. Nutrient Requirements of Beef Cattle (7^{th} Ed.). National Academy Press, Washington, DC. 1996.

6. Beever, D. E. and B. R. Cottrill.: Protein systems for feeding ruminant livestock: A European Assessment. J. Dairy Sci. 1994; 77: 2031-2043.

7. Gomes, M. J., F. D. Hovell and X. B. Chen.: The effect of starch supplementation of straw on microbial protein supply in sheep. Anim. Feed Sci. and Technol. 1994; 49: 277-286.

8. Baldwin, R. L. and S. C. Denham.: Quantitative and dynamic aspects of nitrogen metabolism in the rumen: A modeling analysis. J. Anim. Sci. 1979; 49: 1631-1639.

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

10. Djouvinov, D. S. and N. A. Todorov.: Influence of dry matter intake and passage rate on microbial protein synthesis in the rumen of sheep and its estimation by cannulation and a non-invasive method. Anim. Feed Sci. and Technol. 1994; 48: 289-304.

11. Czerkawski, J. W.: Chemical composition of microbial matter in the rumen. J. Sci. Food. Agric. 1976; 27: 621-627.

12. Makkar, H. P. S., M. Blummel and K. Becker.: In vitro effects of and interactions between tannins and saponins and fate of tannins in the rumen. J. Sci. Food. Agric. 1995; 69: 481-489.

13. Sinclair, L. A., P. C. Garnworthy, J. R. Newbold and P. J. Buttery.: Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 1993; 120: 251-259.

14. Sinclair, L. A., P. C. Garnworthy, J. R. Newbold and P. J. Buttery.: Effect of synchronizing the rate of dietary energy and nitrogen in diets with a similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. J. Agric. Sci. 1995; 124: 463-472.

15. Cole, N. A., R. R. Johnson, F. N. Owens and J. R. Males.: Influence of roughage level and corn processing method on microbial protein synthesis by beef steers. J. Anim. Sci. 1976; 43: 497-503.

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

17. Polan, C. E.: Update: Dietary protein and microbial protein contribution. J. Nutr. 1988; 118: 242-248.

18. Sniffen, C. J. and P. H. Robinson.: Symposium: Protein and fiber digestion, passage, and utilization in lactating cows. J. Dairy Sci. 1987; 70: 425-441.

19. Salter, D. N., R. H. Smith and D. Hewitt.: Factor affecting capture of dietary nitrogen by micro-organisms in the forestomachs of the young steer. Experiments with [^{15}N] urea. Br. J. Nutr. 1983; 50: 427-435.

20. De Visser, H., P. L. Van Der Togt, H. Huisert and S. Tamminga.: Structural and non-structural carbohydrates in concentrate supplements of silage-based dairy cow rations. Rumen degredation, fermentation and kinetics. Neth. J. Agric. Sci. 1992; 40: 431-445.

21. Huber, J. T. and L. Kung, Jr. : Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci. 1981; 64: 1170-1178.

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

23. Broderick, G. A.: Desirable characteristics of forage legumes for improving protein utilization in ruminants. J. Anim. Sci. 1995; 73:2760-2773.

24. Farquhar, A. S.: Kinetics of alfalfa nitrogen and cell wall disappearance from ruminally-incubated dacron bags. Ph.D. Thesis. Iowa State University, Ames. 1985.

25. Yang, J. H., G. A. Broderick and R. G. Koegel.: Effect of heat treating alfalfa hay on chemical composition and ruminal in vitro protein degradation. J. Dairy Sci. 1993; 76: 154-163.

26. Mullahey, J. J., S. S. Waller, K. J. Moore, L. E. Moser and T. J. Klopfenstein.: In itu ruminal protein degradation of switchgrass and smooth bromegrass. Agron. J. 1992; 84: 183-188.

27. Salter, D. N., K. Daneshvar and R. H. Smith.: The origin of nitrogen incorporatedinto compound in the rumen bacteria of steers given protein- and urea-containing diets. Br. J. Nutr. 1979; 41: 197-209.

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

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

30. Van Soest, P. J.: Nutritional Ecology of the Ruminant. Cornell University Press. New York, NY. pp.476. 1994.

31. Orskov, E. R. Protein Nutrition in Ruminants (2^{nd} Ed.). Academic Prees. San Diego, CA. pp.175. 1992.

32. NRC. Nutrient Requirements of Beef Cattle (6^{th} Ed.). National Academy Press, Washington, DC. 1984.

33. Stern, M. D., G. A. Varga, J. H. Clark, J. L. Firkins, J. Huber and D. L. Palmquist.: Symposium: Metabolic relationships in supply of nutrients for milk protein synthesis. J. Dairy Sci. 1994; 77:2762-2786.

34. Williams, P. P. and E. D. William.: Amino acid and fatty acid composition of bovine ruminal bacteria and protozoa. J. Anim. Sci. 1973; 36: 151-155.