Philosophical and Scientific Background for AminoCow®, the Mepron Dairy Ration Evaluator, Version 3.5, May 2006

R. A. Patton[1], W. Heimbeck[2], M. J. Stevenson[3] and R. J. Brinkman[4]

[1] Nittany Dairy Nutrition, Inc, Mifflinburg, PA, USA [2] Degussa AG, Hanau, Germany [3] Degussa Canada, Burlington, Ontario, Canada [4] Unique Software, Inc, Lewisburg, PA, USA

Introduction

AminoCow, the Mepron Dairy Ration Evaluator, was created in response to industry requests for a program that could easily integrate amino acid nutrition for dairy cattle within other practical nutritional considerations. Our goal was to create a practical program based on sound nutrition research and yet tested on commercial farms.

In any endeavor like the AminoCow ration program, there are numerous assumptions that must be made and more weight placed on some studies than on others. It is altogether fitting that these assumptions be challenged and the biases, which go into a work such as AminoCow be explained. As Dr. Robert McCarthy, a renowned ruminant physiologist, was fond of saying: “Always question the assumptions. The weaknesses always lie with the assumptions.” Only when our assumptions have been fully challenged will our logic in constructing the AminoCow program be found wanting. And later, when there are adequate studies, a more accurate model can be produced. We want to emphasize the words “more accurate” because in any area of science where active research is on going such as dairy cattle nutrition, the state of knowledge is in a constant state of flux. Any nutritional requirement and/or calculation of nutrient delivery must be accompanied by knowledge of the newest advances in the field and tempered by common cow sense.

The primary purpose of AminoCow is to provide an educational resource regarding amino acid nutrition of dairy cattle as well as to provide an easy, accurate method to balance rations for amino acids within the confines of other nutrients. Many people may have the opinion that AminoCow was written purely as a commercial venture in order to sell more Mepron®, Degussa’s brand of rumen stable methionine. Others who have used earlier versions of the Mepron Ration Evaluator may believe the present program recommends more methionine than is necessary.

In both cases nothing could be further from the truth.

The amino acid requirements have not changed between versions of the Mepron program. However, database methionine content for some feeds has been revised based on new information. Also, the amount of methionine contributed by microbial synthesis may have changed depending on the quality of the forages and the availability of rumen degradable protein. But these are the only two changes and can both be supported with scientific literature.

The authors as a group believe that if working nutritionists had an easy way to balance amino acids in the diets of dairy cattle, they would find that methionine, lysine and possibly some other amino acids are limiting production as they have been shown to limit production in other species. In this case nutritionists may choose to add some rumen protected amino acids as a method of providing adequate amino acid nutrition. At least some of these amino acids might be Degussa products. In this way it is a commercial venture. However, please be assured the thrust of AminoCow has always been to be an educational vehicle. The emphasis has always been on the complete nutrition of the cow, it has not been centered on amino acids. Additionally, there have been no assumptions made regarding the “recommendations” for methionine that have not been made for other essential amino acids. All assumptions have been made in light of the best scientific information available and cost efficient production.

Requirements

In a recent conference, the discussion turned to the difference between requirements and recommendations. The general consensus of the group was that a nutrient requirement was the bare minimum necessary to maintain animal health and to ensure productivity and reproductive function, while a nutrient recommendation was the amount necessary to ensure adequate production in two standard deviations of an animal population. This struck us as an eminently important distinction: that is to say, the same amount of a nutrient sufficient to sustain life may not be sufficient to maintain adequate levels of production in commercial dairying. The amount by which the requirement and the recommendation differ is due to our lack of knowledge of the nutrient, our inability to adequately predict the delivery of the nutrient in the diet and/or the biological variation of the animals.

In this regard, we prefer to think of the requirements presented in AminoCow as recommendations. Although most of these “requirements” have been developed by the rigors of published scientific trials, many of them have grown out of nutritional practice on commercial dairy farms. This is a hard school, and the referees are not always knowledgeable about nutrition, but the criteria are simple: produce the most at the lowest possible cost while maintaining the health and reproductive function of the animal. This implies there must be compromises between cost and performance. We have tried to reflect this concept in the AminoCow program.

Our own research has confirmed that of our institutional colleagues and Degussa competitors. The fact is that methionine and lysine are either first limiting or co-limiting and must be in the proper proportion. Sufficiency of one essential amino acid without the others is rather futile. Likewise supplying perfectly balanced amino acids without adequate carbohydrates, fiber and minerals will raise only the cost of the ration. For these reasons we urge you to study the following discussion. We will attempt to point out what is known from what is conjecture about dairy cattle nutrition. This will enable you to judge how much we biased the model.

General Considerations—Factors Included in the Model

Many factors need to be considered when designing a model of the dietary requirements and feed intakes of dairy cattle. Dietary and environmental factors that are included and those that are de-emphasized determine to a large degree the relative robustness of the model. The relative completeness of available data to put into the model also helps to determine the accuracy and the ease of use. It is desirable to add as many factors as are clearly understood and predictable to increase both the accuracy of prediction and the robustness of the model against a wide range of conditions. However, the more factors introduced into a model, especially those that have not been rigorously tested, the greater is the chance of errors that bias the prediction. Thus, the model may be robust against many situations, but the accuracy of prediction suffers. But more simply, one can overfeed nutrients so that in almost all instances there will be no nutritional deficiencies, but this reduces the usefulness of the model and increases the cost. In the AminoCow model, unless a factor could be clearly shown to have a major impact on the calculation of nutrient flow, there was no attempt to describe the factor mathematically.

General Considerations - Data Sources

Early on it was decided to use only data from dairy cattle experiments in the development of AminoCow insofar as this was possible. This allowed for great simplification of the model. Many factors, which have large significant correlations across species (i.e., sheep and cattle) and types of animals (i.e., beef steers and dairy cows), become much less significant when considered within types of dairy cattle. Limiting the data to studies with dairy cattle significantly lowers the number of factors that must be modeled. Three types of dairy animals are considered: milking cows, growing heifers and dry cows. Within each of these groups, the assumption was made that dairy breeds were similar in carbohydrate and protein metabolism on a unit of metabolic body weight basis, although frankly, definitive data is lacking. For all nutrients, requirements are based on body weight, body condition and dominant metabolic state of the animal. AminoCow recognizes 5 metabolic states: calves on milk, growing heifers, dry cows, fresh cows and milking cows.

Assumptions - Fiber, RDP and NFC

In the development of the AminoCow model, we also assumed that within types of cattle and production limits, diets would contain the NRC (1989) recommended requirements for NDF and ADF within four percentage points on a dry matter basis. We decided to use the 1989 NRC for fiber because these requirements have produced acceptable production results over a wide range of management conditions. Also, these values encompass the range of dietary fiber normally encountered in commercial dairy rations and make the problem of prediction of dry matter intake easier and perhaps a bit more accurate. This also limited the effects of rumen pH so that pH would not greatly affect predictions of feed intake and digestibility since many studies on dry matter intake and digestibility exist in the literature using these requirements. Most recommendations found in AminoCow are designed to optimize fiber utilization and prevent problems such as lactic acidosis, bloat and digestive upset from occurring. Further, it was assumed acceptable feeding practices and minimum and maximum recommended inclusion rates would be respected. We also expected normal levels of rapidly and slowly degradable intake protein and sufficient fermentable carbohydrate would be added to rations. If adequate RDP for ammonia production is included in the diet without overfeeding non-protein nitrogen sources, then sufficient amounts of ruminally available peptide and free amino acids are also assumed to be available.

A most important consideration is that all metabolic events are described at steady state. By this we mean that feed consumed equals animal performance minus the undigested portion. While this is rarely true on a daily basis, it must approximate being true for the long term (weekly and monthly), or it would be impossible to sustain performance.

Carbohydrate Nutrition of the Dairy Cow

It is the underlying thesis of the AminoCow model that carbohydrate supply and digestion (not only NDF but also starch, sugar, pectins and ß-glucans) affect productive capacity and ultimately affect metabolizable protein and amino acid nutrition. It is the interdependence of these two factors coupled with the changes imposed by the rumen microbial population that makes dairy nutrition both unique and frustrating.

In many ways it is misleading to discuss the protein requirements of the dairy cow in the absence of requirements for carbohydrates and energy (Clark et al., 1992). Increased energy and greater productivity drive the requirement for amino acids higher. The interaction of amino acids and glucose as metabolic fuel for the cow, particularly immediately postpartum, must also be considered (Overton, 1998). Therefore, we will briefly describe carbohydrate nutrition in the dairy cow in relation to aspects of AminoCow.

It has been known for years that increasing grain feeding (i.e., increasing starch) resulted in increased growth rates and milk production. For many years this production improvement was assumed to be related to the increased energy content of the diet. However, it is increasingly clear that at least some of this increase is due to the type of metabolic fuel produced by the addition of starches and sugars (i.e., propionic acid) as well as the increase in microbial protein yield.

In the mid 1970's, it became clear that feeding excessive amounts of grain (i.e., starch) could lead to digestive upsets and founder (Wangsness and Muller, 1981). It then became clear that a cow had a minimum as well as a maximum fiber requirement. In AminoCow this fiber requirement is expressed in two ways - as ADF (cellulose and lignin) and NDF (cellulose, lignin and hemicellulose). In our view, NDF is the best measure of the total fiber in terms of requirement or supply. Because forage NDF is a more effective fiber source than non-forage NDF (Allen, 1997), the amount of NDF provided by forage is an aid in determining the sufficiency of the fiber requirement. In AminoCow this is included as an evaluation tool.

Because ADF is a more resistant fraction due to the high levels of lignin, we believe in its use as a measure of mat-forming ability. In this instance the amount of ADF, not a percent, is a more appropriate measure. It is known that cows fed diets based on alfalfa and corn grain can produce normal amounts of milk and milk components on diets of lower NDF content than when the diet is based on corn silage and high moisture grain (NRC 2001). Interestingly a calculation of ADF tends to be the same for both types of diets.

Adding supplemental starch and sugar to diets of dairy cows alters rumen fermentation (Poore et al., 1993) producing increases in propionic acid production with a lesser proportion of acetic acid. Excess levels of starches are associated with decreased fiber digestion, depressed feed intake and depressed milk fat percent and digestibility (Piwonka and Firkins, 1996). Perhaps this is due to an increase in ruminal lactic acid levels and a decrease in rumen pH. However, even on high starch diets increasing the amount of sugar increases the milk protein percent as well as milk yield (Reis and Combs, 2000).

Increases in ruminally available carbohydrate increase the use of NH_3 (Stern et al., 1978). In cases where rumen ammonia levels are less than adequate, competition between starch and cellulose digesters for NH_3 may explain the observed depression in ruminal cellulose digestion. This is in contrast to the suggestion that the reduced cellulose digestion is due to a pH depression because the depression in cellulose digestion occurs whether rumen pH is depressed or not. It is certain that microbial protein synthesis is controlled by the rate of carbohydrate digestion and NH_3 availability (Hoover and Stokes, 1991).

There has also been much discussion on the contribution ruminally undegraded starch makes to the energy efficiency of dairy cattle. It is assumed that starch digested in the small intestine is more energetically efficient than starch digested in the rumen. However, adding more rumen by-pass starch has resulted in greater FCM yield in some studies, no responses in others and in still others a decrease in FCM production. While there is a large difference in the amount of starch that can bypass the rumen, older literature (Bergen, 1978) suggests that a limit of approximately 1 kg of starch can be digested in the small intestine. Knowlton et al. (1998) in feeding experiments with corn-alfalfa silage diets (55% grain:45% alfalfa silage) found starch sources that were more completely fermented in the rumen (i.e., high moisture corn) also had the highest rate of small intestinal digestibility. In general, it appears that starch that is not fermented in the rumen is also poorly digested in the small intestine. In their experiments only 23% of the starch from dry corn was fermented in the large intestine, while up to 25% of the starch passed into the manure.

The extent to which the animal can capture VFA from the large intestine, which is probably limited, determines the energy efficiency of these starch sources. These same authors postulated that in order for starch to be digested in the small intestine, the crystalline structure of the starch granule had to be destroyed. Reynolds et al. (2001), infusing starch, which was solubilized and therefore no longer crystalline through duodenal canulas, were able to infuse up to 2000 g of starch per day without any loss of starch digestibility. This was approximately the same small intestinal starch flow as used by Knowlton et al. (1998). These experiments suggest that starch needs to be solubilized in the rumen before it can be adequately digested in the small intestine. Because starch that is solubilized in the rumen will be readily available to rumen organisms, these studies suggest that there is little starch digested by the small intestines.

Interestingly, the increase in starch entering the small intestine in the studies of Reynolds et al. (2001) did lower the digestibility of nitrogen. However, neither FCM yield nor milk protein percent were affected by starch infusion in this experiment, although tissue energy retention increased.

It is apparent that not only the amount of starch and sugar contributed to a diet must be modeled, but also the physical form of this carbohydrate, both in regard to source and processing. In the program both a processing factor and a starch fermentability factor have been included, although they have not been linked mathematically.

Non-Fiber Carbohydrate Fractions

There has been a general consensus among working nutritionists, even if not completely embraced by the academic community, that carbohydrate fractions other than fiber are of particular importance for high milk production. It is also increasingly clear that non-fiber carbohydrates (NFC) really do not describe the nutritional effectiveness of these fractions sufficiently. To reflect these important differences in AminoCow, we have divided the NFC according to the scheme proposed by Hall (2000). That is, we have divided the NFC to include these nutritionally relevant fractions: sugars, starch, volatile fatty acids, and fiber fractions soluble in neutral detergent solution. The NDF soluble fractions include primarily pectins and ß-glucans. All of these fractions ferment rapidly and relatively completely but have different nutritional consequences. Sugars and starches ferment to propionic acid, while neutral detergent solubles (SolND) ferment mainly to acetate. Also while sugars, starches and neutral detergent solubles do contribute to microbial growth, VFAs do not.

The relative amount of these carbohydrate fractions also influences the need for rumen degradable protein, the amount of microbial protein synthesized, the amount of rumen by-pass protein and the amino acid quality of this protein, which must be added to fulfill the needs of the animal. Just as important, the amount of sugar and starch as well as their fermentability determines the amount of glucose precursor produced. Glucose (Herdt, 1988) has been found to be the most limiting nutrient for high producing cows. Through its conversion to lactose, glucose indirectly ends up being the osmotic regulator of milk production. For this reason we have added a calculation of the amount of glucose precursor produced (GluPre) to AminoCow.

The majority of the sugar, starch and VFA values used in the feeds database were obtained from Cumberland Valley Analytical Services through the cooperation of Ralph Ward. This data set gave us access to literally thousands of samples for which starches and sugars had been determined using the same methodology in the same laboratory. Our studies (Ward et al., 2003a; 2003b) established that starch and sugar content of forages displayed a wide variability and, except for the starch values of high starch silages (corn, barley, sorghum and wheat silages), were unpredictable. Grains varied only slightly in starch and sugar, but these values do seem to depend on grain maturity.

In AminoCow we have calculated NFC as it is calculated in 2001 NRC:

$$\text{NFC (\%DM)} = 100 - [\text{NDF (\%DM)} + \text{CP (\%DM)} + \text{EE (\%DM)} + \text{ash (\%DM)}]$$

This equation is not universally accepted. An alternative equation makes the correction for nitrogen and/or ash contained in the NDF. Although this may be a more accurate measure of NFC, many laboratories around the world do not have the ability to accurately determine the NDIN and soluble ash necessary to make this calculation. Therefore, we have accepted the definition of NFC, which was easier to determine and more widely accepted.

We have calculated the amount of SolND by the following formula:

$$\text{SolND (\%DM)} = \text{NFC (\%DM)} - [\text{starch (\%DM)} + \text{sugar (\%DM)}]$$

It should be noted that VFA does not enter into this calculation. Although most feed analyses are performed on a dry matter basis, this is impossible with VFA analysis. They are determined on a wet basis because during the drying process, significant amounts of VFA are lost due to volatilization. However, most of the lactic acid does remain after drying. Thus, the SolND fraction can be incorrect by the amount of lactic acid remaining in the silage. Normally this is an inconsequential error. However, lactic acid can be used by some microorganisms to form propionate as well as used by ruminant liver to form glucose. Thus, the amount of glucose potentially formed by the diet may be underestimated by the amount of lactic acid in the diet. On all silage diets this could amount to up to 4 - 6% of the dry matter.

Glucose precursor as used in the AminoCow program is given by the formula:

GluPre = sugar (%DM) + [starch (%DM) * % fermentability]

We have employed this usage because as summarized above glucose is the nutrient most likely to be limiting for high producing cows. Also, starch not digested in the rumen may make only a small contribution to the glucose economy of the cow.

Assumptions of Carbohydrate Use and Recommendations

AminoCow always assumes that either ADF or NDF (preferably both) are balanced close to the amount recommended by the AminoCow program. The recommendations for these fiber amounts were taken from the 1989 edition of NRC. Fiber is required: (1) to provide an environment for rumen microbes and, hence, microbial protein synthesis, (2) to maintain rumen motility, rumination, salivary flow and therefore, pH balance, and (3) to obtain nutrients, principally energy in the form of VFAs, and protein. AminoCow provides various methods of looking at the adequacy of fiber on the “Nutritional Ratios” screen, including Forage:Concentrate Ratio, Forage NDF as % of total NDF, NDF intake as a % of Body Weight, Forage NDF as a % of DM, and Total Forage ADF (weight). Each of these measures is useful in assessing the adequacy of the fiber in the diet, but none of them is sufficient of themselves.

AminoCow also assumes that the other carbohydrate fractions (NFC and its components starch, glucose and SolND) are reasonably well balanced as discussed below, ensuring that glucose is not lacking for milk synthesis.

While we believe the trends in response to feeding various levels of starch and sugar discussed above are correct, there is insufficient data to make firm recommendations. At least part of this is due to different techniques employed for measuring both starch and sugar (Hall, 2000). Therefore, what might be a level of 8% starch for one technique may yield 14% for another, for example. There is also considerable variation among authors when it comes to the amount of starch that is rumen degradable in various feeds (Theurer et al., 1999). For these reasons we have avoided requirement recommendations. True requirements need to await standardization of methodology.

Notwithstanding, if one uses the default values for starch and sugar for feeds found in the AminoCow program, we would recommend levels from 22.0 - 26.0% starch and 3.5 - 5.5% sugar as a percent of dry matter for high producing cows. This would equate to approximately 18% rumen fermentable starch and a glucose precursor content of 18 - 24% of DM. Obviously, this recommendation will depend on the extent to which the program starch and sugar values are similar to the feeds that are being fed. Because the accuracy of prediction of starch and sugar is low, they do need to be analyzed (Ward et al., 2003a; 2003b). Therefore, we urge nutritionists to analyze starch and sugar values with one laboratory, to modify the content using these values and to track the response to different levels to determine for themselves the correct level to use given individual laboratory analyses.

Protein Nutrition in the Dairy Cow

Traditionally ruminant nutrition has emphasized the role of total nitrogen intake (crude protein or N*6.25) in ration formulation. Over the past two decades, there has been increasing emphasis on use of protein fractions for balancing rations. This has led to the popular belief that there is a requirement for both crude protein (CP) and rumen undegradable protein (RUP) (Chandler, 1994). A summary of cow production studies does not support the concept of a requirement for RUP (Santos et al., 1999). In our opinion, there is no evidence of a RUP requirement.

As far as the animal is concerned, her requirements are for essential amino acids (EAA) and for metabolizable protein (MP) (Clark et al., 1992). In this context, MP is defined as the total amount of amino acids (both essential and non-essential) needed for body functions. The sources of these amino acids are microbial protein, feed protein that escapes rumen degradation and endogenously secreted protein. Neither CP nor RUP describes these complex relationships. The microbial population shows a preference for ammonia as a nitrogen source. In fact, many rumen microbial species require NH_3 but are able to make use of (and may require) both peptides and amino acids (Argyle and Baldwin, 1989). It is obvious then, that there are two types of nitrogen requirements for the ruminant animal: a microbial requirement for ammonia and an animal requirement for metabolizable protein and essential amino acids.

Protein Definitions

A large amount of confusion has developed because of failure to grasp the exact meaning of protein fractions. Thus, working nutritionists have often confused and been confused by exactly what is meant by this terminology.

As used in the AminoCow program *metabolizable protein* (MP) or *absorbed protein* is the total amino acid that has been absorbed from the intestine and is ready for further metabolism. MP by definition includes two metabolic processes: (1) the work of breaking protein into its component amino acids and/or small peptides and (2) the work of absorbing these amino acids and small peptides across the intestinal wall. Taken together these processes constitute the true digestibility.

The amino acids that make up MP come from three sources: (1) microbially synthesized amino acids, (2) feed amino acids, and (3) endogenously furnished amino acids. The amino acids in MP are of two types, the essential or indispensable amino acids, and the amino acids, which can be synthesized by the animal, termed the non-essential amino acids. Endogenously furnished amino acids have two main sources: tissue sloughing from the rumino-reticulum, omasum and abomasum as well as the small intestine and amino acids from the addition of enzymes and hormones that function in the work of digestion. Given the techniques available today, MP is not measurable, it must be calculated.

Given that we cannot measure MP, what protein fractions can be measured and what use can be made of them?

For all its inherent analytical problems, the amount and type of true amino acid flowing to the duodenum can be measured. This measurement is the flow of amino acids potentially absorbable from the small intestine and is termed *absorbable protein* (AP). These amino acids are distinguished from absorbed amino acids that are termed MP. Neither the intestinal digestibility nor the sources of these amino acids can be determined from measurements of the duodenal flow. AP should be looked upon as the total protein flowing to the small intestine and is potentially digestible or potentially metabolizable.

The general procedure for determining the source of the amino acids in AP is to calculate how much of this absorbable protein is of microbial origin using factors for microbial markers and applying an assumed average composition of rumen microbial protein. The total amino acids of microbial origin are then subtracted from the total amino acids to calculate a fraction that is composed of feed plus endogenous protein amino acids. With present techniques it is impossible to separate feed amino acids from endogenously secreted amino acids. In practice the endogenously secreted amino acids are calculated using assumed rates of secretion per unit of intake. The calculated endogenous amino acids are then subtracted from the feed plus endogenous pool to calculate the amino acids supplied by feed proteins.

It is also worth a moment to discuss various aspects of apparent digestibility. First, it is necessary to make a distinction between ruminal and intestinal digestion. We propose using the term “degradation” for all ruminal digestion. We suggest nutrients that reach the small intestine and that are absorbed directly from it should be considered truly digested.

The result of rumen degradation is a modification of the feed presented to the intestine for absorption. Especially concerning amino acids, the work of digestion occurs in the small intestine. This is not to trivialize the degradation in the rumen. Obviously, fiber not degraded in the rumen will not be digested in any portion of the digestive system. Likewise, starches not degraded in the rumen may not be digested in the small intestinal tract. However, all amino acids, fats and minerals must be absorbed (digested) from the small intestine. Although there is some debate, most studies indicate that absorption of these nutrients from the large intestine is essentially nothing.

It is also necessary to distinguish between measures of digestibility. This is really an important distinction only in the digestion of amino acids, fatty acids and some minerals. The most common measure of digestion is *apparent digestion*. Apparent digestion is calculated by measuring the amount of nutrient that has been eaten and subtracting the amount of nutrient isolated in the feces. The difference between these measurements is the amount of nutrient apparently digested. There are three problems with this technique: (1) There is no measure of the effects of rumen fermentation and rumen degradation on the flow of nutrients. (2) There is no measure of the contribution of endogenous secretion to the nutrient flow. (3) Secondary fermentation in the cecum and large intestine can alter the amount of nutrient isolated in the feces.

A much better measure is true digestibility. This method requires not only measuring the amount of nutrient entering the duodenum, but also of cannulating the mesenteric vessel of the intestine to measure increases in nutrient flow from the intestine. This technique should be coupled with a radiolabeled assay to determine the amount of nutrient used by the intestinal cells. In calculation, the *true digestibility* is the sum of the nutrient entering the mesenteric blood supply and the nutrient used by the intestinal tissue. See Clark et al. (1992) and Lapierre et al. (2000) for a complete review of the effects of degradation and digestibility on MP and amino acid nutrition in the dairy cow.

This true digestibility is what is important for the nutrition of the animal. However, as one might expect, there is almost no data on the true digestibility of amino acids. This is a very intensive and time-consuming technique. There are two methods that have been used to estimate the digestibility of amino acids. One is to use the apparent digestibility, the other is to use limited data on the digestibility of one or two radiolabeled amino acids and apply it to all amino acids. The first method yields an estimate of approximately 80% for all amino acids with a relatively low standard error. The second method produces a wide range of estimates with a very high standard error. Whether the latter estimates are truly widely variant or whether the difference is due to experimental error cannot be determined at present.

In summary, what is measurable is absorbable protein (potentially absorbed), but what we are interested in predicting is metabolizable protein (absorbed protein), which cannot be measured with present techniques. Likewise, there is a large database existing on apparent digestibility, but what we are interested in is true digestibility, which is difficult and expensive to measure.

Lastly, in monogastric species it is known that the amount of MP required varies with the quality of amino acids. This is to say, less MP is required if the amino acid profile matches the amino acid profile required by the dominant metabolic function of the animal. If MP has an unbalanced amino acid pattern, a greater quantity of MP will be required. An amino acid pattern that exactly matches the amino acid needs of the animal is termed the “ideal protein.” In order to minimize the amount of MP required, the MP must be the “ideal protein.”

The protein part of the AminoCow model was developed with these criteria in mind: (1) to predict the need for total metabolizable protein and essential amino acids for the animal, and (2) to predict the need for sufficient ammonia (i.e., rumen degradable protein or RDP) in the rumen. This approach was taken with the goal of maximizing rumen microbial protein synthesis. Maximizing microbial protein normally ensures both maximization of rumen carbohydrate digestion and minimization of protein cost. However, this is not always true. Depending on diet ingredients and market prices, it may not always be profitable to obtain the last increment of microbial protein. Rather, it may well be better, from both an economic and environmental point of view, to reduce the amount of microbial synthesis and add rumen undegradable protein to ensure amino acid sufficiency for the animal. The decision of whether to maximize microbial protein or not is ultimately an economic one.

AminoCow uses the traditional method of NRC for apportioning amino acids and metabolizable protein. That is, they are used for maintenance, growth and pregnancy. We have also added an additional category—repletion of body condition. This is especially important for heifers that have been undergrown or for early lactation cows that have lost body condition. The sum of the amino acids needed for these metabolic functions equates to the MP and amino acid requirement.

Basic Assumptions of Protein Metabolism

AminoCow assumes that all protein accretion is due to the balance between protein synthesis and protein breakdown. Both processes are occurring continually (Garlick, 1980). When protein synthesis exceeds breakdown, tissue protein is accumulated. When breakdown exceeds synthesis, protein mobilization occurs. The majority of the amino acids from the process of protein breakdown are recycled into newly synthesized protein. All calculations of protein accretion are based on metabolic body weight. Twenty percent of metabolic body weight is assumed to be protein (MacRae, 1989; Rohr and Lebzien, 1991).

In the AminoCow model, protein synthesis is always understood to be under the control of the energy-producing processes of metabolism. In this case both synthesis and breakdown are energy-consuming processes and require about 0.3 Mcal or 1.25 MJ per kg of protein to maintain protein equilibrium. Protein accretion requires 0.6 Mcal or 2.50 MJ/100 g of new protein (MacRae, 1989). Because of the relationship between energy and protein utilization, AminoCow assumes that amino acids are utilized with the same affinity with which energy is used. That is, maintenance is nearly as efficient as lactation, which is more efficient than growth. Therefore, utilization of amino acids for maintenance is more efficient than for lactation, which is more efficient than growth. Because the reproduction requirement is largely a process of fetal growth, amino acids destined for fetal growth are assumed to be used with the same efficiency as growth.

Metabolizable Protein and Amino Acids Requirements for Maintenance

Maintenance requirements for amino acids are divided into two categories: the splanchnic bed (portal drained viscera [PDV] plus the liver) and extra hepatic tissues (Reynolds et al., 1994). In a manner similar to glucose, the PDV utilizes amino acids to a significant amount both as protein precursors and as energy sources with non-essential amino acid use predominating. The degree to which the PDV uses amino acids is apparently related to the dry matter intake and the energy content of the diet. Greater dry matter intake and higher energy in the diet cause greater utilization of amino acids by PDV. Studies with growing heifers and steers indicate that between 25 and 55% of the amino acids absorbed from the lumen of the small intestine are utilized by the gut tissues (MacRae, 1989). The liver also makes extensive use of amino acids for synthesis of protein products and as sources of energy. The liver may remove as much as 25% of the amino acids that appear in PDV (Armentano, 1994). The extent to which these amino acids are removed from the blood supply also depends on the energy level in the diet as well as the type and amounts of amino acids absorbed (Reynolds et al., 1994). Although there are studies that have determined hind limb amino acid use in growing animals, there are virtually no studies we are aware of that have attempted to measure extra hepatic protein turnover in lactating cows. It is obvious, however, that as the amount of muscle, renal, brain, mammary and circulatory tissue increases and that must be maintained, the more amino acids as well as energy must be put into the system to replenish worn out tissue. Also, the extent to which peptides contribute to the amino acid balance of these tissues is only now becoming established (Remond et al., 2000). It should be appreciated that measurement of free amino acid uptake may not indicate the true amino acid usage by tissues because of possible peptide utilization. Peptide contribution to amino acid removal may also be included in future editions of the AminoCow model should data become available.

There are several methods for calculating the metabolic protein requirement for maintenance. Briefly they are: (1) calculation of N loss at maintenance energy in calorimetric chambers, (2) feeding graded amounts of protein with excess energy and measuring protein accumulation, and (3) calculation from physiological studies of isolated organs. Most studies are from feeding graded amounts of protein. In these studies, measurements are regressed to a zero protein accumulation in the whole animal. The 1989 NRC estimates the requirement for metabolizable maintenance protein as the sum of urinary protein plus scruff protein at a 67% utilization rate, as does the CNCPS model (O'Connor et al., 1993). Both sources of N loss are estimated from body size, which results in a very low maintenance requirement. For example, a 650 kg (1430 lb) mature cow would need 119 g of maintenance metabolizable protein (0.25 lb) using the 1989 NRC calculation. 2001 NRC changed to a requirement based on the body weight of the animal, the dry matter intake, the amount of microbial protein synthesized and an endogenous factor. This results in the same cow requiring 449 g of MP for maintenance.

Also, it must be pointed out that the methodology required for these measurements (very low dietary crude protein) may mean that metabolism has been altered so as to conserve amino acids to a greater extent than would be the case if energy and protein were not limiting. Also, because the amount of RUP and its amino acid composition are not always known, this could produce errors. For example, an animal may appear to require more of a protein in which lysine was limiting than of a protein in which all amino acids were balanced. However, it must be agreed that the actual physiological requirements for amino acids have not changed. Rather our ability to measure the protein and correct for changes in physiology may have changed.

AminoCow calculates maintenance requirements on the basis of tissue amino acid metabolism. Computationally, AminoCow expresses the need for amino acids on a metabolic body weight basis as a description of increased metabolic demand for amino acids as body size increases. Twenty percent of metabolic body weight is assumed to be protein. Estimations are that 18% of this protein turns over daily and that 97% of the amino acids in this protein are recycled into protein again (Garlick, 1984; MacRae, 1989). Thus, about 3% of total protein and amino acid turnover must be from dietary sources. AminoCow assumes that the efficiency of amino acid use for maintenance is only slightly more efficient than that for lactation. We are assuming a 70% amino acid utilization rate for maintenance.

Our equation based on these calculations yields a substantially higher metabolizable protein requirement for maintenance than 1989 NRC, but it is lower than that proposed by the Dutch system (Tamminga et al., 1994) and substantially less than 2001 NRC. For example, the same 650 kg mature cow that would require 119 g metabolizable protein in the 1989 NRC system would require 169 g of metabolizable protein in AminoCow and 312 g in the Dutch system. At least a portion of the difference between these models is the requirement of endogenous protein included in the 2001 NRC.

AminoCow assumes the amino acid composition of bovine body crude protein as a percentage to be 1.8% methionine, 6.0% lysine, 6.0% leucine, 3.25% isoleucine, 3.8% valine, 3.4% threonine, 6.6% arginine, 2.4% histidine, 0.6% tryptophan and 3.5% phenylalanine (adapted from Degussa AG, 2001). The total amino acids make up approximately 83.7% of total crude protein. The remaining crude protein is composed of nucleic acids, amines and other nitrogenous compounds. Of the total amino acids in body protein, 44.6% are considered to be essential and 55.4% are considered non-essential amino acids (Ainslie et al., 1993).

Lactation Requirements for Amino Acids and Metabolizable Protein

During lactation, the largest portion of amino acids is used for milk protein synthesis (Reynolds et al., 1994). Therefore, milk protein production is the largest determinate of amino acid and metabolizable protein requirement in lactating cows. AminoCow assumes that the amino acid content of milk protein is 2.7% methionine, 8.1% lysine, 9.8% leucine, 5.4% isoleucine, 6.3% valine, 4.7% threonine, 3.4% arginine, 2.7% histidine, 1.5% tryptophan and 4.7% phenylalanine (Degussa-Hüls, 2000). It is further assumed that amino acids for milk protein synthesis are used in proportion to their appearance in milk protein. Traditional extraction coefficients for individual amino acids have not been used in the AminoCow model. The amount of amino acid taken up by the mammary gland is determined by blood flow and blood amino acid concentration (Cant and McBride, 1995). In fact efficiency of conversion of amino acids to milk protein declines as amino acid supply approaches estimated requirements (Doepel et al., 2004).

Extraction may also be affected by the relative amounts of essential versus non-essential amino acids presented to the mammary gland (Trottier et al., 1995), with less non-essentials extracted when essentials are high. Recent experiments (Bequette et al., 1999) with infusion of amino acid mixtures that were low in histidine indicated that mammary blood flow was increased by 36%, histidine extraction was increased by 43 times and extraction of other amino acids decreased 2 - 5 times. Also, when methionine was infused into the duodenum of lactating cows (Guinard and Rulquin, 1995), mammary blood flow was decreased. In this experiment the total amount of methionine extracted was unchanged. Both these experiments indicate that the quantity of amino acid removed is related to the amount of protein synthesized and not solely to the blood concentration. There is further evidence that glucose concentration in mammary blood can affect the flow and, therefore, the extraction of amino acids from mammary blood supply (Cant et al., 1999). Thus, the use of constant mammary extraction coefficients for each amino acid is not biologically accurate. Until there is a better understanding of the effects of blood flow and both blood amino acid and glucose levels, we believe that it is better to provide a balance of all amino acids. Providing what is believed to be the proper mix of amino acids (Schwab, 1995; 1996) while assuming that individual amino acids are transferred to the mammary gland at the same efficiency is both more biologically sound and easier to model. AminoCow calculates that the amino acids required for milk protein production should reflect their appearance in milk at a 67% utilization rate.

NRC 2001 has adopted an emphasis on the ratio technique (amino acid as a percent of MP) for describing amino acid requirements. This means that MP is essentially the only requirement set. While this fits logically into an ideal protein system, we do not believe that sufficient studies have been done with diets of defined amino quality to be able to adequately set a ratio of amino acids. We believe that ratios of amino acids have meaning only if the gram quantities of amino acids are sufficient and the ideal amino acid distribution is known. For example, if the ratio of lysine in MP is correct, but there is only 10 g of MP, then the ratio means little for productive purposes. Manipulating methionine as a percentage of calculated metabolizable protein within an experimental protocol has increased milk protein percentage (Schwab et al., 1992; Rulquin et al., 1993). However, we were unable to correlate any ratios to either milk protein yield or milk protein percent across 281 separate diets (Patton et al., 2003). The research summarized by Doepel et al. (2004) shows clear response to lysine and methionine individually. Thus we have chosen to place emphasis on meeting individual requirements for amino acids as the parameter that is simplest and most effective.

The MP requirements for AminoCow were determined by regressing data from studies published in the Journal of Dairy Science. The following information was reported or could be calculated from study data before use was made of the study: body weight of cattle, milk production, milk protein yield, MP flow to the duodenum (non-ammonia amino nitrogen) and the amino acid content of the MP. Fifteen studies comprising 62 diets were used. Virtually all the studies were Latin square in design with relatively short collection periods so that there was little or no change in body weight or body condition during the studies. The study references are presented in Appendix Table 1. The data was sorted by MP flow to the duodenum against the production of milk protein. Milk protein was chosen as the most sensitive marker of MP demand in these short-term experiments. This data is presented graphically in Figure 1.

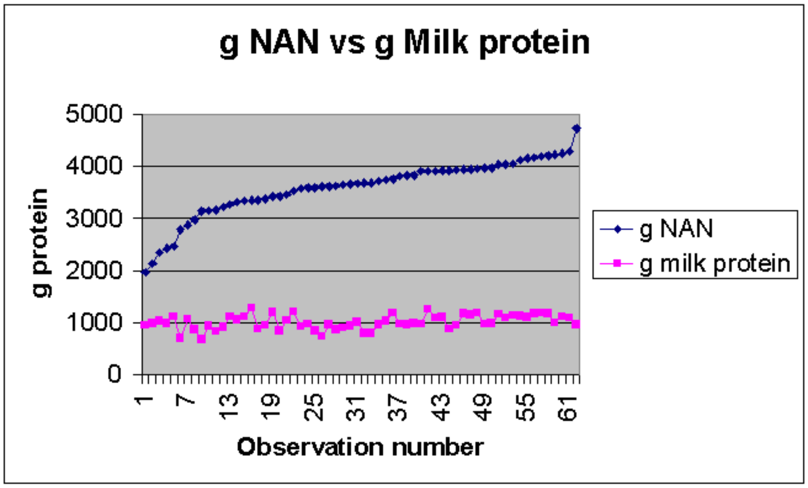

Figure 1: Plot of grams of duodenal protein flow and grams of milk protein.

Studies have indicated that amino acid infusions that mimic either milk or microbial amino acid patterns have increased milk protein production. Likewise, studies in growing monogastrics had shown that when the balance of amino acids was more ideal, the MP requirement was reduced. This suggested that MP required for lactation must be assessed in terms of the amino acid composition of that protein. We wanted to compare the MP supplied in the above studies with the amino acid composition of the AP. Further, we wanted to be able to rank the amino acid composition of the intestinal flow of each diet against the intestinal flow of amino acids of the other diets. We used the amino acid pattern of bovine milk as the standard to compare amino acid flow because this composition is relatively well known and this composition does increase milk protein yield when infused post ruminally.

Quality of protein was assessed against milk protein by taking the amino acid as a percent in the AP in the duodenal flow and dividing it by amino acid percentage in milk protein, then multiplying by 100. This was performed for all ten amino acids believed to be essential plus the total amount of non-essential amino acids. These individual percentages were summed to calculate the amino acid index. Essential amino acids above 100% of milk were added to the non-essential pool. If the amino acid had a value of greater than 100, it was considered to have a value of 100. This is in recognition that essential amino acids in excess of requirement may be modified into non-essential amino acids. Thus, the ideal index was assumed to have a value of 1100. This would equate to 100% of milk protein for each individual amino acid and 100% for the total non-essential amino acids. If AP (NAN) flow was above 3000 g, amino acid composition of that AP really made no difference in the production of milk protein. That is to say, as AP increased above 3000 g, the production of milk protein remained constant. Obviously AP in excess of 3000 g was protein wastage. It was also obvious that below 3000 g of AP flow, there was a sharp inflection point where the amino acid composition of the AP appears to have an effect on the production of milk protein. In order to study the effects of the index and the amount of AP, only studies below 3154 g of flow were included. These resulted in the use of only 10 diets with a minimum of 1954 g and a maximum of 3154 g of AP flow.

Using the 10 diets where there was an effect of amino acid quality (i.e., the lowest duodenal flow for those in the graph above), the best regression model for these rations included the following terms:

Milk protein (kg) = [0.00032159 * AP flow in g] + [0.0024787 * Index value] + [0.01950 * NFC percent of dry matter] – 3.02835.

This model yielded an R^2 of 0.92 with all terms in the model significant. Use of this model produced an estimate of 1774 g of AP required for synthesis of 1 kg of milk protein at amino acid index of

1100 and NFC at 37.5%. This basic relationship defines all the MP requirements in AminoCow, although we did look at other avenues of evidence.

Early Lactation Requirement for Amino Acids and Metabolizable Protein

During initiation of lactation, it is well known that the dairy cow is in negative energy balance because feed intake cannot keep pace with increasing milk production (Emery, 1988). Additionally, it has been estimated that as much as 15% of the glucose necessary to sustain production may be derived from amino acids via gluconeogenesis (Bergman, 1973). Clearly, the early lactation cow is challenged to meet her amino acid needs. Estimates of body protein loss in the first six weeks of lactation vary from 9 - 18 kg (~20 - 40 lbs). While loss of some body protein is normal, excessive protein loss often leads to poor performance and delay in returning to proper reproductive function in dairy cattle (van Saun, 2003, personal communication). To the best of our knowledge this observation has not been experimentally demonstrated. Likewise, the acceptable amount of body protein loss has not been established.

However, while it is logical that metabolizable protein and amino acid requirements should be increased in the early postpartum period, the amount by which they should be increased is less clear. The range that cows under-consume dry matter varies widely among studies (Hayirli et al., 1998) and has been shown to depend on the characteristics of the fresh cow diet, body condition and probably the dietary management in the dry period. In AminoCow we have arbitrarily increased absorbable protein and essential amino acid requirements by 15% during the first two weeks of lactation based almost entirely on field experience. We have consistently found that cows lose less body condition, reach peak milk production more quickly and display faster uterine involution with this program.

Conceptus Requirement for Amino Acids and Metabolizable Protein

There are few studies available for determining use of metabolizable protein by the developing fetus in dairy cattle and none that we are aware of for determining amino acid use. NRC (1989) assumes that total conceptus protein is used with an efficiency of 50%. A study by Bell (1995) would indicate that efficiency is as low as 30%. Further, this group (Bell et al., 1995) suggests that placental protein is accumulated first and that in the last month of gestation, protein use is directed almost completely toward fetal accretion.

In AminoCow conceptus growth (i.e., fetus + placenta) has been calculated as an average daily growth, depending on the breed of cow (Table 1). There is little doubt that conceptus growth does not occur on a linear scale, but rather a logarithmic scale. However, differences in amino acid requirements for fetal growth are small on a daily basis. Also, on a practical basis, pregnant cows housed together would have a relatively large range of conceptus age. Because of these two factors, we have averaged the daily amino acid requirements over the dry period. Assumed daily protein accretion in conceptus growth for the last 40 days of the dry period by breed is presented in Table 1.

Table 1: Expected daily conceptus protein accretion by breed.

Breed	**Daily Protein Accretion (kg)**
Australian Red	0.495
Ayrshire	0.500
Brown Swiss	0.795
Guernsey	0.500
Holstein	0.682
Jersey	0.318
Milking Shorthorn	0.575
Simmental	0.600

These accretion rates are regressed from average birth weights, assuming that 90% of tissue growth occurs in the last trimester of gestation. Metabolic body weight of conceptus is assumed to be the number of days dry times daily protein accretion raised to the 0.7 power. Twenty percent of metabolic body weight is assumed to be protein as above for maintenance. The AminoCow model is based on the assumption that the amino acid content of the conceptus protein is equal to that of the mature animal and that individual amino acids are all used at an efficiency of 35%. The figure of 35% is conservative based on the work of Bell mentioned above. When more data is available, this may need to be changed in future editions of AminoCow.

Growth of Protein Tissue

There have been many attempts to estimate the protein requirements for growing muscle. The majority of these studies use dietary composition regressed against average daily gain. Amino acids then may be estimated by tissue composition (O'Connor et al., 1993).

For lactating cows, daily body weight growth is calculated as the difference between present weight and that of desired mature weight until the animal has attained a third lactation. For 3+ lactations no growth requirements are added. For growing heifers, daily body weight growth is determined as the difference between present body weight and that expected at first parturition divided by the difference in number of months times 30 between present age and expected calving at 24 months. AminoCow converts daily weight gain into metabolic body weight with 20% of metabolic body weight assumed to be accumulated protein. Proteins and amino acids are assumed to be deposited with an efficiency rate of 30%. Amino acid content of deposited protein is assumed to be reflective of the amino acid composition of whole body protein as explained above. Protein and amino acid for body condition repletion are based on the body weight of 1 condition score unit converted to metabolic body weight and divided by the number of days until dry off at an expected lactation of 325 days. For body condition score repletion, the same assumptions regarding amino acid composition and utilization rate as for other growth processes have been made (Patton, 1989).

Finally, when reviewing all the data collected, it became obvious that the MP requirement for all classes of cattle except those in early lactation could be modeled simply by doubling the requirement for essential amino acids. In the case of early lactation cows, calculating 2.3 times the essential amino acid requirement could approximate the MP requirement. Because of the ease of programming, these are the equations that were finally put into use.

Protein and Amino Acid Supply at the Duodenum

Protein and amino acid supply to the duodenum is the sum of three processes—the amount of ruminally synthesized microbial protein that flows to the duodenum, the amount of feed protein that escapes degradation in the rumen and the amount of endogenous protein secreted in response to feed intake. Microbial protein typically makes up from 50 - 65% of the total protein flow in milking cows, while feed protein that escapes ruminal digestion supplies the majority of remainder of the protein, although this may depend on diet quality and roughage amounts. Amino acid supply is assumed to reflect the amount of microbial protein and both the type and amount of escape protein (Clark et al., 1992) as well as a small fraction of endogenous origin.

Microbial Protein Synthesis

Microbial protein synthesis has been shown to be the result of the organic matter actually fermented in the rumen (Dijkstra et al., 1998). However, the fermentability of organic matter is not easily predicted from the organic matter content of the diet or the types of feeds in the diet (Stern et al., 1994; Firkins et al., 1998). This unpredictability is probably due to the interactions between feeds, amounts of different feeds and the rumen microorganisms inhabiting the rumen.

Alternatively there have been attempts to use other dietary parameters to predict microbial protein. NRC (1989) uses the energy in the diet, expressed as NE_L to predict microbial protein. The 1989 NRC equation did not adjust for NE_L in lipid, nor does it discount for VFAs in silage or for the amount of starch and protein that escapes rumen digestion. European systems have developed data for the grams of microbial protein synthesized per kg of feed. In these systems, total microbial protein is calculated as the kilograms of each feed consumed multiplied by the grams of microbial protein that are assumed to be produced by the feed (INRA, 1988; Tamminga et al., 1994). While this strategy gets around the problems of the 1989 NRC system, it fails to take into account the interactions of feeds in the diet. The Cornell Net Carbohydrate and Protein System uses a very mechanistic approach to predicting microbial protein synthesis that attempts to model many of the interactions between feed types and amounts (O'Connor et al., 1993), but without apparently any greater increase in accuracy of prediction (Bateman et al., 2001a; 2001b).

Estimations of microbial protein synthesis per gram of organic matter obtained from in vivo and in vitro studies vary widely with estimates obtained in vivo being considerably less than those obtained in vitro (Dijkstra et al., 1998; Schadt et al., 1999). Both in vivo and in vitro estimation methods have problems. Accuracy of in vivo methods depends on the assumption that the markers used flow uniformly and that microbial markers are not recycled. This method also depends on the assumption that the presence of cannulas does not affect protein flow or amino acid composition. There is good evidence that both of these assumptions are incorrect, but general agreement about how to correct for them is lacking. In vitro measurement assumes the environment of the chemostat is nearly the same as the rumen environment. This is a condition clearly not met, because feed is ground, outflow is controlled, buffer is added to maintain a constant pH and there are no muscular contractions. It seems logical that the amount of microbial protein actually synthesized must be between these two values in the intact animal.

Research reports from the University of Nebraska further complicate the measurement of microbial protein in vivo. The researchers found that the most widely used techniques for estimating rumen degradable protein allowed significant numbers of rumen bacteria to be attached to the protein (Klopfenstein et al., 2000). This resulted in an over prediction of the amount of degradation that protein and protein fractions undergo in the rumen. The same group (Creighton et al., 2000) concluded that the present system used for determination of microbial protein synthesis destroys a significant amount of the purines. Their study suggested the amount of microbial protein was underestimated and the amount of feed protein was overestimated in many studies. Taken together, both of these studies suggest the in vitro numbers may be closer to actual rumen microbial protein synthesis than the numbers determined in vivo.

It has been theorized that other factors can affect microbial protein synthesis. Some factors suggested are dry matter intake, diet digestibility, RUP intake, forage:concentrate ratio, amount of fat in the diet and rumen pH (Titgemeyer, 1997). Other dietary factors suggested as impacting microbial synthesis include crude protein, degradable intake protein (RDP), NFC, NDF, peptides and amino acids (Stern et al., 1994). However, obtaining sufficient data to model the interactions among these factors is almost impossible at the present time.

Dry matter intake has been shown to be the most important factor regulating microbial protein synthesis (Clark et al., 1992; Stern et al., 1994; Firkins et al., 1998). On any given diet, the more feed consumed, the greater is the total amount of microbial protein produced. Some studies indicate that on the same diet, the amount of microbial protein synthesized per kg of dry matter consumed is reduced as DMI rises. This is well known in carbohydrate digestion, but its effect is less well established in microbial protein production. Some studies indicate that there is a reduction of protein synthesis per gram of dry matter consumed (Dijkstra et al., 1998), and others indicate there is no difference (Robinson et al., 1987).

Fermentability of the carbohydrate portion of the diet is the second major factor affecting rumen microbial synthesis, as this leads directly to a prediction of the amount of organic matter truly fermented. As mentioned above, the more organic matter truly fermented in the rumen, the greater is the amount of microbial protein synthesized. As concentrate (i.e., sugars and starches or NFC) is increased, the diet becomes more fermentable. Clearly, the more NFC in the diet, the greater is the microbial synthesis (Hoover and Stokes, 1991). However, excess NFC leads to rumen acidosis and a lowering of rumen pH (Erdman, 1988). Low pH has been proposed as a mechanism for lower microbial synthesis (Firkins et al., 1998). However, in vitro data demonstrates that as pH drops, microbial synthesis may actually be increased due to the relative rate of growth between starch-fermenting microbes and cellulose fermenters (Argyle and Baldwin, 1988). As the pH drops, starch and sugar-utilizing bacteria, which grow at a more rapid rate, begin to predominate, and microbial protein can actually increase. There is a point at which the pH becomes low enough that microbial growth essentially ceases.

This decrease in microbial growth does not seem to be a problem on diets within the range of NDF and NFC recommended in the AminoCow program. Our analysis of published studies (Patton, unpublished) indicates that rumen pH is not a significant influence on the production of microbial protein as long as fiber fractions in the diet are in the general ranges recommended by 1989 NRC. Data sets of literature studies where duodenal amino acid flow is measured included many diets that had significantly higher levels of NFC and presumably, lower ruminal pH than would normally be recommended in commercial situations. Higher than commercial NFC diets are used in many of these studies due to problems encountered with measuring duodenal flow on high forage diets. However, there is no evidence that these high NFC diets have lower than expected microbial flows.

There is general agreement that starch-fermenting organisms make more use of ammonia as an N source than do cellulose digesters. However, both types of organisms can grow with inorganic sources of nitrogen. Clark et al. (1992) observed that the amount of CP in the diet was a significant predictor of the amount of protein flowing to the intestine and that as the percent of RUP in the diet rose, the amount of microbial protein synthesis decreased.

There has been much speculation over the role of proteins, peptides and amino acids in the production of rumen microbial protein (Argyle and Baldwin, 1989). Several studies have shown a positive effect of amino acids on microbial growth in vitro. This has led to a suggestion that there may be a requirement for a definitive amino acid mixture for efficient fermentation by rumen microorganisms. There have been similar suggestions that peptides may be limiting production by rumen microbes (Chen et al., 1987). The review of Baldwin et al. (1994) suggested that the requirement for peptides and amino acids for microbial organisms would be low. Other experiments have suggested somewhat higher requirements for peptides (O’Connor et al., 1993). These measurements have been determined almost entirely in vitro, and for reasons discussed above, it is very difficult to generate requirement numbers from these types of experiments. Because both peptides and amino acids are part of the rumen milieu in normal diets, there may be little advantage to balancing the rumen for these compounds. While rumen microbes appear to have a requirement for amino acids and perhaps peptides, sufficient proof that increased levels aid production is lacking (Baldwin et al., 1994).

On the other hand, it is clear that cattle perform better on diets with several different protein sources than when a single protein source is used. Mixed cultures of rumen microbiota have been shown to digest carbohydrate more completely when ammonia, peptides and amino acids are all present in the media (Argyle and Baldwin, 1989). Rumen bacteria and protozoa are known to hydrolyze protein rapidly. In both species proteases that hydrolyze cysteine bonds predominate. The proteolytic activity of the microorganisms results in rapid accumulation of peptides. Although the rate of hydrolysis exceeds that of peptide utilization, peptides are also rapidly degraded. Under normal feeding conditions ammonia, peptides and amino acids all reach high concentration approximately two hours post feeding. The levels continue to decline until the next meal, which in dairy cattle would be every 2 - 4 hours. Levels of peptides of 100 mg/l were necessary for stimulation of rumen microbial digestion (Argyle and Baldwin, 1989), which was a level lower than encountered on normal diets (Chen et al., 1987).

It is assumed that free amino acids are readily utilized as energy sources or absorbed intact into the microbial protoplasm. Increased microbial growth due to amino acid or peptide supplementation appears to be due to the amount of amino acids or peptides added and not to specific amino acid deficiency (Baldwin et al., 1994). However, supplementation of many diets with DL-methionine or methionine hydroxy analog shows better overall microbial digestive efficiency. Whether this effect is due to methionine as an amino acid, a sulfur source or as a methyl donor is not understood (Lundquist et al., 1982).

All feeds contain mixtures of amino acids, peptides and free ammonia as well as true protein, although poor ensiling may affect both the protein availability and amino acid content compared to the unensiled forage (Llames et al., 1998). This fact, along with the reasonably frequent meal pattern of dairy cattle and the reasonably quick hydrolysis of rumen degradable protein, ensure that dairy cattle fed total mixed rations with several protein sources probably do not require additional peptide sources (Argyle and Baldwin, 1989). As noted earlier, we have made this assumption in the AminoCow model. If more accurate data regarding levels of peptide utilization become available, it will be incorporated into the AminoCow model.

In a series of rather groundbreaking experiments, the relationships between microbial protein synthesis, NFC intake and rumen degradable protein (RDP) were investigated (Stokes et al., 1991a; 1991b; Calsamiglia et al., 1995). These experiments demonstrated that microbial protein synthesis was maximal as diets approached 37% NFC. They also suggested a ratio of NFC to RDP on a weight basis of from 3.0 - 3.2% would result in maximum utilization of the NFC by microbes. This is a ratio that is reasonably close to that suggested by INRA data (1988). These experiments point out the necessity of adequate protein availability in the rumen if microbial protein synthesis is to be maximized.

Major portions of the factors that are thought to influence the synthesis of microbial protein are probably due to effects on DMI. For example, inclusion of both ruminally active and rumen inert fat in the diet lowers microbial synthesis (Stern et al., 1994). It is known that fat hydrolyzed and saturated in the rumen is toxic to cellulolytic bacteria and, thus, lowers protein synthesis. However, inert fat interacts only slightly with rumen bacteria. Nor does inert fat have a direct effect on microbial synthesis. However, both ruminally active and inert fat lower DMI, which in turn lowers total protein synthesis. Likewise, there is greater protein synthesis on high concentrate diets than on high forage diets because of faster carbohydrate fermentability. However, there is also greater DMI on higher concentrate diets. At least a portion of the increased microbial synthesis on high concentrate diets must be attributed to greater DMI. Studies demonstrate that as the amount of RUP in the diet rises, microbially synthesized protein flow decreases (Clark et al., 1992; Oldick et al., 1999). This decrease in microbial protein is proportional to the increase in RUP so that total absorbable protein flow is largely unchanged (Santos et al., 1999). As more RUP is provided, the amino acid profile of the protein flowing to the duodenum becomes more reflective of the amino acid composition of the RUP protein.

Modeling Microbial Synthesis in AminoCow

From the standpoint of modeling rumen protein synthesis, the difficulty is in determining which factors have a significant effect on rumen microbial synthesis and using this data to predict the amount of organic matter truly digested in the rumen. In our review of published literature studies (Patton, unpublished), when cattle were fed the same ration but differing in small amounts of non-organic additives (vitamins and minerals primarily), there was a range of approximately 130 g of rumen microbial protein synthesis in the same cows fed the same diets. This range is a very small percent (6.05%) of the mean. Unfortunately, this is a greater difference in microbial synthesis than was often observed after significantly greater dietary manipulation of starches and proteins.

Because the amount of microbial protein synthesis depends on the amount of dry matter consumed, the characteristics of the carbohydrate portion of the diet, the ruminally available nitrogen and the amount of organic matter (OM) truly fermented, it was impossible to find terms that would describe all these functions satisfactorily.

In our literature studies OM%, NDF%, ADF% or CP% were not useful as predictors of microbial protein. Using regression analysis of studies with reported dietary information and measured microbial production, the only significant predictors of rumen microbial synthesis were total dry matter intake and a term for the laboratory in which the experiment was conducted. The term for laboratory reflects differences in cows, climate and/or techniques. However, many other factors are known to have an effect on microbial protein production. The problem becomes how to best model these interactions.

NRC (1989) uses an equation based on the NE_L content of the diet to predict microbial protein. This has several appeals because it is reflective of the digestibility of the diet and the energy value of the carbohydrates, and computationally it is relatively easy to sum the components for total NE_L intake. Therefore, we adopted the basic model of 1989 NRC but made the following adjustments. The 2001 edition of NRC uses a truly digestible nutrient system to predict the NE_L content of feeds. This system has advantages because it predicts different energy contents of feeds based on a more complete profile of nutrient values and the factors that affect them. This would include the lignin content of the fiber and the amount of protein bound to both ADF and NDF (NRC, 2001, section on Energy, pp.13-27). The 2001 edition of NRC also included a processing factor that made it possible to reflect the effects of different processing methods on the fermentability of the NFC portion of feedstuffs. Taken together the NRC system reflects differences in feed quality and processing which affect the NE_L prediction that had been missing in earlier versions of our program.

We adopted the following system for calculating the energy available for microbial protein synthesis in the AminoCow model and termed it "microbial energy". Microbial energy was the NE_L content of the diet as calculated by 2001 NRC with the following adjustments. NE_L content of the diet for microbial synthesis was reduced by the amount of fat over 3% of the DMI as suggested by Erdman (1995). Also NE_L content of silages for microbial synthesis was reduced by the amount of VFA in silages. When the diet contained over 30% RUP, this amount of energy in this protein was also discounted, as our studies indicated that diets with higher RUP than this failed to produce the same level of microbial protein as similar diets without this level of RUP. The processing factors used in the NRC publication were used in the AminoCow model without modification, except in the case of grain processing. The processing factors of grains were modified to reflect the effects of the milling process, as described by Theurer and his colleagues (Theurer, 1986; Olivera et al., 1995; Theurer et al., 1999).

Thought was given to reducing the NE_L estimate by the amount of rumen undegradable starch. This was rejected for two reasons. First, the NE_L values assigned to the feed already reflect these processes to some degree. For example, cracked corn has a lower NE_L value than ground corn because of processing differences. Second, there is insufficient data to be able to predict just how much of the starch in a feedstuff which is undegraded in the rumen can be subsequently digested in the small intestine. When more data is available, these values may need to be changed.

Clark et al. (1992) reported that the distribution of amino acids in microbial protein varies greatly. However, most of the studies that Clark cites are in vivo studies, and there is some doubt as to the ability to adequately separate particle-associated bacteria and liquid-associated bacteria in the correct proportion. This technique may also have resulted in contamination of microbial protein with feed proteins. In chemostat studies, amino acid distribution of microbial protein is within the 15% coefficient of variation normally found in biologic populations, but how close the chemostat is to true rumen conditions is also debatable (Dijkstra et al., 1998). There are known differences in the amino acid content of separate rumen microbial species. How greatly this might affect the flow of amino acids to the duodenum has not been studied extensively. Also, the ability to predict which species of rumen microbes might dominate on any given diet simply does not exist.

AminoCow uses a weighted average value of seven compendium studies to predict amino acid content of the rumen microbes (ARC, GEH, NRC (1989; 2001), CVB, INRA and CPM). In the AminoCow model, rumen microbial protein is expected to contain: 1.68% Met, 6.55% Lys, 6.90% Leu, 4.90% Ile, 4.90% Val, 4.45% Thr, 3.80% Arg, 1.75% His, 1.35% Trp and 4.30% Phe. Of the total microbial crude protein synthesized, 60% is expected to contain true amino acid protein. The other portion of the crude protein is assumed to be nucleic acid and cell wall associated nitrogen fractions.

Rumen Degradable Protein Requirements

The work at West Virginia mentioned above, Stokes et al. (1991a; 1991b) clearly established the relationship between the amount of NFC in the diet and a requirement for degradable protein.

Further, the necessity of a mix of nitrogen sources for maximizing the microbial protein has been established. We have continued the use of the RDP term (or DIP) as defined by Stokes et al. (1991a; 1991b). In these studies, microbial protein synthesis was optimized when RDP was 0.3125 of the total NFC.

In AminoCow this relationship has been used to calculate the RDP requirement. Although this is presented as a cow requirement, it is really a microbial requirement that leads to maximization of microbial protein synthesis. There is a further assumption that non-protein nitrogen additions will be within those guidelines normally imposed, i.e., a maximum of 0.5% of total dry matter intake. If total NPN has been limited, and natural protein sources make up the remainder of the protein supplementation, AminoCow assumes that sufficient peptides and amino acids exist to optimize microbial growth. Therefore, no requirements for peptides or free amino acids have been generated. Peptides and amino acids are assumed to be part of the RDP requirement. We realize this may not always be true in cases of damaged feeds. In the use of the Balance command of AminoCow, it is always assumed that microbial protein synthesis is of primary importance and that this is best accomplished by balancing the NFC portion of the diet and by providing sufficient RDP to maximize the digestion of NFC.

To repeat the ideas expressed above, we would always like to balance rations by maximizing microbial protein production. However, we have noticed that in some milk markets, this may not always optimize economic returns. Depending on local market conditions, it may be more advantageous to meet the MP and amino acid needs of the cow by adding RUP sources and using less RDP. For this reason we added the ability to adjust the amount of microbial protein for deficiencies in RDP. The nutritionist can then evaluate the economic impact of meeting the total RDP need, adding more RUP or in some situations, leaving the protein fractions as they are.

Reduction of Microbial Protein Synthesis

It became obvious when working with commercial diets that for a variety of reasons the recommended level of RDP to maximize rumen microbial synthesis was not always present. Based on animal performance, we suspected that microbial protein flow was being overestimated in the cases where RDP was deficient. Studies of published literature confirmed this supposition. Therefore, using data from published studies that reported NFC, RDP and microbial yield, we developed the following equation to predict the reduction in yield of microbial protein when RDP was below recommended levels:

$$\text{Microbial yield reduction} = (0.36 * \text{RDP deficiency g}) + (0.247 * \text{RDP deficiency g})^2$$

This equation had an R^2 of 0.73 for microbial protein with published studies. The implications of this formula are clear. If the RDP is not much lower than the requirement, then the reduction in microbial protein synthesis is very small. We theorize this is probably because at low RDP deficiency levels, urea recycling permits maintenance of normal levels of microbial synthesis, but as the RDP levels decrease, urea recycling is not sufficient to maintain predicted microbial synthesis levels. Thus, as RDP supply decreases, microbial synthesis decreases at an ever increasing rate.

Rumen Undegradable Intake Protein

A variable amount of between 40 - 55% of amino acid flowing to the duodenum is of feed origin (Clark et al., 1992). The measurement of this escape protein has been performed in several ways and results in different estimations for the same feed source. The data of NRC (1989) is measured using the dacron bag technique and has errors associated with sample preparation, wash out and microbial infiltration and attachment to the feedstuff within the bag. 2001 NRC presents more detailed protocols for these procedures to increase the accuracy of determination. The repeatability of this data is extremely high and provides a relative ranking of protein degradability. Another measurement of protein degradability has been obtained using in vivo methods in which microbial protein is estimated and subtracted from the total amount of protein flowing to the duodenum. This technique provides a much more variable and usually higher estimate of protein degradability partly because of the problems of measuring microbial protein synthesis rates and flows as detailed above. In our studies, the estimated amount of feed protein flowing in the in vivo system was an average of 20% higher than that measured using in sacco methods (Patton, unpublished).

The 2001 NRC and CNCPS (Sniffen et al., 1992) systems advocate use of chemical separation of proteins into fractions where the A fraction is completely rumen degradable, the C fraction is totally ruminally undegradable and the B fractions are potentially degradable at various rates. This system attempts to relate chemical separations to nutritionally relevant protein fractions. The problem for the working nutritionist is basically the same, however. How does one predict the degradability of the B fraction of the protein? In reality, whether it is measured as the whole protein or part of the protein, we are really searching for the same number. The distinction of protein fractions is only important if there are significant differences between the amino acid composition of the protein fractions, which apparently are not there (Schwab, 1996).

There may be some concern as to why AminoCow did not adopt the protein fractionation system. There are basically three reasons for this. One reason is the low repeatability of these measurements (Terramoccia et al., 2000). Even 2001 NRC suggested an error of from 25 to 50% of protein fractions for various common forages (NRC, 2001 Table 15-2a pp. 290-299). A second reason is the errors associated with the techniques of determining these fractions. Mathis et al. (2001), in a collaborative study with laboratories at 14 locations and utilizing the same forages at each laboratory, found differences of approximately 50% (± 25% SE) for A, B and C fractions. The last reason is that using the protein fraction system, dry matter intake and increased rate of solids passage should have the largest effect on the percent of the B fraction that is undegraded. We were not able to confirm that rate of passage affected the amount of RUP flow in our literature studies (Patton and Stevenson, 2000).

Because of the inverse relationship between RUP and microbial protein synthesis, many of the same factors that are thought to affect microbial protein are thought to affect RUP. However, because of the relatively great differences in protein degradability among feeds, the most important factor in determining delivery of RUP amino acids to the duodenum is the amount of individual protein sources consumed (Clark et al., 1992; Stern et al., 1994). Volden (1999) has suggested that the amount of free amino acids that escape degradation is related to the level of the individual amino acid source rather than the amount of DMI. In our literature studies, there was no significant relationship between total dry matter intake and differences in protein degradability of individual feed sources (Patton and Stevenson, 2000). There was a slight effect of feed fermentability (based on either NFC or NDF content) on the percent of the protein that was undegraded in the rumen. Feeds of higher fermentability had a lower percent of undegraded protein. This was particularly true of forage proteins. Although this trend was statistically significant, the differences were slight and of little practical importance. In fact, there is a small but growing body of evidence that indicates protein degradability is conferred by overall dry matter digestibility of the feed (Erasmus et al., 1994; Belyea et al., 1999; Stevenson et al., 1999). Therefore, processes such as lignification, grinding, flaking and heating can change protein degradability by changing ruminal dry matter digestibility. We have not attempted to model these processes in the AminoCow program.

The term protein solubility has been used in the same sense as protein degradability, but they are clearly not the same. A protein may be degradable but not soluble and soluble but not very degradable (Stern et al., 1995). Solubility is a physical characteristic of protein, but not a biological characteristic. Protein solubility has been added to this version of AminoCow for monitoring purposes only, and no requirements are generated. While it is theoretically correct and nutritionally desirable to coordinate rapidly fermentable carbohydrate with rapidly degradable protein, a more biologically descriptive measure than soluble protein appears to be necessary.

There is some debate about the effect of ruminal degradation on the amino acid composition of RUP. In general, studies have shown no or very small statistical differences between the amino acid composition of feedstuff protein before rumen incubation and the residue after incubation (Rulquin and Verite, 1993). In Degussa studies (Stevenson et al., 1999), we found numerically significant differences between pre and post incubated soy proteins, but the differences were so small as to be of little practical importance.

The vast majority of feed amino acid values in AminoCow are from the Degussa database (2001) or from laboratory results developed post publication of this program. A few values are taken from the WPSA European Amino Acid Table (1992) for feeds not normally encountered in US practice. All amino acid values are expressed on a percent of dry matter basis. It is further assumed that within a given plant or animal species amino acid content of protein is genetically set and does not vary greatly in percentage composition. Therefore, amino acid amount, but not distribution, varies in direct proportion to the amount of true protein present in the plant. When there is sufficient data for development of a significant regression equation for amino acid against crude protein, this data is included in the model (as AAByFormula). When this data is not available, calculation of amino acid content is made by amino acid composition of the protein. For the majority of feedstuffs, AminoCow uses undegradable protein values from NRC (1989). Most of the rest are from Spartan 2 (VandeHaar et al., 1992), although a few are based on Degussa experiments. In general, these values are not greatly different from those published elsewhere.

Duodenal Flow of Endogenously Secreted Protein

Earlier versions of the Mepron Dairy Ration Evaluator lacked a calculation for the contribution of endogenously secreted protein to the total MP flow, although the amino acids in this flow were accounted for. While this generally made little practical difference, it often made it difficult for users to compare results from other models to those of our model. Using regression analysis on our previously described database, we obtained a suggested value of 11.8 g of endogenously secreted protein for each kg of dry matter intake for milking cows. This figure compared very favorably with the 2001 NRC and that of a summary of swine data (Jansman et al., 2002). We have decided to use the formula of Jansman et al. because it has the added advantage of having amino acid composition of the endogenous protein associated with it. Compositional data is lacking in our own estimates and that of NRC 2001. We must await further studies in order to discover whether the amino acid composition of bovine endogenous protein is significantly different from porcine.

In the AminoCow model endogenous protein is assumed to be secreted at the rate of 11.87 g per kg of dry matter intake and to have an intestinal digestibility of 80%. This will increase the MP flow about 190 g compared with earlier editions of the Mepron Dairy Ration Evaluator for the average ration. The endogenous protein is calculated to supply: 0.11 g of methionine, 0.40 g of lysine, 0.49 g leucine, 0.38 g of isoleucine, 0.54 g of valine, 0.61 g of threonine, 0.39 g of arginine, 0.19 g of histidine, 0.14 g of tryptophan and 0.34 g of phenylalanine for each kg of dry matter intake.

Intestinal Digestibility of Proteins and Amino Acids

Stern et al. (1995) have presented data on different digestibilities of protein from various sources. Their data indicates a wide range of potentially different true digestibility for the same protein type. While it is clear that different proteins are digested with different efficiencies, it is less clear whether amino acids within the same protein source have different digestibilities as has been suggested for swine.

There are thought to be differences in digestibilities among amino acids that reach the intestine of swine (NRC, 1988). However, exceptionally little work has been done on amino acid digestibilities in mature dairy cattle (Hanigan et al., 1998). The pathways of amino acid absorption in cattle are believed to be those as reviewed by Baumrucker et al. (1989). A distinction must be made between the apparent digestibility and the true digestibility of amino acids. Data suggests that between 65-75% of amino acids flowing to the duodenum are apparently digested. However, these values are complicated by the secretion of endogenous protein, post ruminal fermentation and errors in measurement associated with the cannula technique. True digestibility is probably in the range of 55 - 90% of amino acids entering the duodenum (Erasmus et al., 1994) depending on protein source and dietary interactions. There are no controlled studies that we are aware of that would suggest that amino acids from the same protein source are absorbed with different efficiencies in cattle. In swine (NRC, 1988; Stein et al., 1999), different digestibilities are calculated for different amino acids among different feeds. Amino acid digestibility in swine has been shown to be affected by age of the animal, metabolic state, feed processing and concentration of amino acid within the intestine.

2001 NRC has included digestibility values for the RUP of various feedstuffs (Tables 15-2a and b, pp. 290-302) determined from the mobile nylon bag technique. This resulted in many grains having substantially higher digestibilities. We are leery of this data for several reasons, not the least of which is that for rumen protected amino acids, very different estimates of digestibility are obtained depending on whether the material is sequestered in the mobile bag or not (Berthiaume et al., 2000). Later studies by this group (Berthiaume et al., 2001) confirmed the only way to measure true protein digestion was to cannulate the mesenteric vessels of the small intestine and measure the appearance of individual nutrients. Lastly it seems illogical to have digestibilities for dairy cattle that are substantially higher than for swine within the same feed. This is especially true when considering that swine have a longer, more heavily illiated intestinal tract without the interference from the amount of fiber fractions.

Considering the lack of knowledge about either protein or amino acid digestibility in the bovine, we have chosen to use conservative values for protein digestibility and assume that amino acids have approximately the same digestibility as the protein from which they came. The AminoCow model uses a true digestion coefficient of 80% as a default value for all feed proteins. This value is at the low end of the reported range for most feed products, but is at the high end of the range for heated animal and heated vegetable proteins. The protein digestibility is editable within the program. A default value of 80% digestibility for microbial protein is also used, which is at the low end of the normal range for protein digestibility. This value is also editable in the Setup file. Updated protein and amino acid digestibilities will be included in AminoCow as soon as sufficient data is accumulated to make meaningful correlations.

Calculation of Absorbable Protein and Metabolizable Amino Acids

Calculation of absorbable protein is straightforward. Total absorbable protein equals the sum of true microbial protein plus feed escape protein plus endogenously secreted protein.

$$\text{Prot}_{ab} = (\text{McrbSyn} * 0.6) + \Sigma\,(\text{Feed}_i * \text{Cp}_i * \text{UIP}_i) + \text{Endog}$$

Where Prot_{ab} = Total absorbable protein

McrbSyn * 0.6 = Total microbial synthesis of which 60% is assumed to be true protein

$\Sigma\,(\text{Feed}_i * \text{Cp}_i * \text{UIP}_i)$ = sum the 1 to ith feedstuff for the dry matter of each individual feed multiplied by the crude protein as a percent of dry matter multiplied by the UIP as a percent of crude protein

Endog = Total flow of endogenously secreted protein

Because amino acids in feeds are expressed on a percent of dry matter basis, calculation of amino acids from feed is as below.

$$\text{Feed Amino Acid}_{jtot} = \Sigma\,(\text{Feed}_i * \text{Amino Acid}_j)$$

Where $\text{Feed Amino Acid}_{jtot}$ = Total of the 1 to jth amino acid

$\Sigma\,(\text{Feed}_i * \text{Amino Acid}_j)$ = sum of the 1 to ith feedstuff in dry matter multiplied by the amino acid as a percent of dry matter

Amino Acids from rumen microbial synthesis are calculated as

$$\text{Micrb AA}_j = \text{McrbSyn} * 0.6 * \text{AminoAcid}_{jmcrb}$$

Where MicrbAA_j = the 1 to jth amino acid

McrbSyn * 0.6 = Total microbial synthesis * 60% assumed to be true protein

AminoAcid_{jmcrb} = the percent of the 1 to jth amino acid from the assumed microbial protein content

Amino acids from endogenous protein are calculated as

AAend = DMI * AAkgend

Where AAend = endogenous amino acid contribution
DMI = total dry matter intake
AAkgend = amount of amino acid secreted per kg of dry matter intake

Microbial synthesis is calculated from the microbial energy number using a modification of the 2001 NRC system for determining truly digestible nutrients. This number is converted to microbial protein using the 1989 NRC equations.

Mineral and Vitamin Requirements

Requirements for both macro- and microminerals have been taken from the 1989 NRC. We have continued to use these recommendations for two reasons. First, these requirements have proven their accuracy to the authors over a wide range of conditions worldwide. Second, the 2001 NRC, while basically maintaining the recommended levels of minerals, has added a digestibility factor for various sources of minerals. Insofar as we are aware, mineral digestibility will depend on numerous factors such as pH of the intestine and interactions of and mineral complexes formed from various dietary minerals. While the 2001 NRC may eventually prove to be valid, we saw no reason to change from a system that was producing acceptable results on a worldwide basis to a system that may or may not work outside of the US. However, we readily grant our expertise is not mineral nutrition. Certainly as data is forthcoming, this subject will be reviewed.

Vitamin D recommendations are also from the 1989 NRC. Vitamin A recommendations are from the writing of Adams (1980) while vitamin E requirements are taken from work at Ohio State University (Weis et al., 1991; 1997). Cation-Anion balance has been calculated by the formula as recommended by Beede et al. (1992) as:

Cation-Anion Balance = ((Na%DM/0.023) + (K%DM/0.039)) - ((Cl%DM/0.0355) + (S%DM/.016))

Our experience using this equation is that a balance of -5 to -10 is usually sufficient to prevent milk fever.

Conclusion

The AminoCow model was constructed using published data about all aspects of dairy cattle nutrition but targeting protein and amino acid nutrition. Nutritional factors that were critical to the flow and utilization of protein and amino acids were identified. Those factors that did not materially affect the prediction of protein and amino acid flow and utilization were purposely estimated with a constant term. Carbohydrate nutrition, especially fiber nutrition, has been given great emphasis as a necessary starting point for adequate cattle nutrition. This data was then evaluated against responses on commercial dairy farms in various geographical locations worldwide. This process has provided a reasonably accurate, but also relatively simple model of nutrition in the dairy cow.

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Appendix Table 1: References for studies used in development of metabolizable protein requirements for lactation in AminoCow.

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