## The Strategic Use of Ruminally Protected Amino Acids in Dairy Nutrition

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## Introduction

When speaking with dairy nutritionists working at the field level about amino acid (AA) balancing, several comments are often heard. They are:

- 1. I tried balancing for amino acids and nothing happened.
- 2. I saw a milk protein increase initially, but it faded away pretty quickly.
- 3. My farmers don't get paid for milk protein, so we don't worry about amino acids.
- The only time I see a milk protein response is when I use (pick one: fish meal, blood meal, any of 10 different animal protein blends, Smartamine, Mepron). Nothing else works.
- 5. Every time I lower crude protein, the cows drop in production.

These situations are real. I am sure that many of you have made or heard the same or similar comments. Why is this? I suspect it is because balancing rations for AA, although simple operationally, is affected by factors that are not always straight forward. What we as working nutritionists need to know are the situations in which we will optimize the response to amino acid balance. Please notice that I use the term amino acid balance and not ruminally protected amino acids (RPAA). This is important because what matters is not the source of AA but the balance. And please notice that I said that it is not straight forward. AA balance is the result of many nutritional interactions, and we need to understand them as well as possible in order to obtain consistent results.

Although the title of this talk has the phrase "ruminally protected amino acids" in the title, we must admit there are only a few research-proven rumen protected amino acids. Currently only methionine is available as a commercial product, with several different products available. Often, but not always, RPAA are the least expensive source of methionine. Recently an RP-lysine product has been made available. Research will need to be done on this product, however. Should it prove to be truly RP-LYS, then the development of AA balance for dairy cattle will proceed at a much more rapid rate. For now when we talk about RPAA, we have to include the AA contributed by RUP sources.

What we propose to do in this article is to (1) suggest situations where amino acid balance is critical; (2) briefly describe what we know about amino acid (AA)

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nutrition of the dairy cow; (3) point out where we need more knowledge and how this lack of knowledge could contribute to inconsistent results; and (4) describe how we go about balancing for AA.

### When AA Balance Is Critical

In our opinion, there are times when AA balance is critical. They are:

- When attempting to reduce the amount of protein fed, thereby reducing the cost of the diet and increasing the space in the diet for higher energy feeds. We need to keep in mind that for the high producing dairy cow, energy is usually more in deficit than AA. Lowering protein in the diet will also reduce the spilling of nitrogen into the environment and lower the threat of regulation. This becomes an extra bonus and for herds under environmental regulation may be a primary use of AA balance.
- 2. For fresh cows, both to minimize BC loss as well as increase milk production.
- 3. To increase milk protein.

#### The Basics of AA Balance

Lactating dairy cows DO NOT have a requirement for crude protein or rumen bypass protein. Like other species, they have a requirement for amino acids (National Research Council, 2001). In most situations, by selecting proper protein sources and judiciously using RPAA, it should be theoretically possible to balance the amino acid needs of the cow while reducing crude protein intake. Broderick and his colleagues (2008) published a study that demonstrated that a ration with 16.1% CP and added RP-Met resulted in the same amount of milk as a 17.3% CP ration without RP-Met, and both rations resulted in higher milk production than an 18.3% ration (Table 1). There are current studies underway to further refine this relationship. Another study (Armentano et al., 2005) was done with small, high producing herds in Wisconsin. In this study ration crude protein (CP) was reduced about 1%, but MET and LYS were maintained. Milk production remained unchanged in these herds, but milk protein tended to be increased. In practice, we have not been able to reduce the crude protein content of the diet below 16.7% on a consistent basis without losing milk production. However, this has generally saved the dairy producer between 5 and 60 cents per cow per day depending on available protein sources and the reduction from previous dietary protein levels.

We are all aware that fresh cows, because they cannot consume sufficient feed, must depend on gluconeogenesis from AA as a significant source of glucose (Vanhatalo et al., 2003). Following the responses suggested by the data of Santos et al. (1998), we routinely add more glucogenic AA during the first weeks after freshening. This has consistently resulted in more milk production, both initially and at peak, as well as less loss of body condition. Exactly how much AA will be required will take some extra calculation depending on the diet and the types of protein available. Better requirements for AA at this stage of lactation are currently under active investigation at both Cornell and Michigan State Universities. Our personal recommendations for this calculation will be detailed below.

Because of changes in milk pricing systems with greater emphasis on milk protein, there is more interest in optimizing milk protein percent. Normally, the reason RPAA are first used in the diet is to try to increase milk protein percent. Although this response has been achieved in experiments documented in the literature (Rulguin et al., 1993; Schwab et al., 1992a and 1992b), it appears to be very difficult to obtain routinely on commercial dairy farms. This does not mean that rations should not be balanced for AA so as to optimize milk protein, but it must be recognized there are significant other influences. Milk protein percent is affected by genetics, stage of lactation, and season of the year. Nutritionally, the levels of starches and sugars in the diet are the most important determinant of milk protein percentage. In general the higher the levels of NFC, the greater will be the percentage milk true protein (National Research Council, 2001). As high NFC often results in higher milk production, maintaining higher NFC levels is a significant benefit for the dairy producer. Other nutritional factors that influence milk protein are the balance of AA and the amount of fat in the diet. In general, the higher the level of fat, the lower will be the milk protein (Palmquist and Moser, 1981). Further, we need to realize that although fresh cows may respond well to improvements in AA balance, once cows are past peak lactation, they have settled on a metabolic set point for production of milk, milk lactose and milk protein. Barring drastic deficiencies in amino acids or glucose, the production of milk components will be very difficult to change.

## Sources of Variability in Response to AA Balance

In the discussion above, we have hinted at several reasons why AA balancing is not always successful. Now we would like to discuss some of them in a little more depth.

## The Ruminant "Problem"

The ruminant animal has evolved to consume large quantities of grass and to convert this grass to high quality meat and milk protein. Thus, the ruminant animal has not only evolved with a microbial population that digests cellulose and hemicellulose, but that also converts the crude protein in grass, which is largely non-protein nitrogen, unusable by the animal, into microbial protein which the cow can then utilize. While this relationship is economically beneficial to farmers, it has made studying the protein and amino acid requirements very difficult. It is not possible to feed a protein-free or amino acid-defined diet to cattle and observe the milk production or body gain. On the contrary, the action of the rumen microbes has made reasonable production possible on diets without any true protein whatsoever. Researchers have known for years that the amount of microbial protein actually synthesized depends on two factors: the amount of organic material that is actually fermented and an adequate concentrate of rumen ammonia (National Research Council, 2001). At present, it is not possible to precisely predict the amount of organic matter that will be fermented, although this is becoming

more precise as we understand better the factors that affect fermentability. But we must recognize that the production, or lack of production, of microbial protein is one factor that can influence the results that we obtain with AA balance. As nutritionists we must recognize that we are always feeding two systems. One is microbial which, although it can use some AA and peptides, requires ammonia. The other is a mammalian system that requires a supply of both total AA, the metabolizable protein (MP) and essential AA (those that the mammalian body cannot synthesize or cannot synthesize in sufficient quantities).

#### The Mammalian "Problem"

Many nutritionists have the idea that supplying more AA will result in greater flow to the mammary gland and thus more milk protein. Unfortunately this may not be the case. Once absorbed into the cow, amino acids can contribute to protein, but the majority of the protein is deaminated and used for energy. In fact, the growing fetus depends on AA for up to about 50% of its glucose (Bell et al., 1995) as does the periparturient cow. There is no doubt that there are situations in mid-lactation cows where there is competition for AA between energy and protein secretion. In these situations protein can end up being fed for energy and not for amino acids. This is obviously a very expensive proposition, as even at today's prices protein is more expensive than energy. When there is more demand for energy than for AA, the liver actively deaminates excess AA, increasing urinary nitrogen excretion and MUN in milk. At least a portion of this nitrogen will be recycled through the saliva and directly through the rumen wall into the rumen contents (National Research Council, 2001). Unfortunately, it is not possible to model this urea recycling at present. Likewise, we must recognize that lysine and the branch chain amino acids do not directly contribute to glucose supply and add to urinary excretion. This results in more nitrogen being spilled into the environment.

We all recognize that the cow receives her AA from two sources, the microbial protein that is washed to the abomasum and the protein that escapes rumen degradation (i.e., the rumen undegradable protein or RUP). Both these sources must be absorbed as free AA from the small intestine. There is some debate on the factors that influence both the RUP amount and the AA composition of this protein (National Research Council, 2001). This again will require a nutritional model to calculate the RUP and its AA composition and to predict the overall AA economy of the animal. There also seems to be little doubt that the cow, like other species, uses AA more efficiently when they are in great deficit compared to when they are only slightly deficient (Hanigan, 2005). Whether this is a protective mechanism or is a reflection of greater demand for energy is unknown.

Although the liver appears to limit the concentration of AA in blood (Berthiaume et al., 2001), the mammary gland has a great ability to alter blood flow as well as extraction coefficients so as to obtain AA for a more or less constant supply of AA for milk protein synthesis when blood concentrations are low (Bequette et al., 2000;

Vanhatalo et al., 2003). It appears that by this mechanism evolution has protected the calf from great variation in milk protein content.

### The Requirement "Problem"

Because of the problems with the microbial production of proteins and the impossibility of feeding a protein-free diet, the AA requirements of dairy cattle are also subject to interpretation. The 2001 NRC gives requirements for AA as percentages of MP, which was based on a similar system previously suggested by French researchers. Methionine and lysine are the AA most likely to be limiting or to be co-limiting in typical North American diets (Schwab et al., 1992a and b), with histidine (Vanhatalo et al., 1999) being limiting or co-limiting on grass diets. This has been demonstrated by infusing graded levels of these AA into the duodenum of milking cows and observing an increase in milk protein percentage. While the ratio requirement is satisfying at first, these would be the only nutrients whose requirement was determined by ratio and not by weight. We (Patton et al., 2003) found in a meta-analysis of experiments that neither MET or LYS as a percent of MP were correlated with milk protein percent (Figures 1 and 2). Further, longer term experiments attempting to increase the milk protein by increasing MET and LYS as a % of MP with rumen protected methionine (RPMET) and LYS from blood meal have achieved little or moderate success in terms of milk protein percentage (Bucholtz et al., 2008). In another experiment with similar results (Benefield et al., 2006) it appeared that because of higher than expected DMI, estimated gram requirements were in excess, and this may have been the reason for the failure, but it certainly was not the result of low MET or LYS as % of MP in either of these cases. For this reason, we favor balancing for grams of AA. In any case, nutritional science has not yet established an expression of AA requirement that consistently correlates with high milk protein production. Clearly this relationship needs to be looked at more closely under commercial dairy conditions.

It may be worthwhile to focus on why field results do not match expectations created by scientific experiments. Many people have speculated that the big difference is because of tighter control and better management in the research setting. While this may have an influence, I suspect it is the experimental design that makes the big difference. Most AA balance experiments are done for short periods of time, either as Latin square experiments with 2 to 4 week periods or as infusion experiments with even shorter periods. Over short time periods, cows can make many metabolic adjustments to maintain their production. These experiments, although designed to show the maximum difference possible, are very different from what happens over longer periods of time. This could explain the initial protein increases with later decreases when AA balance is "made better." And it might also explain why it is not possible to balance rations longer term for less than 16.7% CP and still maintain production.

## The Model "Problem"

Because of the complexities involved in predicting microbial protein and in predicting the amount and AA composition of RUP, we must use a model to integrate all

these factors. We need to take a few moments to talk about models. If we look at the 3 most widely used models (AminoCow, CPM and NRC), we find that there are differences. As an example, a simple ration has been entered into all three ration programs with the same nutrient composition of CP, ADICP, NDICP, NDF, ADF, fat, and ash. Program defaults are then used to calculate the predicted flow of metabolizable protein (MP), microbial protein, methionine and lysine to the small intestine. This comparison is presented in Tables 2 and 2a. Notice that although the CP is identical, there are differences in the prediction of MP, microbial protein and flows of both MET and LYS, both in grams and as a % of MP. A similar situation exists if we consider metabolizable AA requirements for MET and LYS (Table 2b). Does this mean that one model is "better" than the others? Hardly! There are only so many studies on the flow of AA in dairy cattle, and the authors of these models are aware of them all. And, while they would all like the models to agree perfectly, these authors all believe their model best describes the nutritional situation. All are better than a blind guess.

It should also be kept in mind that different models require different inputs. Often these tests have not been made because of cost considerations, and there is reliance on default values as in the comparisons above. Defaults by definition are averages, and they may or may not adequately reflect the feed fed. The studies simply have not been done to determine whether these inputs add to the accuracy of the model or only to the complexity.

The point is that all models are imperfect and are in a constant state of development. For any given ration one or all of these models will be very incorrect. It is simply the nature of models. What working nutritionists need to do is to pick a model and learn to understand it. Then they should go about using AA balance in an organized way, noting the reaction of the cows when making changes. If they suspect that a model is consistently biased in over or under predicting the response to amino acid balance, they need to consider that the prediction of microbial protein production, the prediction of RUP or even amino acid requirements could influence the reported AA balance.

#### The DMI Problem

We have saved the biggest problem for last, but happily, it is also the easiest to remedy. If you are going to balance for AA, you must know the true dry matter intake. In Table 3 are presented estimated MP, metabolizable MET and metabolizable LYS for the exact ration we have compared for model differences, but this time we will use only the NRC model but with the predicted DMI from the other models. First of all, we need to recognize that the DMI prediction of each of these models has been developed from many published studies; so we can be sure that it is more than a guess. Yet there is more than 6 lb difference in the predicted intake for the same cow! How much milk is there in a difference of 6 lb of DMI? Obviously this difference in DMI changes the flow of AA more than the difference predicted among models!

There is a related, but different problem, and that is the problem of the one group TMR. Most of these rations have been balanced for the "high producing cows,"

generally from 90-120 lb of milk. These cows are usually only 15-20 % of the herd. By definition all the other cows in the herd are overfed, not only for amino acids, but also for every nutrient except perhaps fiber. While balancing for AA in this ration may elicit a response in the high producing cows, its effect will be diluted by the other 80% of the herd, and no clear response will be observed. The simple truth is that AA balance is a technology that only benefits the dairy producers that are business-oriented, those trying to increase feed efficiency. These are the dairy producers who are willing to track DMI on a daily basis and are willing to group cows and feed them according to production.

The take-home message is this: If you do not know the DMI for a group of cows with reasonable certainty, then do not balance for AA. It cannot be done.

## What Does This Mean?

By now, this may all seem too hard and too complicated. That was NOT the point we are trying to make with the previous discussion. The point is that because there are so many things that can affect the response to AA balance, just trying it once and deciding it does not work is not sufficient. Rather, as professionals, we need to think about all the other contingencies and then move to make sure that some confounding effect is not interfering. In short we need to learn to tweak the rations to get the most for our clients. Most importantly, we must encourage clients to measure DMI.

It will take an investment of time and study to understand some of the complexity of AA balance. But once these contingencies are understood, AA balance can be accomplished rather easily with a minimum of work for the nutritionist and an optimum of profit for the client.

## How to Balance for Amino Acids

Because of all the different herd situations where AA balance could be used, we will give a general recipe for working with AA balance according to the critical situations mentioned above.

## **Recipe to Reduce Ration Crude Protein**

- 1. Measure dry matter intake, obtain weight of cows and actual production of milk and protein for the group.
- 2. Pick a model with which you are comfortable. AC, CPM and NRC have all been research proven.
- 3. Increase the milk production 3 lbs more than is actually obtained.
- 4. Using available protein sources, including RPAA, balance the AA required at +1 for MET, +1 for HIS and +5 for the other AA. We tend to use RPMet as the MET source and expeller soy for our LYS source, although this is a choice and not a requirement.

5. For AC, meet the MP requirements. For CPM and NRC, the amount of MP can be about 100 gm deficient. For AC, do not worry about the RDP unless the deficit is greater than 0.8 lb. For CPM ignore the peptide balance; if NPN sources are limited to 100 g (0.25 lbs), peptide balance will not have an effect (Argyle and Baldwin, 1989). For NRC meet the RDP requirement.

## Recipe for Fresh Cow AA Balance

The following is the way that we balance rations for the fresh cow group (from 14-18 DIM). This has been successful in many localities.

This will be an exception to the measure DMI rule. For fresh cow groups we will assume that for mixed groups of primiparous and adult cows, Holsteins will produce 78 lb on average at 14 days in milk (DIM) and Jerseys will produce 57 lbs. Use the model predicted intake for these cows and DIM.

- 1. Enter the ration for fresh cows based on the production above and the days in milk above.
- 2. Note the grams of MP in excess of requirement. For CPM and NRC, add 100 g.
- 3. Amount of excess MP required for gluconeogenesis can be calculated in the following way.

Glucose required = milk production \*.05 / .5; (or the lbs of milk lactose divided by 0.5, adapted from Orskov (1986).

Dietary glucose supply (w/o gluconeogenesis from AA) = lbs dietary starch \* 0.5 (dietary starch is impossible to obtain from NRC. It can be crudely estimated at 0.7 \* NFC. For NRC, NFC will also need to be calculated.)

- 4. Subtract the glucose required from the glucose supplied in the diet. This equals the amount of glucose that must come from gluconeogenesis of AA.
- The amount of glucose from AA /.55 = amount of excess MP that must be provided. (This assumes that MP is converted to glucose with an efficiency of 55%.) If MP is given in grams, convert above number to grams by multiplying by 454.
- 6. Add a protein source, preferably a high RUP source such as corn gluten meal, expeller soy or a high quality blended RUP source. We tend to shy away from pure blood meal and pure fish meal because of intake problems at this stage of lactation.

## Maximizing Milk Protein

- 1. Measure dry matter intake, obtain weight of cows and actual production of milk and protein for the group.
- 2. Enter the ration into the computer program with which you are comfortable. Evaluate the ration with the milk protein percent actually produced and for the desired milk protein.
- 3. Make sure that dietary starch levels are between 23.5 and 25.5% of dry matter and ration sugar is 3.5-5.5% of dry matter. (For CP these will be estimated at 2% higher. For NRC use an NFC of 38.5-42.5.)

- 4. If actual production of milk protein and feed consumption indicates that g quantities AA are deficient, then something is in error. We would lower our expectations of getting a response to AA balance. (For NRC gram quantities can be calculated as MP required times 2.5% for MET, 7.4% for LYS, and 2.8% for HIS. In practice we reduce these by 0.4% with good effect.)
- 5. Make sure required grams for MET, LYS and HIS are supplied.
- 6. Adjust the ratio of MET to LYS at a 1:3 ratio if either are in excess and cannot be lowered.

## Summary

- 1. Balancing rations for AA is not difficult, but given the tools we have, it is not straightforward. Extra calculation may be required.
- 2. Because of the complexities of supplying AA to the small intestine of a cow, models must be used to predict this supply.
- 3. Pick a model with which you are comfortable. Learn to use it to get the results that you are expecting.
- The three situations where AA balance is critical are (1) in reducing protein consumption, (2) in balancing fresh cow diets, and (3) in increasing milk protein. Reducing dietary protein is the best use of AA balance.
- 5. Do not attempt AA balance unless the actual DMI is known for each group of cows.
- 6. Because many things can affect the response to AA balance, especially energy from NFC, check to make sure the energy from NFC is adequate. Adding energy from fat will reduce the milk protein percentage, although more pounds of milk may be produced.
- 7. Investment of time and study to understand the complexities of AA balance will result in more profit for clients while reducing the burden on the nutritionist.

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excretion.				
	Dietary CP (%), RPMET (g/day)			
Item	18.6, 0	17.3, 5	16.1, 10	14.8, 15
DMI (lb/ day)	51.5	51.5	52.4	52.1
Milk yield (lb/day)	87.3b	91.5a	91.5a	87.3b
Milk fat (%)	3.55	3.58	3.40	3.31
Milk true protein (%)	3.02	2.98	2.97	3.04
Milk fat (lb)	3.01ab	3.28a	3.15ab	2.90b
Milk protein (lb)	2.53b	2.71a	2.71a	2.64ab
MUN (mg.dl)	15.5a	11.8b	9.5c	7.9d
Urinary N excretion (g/day)	260a	207b	188c	150d

Table 1.Effect of reducing CP by replacing dietary soybean meal with high moisture<br/>corn plus rumen protected methionine (RPMET) on milk production and N<br/>excretion.

<sup>abcd</sup> Items within row with differing superscripts differ (P<.05).

After Broderick et al., 2008. J. Dairy Sci. 91:1092-1102.

Table 2.Diet (dry matter basis) and nutrient analysis used to predict metabolizable<br/>protein (MP), microbial protein (MCRB), methionine (MET) and lysine (LYS)<br/>flows to small intestine by three nutritional models.

Cow: 3rd lactation, producing 95 lb of 3.6% fat, 3.2% true protein, 180 DIM, 3.2 BCS

Diet:	Lb DM consumed
Alfalfa hay, 18% CP, 42% NDF	5.000
Corn silage, 8.5% CP, 44% NDF	28.000
Corn grain, finely ground"	11.000
Soybean meal, solvent 48% CP	10.000
Dicalcium phosphate	0.412
Limestone, finely ground"	0.500
Trace minerals	0.030
Vitamin A,D, E premix	0.098
Magnesium oxide	0.039
Salt, white	0.253
Fat, calcium salt"	0.792
Total	56.100
Analysis	
NEL (Mcal/lb)	0.77
Crude protein (% DM)	17.47
ADICP (% DM)	0.67
NDICP (%DM)	1.13
ADF (% DM)	16.00
NDF (% DM)	29.40
Lignin (% DM)	2.13
Fat (% DM)	3.99
Ash (% DM)	7.22
Calcium (% DM)	0.92
Phosphorus (% DM)	0.25

diet.						
Model <sup>1</sup>						
Item	AC	CPM	NRC			
Crude protein (% DM)	17.5	17.5	17.5			
MP (g/day)	2798	2916	3005			
MCRB (Crude protein, g/day)	2465	2645	2138			
MET (g/day)	66	76	64			
MET (% of MP)	1.89	1.99	1.76			
LYS (g/day)	236	244	229			
LYS (% of MP)	6.77	6.83	6.32			

**Table 2a.**Prediction of metabolizable protein (MP), microbial protein (MCRB),<br/>methionine, and lysine flows to the small intestine for a cow fed a simple<br/>diet.

 Table 2b.
 Differences in MP, metabolizable MET and metabolizable LYS requirements for the cow described above.

Model				
AC	CPM	NRC <sup>2</sup>		
2217	2931	2945		
60	53	74		
182	172	212		
	AC 2217 60 182	AC         CPM           2217         2931           60         53           182         172		

<sup>1</sup> Computed using AC (AminoCow, the Mepron Ration Evaluator, version 3.5.2); CPM (CPM Dairy Ration Evaluator, version 3.0.8.01 NRC (Nutrient Requirements of Dairy Cattle, Seventh Revised Edition, (version

1.0)

<sup>2</sup> Calculated for NRC by multiplying the MP requirement by 2.5% for MET and 7.4% for lysine as recommended in NRC 2001.

Table 3.	Comparison of g of metabolizable protein, metabolizable
	methionine and metabolizable lysine predicted by the NRC model
	for dry matter intakes predicted by three models.

	Model				
Item	CPM	AC	NRC	Dif	% Dif
DMI predicted (lb)	53.3	56.1	59.8	6.5	10.9
MP predicted (g)	2858	3005	3216	358	11.1
MET predicted (g)	50	53	56	6	10.7
MET predicted (%MP)	1.77	1.76	1.75	-0.02	-1.1
LYS predicted (g)	182	190	202	20.0	9.9
LYS predicted (%MP)	6.36	6.32	6.28	-0.08	-1.3



Figure 1. Relationship of methionine as % of MP against milk protein % for 281 published diets where methionine was measured at the intestine.



Figure 2. Relationship of lysine as a % of MP to milk protein % for 281 published diets where lysine was measured at the intestine.

# **SESSION NOTES**