CONCEPTS FOR MAXIMIZING MICROBIAL PROTEIN SYNTHESIS AND BYPASS PROTEIN UTILIZATION

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INTRODUCTION

Assessment of the need for protein supplementation is an important aspect of modern ruminant production systems because protein supplements are generally the most expensive ingredient in the ruminant diet. Traditionally, protein requirements for ruminants have been defined on the basis of dietary crude protein (CP) concentration. Crude protein is determined by multiplying the total nitrogen (N) in a diet by 6.25; however, this system does not account for differences that exist between nitrogenous components of different feedstuffs, nor the fate of these compounds upon ingestion by the animal. During the 1970's, several fundamental concepts were developed related to protein supplementation of ruminants. These include recognition of the following aspects of digestive physiology and metabolism of the ruminant:

1) Digestion in ruminants is a complex process which is initiated in the rumen (by ruminal microorganisms) and completed in the small intestine.

2) The protein needs of the host ruminant for maintenance, protein deposition, fetal development, lactation or wool growth are met by amino acids absorbed from the small intestine.

3) A relationship (called a yield coefficient) exists between microbial protein synthesized in the rumen (and made available for digestion and absorption by the small intestine) and energy available to ruminal microbes for biosynthetic purposes.

4) The value of dietary urea (or other NPN source) as a protein supplement for ruminants is dependent upon its degradation to ammonia in the rumen and subsequent incorporation into microbial protein.

5) Ruminal microbial protein synthesis is insufficient to meet the protein requirements of young, rapidly growing ruminants. This is also the case for very productive, lactating dairy cows.

6) Dietary nitrogen is degraded in the rumen to a degree dependent on source, processing and rumen environment.

7) Protein that is digested in the small intestine of the ruminant can be of either microbial or dietary origin. Dietary protein which escapes proteolysis and deamination in the rumen is called "bypass" or "undegraded dietary protein (UDP)".

8) Nitrogen requirements of ruminal microbes (for production of microbial protein and optimization of ruminal digestion of organic matter) are separate from those at the tissue level of the host animal.

Recognition of these considerations led to the development of metabolizable protein systems (ARC, 1980; NRC, 1985) for expressing the protein requirement of ruminants. In these systems, protein requirements are based upon estimation of the amino acids absorbed from the small intestine. Several models exist to estimate the contribution made by amino acids supplied by microbial protein synthesized in the rumen along with dietary protein which has escaped ruminal degradation (ARC, 1980; NRC, 1985; Fox et al., 1980; Satter, 1980; Trenkle, 1980; Van Soest et al., 1980). This paper will review the key concepts upon which these new systems are based. It will also identify advantages that these new systems offer as well as problems inherent to their current use.

GENERAL PRINCIPLES

Feedstuffs ingested by the ruminant are exposed to a myriad of hydrolytic activities of microbial origin (Prins, 1977). Complex carbohydrates are digested to sugars which, in turn, are fermented by the anaerobes occupying the rumen to volatile fatty acids (VFA) and other end products. Fermentation of carbohydrate results in the synthesis of adenosine triphosphate (ATP). Proteolytic microbes in the rumen degrade ingested dietary protein to varying degrees resulting in release of peptides and amino acids, some of which are directly incorporated into microbial protein. Some ruminal microbes also degrade amino acids to carbon skeletons and ammonia by the process of deamination. Urea, originating endogenously or from the diet, is hydrolyzed to ammonia. Ammonia also arises from lysis of bacterial cells and the release of bacterial proteins which are then subjected to proteolysis and deamination. Ruminal ammonia can be: 1) incorporated into microbial protein, 2) absorbed through the rumen epithelium or 3) flushed to the omasum (Owens and Bergen, 1983). Thus, ammonia is the major product of protein and NPN catabolism, and also the main substrate for microbial protein synthesis. ATP is required for the biosynthesis of precursor monomers and their polymerization into macromolecules. The amount of microbial protein synthesis associated with cellular growth is proportional to the amount of carbohydrate fermented, provided there are no other nutritional constraints. Urea is recycled to the rumen via saliva or by diffusion across the rumen epithelium. Nitrogen recycling to the rumen serves to conserve N within the metabolic scheme of the ruminant whenever dietary N is limiting. Nitrogen is conserved because urinary excretion of urea is decreased when plasma urea concentration is low, thereby funneling urea to the rumen. When the rumen ammonia pool is large relative to the energy available to fuel the incorporation of ammonia into microbial protein, ammonia overflow results and leads to inefficient N retention. Protein reaching the small intestine for digestion and amino acid absorption is comprised of bacterial protein and dietary protein which escapes degradation in the rumen. Both sources of protein are used to meet the requirements of the host ruminant for maintenance and production.

Ingraham et al. (1983) listed the following general properties of a fermentation:

1) Almost all ATP is generated by substrate level phosphorylation.

2) Oxidative and reductive reactions occur in a fermentative pathway, but a strict oxidation-reduction balance is maintained. The average oxidation state of the products is the same as that of the substrate. Although some substrate carbon is assimilated into cell material, this amount is relatively small.

3) In order for both oxidation and reduction of the substrate to occur, substrates of fermentation are usually at an intermediate state of oxidation. The substrates of fermentation are usually sugars.

4) Most pathways of bacterial fermentation involve pyruvate as a metabolic intermediate, and the diversity of end products produced in various bacterial fermentations depends largely on the reactions by which pyruvate is metabolized.

5) Because the yield of ATP from fermentations is relatively low, large quantities of substrate are utilized when growth occurs as a consequence of fermentative metabolism. As a result, most of the carbon from the metabolized substrate can be recovered in fermentation products.

A thermodynamic constraint is placed on microbial cell growth by anaerobic, fermentative metabolism (Hungate, 1966). The amount of microbial protein which can be synthesized depends on the quantity of ATP derived from the energy substrate and the amount and nature of food derivatives which can be directly incorporated into cells. Respiration results in a far greater yield of ATP than fermentation. Whereas aerobic organisms (which carry out respiration of their energy substrates) can use as much as 60 to 70 % of their energy substrate to synthesis of cellular material, most anaerobes (which ferment their energy substrates) incorperate only 10 to 20% of the carbon derived from metabolized carbohydrate. For this reason, the extent of ruminal microbial protein synthesis is directly related to digestion, and subsequent fermentation, of organic matter in the rumen.

ATP generated during energy yielding reactions is used by the cell for maintenance and synthetic processes. The greater the proportion of ATP used for biosynthesis, the greater the yield of microbial cells (Pirt, 1975). Energy expenditure for maintenance is a function of the rate at which a microorganism grows (Stouthamer and Bettenhausen, 1973). Yield (YATP; grams of bacterial dry matter per mole of ATP) will approach a maximum as rate of growth approaches infinity and time for maintenance approaches zero. In vitro studies with mixed ruminal bacteria in continuous culture have established that a positive relationship between yield coefficients and growth rate exists (Isaacson et al., 1975). Factors which affect the efficiency of microbial protein in vivo include: 1) type of substrate 2) turnover rate of liquids and solids 3) preservation method (silages are associated with much lower yields than other forms of roughage), 4) synchronization of release of energy, rate

of growth and nitrogen degradation and 5) source of N for microbial protein synthesis (Orskov, 1982).

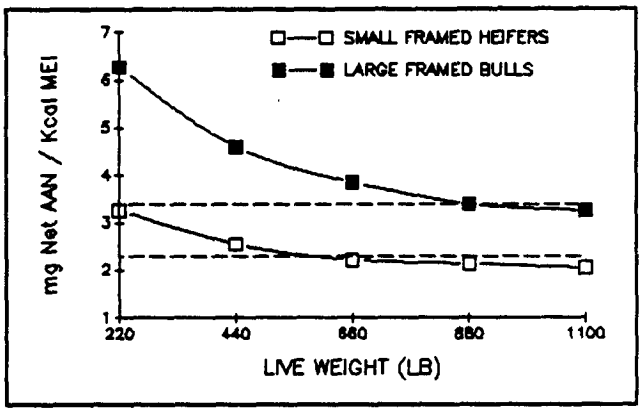
Key Concepts

Several key concepts enable understanding of the theoretical justification for balancing ruminant diets for protein using the metabolizable protein approach. In general, this approach involves calculation of the amount of microbial protein synthesized in conjunction with a given level of energy intake. An accurate yield coefficient (corrected for protein composition of the cell) is needed to accomplish this calculation. Microbial amino acid - N synthesized in the rumen is compared with total tissue needs (following estimation of digestibility of microbial protein and the efficiency of microbial amino acid - N absorption). When the quantity of microbial amino acid - N is greater than tissue requirements, then overall N requirement of the ruminant is equivalent to the amount of ruminally degraded N (ammonia) needed by rumen microorganisms. If, however, synthesis of microbial amino acid - N is less than tissue needs, then production can be maximized only by providing amino acid - N in the form of undegraded dietary protein. (Undegraded dietary protein, UDP, is used interchangably in this text with the term, "bypass" protein.) The metabolizable protein approach attempts to quantify amino acid - N absorbed by the small intestine and available to the tissue of the host ruminant. Application of this method requires knowledge about the level of energy intake, ruminal degradability of dietary protein and total tissue N requirement.

Effect of energy intake on the rate of protein retention has been reported by several authors (Balch, 1967; Andrews and Orskov, 1970) who demonstrated that optimum protein concentration in the diet of young ruminants is related to energy intake. A similar relationship between energy intake and milk production has been observed (NRC, 1988). The expression of an animal's protein requirements in relation to energy intake is fortuitous because microbial protein yield can also be expressed as a function of energy intake (Orskov, 1977); yield coefficient for ruminal microbes can be expressed in relation to digestible organic matter or TDN intake, or on a caloric basis as related to metabolizable or net energy intake. By superimposing microbial yield on ruminant requirements, it is possible to estimate the level of production achieved if microbial protein is the only source of protein available to the animal, as is the case when a semi-purified diet is fed containing NPN as the only source of N.

Orskov (1982) presented the effect of body weight on requirements of cattle growing at one kg per day (figure 1). The area between the dashed lines represents the range of net microbial amino acid-N observed with different types of diets under varying conditions. The requirement of bulls of large breeds for protein always is greater than the quantity of microbial protein synthesized in the rumen because of the high rate of protein deposition in these animals. However, the requirement for protein decreases with increased body weight. This is because the composition of gain contains an increasing proportion of fat, and because gain relative to maintenance becomes less in the larger animal such that food intake per unit gain is greater in the heavier animal (Orskov, 1980). Differences between the two types of cattle are extreme because large beef breeds have a greater genetic

Figure 1. The effect of live body weight on requirements of cattle growing at the rate of one kg per day. The area between the horizontal dashed lines represents the range of net amino acid-nitrogen contribution from microbial protein. The lower limit of microbial yield is representative of cattle fed silage diets whereas the upper limit is representative of cattle fed roughage diets. The microbial yield of cattle fed concentrate diets is intermediate to these two extremes. Adapted from Orskov (1982).



potential for protein deposition than do small breeds, as do bulls compared to heifers. Previous and current plane of nutrition also affect the rate of protein deposition at a given body weight. At lower rates of gain, protein requirements of steers and lambs are lower than they would be if gain was more rapid (Orskov, 1977). It has been concluded that protein supplementation with ruminal bypass protein is required to maximize growth of young, rapidly growing cattle up to approximately 500 lb (Orskov, 1977). Cattle weighing 500 to 800 lb may respond to bypass protein supplementation if the basal diet consists of silages or concentrates. Heavier feed-lot cattle are unlikely to respond to supplementation with bypass protein because microbial protein synthesis should be sufficient to meet the tissue requirements of the host, provided the microbial requirement for ruminally degraded protein is met. Thus, the diet of heavier steers should be balanced with ruminally degraded protein (RDP) to meet the requirements of the ruminal microbes for ammonia.

As with the growing ruminant, the adequacy of microbial protein to meet the protein requirements of the lactating dairy cow depends on level of production. In dairy cattle, the need for amino acid-N relative to ME intake is increased by high levels of production and extreme mobilization of body fat (Miller et al., 1977). The response of lactating cows to differences in protein degradability depends on the extent of negative energy balance in the cow (Orskov, 1982). Microbial protein synthesis should be adequate when cows are yielding no more than 20 lb of milk daily.

The effect of pregnancy on protein requirement of beef cows is illustrated in figure 2. Due to the relatively high energy requirement for fetal development, there is little increase in the requirement for protein expressed on an energy basis (Orskov, 1982); however, if the pregnant cows are fed at maintenance (as are many pregnant cows in Florida during the winter months), then a protein deficiency is imposed which increases with increasing stage of pregnancy. This point underscores the important influence that negative energy balance can have on the requirement of ruminants for supplementation with a bypass protein source.

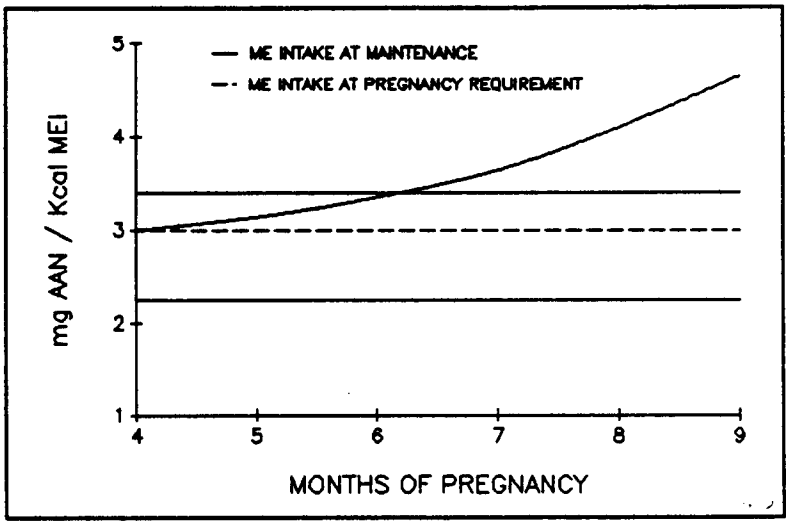


Figure 2. The effect of pregnancy in cows on the adequacy of microbial protein synthesized in the rumen to meet the protein requirements of the pregnant beef cow. The area between the horizontal solid lines represents the range of net amino acid-nitrogen contribution from microbial protein. The protein requirement is given for ME intake to meet the requirement of pregnancy and at maintenance. Adapted from Orskov (1982).

Digestion in the rumen is essential for feed utilization by the ruminant, and rumen microorganisms need RDP in the form of amino or ammonia N to optimize ruminal digestion. If insufficient RDP is available, the rate of digestion may be negatively affected. A reduction in feed intake also may be decreased (Owens and Bergen, 1983), or feed intake may be reduced.

NPN is utilized only as a source of RDP to meet the requirement of the rumen microbes. Thus, NPN supplementation is effective only if ammonia arising from NPN is incorporated into microbial protein. This point was demonstrated nicely by the classic work of Satter and associates (Satter and Slyter, 1974; Satter and Roffler, 1975). When incremental levels of urea were added to continuous cultures of mixed ruminal microbes, microbial protein synthesis increased linearly up to a point dependent upon the energy content of the diet. After this CP concentration, was reached, there was no further increase in the amount of microbial protein synthesized. Up until that same critical threshold CP concentration ammonia concentration in the fermentors remained low; however, ammonia concentration progressively increased at CP concentrations greater than the threshold level. Threshold concentration of CP was decreased when lower energy diets were used, and increased when higher energy diets were fed. Interpretation of these results led to the conclusion that, at low CP concentrations below the threshold CP concentrations, RDP (ammonia concentration) limited microbial growth and incremental addition of urea resulted in a proportional increase in microbial protein synthesis. At the threshold CP concentration, the requirements of rumen microbes for energy and ammonia were being met and energy and ammonia available to the microorganisms were in balance. As CP concentration increased above the threshold CP concentration, energy availability was limiting in relation to the ammonia supply. This resulted in ammonia accumulation in the fermentor.

Practical implications of this research are demonstrated in figure 3. With a diet that is approximately 80% TDN and CP concentrations greater than 12%, urea does not contribute to the metabolizable protein concentration of the diet. This is because RDP (ammonia) concentrations exceed the energy

available to fuel incorporation of ammonia into microbial protein. Above 12% CP, natural dietary protein source contribute to metabolizable protein only to the extent to which they are resistant to degradation within the rumen. The threshold CP concentration would be raised by an increase in energy content of the basal diet, or by factors which increase the efficiency of microbial protein synthesis (e.g., increased dilution rate). Likewise, a decline in the threshold CP concentration of 12% would result from a decrease in energy content of the diet or factors which decrease efficiency of microbial protein synthesis (e.g., dietary defficiency of a micronutrient).

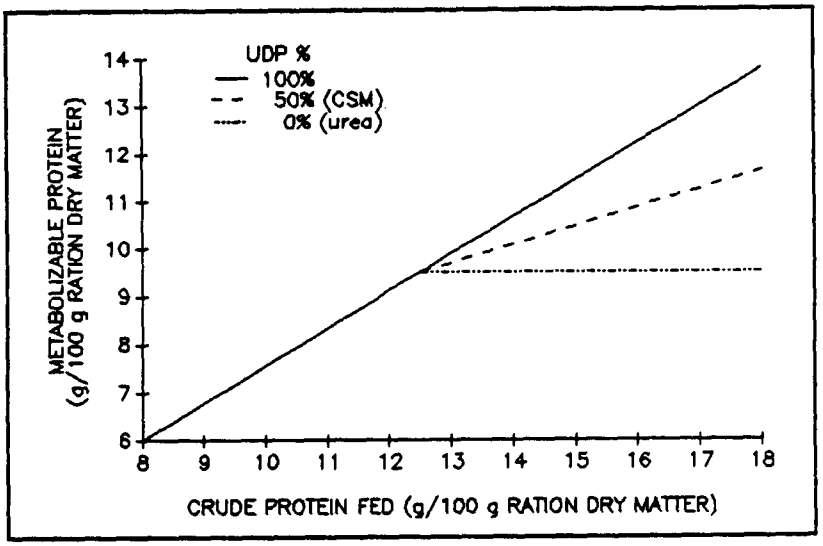


Figure 3. Effect of ruminal degradability of a protein source on the equivalency of crude protein and metabolizable protein for meeting the protein requirements of the ruminant (adapted from Satter and Roffler, 1977). Inefficient utilization of urea at CP concentrations greater than the threshold concentration of 12% results because insufficient energy is available to rumen microorganisms to fuel microbial protein synthesis. The basal diet represented in this figure is approximately 80% TDN.

Considerations Affecting Application of Metabolizable Protein Systems

Sound theoretical principles provide a rationale for development of metabolizable protein systems. Appreciation of these principles facilitates strategic decision making by the livestock producer and feed manufacturer i.e., just weaned cattle and high producing dairy cows are likely to respond to supplementation with a bypass protein source, whereas mature beef cows supplemented with molasses and feed-lot cattle in the terminal stage of finishing are not. Unfortunately, there are a number of considerations which affect absolute quantification of metabolizable protein in a diet. These considerations include:

1) Although fat contributes to the energy content of a diet, fat is not fermented in the rumen. Current metabolizable protein systems overestimate microbial protein synthesis with diets that have even a moderate concentration of fat.

2) Protein degraded in the rumen is not necessarily equivalent in capacity to support efficient microbial protein synthesis. Amino acids and peptides are taken up by many bacteria (Wallace and Cotta, 1988) and appreciable cellular incorporation of amino acids has been observed in vivo (Nolan and Leng, 1972). Amino acids and peptides increase the growth rate and yield of rumen bacteria

(Maeng and Baldwin, 1976; Argyle and Baldwin, 1989). RDP derived from natural protein sources may support greater microbial yields than urea, thus explaning the apparent superiority of soybean meal as a source of RDP when compared with urea (Polan, 1988).

3) A number of other factors also affect microbial yield coefficients. Reported microbial yield coefficients have a wide range. Deviation of the actual yield coefficient from the constant value used by current metabolizable protein systems (ARC, 1980; NRC 1985) markedly affects the accuracy of estimates of required UDP.

4) Different sources of UDP may offer substantially different amino acid profiles for absorption by the small intestine. Adequacy of a bypass protein supplement for meeting tissue requirements depends on how well the amino acids absorbed by the small intestine complement the specific amino acid requirements of the host animal. These may change due to physiological state and level of production.

5) The data base specifying ruminal degradability of commonly used protein sources under specific conditions is not complete. Methods for determining ruminal degradability have not been standardized.

6) Treatments that increase resistance of natural proteins to ruminal degradation may also increase the resistance of those proteins to digestion in the small intestine. Some treatments suffer in their ability to reproducibly affect ruminal degradability.

7) Over-reliance on protein sources that possess a large proportion of their total protein concentration as UDP can result in an inadequate supply of RDP to meet the requirements of the rumen microflora. Decreased digestibility or intake may result from overestimation of ruminal degradability of dietary protein.

Conclusion

Although limitations affect the ability of the new metabolizable protein systems to quantify the absolute amount of amino acids absorbed from the small intestine, there are several important advantages to these systems. They enable a more accurate evaluation of the contribution made by NPN to the protein nutriture of the ruminant. They also recognize the need to supplement with bypass protein when the requirement of the host animal for protein is greater than the contribution of rumen microbial protein. Orskov and Macleod (1982) nicely summarized the current status of protein evaluation for ruminants:

> "No doubt, all research workers who have been involved in the formulation of changes in the evaluation of different protein sources for ruminants have been aware of weaknesses due to inadequate data, and it could well be argued that the systems they suggested were 'premature'. It is often difficult to decide just when a change to practical systems of protein evaluation should be

introduced as a result of better understanding. However, if the new concepts are based upon a sound logic according to new knowledge, and if the current system of evaluation is no longer adequate, then the introduction of a new system can be justified, even if the data upon which it is based are not complete, provided that:

1) Existing data, when re-analysed by the new system, predict the animal response at least as well as the old system.

2) The new system encourages new data to be generated by the new concepts proposed.

3) The new data can be readily incorporated to improve the precision of the system."

Bibliography

Andrews, R.P. and E.R. Orskov, 1970. The nutrition of the early weaned lamb. II. The effect of dietary protein concentration, feeding level and sex on body composition at two liveweights. J. Agric. Sci. 75:19.

ARC. 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureau, Slough, U.K.

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

Fox, D.G., C.J. Sniffen and P.J. Van Soest. 1982. A net protein system for cattle : Meeting protein requirements of cattle. P 280. In Protein Requirements for Cattle : Proceedings of an International Symposium, F.N. Owens, ed. MP-109. Stillwater, Okla: Division of Agriculture, Oklahoma State University.

Hungate, R.E. 1966. The Rumen and its Microbes. Academic Press, New York.

Ingraham, J.L., O. Maaloe and F.C. Neidhardt. 1983. Growth of the Bacterial Cell. Sinauer associates, Inc., Sunderland, U.K.

Isaacson, H.R., F.C. Hinds, M.I. Bryant and F.N. Owens. 1975. Efficiency of energy utilization by mixed rumen bacterial in continuous culture. J. Dairy Sci. 58:1645.

Maeng, W.J. and R.L. Baldwin. 1975. Factors influencing rumen microbial growth rates and yields : Effects of urea and amino acids over time J. Dairy Sci. 59:643.

Miller E.L., C.C. Balch, E.R. Orskov, J.H.B. Roy and R.H. Smith. 1977. Comparison of calculated N requirements for ruminants with the results of practical feeding trials. P. 137. In: Proceedings of the Second International Symposium on Protein Metabolism and Nutrition, the Netherlands, S. Tamminga ed. Centre for Agricultural Publishing and Documentation, Wageningen, Netherland.

Nolan, J.V. and R.A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Brit. J. Nutr. 27:177.

NRC. 1988. Nutrient Requirements of Dairy Cattle, Sixth Edition. National Academy Press, Washington, D.C.

NRC. 1985. Ruminant Nitrogen Usage. National Academy Press, Washington, D.C.

Orskov, E.R. 1976. Factors influencing protein and non-protein nitrogen utilization in young ruminants. p. 457. In: Protein Metabolism and Nutrition, D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan, ed. Butterworths, London, U.K.

Orskov, E.R. 1977. Dietary protein energy relationships for growth in young ruminants. p. 110. In: Proceedings of the Second International Symposium on Protein Metabolism and Nutrition. The Netherlands, S. Tamminga, ed. Center for Agricultural Publishing and Documentation, Wageningen, Netherland.

Orskov, R.R. 1980. Possible nutritional constraints in meeting energy and amino acid requirements of the highly productive ruminant. p. 309. In: Digestive Physiology and Metabolism in Ruminants, Y. Ruekebusch and P. Thivend, ed. AVI Publishing Company, Inc., Westport, CT.

Orskov, E.R. 1982. Protein Nutrition in Ruminants. Academic Press, New York

Orskov, E.R. and N.A. Macleod. 1982. Validation and application of new principles of protein evaluation for ruminants. p. 76. In: Protein Contribution of Feedstuffs for Ruminants, E.L. Miller, I.H. Pike and A.J.H. Vans, ed., Butterworth Scientific, London, U.K.

Owens, F.N. and W.G. Bergen. 1983. Nittogen metabolism of ruminant animals: Historical perspective, current understanding and future implications. J. Anim. Sci. 57 (Supplement 2):498.

Pirt, S.J. 1975. Pricibles of Microbe and Cell Cultivation. John Wiley & sons, New York.

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

Prins, R.A. 1977. Biochemical activites of gut microorganisms p. 73. In: Microbial Ecology of the Gut, R.T.J. Clarke and T. Bauchop, ed, Academic press, New York.

Satter, L.D. and L.L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Brit. J. Nutr. 32:199.

Satter, L.D. and R.E. Roeffler. 1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

Satter, L. 1982. A metabolizable protein system keyed to ruminal ammonia concentration - The Wisconsin System. p. 245. In: Protein Requirements for Cattle: Proceedings of an International Symposium, F.N. Owens, ed. MP-109. Stillwater, Okla: Division of Agriculture, Oklahoma State University.

Stouthamer, A.H. and C.W. Bettenhausen 1973. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. A re-evaluation of ATP production by measuring molar growth yields. Biochem. Biophys. Acta. 301:53.

Trenkle, A. 1982. The metabolizable protein feeding standard. p. 238. In: Protein Requirements for Cattle: Proceedings of an International Symposium, F.N. Owens, ed. MP-109. Stillwater, Okla,: Division of Agriculture, Oklahoma State University.

Van Soest, P.J., C.J. Sniffen, D.R. Mertens, D.G. Fox, P.H. Robinson and U. Krishnamoorthy. 1982. A net protein system for cattle: The rumen submodel for nitrogen. p. 265. In Protein Requirements for Cattle: Proceedings of an International Symposium, F.N. Owens, ed. MP-109. Stillwater, Okla: Division of Agriculture, Oklahoma State University.

Wallace, R.J. and M.A. Cotta. 1988. Metabolism of nitrogen-containing compounds. p. 217. In: The Rumen Microbial Ecosystem, P.N. Hobsen, ed. Elsevier Applied Science, London, U.K.