AMINO ACID NUTRITION OF LACTATING COWS

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Introduction

It has been known for decades that absorbed amino acids (AA), and not protein per se, are the required nutrients. Used principally as building blocks for the synthesis of proteins, absorbed amino acids are vital to the maintenance, growth, reproduction, and lactation of dairy cattle. It is also understood from poultry (NRC, 1994) and swine (NRC, 1998) research that an ideal profile of absorbed essential AA (EAA) exists for maintenance, growth, and lactation. While these ideal profiles remain to be established for dairy cattle, it is known that feeds vary in AA composition and that the ingredient composition of the diet affects the AA composition of duodenal protein.

To advance research on AA requirements, and to allow for improved ration formulation as new information on AA requirements becomes available, the protein model of NRC (2001) was extended to one that would most accurately predict the profile and flows of EAA to the small intestine. The focus was on EAA because there was no evidence that nonessential AA (NEAA) (as a group) would ever become more limiting than the EAA. Thus, there was no apparent reason to consider NEAA.

The purpose of this paper is to review current knowledge regarding limiting AA, required concentrations of limiting EAA in metabolizable protein (MP), and responses of lactating cows to ruminally protected AA. Some comments on the economics of using a ruminally protected Met product are also provided.

Factors Affecting the Profile of Absorbed Amino Acids

It is now clear from a summary of two large data sets (Rulquin *et al*., 1998; NRC, 2001) that two factors account for most of the variation in AA profiles of duodenal protein. These are the proportional contribution that RUP makes to total protein passage and the AA composition of that RUP. This would be expected because feed proteins vary in AA composition and usually differ from ruminally synthesized microbial protein (Table 1).

Limiting Amino Acids

Methionine (Met), lysine (Lys), and histidine (His) have been identified most often as the most limiting AA for dairy cattle. As expected from the above discussion, the extent and sequence of their limitation appears to be affected primarily by the amount of RUP in the diet and its AA composition.

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Methionine has been shown to be first limiting for growth and milk protein production when dairy cattle were fed high forage or soybean hull-based diets and intake of RUP was low. Methionine has also been identified as first limiting for growing cattle and lactating cows that were fed a variety of diets in which most of the supplemental RUP was provided by soybean protein, animal-derived proteins, or a combination of the two. In contrast, Lys has been identified as first limiting for growth and milk protein synthesis when corn and feeds of corn origin provided most or all of the RUP in the diet (NRC, 2001). Relative to concentrations in microbial protein, feeds of corn origin are low in Lys and similar in Met whereas soybean products and most animal-derived proteins are similar in Lys and low in Met (Table 1).

Methionine and Lys have been identified as co-limiting AA for milk protein synthesis when cows were fed corn silage-based diets with little or no protein supplementation (NRC, 2001). And more recently, His has been identified as first limiting for milk protein production when dairy cows were fed grass silage-cereal (barley and oats) based diets, with or without feather meal as the sole source of RUP supplementation (Kim *et al*., 1999, 2001; Vanhatalo *et al*., 1999).

It should not be too surprising that these AA have all been shown to be first limiting. First, all have been identified as being among the most limiting AA in microbial protein. Methionine has been identified as first limiting and Lys as second limiting in microbial protein for nitrogen retention of both growing cattle and growing lambs. Histidine has been identified as possibly third limiting for sheep.

Second, concentrations of Met and Lys in most feed proteins are lower than in microbial protein (Table 1). Thus, most feed proteins are not complementary to microbial protein and instead, when they are fed, will accentuate rather than eliminate deficiencies of Met and Lys in MP. This also appears to be why Met and Lys become more limiting (relative to the other EAA) with increasing intakes of complementary sources of RUP.

Third, Lys is more vulnerable to heat processing than the other EAA. Over-heating decreases Lys concentrations and can decrease the availability of the remaining Lys.

Required Concentrations of Limiting EAA in Metabolizable Protein (MP)

It was the opinion of the NRC (2001) committee that knowledge was too limited, both for model construction and model evaluation, to put forth a model that "quantifies" AA requirements for dairy cattle. However, an alternate and first step to that approach is to begin to define the ideal content of EAA in MP. This requires establishing dose-response relationships between changes in concentrations of EAA in MP (at least those considered to be the most limiting) and animal responses. As mentioned, the NRC (2001) model predicts concentrations of EAA in MP. Because studies have evaluated milk protein responses to changes in concentrations of Lys and Met in duodenal protein, the prerequisites were in place to use the model to define the requirements for Lys and Met in MP for lactating cows.

The approach that was used was that described by Rulquin et al. (1993). Experiments were identified in which one or more levels of either Lys or Met were infused continuously into the abomasum or duodenum or fed in ruminally-inert form. To calculate the concentrations of Lys and Met in MP, all cow and diet data were entered into the model. Contributions of supplemental Lys and Met to predicted flows of metabolizable Lys and Met from the basal diet were calculated as described in the publication. Also described are the calculations that allowed the pooling of data from different experiments.

Figure 1 shows the plot of predicted concentrations of Lys in MP and the corresponding responses for milk protein content. The final regression analysis was limited to data where Met was adequate or near adequacy (1.95% or more of MP). This was done to help ensure that Met did not become more limiting than Lys. Using this restricted data, it was observed that a rectilinear model was slightly superior to quadratic models for describing the relationship between changes in milk protein content and content of Lys in MP. The breakpoint estimate for the required concentration of Lys in MP for maximal content of milk protein is 7.2%.

Figure 1 also shows the corresponding plot for Met. In this case, the final regression analysis was limited to data where Lys was adequate or near adequacy (6.50% or more of MP). As in the development of the dose-response plot for Lys, this was done to help ensure that Lys did not become more limiting than Met. Again, the rectilinear model was superior to the other models for describing milk protein responses to increasing amounts of Met in MP. The breakpoint estimate for the required concentration of Met in MP for maximal content of milk protein is 2.4%.

In summary, the model indicates optimal use of MP for maintenance plus milk protein production when Lys and Met approximate 7.2% and 2.4% of MP, respectively. Therefore, the optimum ratio of Lys and Met in MP is 3.0/1.0 using this model. A unique and practical feature of this approach for determining the required concentrations of EAA in MP is that the "requirements" were arrived at by using "real" production data and the NRC (2001) model. In other words, the requirements are specific to the use of the NRC (2001) model. An analysis of the same production data with another model may result in different-looking dose-response plots, and therefore, different "requirements" for Lys and Met in MP.

As might be expected from the previous discussion on limiting AA, it is not possible with available protein supplements to achieve what the model indicates are the "optimum" levels of 7.2% Lys and 2.4% Met in MP. For those of you who have evaluated some of your diets with NRC (2001), you know that to be the case. Table 2 shows NRC (2001) evaluations of four diets. All were being fed to high producing cows. Other than low milk proteins, the producers were happy with the diets. Several observations are worth noting. First, in all cases, predicted concentrations of Lys and Met in MP fall rather short of the apparent optimum requirements of 7.2 and 2.4%. Second, the ratio of Lys and Met in MP is similar for all diets (3.41-3.66/1.00) but considerably higher than the apparent optimum 3.0/1.0 ratio. From these two observations, we would conclude that Met is the first limiting AA in all cases. And finally, in spite of the fact that the four diets differ considerably in ingredient composition, the adverse effect that increasing levels of dietary RUP has on Lys and Met concentrations in MP is evident.

Responses to Rumen-Protected AA

The responses of dairy cattle to improved concentrations of Lys and Met in MP have been reviewed (NRC, 2001). In all experiments, levels of Lys and Met in MP were increased by intestinal infusion of the AA or by feeding them in a ruminally-protected form. Growing cattle respond to improved Lys and Met nutrition with variable increases in body weight gains and feed efficiency and variable decreases in urinary N excretion. The most common responses of lactating cows to improved Lys and Met nutrition are variable increases in content and yield of milk protein, milk yield, and feed intake. The literature review for lactating cows revealed the following five observations. First, milk protein content is more responsive than milk yield to improvements in Lys and Met nutrition. This is particularly true in post-peak lactation cows. Second, increases in milk protein percentage are independent of milk yield. Third, increases in milk protein concentrations are just as great if not greater during later lactation as they are in mid lactation. Fourth, increases in milk protein production to increases in MP of either of the two AA are the most predictable when the amounts of the other AA in MP is near or at estimated requirements. And last, milk yield responses to increased amounts of Lys and Met in MP are most often observed when cows are in the first 2 to 3 months of lactation.

That milk protein percentage is more sensitive than milk yield was demonstrated nicely in a single experiment by Chapoutot *et al*. (1992). The authors used a multiple switch-back experiment as a way to evaluate the responses of individual cows to ruminally protected Lys and Met. Of the forty cows that participated in the experiment, 37 responded with increased content of milk protein, 31 responded with greater protein yield, and 16 responded with more milk.

There are also several reports of increased percentages of milk fat with increased amounts of Met or Met plus Lys in MP (NRC, 2001). As noted in NRC (2001), these increases have almost always been observed in conjunction with increases in milk protein. Unlike milk protein responses, milk fat responses to improved Met and Lys nutrition have not been predictable. The reasons as to why improved Met and Lys nutrition may increase milk concentrations are not clear. It has been suggested that correcting a Met deficiency may enhance *de novo* synthesis of short- and medium-chain fatty acids in the mammary gland (Pisulewski *et al*., 1996). There also is limited evidence of increased formation or secretion of chylomicrons and VLDL with improved Lys and Met nutrition. In either case, the mammary gland would experience an increased supply of fatty acids.

Experimental data still limited as to the magnitude of the production responses that one can expect with early lactation cows when the only change that is made is one of more adequate concentrations of Lys, Met or both in MP. In an attempt to answer that question, Garthwaite *et al*. (1998) summarized 11 experiments in which ruminally protected forms of Met or Lys plus Met were fed. When supplementation commenced 7 to 21 days before calving, the cows responded with an average of +1.7 kg milk, +0.06 percentage units milk protein, +79 g milk protein, +0.10 percentage units milk fat, and +85 g milk fat during the first 28 to 112 days of lactation. When the data were removed of two experiments in which there was evidence of over-feeding of rumen-protected Met, the average responses to supplemental AA were +2.3 kg milk, +0.09 percentage units milk protein, +112 g milk protein, +0.10 percentage units milk fat, and +116 g milk fat. When AA supplementation commenced 0 to 35 days after calving, the cows responded with an average of +0.7 kg milk, +0.16 percentage units milk protein, +79 g milk protein, +0.02 percentage units milk fat, and +48 g milk fat during the next 21 to 119 days of lactation.

Availability of Ruminally Protected Amino Acids

At this time, commercial products are limited to Met-Plus™ (Nisso America, Inc.), Mepron® M85 (Degussa Corporation), and Smartamine™ M (Aventis Animal Nutrition). In all cases, these are ruminally protected Met products. The three products are distinctly different in the protection technology that is used.

Met-Plus™ is an example of a lipid-protected product. It is a matrix compound that contains 65% DL-methionine embedded in a mixture of calcium salts of long-chain fatty acids, lauric acid, and butylated hydroxytoluene (BHT); BHT is a preservative for the fatty acids. Similar to other lipid-coated products that have been on the market, the technology relies on achieving a balance between ruminal protection vs. intestinal release so as to maximize the amount of Met available for intestinal absorption while minimizing losses in the rumen and in feces.

Mepron® M85 is an example of a surface-coated, carbohydrate-protected product. The small pellets have a diameter of 1.8 mm, a length of 3-4 mm, and an approximate density of 1.2 g /cm^3. The pellets consist of a core of DL-Met and starch coated with several thin layers of ethylcellulose and stearic acid. The final product contains a minimum of 85 % Met, and approximately 8.5% non-structural carbohydrates, 3.5% NDF, 1.5 % ash, 1.0 % moisture, and 0.5% crude fat. The technology is a combination of coating materials and application that allows for a large payload of Met. Because enzymatic digestion of the ethyl cellulose is minimal, degradation of the product occurs primarily through physical action and abrasion. The result is a product that results in a slow degradation in the rumen and a slow release of Met in the intestine.

Smartamine™ M is an example of a lipid/pH-sensitive polymer-protected product. It is a surface-coated product that contains a minimum of 75% DL-Met. The small 2-mm pellets consist of a core of Met plus ethylcellulose which is covered with a coat of stearic acid containing small droplets of poly (2-vinylpyridine-co-styrene). The copolymer contributes 3% by weight of the final product. The presence of the copolymer appears to alter the steriochemistry of the stearic acid such that the surface-coating becomes enhanced in its resistant to ruminal degradation. The presence of the copolymer, as a result of its solubilization at low pH, also allows for a rapid release of the Met in the abomasum.

The "Economics" of Using a Ruminally Protected Met Product

Understandably, the economics of using a ruminally protected Met product will vary from farm to farm. Thus, there will be no attempt in this paper to arrive at some average benefit-to-cost ratios for using the products. However, it has been our experience and observation that the economics of using a ruminally protected Met product can be very favorable. This is particularly true if the products are used in conjunction with an overall feeding strategy that is clearly aimed at maximizing the efficiency of milk protein production. In part, it is because of economics that some nutritionists have dramatically increased their use of the products.

There are two key factors that influence the economics of feeding a rumen-protected Met product. First and foremost, there must be both a willingness and confidence of both the producer and the nutritionist to put "science into practice" and to use the new models that have been developed that predict concentrations of AA in MP. There must be a willingness "to bend" on the protein supplements that are fed and to select high-RUP supplements that complement the use of a ruminally protected Met product. There must be willingness to accept the fact that improving the profile of EAA in RUP, and thus in MP, reduces the need for RUP. And second, the economics are enhanced considerably if the producer is paid for milk protein. The cost of ruminally protected Met products should not be the determining factor as their cost to deliver a gram of MP-Met is considerably less than high-RUP supplements (Table 3).

Let us share just one example to make our point. Approximately 75 of our dairy cows at the University of New Hampshire were fed diet 4 in Table 2 for 11 consecutive months. During that 11-month period, milk true protein concentrations ranged between 2.70 and 2.83%. Milk fat concentrations averaged 3.4 to 3.7%.

At the end of that period, the ingredient composition of the diet was changed to the following (% of DM): 30.9% corn silage, 12.3% grass silage, 6.0% alfalfa hay, 19.1% ground corn, 9.4% ground barley, 3.7% soy hulls, 7.4% soybean meal, 3.7% canola meal, 0.14% urea, 2.2% of a high quality animal protein blend that contained 80% blood meal, 0.075% Smartamine M, 0.100% Rhodimet AT88 (MHA, Aventis Animal Nutrition), 1.9% fat supplements, and 3.1% minerals and vitamins. A comparison of these values with those in Table 2 will indicate that the biggest changes were the substitution of the expeller soybean meal with the high blood meal product (to further increase Lys in MP), the addition of a ruminally protected Met product to the diet (to achieve the desired 3.0/1.0 Lys to Met ratio in MP), a replacement of some of the soybean meal with canola meal and urea (to provide a more diverse mix of RDP sources and to lower the cost of RDP), and a reduction in the amounts of RDP and RUP in the diet (to eliminate some of what the Dairy NRC Model indicated to be a surplus and to off-set the higher cost of the high blood meal product). The new diet contained 17.2% CP as compared to 18.1% for the old diet. The NRC evaluation of the diet indicated 10.6% RDP (instead of 10.8%) and 6.6% RUP (instead of 7.3%). Because of the decrease in RUP, predicted MP flows to the small intestine were decreased from 3071 g/d to 2809 g/d. However, the predicted concentrations of Lys and Met in MP increased from 6.34% and 1.73% (Table 2) to 6.55% and 2.20%. Therefore, even though predicted passage of MP was decreased, the predicted flow of MP-Met (previously the "weakest link") was increased from 53 to 61 g/d, a 16% increase.

The cows were switched gradually over a 10-day period to the new diet. For the 2-wk period preceding the transition to the new diet, milk protein concentrations averaged 2.82%. Although considered to be very low, this level of milk protein was at the high end of the range (2.70% to 2.83%) for the preceding 11-month period. One week after the change, milk protein concentrations had increased to 3.01%. At the end of wk 2, protein increased to 3.06% and by wk 4 it had increased to 3.13%. Thereafter, and for the next couple of months while it was being monitored, milk protein stabilized between 3.12 and 3.16%. As expected because of the decrease in ration CP, milk urea N decreased from an average of 14.5 to to an average of 12.4 mg/dL. Milk fat concentrations also tended to increase. Milk yields also may have benefited but it was difficult to determine that as the cows on average were advancing in days-in-milk. And finally, a cost analysis of the diet indicated no increase in daily feed costs. For these calculations it was assumed that DM intake was not affected. However, based on the increases in milk protein and milk fat concentrations that were obtained, milk income was increased nearly $0.70 per cow per day.

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Table 1. A comparison of the essential amino acid (EAA) profiles of body lean tissue and milk with that of ruminal bacteria and protozoa and some common feeds.

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	EAA
					(% of total EAA)						(% of CP)
Animal products											
Lean tissue[a]	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1	-
Milk[b]	7.2	5.5	11.4	19.5	16.0	5.5	10.0	8.9	3.0	13.0	-
Rumen microbes											
Bacteria[c]	10.4	4.1	11.5	15.9	16.5	5.1	10.1	11.3	2.7	12.4	-
Protozoa[d]	9.3	3.6	12.7	15.8	20.6	4.2	0.0	10.5	2.8	9.7	-
Forages[e,f]							10.7				
Alfalfa hay	12.5	4.7	10.3	17.9	12.4	3.8		10.6	3.6	12.7	41.2
Alfalfa silage	10.9	4.7	11.1	17.9	12.1	3.8	11.6	10.7	2.7	14.1	35.6
Corn silage	6.2	5.7	10.6	27.2	7.9	4.8	11.7	10.1	1.4	14.1	31.6
Grass hay	11.7	4.9	10.0	18.8	10.5	3.9	12.1	10.9	3.7	13.6	33.1
Grass silage	9.4	5.1	10.9	18.8	10.1	3.7	11.8	10.2	3.3	15.0	32.6
Grains[e]							13.4				
Barley	13.4	6.1	9.2	18.5	9.6	4.5		9.1	3.1	13.0	37.7
Corn	11.5	7.8	8.2	27.9	7.1	5.3	13.5	8.8	1.8	10.0	40.1
Oats	16.6	5.9	9.1	17.7	10.1	4.2	11.5	8.4	2.9	12.6	41.2
Sorghum	9.4	5.7	9.3	31.9	5.4	4.2	12.5	7.8	2.5	11.6	42.8
Wheat	13.6	7.1	9.6	19.3	8.1	4.6	12.3	8.4	3.5	12.3	34.4
Plant proteins[e]							13.3				
Brewers grains	14.7	5.1	9.8	20.0	10.4	4.3		9.1	2.5	12.1	39.2
Canola meal	16.5	6.6	9.0	15.9	13.2	4.4	11.7	10.4	3.4	11.1	42.6
Corn DDG w/sol.	10.7	6.6	9.8	25.4	5.9	4.8	9.5	9.1	2.3	12.4	37.8
Corn gluten meal	7.1	4.7	9.1	37.2	3.7	5.2	12.9	7.5	1.2	10.3	45.2
Cottonseed meal	26.0	6.6	7.3	13.8	9.7	3.7	14.1	7.6	2.8	10.0	42.6
Linseed meal	20.9	4.8	11.0	14.5	8.7	4.2	12.5	8.9	3.7	12.3	42.2
Peanut meal	27.6	6.0	8.1	15.9	8.3	2.9	11.1	6.7	2.4	9.8	40.1
Soybean meal	16.2	6.1	10.1	17.2	13.9	3.2	12.1	8.7	2.8	10.2	45.3
Sunflower meal	20.8	6.2	9.9	15.2	8.0	5.6	11.6	8.7	2.9	11.7	42.2
Animal proteins[e]							11.0				
Blood meal	7.8	1.3	2.2	22.7	15.9	2.1		7.7	2.8	15.4	56.4
Feather meal	16.2	2.7	11.4	19.9	6.0	1.8	12.1	11.1	1.7	17.6	42.7
Fish meal	13.1	6.4	9.2	16.2	17.2	6.3	11.6	9.4	2.4	10.8	44.5
Meat & bone meal	19.5	5.3	7.7	17.2	14.5	3.9	9.0	9.1	1.6	11.8	35.7
Whey, dry	5.0	4.5	12.1	21.2	17.6	3.3	9.4	14.1	3.5	11.7	42.2
							7.0				

[a] Average values of empty, whole body carcasses as reported in three studies.
[b] Average values as reported in three studies.
[c] The mean of average values from over one hundred dietary treatments.
[d] Average values from fifteen literature reports.
[e] Calculated from values presented in NRC (2001).
[f] Legume and grass hays and silages are mid-maturity.

Table 2. NRC (2001) evaluation of four commercial diets.

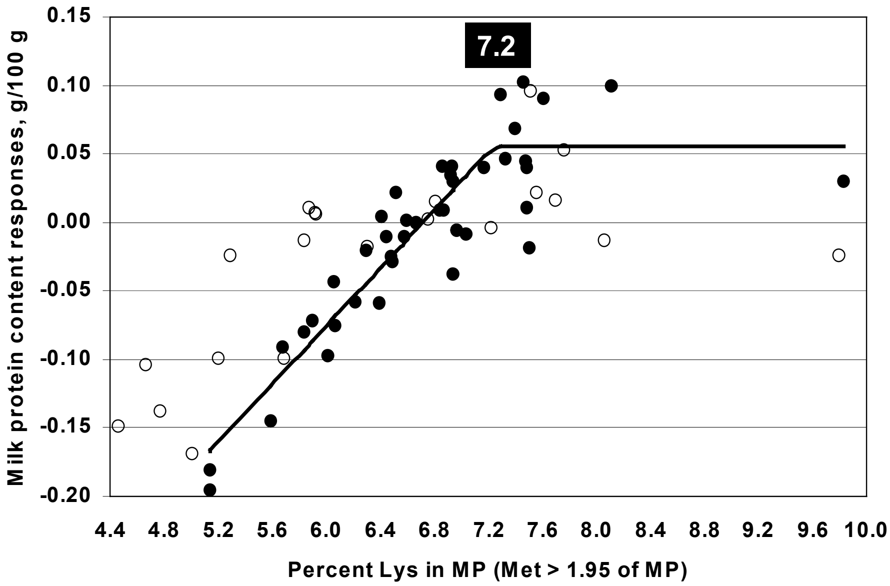
Ingredient	1	2	3	4
Alfalfa hay	18.4	13.0	9.7	9.6
Oat hay	--	0.7	--	--
Alfalfa silage	7.9	--	9.3	--
Corn silage	13.1	--	27.6	29.8
Grass silage	--	--	--	9.6
Oat silage	--	19.6	--	--
Almond hulls	2.8	9.1	--	--
Soyhulls	--	9.4	3.4	4.8
Wheat midds	--	--	5.0	--
Citrus pulp	5.2	--	--	--
Barley grain	6.4	--	--	7.4
Corn, ground	--	--	14.2	15.4
Corn, steam-flaked	8.3	--	--	--
Corn, hominy	7.9	--	--	--
Corn, rolled high moisture	--	10.5	--	--
Wheat, rolled	--	6.4	--	--
Cookie byproduct	--	--	8.1	--
Sugarcane molasses	2.4	--	--	--
Cottonseed, whole	8.8	10.1	--	--
Soybeans, roasted	--	--	4.6	--
DDG with solubles	--	--	5.7	--
Brewers grains, wet	5.4	--	--	--
Soybean meal	--	--	--	11.6
Canola meal	5.0	8.6	6.0	--
Soybean meal, expellers	--	6.1	--	6.4
Soybean meal, nonenz. browned	1.7	--	--	--
Blood meal	0.6	--	0.8	--
Fish meal	1.2	--	0.8	--
Feather meal	--	--	0.8	--
Whey	0.2	2.1	--	--
Fat supplements	0.8	0.9	0.3	1.9
Minerals and vitamins	4.0	3.4	3.9	3.5
NRC (2001) evaluation of diets	--	--	--	--
CP, %DM	18.0	18.0	18.1	18.1
RDP, %DM	12.1	11.4	11.4	10.7
RUP, %DM	5.9	6.6	6.7	7.4
Lysine, %MP	6.57	6.43	6.24	6.34
Methionine, %MP	1.90	1.81	1.83	1.73
MP-lysine, g/day	176	177	173	195
MP-methionine, g/day	51	50	51	53

[1] Parameters used for NRC evaluation of the diets were: 635 kg BW and 25.0 kg/d DMI

Table 3. Cost of providing one gram of metabolizable methionine (MP-Met) from protein supplements versus a protected methionine product.[1]

Supplement	Cost per short ton	CP in DM[2]	Met in CP[2]	RUP in CP[2]	RUP dig.[2]	Amount of supplement required to supply 1g of MP-Met	Cost of supplying 1g of MP-Met
	($)	(%)	(%)	(%)	(%)	(g)	($)
Blood meal	525.00	95.5	1.17	77.5	80.0	144	0.083
Expeller SBM	225.00	46.3	1.45	69.0	93.0	232	0.057
Fish meal	525.00	68.5	2.81	65.8	90.0	88	0.051
Nonenzymatically browned SBM	280.00	50.0	1.32	79.4	93.0	191	0.059
Protein blend[3]	388.75	65.1	1.68	72.9	89.0	141	0.060
Protected Met[1]	9,100.00	75.0	100.00	90.0	90.0	1.6	0.016

[1] Smartamine™ M (Aventis Animal Nutrition, Antony, France)
[2] Values from NRC (2001). The RUP concentrations in CP are NRC (2001) predicted values for a 50% forage/50% concentrate diet with a DMI of 4% BW.
[3] Protein blend = combination of 25% blood meal, 25% expeller SBM, 25% fish meal, and 25% nonenzymatically browned SBM.



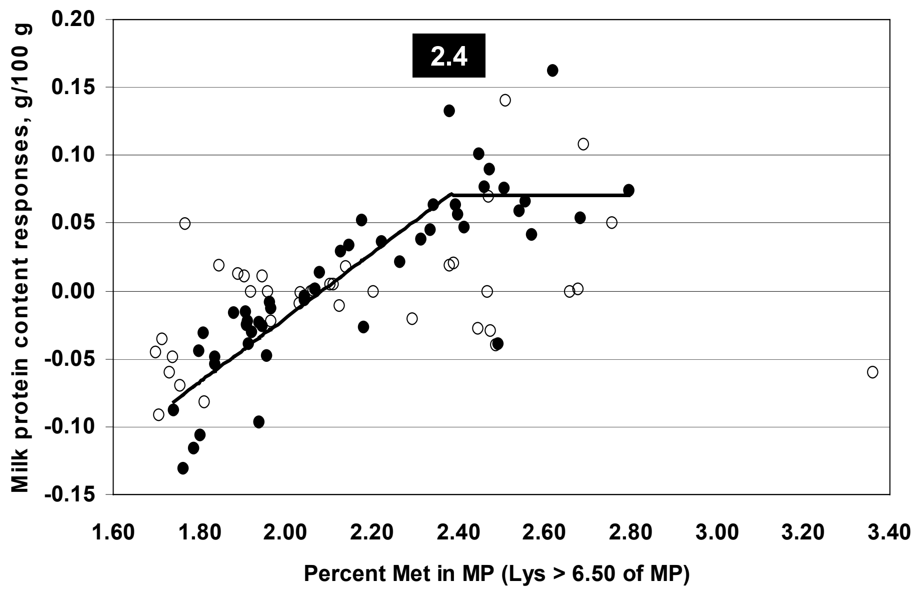


Figure 1. Milk protein content responses as a function of percents of lysine (Lys) and methionine (Met) in metabolizable protein (MP). For the Lys plot, regression analysis was limited to those observations where the corresponding Met values were 1.95% or more of MP. For the Met plot, the regression analysis was limited to those observations where the corresponding Lys values were 6.50% or more of MP.