# **Challenges in Protein Nutrition for Dairy Cows**

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### Take Home Messages

- Ideally, rations for dairy cows should be balanced for amino acids (AA), not for protein per se. As requirements are not clearly established for all AA, the best alternative is to balance for metabolizable protein (MP) with the right proportions for at least lysine and methionine, two AA with more known requirements.
- To meet the requirements for MP without an excess of nitrogen, rations must also be balanced for both rumen undegradable protein (RUP) and rumen degradable protein (RDP).
- Nutritional strategies can be used to manipulate milk protein concentration, despite the fact that it is quite a stable component in milk.
- The goal in protein feeding should be to optimize nitrogen utilization efficiency, which means minimizing total nitrogen intake while still meeting the requirements for milk protein synthesis, therefore reducing feeding costs and nitrogen excretion into the environment.

### Introduction

For many years, the Canadian dairy industry focused on producing low-fat, high-protein milk to keep up with consumer demand for healthy, low-fat dairy products. In more recent years, a massive surplus of skim milk powder across the country has created the demand for milk with higher fat content and lower solids-not-fat (SNF) content. The surplus of skim milk powder has occurred as a result of the importation of milk protein concentrates and related products, the development of technologies allowing more complete use of processing by-products such as whey, and the continued production of

milk with high protein, low fat content. Producers are now encouraged, through a penalty system, to reduce their SNF:fat ratio. This scenario demonstrates the need for producers to be able to rapidly change, through nutritional means, the composition of the milk they ship. While milk protein content is difficult to manipulate, it is possible to make small changes, both up and down, to accommodate the changing needs of the consumer. Understanding how the cow uses the proteins she consumes is critical to being able to make the desired changes in milk protein content in the most efficient manner possible.

### Protein Requirements

To synthesize milk protein, as any other protein, cows use free amino acids (AA). These AA are derived from food protein that escapes ruminal degradation and from microbial protein that is formed in the rumen. Both are then digested and absorbed across the small intestine and circulated to all tissues, including the mammary gland, via blood circulation. Although the cow's requirement is for AA, not for protein per se, protein supply and requirement for dairy cows were first defined as crude protein (CP). However, over the years it became clear that a more refined system was needed to accurately predict milk protein production. The reason for this is that CP simply provides an estimation of total nitrogen intake, and does not reflect the quality of the protein fed nor the site or extent of digestibility of that protein. Consequently the concept of protein degradability in the rumen was introduced, with requirements being established for rumen degradable protein (RDP) and rumen undegradable protein (RUP) derived from feed.

Rumen degradable protein can be considered a requirement for the rumen microbes, and not a requirement for the cow itself. The microbes, through enzymatic action, break down the RDP into ammonia, AA, and peptides. In addition to RDP, the microbes also degrade sloughed epithelial cells and endogenous salivary proteins. The microbes use the ammonia (available from RDP and urea recycling), AA and peptides in conjunction with available carbohydrates for their growth. They are part of the rumen outflow, and microbial protein may contribute more than 50% of the protein flowing to the small intestine (Clark et al., 1992). A deficiency of RDP leads to poor microbial growth reducing microbial protein synthesis, carbohydrate digestion and feed intake, and consequently milk production (Schwab and Boucher, 2005). Conversely, excess RDP is linked to reproductive dysfunction (NRC, 2001) and increased nitrogen excretion in urine.

Although microbial protein has an AA profile that meets the need for milk protein synthesis, the amount flowing to the small intestine is insufficient for today's high producing cow, necessitating the feeding of RUP (NRC, 2001). Sources of RUP such as fish meal, blood meal, soybean meal and corn gluten meal often present an imbalance for one or two AA and therefore, unless attempts are made to balance for AA supply, supplementation with high RUP sources may actually lower the quality of protein (i.e. AA profile) available for absorption in the small intestine. When cows are supplemented with RUP, it is critical that RDP does not become limiting. This will lower the yield of microbial protein resulting in a decrease in the total flow of protein to the duodenum or providing to the animal an imbalanced profile of AA thus possibly reducing milk production (Santos et al., 1998).

While setting requirements for RDP and RUP is an improvement over just using CP, there is not a strong relationship between RDP and RUP supply and milk protein output. Regression equations that predict milk protein output using estimated intakes of RDP, RUP, and dry matter intake show that these factors only account for half of the observed differences in milk protein output (NRC, 2001). Obviously, a more predictive measure of protein intake is required. Metabolizable protein (MP) is now the standard measure of protein supply and requirement for the dairy cow (NRC, 2001). Metabolizable protein is the protein supply available to the animal, i.e. digested in the small intestine, and is assessed either as total protein or AA flows. It is comprised of digestible microbial protein, RUP, and endogenous proteins including sloughed cells from the gastrointestinal tract and protein secretions into the lumen of the gut.

Dairy farmers should be fully aware that there is no direct relationship between MP and CP. Examination of the following two diets demonstrates this point (Table 1).

	Low MP	High MP
Ingredient Composition (% DM)		
Alfalfa hay	25.0	25.0
Barley silage	38.3	38.4
Barley grain	30.6	17.3
Corn grain		13.4
Canola meal	5.0	
Corn gluten meal		1.9
Soybean meal		1.2
Blood meal		1.2
Fish meal		1.1
Urea	0.54	
Vit-Min mix		0.6
Nutrient Composition and intake		
Net energy of lactation, Mcal/kg	1.48	1.48
Crude protein, % DM	17.5	17.5
Dry matter intake, kg/day	26.1	26.1
Metabolizable protein, g/day	2197	2674

Table 1: Crude protein and metabolizable protein of two lactating cow diets.

The two diets are equal in terms of net energy and crude protein content, but when fed at 26 kg/day, the low MP diet supplies 477 g/d less MP than the high MP diet. This translates into a predicted difference in milk protein yield of 342 g/d or 10.7 kg of milk/day. The bottom line is that a high CP diet does not necessarily translate into a high quality diet in terms of protein or AA availability to the cow.

Metabolizable protein is comprised of 20 common AA, 10 of which are considered essential (the animal must obtain them from the diet) and 10 that are considered nonessential (the animal can synthesize these in the body from the essential AA and other compounds). Considering that a significant portion of MP is RUP, which may be highly variable in its constituents, the variability in essential AA profile of MP is huge. To understand this concept, examine the following two diets (Table 2).

	Diet A		Diet B	
Ingredient Composition (% DM)				
Alfalfa hay	24.9		24.9	
Barley silage	38.3		38.3	
Barley grain	9.6		17.2	
Corn grain	22.0 12.3			
Corn gluten meal	3.1			
Soybean meal			3.6	
Blood meal			1.2	
Fish meal			2.0	
Feather meal	1.4			
Vit-Min mix	0.6		0.6	
Nutrient Supply				
Metabolizable protein, g/day	2676		2673	
Digestible AA, g/day		% MP		%MP
Histidine	53	1.98	62	2.32
Leucine	261	9.75	230	8.60
Lysine	153	5.72	179	6.70
Methionine	51	1.91	50	1.87
Phenylalanine	135	5.04	133	4.98
Threonine	127	4.75	129	4.83

 Table 2: AA profile of two lactating cow diets that supply equivalent amounts of metabolizable protein.

You can see that the diets supply equivalent amounts of MP but substantially different amounts of digestible histidine, leucine, and lysine. In diet A, availability of histidine and lysine are much lower than in diet B, while in diet B, leucine is lower than in diet A. We will discuss in the next sections how this could impact milk protein synthesis. Because milk protein is relatively consistent in its composition and its synthesis requires that AA are available in specific proportions and amounts, if any one AA is in short supply relative to the other AA, then milk synthesis will be decreased. So, although the two

diets supply equal amounts of MP, the potential for milk protein synthesis is different.

# Amino Acid Supply and Requirements

Ideally, because the tissues, including the mammary gland, are using AA to synthesize proteins, diets for lactating cows should be formulated on the basis of AA supply, not just MP supply. Models have been developed to predict the flow of digestible AA to the small intestine (CNCPS; NRC, 2001), and indeed, programs such as the Cornell Net Carbohydrate and Protein System (CNCPS, 2000) relate milk protein production potential to digestible AA intestinal supply. However, the availability of AA at the mammary gland is not the same as the amount absorbed from the small intestine due to modulation of AA supply by the gut and the liver. For example, histidine, methionine and phenylalanine are catabolized (removed) extensively by the liver, whereas isoleucine, leucine, and valine are removed in very limited amounts by the liver but are catabolized by gut tissues (Lapierre et al., 2005 & 2006). Removal of AA by the splanchnic tissues (gut and liver) can be interpreted in 2 ways. One, they actively control AA supply to the peripheral tissues and thus regulate mammary gland AA supply and milk protein production, or two, they act passively, removing excess AA that are not required by the peripheral tissues. Either way, their impact needs to be considered when determining the requirements of the dairy cow for AA.

Within the mammary gland, metabolism varies among the AA. In general, the essential AA have been divided in two groups (Mepham, 1982). The AA of Group 1 are taken up by the mammary gland in amounts equal to their output in milk protein (histidine, methionine, phenylalanine, tryptophan), whereas those in Group 2 are taken up in excess of their output in milk protein (arginine, isoleucine, leucine, lysine, valine). For those that are taken up in excess, this implies that there is a use for these AA other than for milk protein synthesis. One role for these AA would be the synthesis of nonessential AA. Indeed, the nitrogen from lysine taken up in excess has been utilized for the synthesis of aspartate and glutamate within the mammary gland (Lapierre et al., 2003). This uptake of AA from Group 2 in excess of milk protein output does not seem, at least on an individual basis, to be absolutely necessary to maintain milk production (Bequette et al., 1996; Lapierre et al., 2005). Conversely, some of the nonessential AA are taken up by the gland in lesser amounts than their output in milk protein, implying that these AA must be synthesized within the gland.

Although these general relationships exist, the uptake to output ratio is not static, but varies with MP supply, at least for the AA of Group 2. For example, as MP supply was increased from 1922 to 2517 g/d, the uptake to output ratio of histidine, methionine, and phenylalanine remained relatively constant,

whereas the ratio increased substantially for isoleucine, leucine, and valine (Raggio et al., 2004). The increase in uptake to output ratio with increasing MP supply suggests that the catabolism of these AA within the mammary gland is increased at high protein supply.

# Amino Acid Efficiency of Use

Current prediction models such as NRC (2001) and CNCPS (2000) use fixed factors of conversion of available MP or AA supply to milk protein. Metabolizable protein is assumed to be used with an efficiency of 67% (NRC, 2001). This means that once the maintenance requirement has been removed, for every 1 kg of protein absorbed across the digestive tract, 0.67 kg of milk protein is synthesized, until requirements are met. However, biological systems do not operate on fixed factors, and as we have already seen, supply of AA at the intestine does not necessarily equate with supply at the mammary gland. The use of fixed efficiency factors contributes to the underprediction of milk protein secretion at low protein intakes.

Using the results of 59 trials and 217 treatments in which AA were infused postruminally, we (Doepel et al., 2004) determined mathematically the milk protein yield response to metabolizable AA supply. This is demonstrated in Figure 1 using a non-linear (logistic) model and lysine as a representative AA. Initially, milk protein yield increases at a constant rate with increasing AA supply, but then as AA supply increases further, milk protein yield increases at a decreasing rate. As we approach requirement, there is a diminishing rate of return (see Table 3 and discussion below).

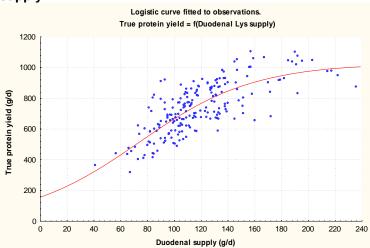


Figure 1: Relationship between milk protein yield and duodenal lysine supply

From these observations, we can conclude that the efficiency of AA use varies with metabolizable AA supply. Variable efficiency factors should be incorporated into milk protein prediction schemes as they will improve our ability to predict milk protein yield in response to supplemental protein.

From this same database, we also calculated the optimal amounts of digestible AA for the lactating cow (Table 3). The recommendations for lysine and methionine, expressed as a percentage of MP, are in agreement with currently accepted recommendations (NRC, 2001). This suggests a certain reliability for our estimations for the other AA.

AA	% EAA <sup>1</sup>	%MP <sup>2</sup>
Arginine	9.6	4.6
Histidine	5.1	2.4
Isoleucine	11.1	5.3
Leucine	18.5	8.9
Lysine	15.0	7.2
Methionine	5.3	2.5
Phenylalanine	11.4	5.5
Threonine	10.4	5.0
Valine	13.6	6.5

Table 3: Optimal relative amounts of digestible AA supply.

<sup>1</sup>EAA: essential AA, excluding Trp.

<sup>2</sup>MP: metabolizable protein, assuming that EAA represent 48% of MP.

Adapted from Doepel et al., 2004

Using the data from Figure 1 and Table 3, we calculated the efficiency of conversion of AA into milk protein at various levels of AA supply relative to the optimum level (Table 4). The fixed efficiency factors of CNCPS (2000) are also shown for comparison.

AA		CNCPS, 2000			
	50%	75%	100%	125%	(fixed)
Arg	0.71	0.57	0.49	0.44	0.35
His	1.09	0.88	0.76	0.68	0.96
lle	0.86	0.72	0.65	0.58	0.66
Leu	0.83	0.70	0.61	0.55	0.72
Lys	0.90	0.76	0.68	0.60	0.82
Met	0.89	0.75	0.66	0.59	1.00
Phe	0.75	0.61	0.53	0.48	0.98
Thr	0.82	0.67	0.60	0.55	0.78
Val	0.86	0.71	0.62	0.56	0.62

# Table 4: Efficiencies of utilisation<sup>1</sup> of amino acids (AA) for lactation after discounting the maintenance<sup>2</sup> requirements from total AA supply.

<sup>1</sup>Calculated from AA in milk as a function of AA available for milk.

<sup>2</sup>Maintenance requirements included scurf protein, urinary protein, metabolic fecal protein, and endogenous protein (NRC, 2001).

Adapted from Doepel et al., 2004

It can be concluded that efficiency of use varies among individual AA, and also varies with metabolizable AA supply. It is clear from this data that to advance in protein nutrition of the lactating cow, we must consider the individual AA and not just MP as a single entity. If we can achieve the proper balance and supply of AA, then efficiency of use will be maximized and nitrogen excretion into the environment, overfeeding of protein, and feed costs will be reduced.

### Nutritional Manipulation of Milk Protein

For many years in Canada the emphasis on dairy producers was to increase milk protein content and decrease milk fat. Now the emphasis has reversed, with there being a need to maximize milk fat and reduce milk protein. The ability to alter milk components rapidly, either up or down, must occur through nutritional strategies.

### **Reducing Milk Protein Content**

The use of fat in the diet of lactating cows normally reduces milk protein content by about 5 to 10%. Cant et al. (1991) fed 1<sup>st</sup> lactation heifers diets with either no added fat or with 4% added fat in the form of yellow grease and found that milk protein decreased from 3.15% to 3.0% with the inclusion of the dietary fat, and milk fat content increased from 3.48% to 3.71%. The overall effect of the dietary fat addition on the SNF:fat ratio ([protein + lactose]/fat) was a decrease from 2.319 to 2.100. While the reduction in milk protein may

be advantageous for the producer in today's economic market, it is not as desirable for the processor, as the change in milk protein was associated with a decrease in casein content and an increase in whey, both of which would have undesirable effects on the manufacture of cheese.

### **Increasing Milk Protein Content**

Milk protein content and yield can be increased by increasing energy intake through manipulation of the forage:concentrate (F:C) ratio. Macleod et al. (1983) observed an increase in milk protein content from 3.12% to 3.22% when the F:C ratio of the diet was decreased from 65:35 to 50:50. It must be kept in mind that a decrease in the F:C ratio also tends to be associated with a reduction in milk fat content. In the study of Macleod et al. (1983), milk fat content decreased from 3.72 to 3.68.

Numerous studies have shown that increasing the supply of digestible AA via postruminal infusions increases milk protein content and yield (Doepel et al., 2004). However, recovery of the supplemental protein in milk protein is low and thus one must consider the economic and environmental implications of this strategy.

An adequate balance of individual AA could be the best way to increase protein concentration in milk without reducing the efficiency of nitrogen utilization. If a single AA is limiting in the diet being fed, the other AA that are in excess will be catabolized into urea and not used for protein synthesis. In theory, additional supplementation of this single AA would increase milk protein synthesis, because the other AA that were previously in excess would now be incorporated into milk protein due to the improved balance of AA. Indeed, the requirements for Lys and Met as a proportion of MP have been established by the relation between milk protein concentration and the proportion of each of these AA in the diet (NRC, 2001 - see page 84, Fig. 5-12). The challenge facing dairy nutritionists is to refine the assessment of the supply and requirement for individual AA to obtain, for each of them, the best match through diet manipulation. If diet manipulation is not enough, rumen protected forms of AA can be added to the diet. At the present time, only Met is commercially available as a rumen-protected supplement. As more is known on the actual requirements of individual AA and as technology evolves, there might be enough incentive to develop other products to fulfill this task.

### Summary

The goals in protein nutrition of dairy cows are to produce milk with a desirable protein content and to optimize AA utilization and efficiency, thus minimizing feed costs and maximizing economic returns. Ideally, lactating cow diets should be balanced for AA. However, given the current state of

knowledge, the best strategy is to balance for metabolizable protein, with adequate proportions of lysine and methionine. Recommendations for the essential AA are given but will need to be confirmed by other studies. Variable efficiency of use of MP or better, individual AA, will need to be incorporated into predictive models to avoid under-prediction of milk protein yield at low protein intake, and vice-versa, over-prediction at high intakes. The ability to alter milk protein content is limited, but present, and as we increase our knowledge of AA metabolism, we will be better prepared to produce milk with a composition that is desirable by consumers and the economic market.

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