Amino Acid Balancing in the Context of MP and RUP Requirements

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Introduction

The protein requirements of lactating dairy cows continue to be refined. In NRC (1971, 1978), dietary requirements were expressed as CP and metabolic requirements as digestible protein. In NRC (1989), dietary requirements were expressed as CP or degraded intake CP (DIP) and undegraded intake CP (UIP), and metabolic requirements as absorbed protein. Mean values of ruminal degradability for common feeds, derived from in vivo and in situ studies using sheep and cattle, were reported. A fixed intestinal digestibility of 80% for RUP and microbial true protein was used for predicting passage of absorbed protein. In NRC (2001), dietary requirements are expressed as rumen-degradable CP (RDP) and rumen-undegradable CP (RUP) and metabolic requirements are expressed as metabolizable protein (MP). In contrast to NRC (1989): 1) microbial CP flows are predicted from intake of total digestible OM intake instead of NE intake, 2) a mechanistic system is used for predicting the RDP and RUP content of feeds that recognizes that the proportional content of these two fractions is not constant and is affected by DM intake and diet composition, 3) variable estimates of digestibility are assigned to the RUP fraction of each feed, and 4) flows of digestible essential amino acids (EAA) and their content in MP are predicted. Amino acid requirements were not established, but dose-response curves that relate measured milk protein content and yield responses to changes of predicted percentages of lysine (Lys) and methionine (Met) in MP are provided.

The purpose of this paper is to emphasize the importance of providing the lactating cow with the correct balance of AA in MP, to explain why some AA are more limiting than others, to review how one balances for Lys and Met in the context of balancing for MP and RUP and some production responses that have occurred when the practice is implemented, and to share some preliminary data aimed at extending the application of NRC (2001) to predict lactation responses from changes in supply of MP-Lys and MP-Met.

Why AA Balancing is Important

Absorbed AA, not protein per se, are the required nutrients. In ruminants, absorbed AA are provided by ruminally-synthesized bacteria, RUP, and endogenous protein. Amino acids are the building blocks for tissue and milk proteins. They are joined together in proteins according to a predetermined genetic code. Therefore, the AA composition of a protein is the same every time

it is synthesized. Hence, the AA composition of synthesized proteins is not affected by the amount or profile of absorbed AA.

While the profile of absorbed AA does not affect the AA composition of synthesized proteins, the profile of absorbed EAA does affect the amount of protein that can be synthesized. This is widely documented in swine (NRC, 1998) and poultry (NRC, 1994). When EAA are absorbed in the profile as required by the animal, their efficiency of use for protein synthesis is maximized and the requirement for total absorbed AA is reduced to a minimum. As a result, catabolism of "left-over" AA is minimal and urinary N excretion is lessened. In contrast, efficiency of use of AA for protein synthesis is less than maximal when the profile of absorbed EAA is less than ideal. In this case, it's the supply of the first-limiting EAA that determines the extent of protein synthesis, not MP (total AA) supply.

Limiting Amino Acids

Limiting AA are those that are in shortest supply relative to requirements. Methionine (Met), lysine (Lys), and histidine (His) have been identified most often as the most limiting AA for lactating dairy cows.

As reviewed in NRC (2001), Met is typically first limiting when most of the RUP is provided by soybean protein, animal-derived proteins, or a combination of the two. This is because of their low concentrations of Met as compared to milk and bacterial protein. As shown in Table 1, Met in milk and bacteria are 2.6-2.7% of CP; in soybean, blood, feather, and meat meals, Met is only 0.8-1.4% of CP. In contrast, Lys is first-limiting when corn and feeds of corn origin provide most or all of RUP (NRC, 2001). Again, these findings are not surprising. Lysine in milk and bacteria are 7.6 and 7.9% of CP, respectively, whereas in corn silage, corn, corn distillers, and corn gluten meal, Lys is only 1.7-2.8% of CP.

As might be expected from the data presented in Table 1, Met and Lys have been identified as co-limiting AA for milk protein production when cows were fed corn silage-based diets containing complementary feed proteins (NRC, 2001). This is because only a small number of feeds have concentrations of either Lys or Met in CP that is as high as the concentrations observed in milk and bacterial protein. Thus, while several feeds are complementary to each other [e.g., corn (low Lys, high Met) and soybean meal (high Lys, low Met)], their combined use cannot be expected to eliminate Met and Lys as the most limiting AA. This is especially when corn-based diets are fed.

More recently, His has been shown to be more limiting than Lys or Met when cows are fed grass silage based diets (Kim et al., 1999, 2000, 2001a, 2001b; Huhtanen et al., 2002; Korhonen et al., 2000; Vanhatalo et al., 1999). In all cases, the diets were devoid of corn and contained barley and oats as the supplemental

energy feeds. The diets were fed with or without feather meal as the sole source of supplemental RUP. While these diets are not common in the United States, these findings make an important contribution to our understanding of AA requirements and highlight the impact that diet composition has on the sequence of AA limitation. Why did the cows respond to abomasal and intravenous infusions of His?

One cannot be certain as to why His was more limiting than Lys and Met, but the data presented in Table 1 gives at least two clues. First, His may be more limiting in ruminally synthesized bacteria than either Lys or Met for milk protein synthesis. As noted in Table 1, His is 2.0% of CP in rumen bacteria and His is 2.7% of CP in milk. In contrast, concentrations of Lys and Met are both very similar in rumen bacteria and milk (7.9 and 7.6%, and 2.6 and 2.7%, respectively). This is mentioned because in these experiments, it is expected that bacterial protein constituted a larger percentage of total MP than in cows fed corn-based diets. The CP of grass silage, barley, and oats contains considerably less RUP than corn silage and ground corn (NRC, 2001). A smaller contribution of RUP to MP means that the AA composition of RUP has less of an effect on the AA composition of total MP than feeds that are less degradable and have a higher content of RUP in CP. And second, the His content of all of these feeds is low. The His content of barley and oats is lower than the His content of corn (2.3-2.4 vs. 3.1% of CP). The His content of feather meal is considerably lower than the His content of other protein supplements (1.2 vs. 2.0-2.8% of CP). The high content of His in blood (6.4% of CP) is noteworthy and may give blood meal an additional advantage over other protein supplements, particularly when higher forage, lower corn diets are fed.

Ideal Profile of EAA in MP

Based on the above discussion, it seems important that the ideal concentrations of Lys, Met, and His in MP be determined for lactating cows. If these concentrations were known, and diets could be formulated to achieve these ideal concentrations, then the efficiency of use of MP for milk protein production should be maximized. In other words, more milk protein could be produced with the same supply of MP, or the same amount of milk protein could be obtained with less MP.

Both results have been obtained. As reviewed in NRC (2001), and has now been observed on many farms, increasing intestinal supplies of Lys and Met, when they are limiting, increases yields of milk protein. The result is an increased conversion of feed N to milk N. In other cases, it has been shown that milk protein yields can be maintained by feeding less RUP if concentrations of Lys and Met in MP are increased (Noftsger and St-Pierre, 2003; Socha et al., in preparation; farm observations). Again, the result is an increased conversion of feed N to milk N.

Progress has been made in determining the ideal concentrations of Lys and Met in MP. As reviewed at the Four-State Applied Nutrition and Management Conference in 2001 (Schwab, 2001), the NRC (2001) publication contains dose-response plots that relate measured milk protein content and yield responses to changes in predicted percentages of Lys and Met in MP. The plots for milk protein content are shown in Figure 1. The reader is referred to either NRC (2001; p. 81-85) or Schwab (2001) for an explanation of how the plots were generated. It should be noted that the Lys plot was generated from only that portion of the Lys data set where predicted Met was 1.95% or more of MP (solid circles) and that the Met plot was generated from only that portion of the Met data set where predicted Lys was 6.50% or more of MP (solid circles). This was done to help ensure that milk protein responses to supplemental amounts of either AA were not prevented by a deficiency of the other.

The breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk protein were 7.2% and 2.4%, respectively (3.0:1.0 ratio). The breakpoint estimates for the required concentrations of Lys and Met in MP for maximal yield of milk protein were 7.1% and 2.4% (plots not shown). Examination of the dose-response plots indicates little or no expected loss in content or yield of milk protein when Lys and Met in MP are 6.9% and 2.3%, respectively. In fact, our research and field experience indicates no advantage of exceeding these concentrations. Therefore, we consider the latter values (6.9 and 2.3% of MP) to be the absolute highest targets to aim for in diet formulation when using NRC (2001) as the diet evaluation model. However, even these concentrations are difficult to achieve, particularly in high producing cows fed corn based diets. As a result, our "practical recommendations" for percentages of Lys and Met in MP in this case are 6.6 and 2.2, respectively (Figure 2).

For those that use CPM-Dairy or CNCPS as a diet evaluation model, we suggest practical targets of 6.7-6.8% for Lys in MP and 2.2-2.3% for Met.

Balancing for AA in the context of MP and RUP

Based on the above discussion and the senior author's field experience over the past 2 years, an effective method to balance for Lys and Met involves a 2-step approach.

The first step is to decide what your target values will be for Lys and Met in MP. Will they be 6.6 and 2.2%, or something lower? Whatever you decide, remember two things. First, maintain a Lys:Met ratio in MP of 3.0:1.0 if using NRC (2001) or 3.1:1.0 if using CNCPS or CPM-Dairy. Having a Lys:Met ratio in MP that is either higher (usually the case) or lower (seldom occurs) than 3:1 means that one of the AA is being supplied in excess of need (see Table 2). This has no benefit to the cow. And second, try to get Lys and Met in MP as close to 6.6 and 2.2% as possible. Doing so has the net effect of increasing passage of the

first two limiting AA without the need to increase passage of MP to the small intestine (Table 2). We believe that it is cost effective, even with the current low milk prices, to use the high-Lys protein supplements (soy products and fish and blood meals) in combination with a rumen-protected Met product (RPMet) to get levels of Lys and Met in MP as close to 6.6 and 2.2% as possible (Schwab et al., 2003).

A question that is frequently asked is "what is more important, AA ratios or AA concentrations"? The reason for this question appears to be the result of the frequent emphasis on the 3:1 Lys/Met ratio. In one of the dairy publications about 3 months ago, the following statement was made – "A by-product such as corn gluten meal has a high level of methionine that can be used in combination with blood meal and fish to achieve the desired ratio of 3:1 lysine-methionine". The author was correct in what he said. However, the emphasis was on AA ratios, with no consideration given to how that strategy affects concentrations of Lys and Met in MP. Purchasing the usually more expensive fish and blood meals only to dilute out their high content of Lys (7.7-9.0% of CP, Table 1) with a low-Lys protein supplement such as corn gluten meal (1.7% of CP) is counter-productive and does not achieve the desired goal of high Lys and Met concentrations in MP. If the decision is made to feed the higher RUP, higher Lys products such as soybean, fish, and blood meals, then the most effective Met supplement is RPMet. Use of a RPMet supplement allows Met in MP to be increased without decreasing Lys.

In a recent paper (Schwab et al., 2003), we made the comparison of achieving a 3:1 Lys-Met ratio in MP using soybean meal, blood meal, and a RPMet product as the primary sources of supplemental AA in a corn based ration vs. using soybean meal, corn distillers grains and corn gluten meal. Diet RDP and RUP concentrations were kept constant between the two diets. In the first case, the NRC (2001) predicted concentrations of Lys and Met in MP were 6.6 and 2.1% (3:1/1.0 ratio) and MP-Lys and Met flows were 191 and 61 g/d. (Note: a 3:1 ratio could have been obtained by including more RPMet in the diet). In the second case, the predicted concentrations of Lys and Met in MP were 5.8 and 1.9% (3.0:1.0 ratio); MP-Lys and Met flows were 170 and 56 g/d.

Field experiences

Presented in Table 3 are the NRC (2001) evaluations of the "before" and "after" diets for six dairy farms in which diet changes were made to increase flows of MP-Lys and Met without increasing flows of total MP. In all cases, Lys concentrations in MP were increased by introducing blood meal and reducing or eliminating distillers grains or a protected soy product. Methionine concentrations in MP were increased to obtain the desired 3:1 Lys-Met ratio in MP by adding a RPMet product (Smartamine M) to the diets. Also presented in Table 3 are the "before" and "after" milk protein and fat percentages. There was no attempt to measure changes in milk yield for most of the herds.

There are three noteworthy observations. First, as evidenced by the high Lys/Met ratios, Met was more limiting than Lys in all of the "before" diets. Therefore, we would conclude that all of the herds would have benefited with higher milk protein concentrations by adding RPMet to the diets. Second, as a result of increasing Lys in MP as well as adding enough RPMet to the diets to achieve a Lys/Met ratio in MP equaled or approximated the desired 3.0/1 ratio, flows of MP-Met were increased 15 to 30%. These are significant increases in the availability of an apparent limiting nutrient and explains why the producers not only observed some rather significant increases in content of milk protein but why in most cases they also thought they observed higher milk yields. And third, in all cases, the diet changes resulted in variable increases in milk fat percentages. This has been an often observed result when Met concentrations in MP are increased (NRC, 2001).

University of New Hampshire Experience

The following discussion describes the "before" and "after" diets for herd 6 and the rationale for the changes that were made. Herd 6 is the University of New Hampshire Research Herd.

For about an 11-month period ending 2 years ago, 75 of our dairy cows were fed a diet containing (DM basis): 29.8% corn silage, 9.6% alfalfa hay, 9.6% grass silage, 15.4% ground corn, 7.4% barley, 4.8% soy hulls, 11.6% soybean meal, 6.4% expeller soybean meal, 1.9% fat, and 3.5% minerals and vitamins. During that period, milk true protein varied between 2.70 and 2.83% and milk fat between 3.4 and 3.7%.

At the end of the 11-month period, the diet was changed. The new diet contained (DM basis): 30.9% corn silage, 12.3% grass silage, 6.0% alfalfa hay, 19.1% corn, 9.4% barley, 3.7% soy hulls, 7.4% soybean meal, 3.7% canola meal, 0.14% urea, 2.2% of a highly digestible animal protein blend (Venture Milling) that contained 0.075% Smartamine M and 0.10% Rhodimet AT-88, 1.9% fat, and 3.1% minerals and vitamins. The animal protein blend replaced the expeller soybean meal to increase Lys in MP. Smartamine M was added to achieve the desired 3.0/1.0 Lys to Met ratio in MP. The canola meal and urea replaced some of the soybean meal to provide a more diverse mix of RDP and to lower the cost of RDP. And finally, RDP and RUP were decreased to eliminate some of what NRC (2001) indicated to be a surplus and to offset the higher cost of the animal protein blend. The new diet contained 17.2% CP as compared to 18.1% for the old diet. According to NRC (2001), the new diet contained 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 to 2809 g/d (8% reduction). RDP was lowered by 2%. However, predicted concentrations of Lys and Met in MP increased from 6.34% and 1.73% to 6.55% and 2.20%, respectively. Therefore, even though predicted passage of MP was 8% less, the

predicted flow of MP-Met (previously the "weakest link") was increased from 53 to 61 g/d, a 16% increase. Predicted MP-Lys flows decreased from 195 to 184 g/d. This was not considered to be a problem because amounts greater than what would be needed to achieve a Lys/Met ratio of 3.0/1.0 using NRC (2001) would be considered to be a surplus.

The cows were switched gradually over a 10-d 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 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 an average of 12.4 mg/dL. Milk fat concentrations also increased. It appeared that milk yields increased but it was difficult to determine that as the cows (on average) were advancing in days-in-milk.

The economics of the diet changes were most favorable. Because we assumed that milk yield was not increased, we also assumed that DM intake was not affected. A cost analysis of the diet indicated an approximate 5 cents per cow per day increase in feed costs. However, because of the increases in milk protein and milk fat concentrations, milk income was increased by $0.70 per cow per day. The increased income-over-feed-costs (IOFC) was $0.65 (assuming no increase in milk yield or feed intake).

Predicting Production from MP-Lys and MP-Met

Is balancing for Lys and Met in MP always profitable? The use of high quality protein and RPMet supplements to increase intestinal flows of MP-Lys and Met continue to be questioned as to their economic value. Do we keep the high-Lys, high-digestible blood, fish and soybean meals and the protected Met supplements in the diets at our normal inclusion levels, or just feed less? Or, do we let cost per ton of feed take center stage and scrap these feeds? Unfortunately, the current diet evaluation models that predict passage of MP-AA to the small intestine (e.g., NRC, 2001; CNCPS, and CPM-Dairy) in their present form are not useful in predicting the effect that changes in supplies of MP-Lys and MP-Met have on milk and milk component production. Until such systems are in place, it will remain difficult to predict the effect that changes in protein supplementation strategies have on milk and milk protein yields and the resulting effects on IOFC.

Schwab et al. (2003) used the NRC (2001) model in conjunction with published experiments to examine the relationships between predicted supplies of MP, MP-Met, and MP-Lys and yields of milk and milk protein. That effort has been extended for this paper.

Over 300 diets from experiments published in the Journal of Dairy Science were entered into the NRC (2001) model. In most of these experiments the objective was to compare the effects of feeding different protein supplements on milk production and milk composition, and in some cases, passage of N fractions to the small intestine. Relevant data from the Summary and Duodenal Amino Acid Supply Reports were recorded.

To generate plots of measured yields of milk and milk protein vs. predicted supplies of MP, data were restricted to diets in which NE-allowable milk was higher than MP-allowable milk, and actual milk yield was between minus 6 kg and plus 6 kg of MP-allowable milk. The former restriction was imposed to help ensure that MP was more limiting than NE. The latter restriction was imposed to avoid the use of experiments in which factors other than MP or NE limited lactation performance or situations where excessive protein mobilization may have been occurring.

To generate plots of measured yields of milk and milk protein vs. predicted supplies of MP-Lys and MP-Met, data were restricted to diets in which MP balance was within -250 and +100 g/d of zero balance. This was done with the hope of further ensuring that Lys and Met were limiting. For the Met plots, we imposed the restriction that that the Lys/Met ratio in MP had to be greater than 3.0/1.0 to make more certain that Met was more limiting than Lys. For the Lys plots, we wanted to add the restriction that the ratio of Lys to Met in MP had to be less than 3.0/1.0 to ensure that Lys was more limiting in MP than Met. However, only in a few cases was the ratio of Lys to Met in MP less than 3.0/1.0. Therefore, to give ourselves an adequate number of data points from which to get some idea of the relationship between yields of milk and milk protein vs. predicted supplies of Lys, diets yielding predicted Lys/Met ratios up to 3.25/1.0 were used.

The resulting plots are presented in Figure 3. There are at least three observations that are worthy of mention. First, in all cases (for MP, MP-Met, and MP-Lys), it appears that protein yields can be predicted more accurately than milk yields. This would be expected because of the changes in milk protein percentages that often occur with changes in protein nutrition. Second, as expected, predicting yields of milk and milk protein from intestinal supplies of the most limiting AA is more precise than predicting yields from MP supply. Third, and while the current data is too limited and not adequate for this exercise, it appears that a very strong relationship exists between milk and milk protein yields and predicted MP-Lys supplies. This should probably be expected given the fact that Lys, unlike Met, has only one function in the body, i.e., protein synthesis.

Conclusions

Balancing diets to optimize Lys and Met nutrition is important to maximizing milk and milk protein yields. As expected, it appears that establishing relationships between predicted supplies of the most limiting AA in the diet and milk and milk protein yields will allow for more accurate prediction of changes in milk protein production when changes in protein nutrition are made. Of concern is the lack of a rumen-protected Lys product and therefore, the inability to achieve desired concentrations of Lys in MP when high corn diets are fed.

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Table 1. A comparison of the essential amino acid composition of body lean tissue, milk, and ruminal bacteria with that of some common feeds[1].

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
	- - - - - - - - - - - - - - - - - - (% of CP) - - - - - - - - - - - - - - - - - -									
Lean tissue	6.6	**2.5**	2.8	6.7	**6.4**	**2.0**	3.5	3.9	0.6	4.0
Milk	3.4	**2.7**	5.8	9.2	**7.6**	**2.7**	4.8	3.7	1.5	5.9
Bacteria	5.1	**2.0**	5.7	8.1	**7.9**	**2.6**	5.1	5.8	-	6.2
Alfalfa silage	3.9	**1.7**	3.9	6.4	**4.4**	**1.4**	4.2	3.8	0.9	5.0
Corn silage	2.0	**1.8**	3.3	8.6	**2.5**	**1.5**	3.8	3.2	0.4	4.5
Grass silage	3.1	**1.7**	3.6	6.1	**3.3**	**1.2**	4.4	3.3	1.1	4.9
Barley	5.1	**2.3**	3.5	7.0	**3.6**	**1.7**	5.1	3.4	1.2	4.9
Corn	4.6	**3.1**	3.3	11.2	**2.8**	**2.1**	4.6	3.6	0.7	4.0
Oats	6.8	**2.4**	3.8	7.3	**4.2**	**2.9**	5.2	3.5	1.2	5.2
Wheat	4.7	**2.4**	3.3	6.6	**2.8**	**1.6**	4.6	2.9	1.2	4.2
Brewers grains	5.8	**2.0**	3.9	7.9	**4.1**	**1.7**	4.6	3.6	1.0	4.8
Canola meal	7.0	**2.8**	3.8	6.8	**5.6**	**1.9**	4.1	4.4	1.5	4.7
Corn DDG w/sol	4.1	**2.5**	3.7	9.6	**2.2**	**1.8**	4.9	3.4	0.9	4.7
Corn gluten meal	3.2	**2.1**	4.1	16.8	**1.7**	**2.4**	6.4	3.4	0.5	4.6
Cottonseed meal	11.1	**2.8**	3.1	5.9	**4.1**	**1.6**	5.3	3.2	1.2	4.2
Soybean meal	7.3	**2.8**	4.6	7.8	**6.3**	**1.4**	5.3	4.0	1.3	4.6
Sunflower meal	8.2	**2.6**	4.1	6.4	**3.6**	**2.3**	4.6	3.7	1.2	5.0
Blood meal	4.4	**6.4**	1.3	12.8	**9.0**	**1.2**	6.9	4.3	1.6	8.7
Feather meal	6.9	**1.2**	4.9	8.5	**2.6**	**0.8**	4.9	4.7	0.7	7.5
Fish meal	5.8	**2.8**	4.1	7.2	**7.7**	**2.8**	4.0	4.2	1.1	4.8
Meat meal	7.1	**2.1**	3.0	6.3	**5.4**	**1.4**	3.6	3.4	0.7	4.4

[1] Amino acid values for lean tissue, milk, and ruminal bacteria are from O'Connor et al. (1993) and amino acid values for feeds are from NRC (2001).

Table 2. Effect of NRC (2001) predicted percentages of Lys and Met in MP on calculated flows of MP-Lys and MP-Met, and the amounts of MP-Lys and Met that can be used for protein synthesis.

Predicted Lys/Met in MP	Lys/Met ratio	Flows[1]		Used for protein synthesis[2]	
		MP-Lys	MP-Met	MP-Lys	MP-Met
(%)		- - - - - - - - - - (g/d) - - - - - - - - - - - - - - -			
6.4/1.7	3.8/1	179	48	144	48
5.8/1.7	3.4/1	162	48	144	48
5.7/1.9	3.0/1	160	53	159	53
5.8/2.1	2.8/1	162	59	162	54
6.3/2.0	3.2/1	176	56	168	56
6.6/2.2	3.0/1	185	62	186	62

[1] Calculations are based on a predicted MP supply of 2,800 g/d.

[2] Based on the assumption that the optimum Lys/Met ratio in MP is 3:1 and the understanding that any AA supplied in excess of need for protein synthesis is not used for protein synthesis and therefore, is catabolized and used for energy.

Table 3. NRC (2001) evaluations of "Before" and "After" diets for six commercial farms in which concentrations of Met and Lys in MP were increased.

	Herd 1		Herd 2		Herd 3	
Item	Before	After	Before	After	Before	After
CP, %	17.7	17.8	18.3	18.6	18.3	18.1
RDP, %	10.9	11.2	11.0	11.2	11.4	11.3
RUP, %	6.8	6.6	7.3	7.4	6.9	6.8
MP, g/d	3054	2984	3159	3131	3062	3040
Lys, %MP	5.78	6.53	5.74	6.20	5.84	6.18
Met, %MP	1.65	2.17	1.68	2.08	1.68	2.01
MP-Lys, g/d	177	195	181	194	179	188
MP-Met, g/d	50	65	53	65	51	61
Lys/Met	3.5/1	3.0/1	3.4/1	3.0/1	3.5/1	3.1/1
Milk protein, %	3.06	3.33	2.99	3.12	3.02	3.22
Milk fat, %	3.81	3.92	3.56	3.66	3.61	3.72

	Herd 4		Herd 5		Herd 6	
Item	Before	After	Before	After	Before	After
CP, %	19.1	18.2	17.6	17.0	18.1	17.2
RDP, %	12.0	11.2	10.4	10.3	10.8	10.6
RUP, %	7.1	6.9	7.2	6.7	7.3	6.6
MP, g/d	3107	3030	3073	3035	3071	2809
Lys, %MP	5.84	6.25	6.34	6.76	6.37	6.55
Met, %MP	1.67	2.04	1.73	2.35	1.73	2.20
MP-Lys, g/d	182	189	195	193	184	174
MP-Met, g/d	52	62	53	61	50	58
Lys/Met	3.5/1	3.0/1	3.7/1	3.2/1	3.7/1	3.0/1
Milk protein, %	3.00	3.20	3.20	3.50	2.82	3.16
Milk fat, %	3.49	3.64	3.90	4.30	3.32	3.78

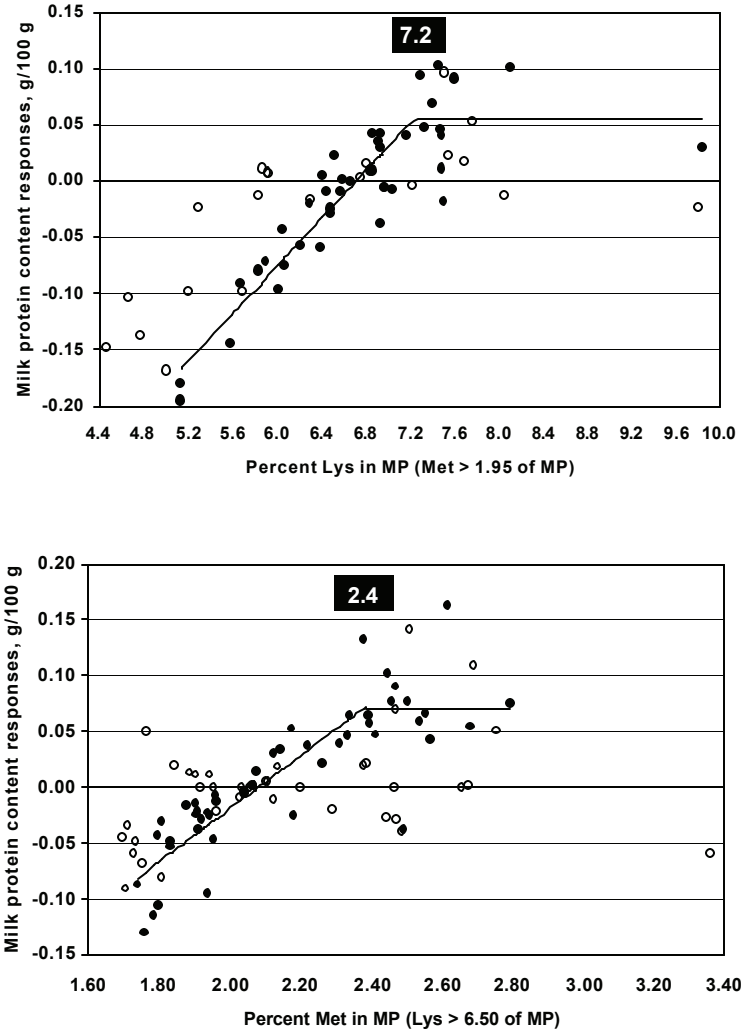
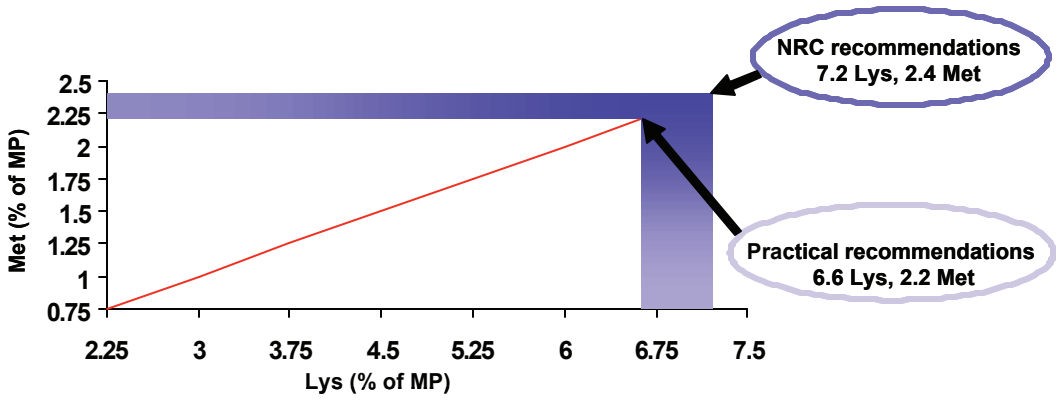


Figure 1. Milk protein content responses as a function of percent Lys and Met in MP. For the Lys plot, the regression analysis was limited to data where Met was predicted to be 1.95% or more of MP (solid circles). For the Met plot, the regression analysis was limited to data where Met was predicted to be 6.50% or more of MP (solid circles) (NRC, 2001).

Figure 2. Optimum versus practical levels of Lys and Met in metabolizable protein.



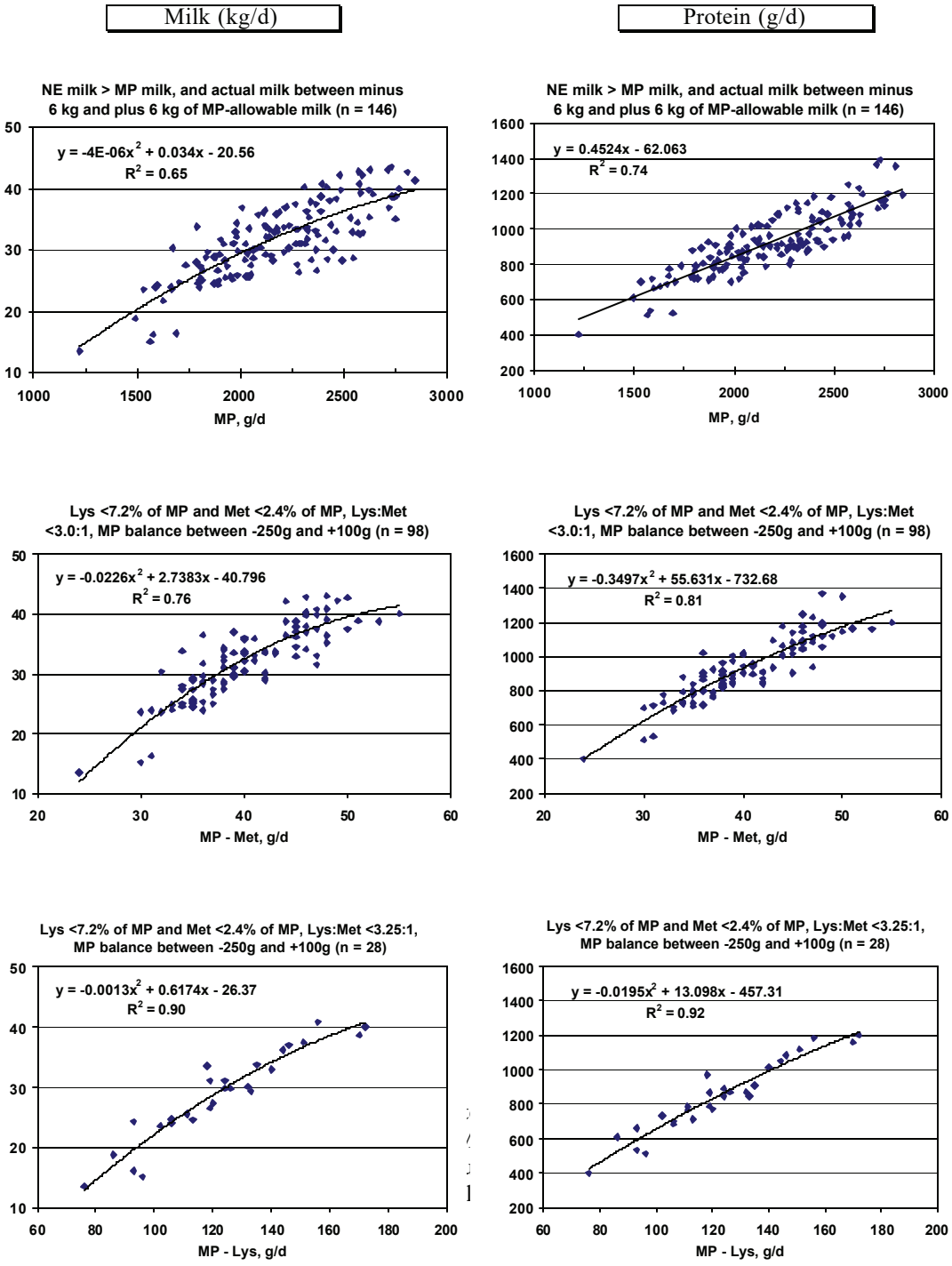


Figure 3. Plots of measured milk and milk protein yields vs. NRC (2001) predicted flows of metabolizable protein (MP) and MP–Lys and MP–Met. Data were selected from a database involving 321 diets fed to Holstein cows without AA supplementation (restrictions used for selecting data are indicated above each of the plots).

Methionine Supplementation Options

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Introduction

Methionine (Met) and lysine (Lys) have been identified most often as the two most limiting amino acids (AA) for lactating dairy cows. This is largely because of their low concentrations in feed protein as compared to their concentrations in milk and ruminally synthesized bacterial protein. The NRC (2001) suggested concentrations of Lys and Met in metabolizable protein (MP) for maximal use of MP for milk protein production are 7.2 and 2.4%, respectively. Under almost all circumstances, these concentrations cannot be achieved, and as a result, "practical recommendations" for Lys and Met in MP of 6.6 and 2.2% are being used by the authors (Schwab et al., 2003). These concentrations can generally be achieved in corn-based rations by using a combination of high-Lys protein supplements (e.g., blood, fish, and soybean meals) and a rumen-protected Met (RPMet) product and limiting intake of rumen-undegraded intake protein (RUP) to requirement levels. Not using a RPMet product requires the mix-and-matching of protein supplements to achieve the desired Lys:Met ratio in MP of 3.1 and as a result, lowers the concentrations of both Lys and Met in MP that are achievable (Schwab et al., 2003)

The purpose of this paper is to review the Met products that have been suggested to provide Met to ruminants. These include RPMet products and two forms of 2-hydroxy-4-methylthio butanoic acid (HMB). A description of the products and methods for determining their metabolic availability to the host animal are reviewed.

Ruminally Protected Methionine

History of Development

Interest in protecting free Met from ruminal degradation dates back to the 1960's and early 1970's when it became apparent from abomasal, intestinal, and intravenous infusion trials that the profile of absorbed Met was not always optimum in ruminants (Chalupa, 1975; Schwab, 1995). These trials indicated that the sulfur AA were clearly first limiting for wool growth and body weight gains of sheep and that Met was a limiting AA for growing cattle and lactating dairy cows. Thus, several laboratories began to devise procedures to protect Met from ruminal degradation (Chalupa, 1975). Subsequent interest developed in protecting Lys when it was discovered to be second limiting for growing lambs and either first or second limiting for growing cattle and lactating dairy cows.

Many approaches have been evaluated to physically protect Met and Lys from ruminal degradation. Initial attempts focused on protecting Met with lipids, often in combination with inorganic materials and carbohydrates as stabilizers, softening agents and fillers. For example, in the 1960's Delmar Chemicals of Canada developed a product in which a core of DL-Met, colloidal kaolin, and tristearin was wrapped in a film of tristearin. The product contained 20% Met. A few years later, Rumen Kjemi a/s of Oslo, Norway introduced a somewhat more efficacious product (Ketionin®) that had a higher intestinal release of the encapsulated Met. The product contained 30% DL-Met, 2% glucose, 4% stabilizing, antioxidant, and flavoring agents, 6% $CaCO_3$, and 58% tristearin and oleic acid.

Several other lipid protected Met products have also been evaluated (Loerch and Oke, 1989; Schwab, 1995). The greatest challenge with using lipids as the primary encapsulating material is to identify a combination of materials and process that had both a high ruminal escape and intestinal release of Met.

The most effective approach to date has been to surface-coat Met with enzyme-resistant, pH-sensitive synthetic polymers that are insoluble in the more neutral pH environment of ruminal digesta but highly soluble in the acidic abomasum. This approach provides for a post-ruminal delivery system that is independent of enzyme function and, instead, relies on the pH differences between the rumen and the abomasum for ruminal protection and intestinal release. Polymer-protected Met has higher ruminal protection and intestinal release coefficients than other products. The patent rights for the use of pH-sensitive polymers for protecting nutrients from ruminal degradation is currently held by Adisseo, Inc., Antony France.

Another method that has been explored for increasing supplies of Met to ruminants is the use of analogs and derivatives of Met. Amino acid derivatives are free AA to which a chemical blocking group has been added to the α-amino group or in which the acyl group has been modified. Some examples of Met derivatives that have been shown to have some resistance to ruminal degradation are isopropyl-DL-Met, t-butyl-DL-Met, N-stearoyl-DL-Met, N-oleoyl-DL-Met, and capryl-caproylic-DL-Met (Loerch and Oke, 1989). There is evidence that extent of ruminal escape is greater with shorter chain rather longer chain alkyl esters. Although these and other derivatives show promise, most have not been investigated adequately to determine the extent to which they will increase post-ruminal supplies of absorbable Met.

Amino acid analogs are generated from the substitution of the α-amino group of the AA with a non-nitrogenous group such as a hydroxyl group. The most studied AA analog is Met hydroxy analog (MHA; DL-α-hydroxy-γ-mercaptobutyrate) or more appropriately called 2-hydroxy-4-methylthio butanoic acid (HMB). Studies indicate that free HMB is more resistant to ruminal

degradation than free Met, that it can be absorbed from the rumen and omasum through passive diffusion, and that ruminants have the enzymes for the conversion of HMB to Met. However, because of observed minimal effects on blood Met concentrations and milk protein concentrations when fed to lactating dairy cows fed Met-deficient diets, it appears that its ability to substitute for absorbed Met in dairy cows is minimal.

Recent research has shown that several esters of HMB enhance ruminal escape of HMB, at least in part because of their apparent ability to be absorbed across the rumen wall. The isopropyl ester of HMB has been shown to have an excellent replacement value for absorbed Met (Robert et al., 2001b, Schwab et al., 2001).

Commercial RPMet Products

Met-Plus™ (Nisso America, Inc.). This is an example of a lipid-protected product. It is a matrix compound that contains 65% DL-Met 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. However, like other lipid-coated products, 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 (Degussa Corporation, Germany). This 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 Met and starch coated with several thin layers of ethylcellulose and stearic acid. The final product contains a minimum of 85 % DL-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 (Adisseo, Inc., Antony France). This 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 DL-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 stereochemistry 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 rapid release of the Met in the abomasum.

Methionine Analogs

Background

Koenig et al. (1999) reported that 50% of HMB escaped ruminal degradation and became available for postruminal absorption in early lactation dairy cows. The approach was to feed the cows a pulse-dose of 90 g of Alimet (an 88% aqueous solution of HMB, Novus International, Inc. St. Louis, MO) and 600 ml of Cr-EDTA, a liquid phase marker, mixed with 2 kg of ground corn grain. Ruminal escape of HMB was calculated from the fractional rate constants for ruminal disappearance of HMB and passage of liquid. Subsequent studies using a dual effluent continuous culture system indicated ruminal escape values of 22 to 43% for HMB (Vázquez-Añón et al. (2001). Other research has shown that HMB can be absorbed across ruminal and omasal epithelium (McCollum et al., 2000), and that if absorbed, it can be converted to Met (Belasco, 1972, 1980; Papas et al., 1974; Wester et al., 2000a,b). These findings led to the use of HMB as a substitute for RPMet for lactating dairy cows.

However, the extent to which dietary HMB substitutes for absorbed Met for milk protein production in lactating cows remains questionable. Many studies have indicated that content of protein in milk is sensitive to adequacy of Met in MP and that milk protein percentage increases when the content of Met in MP is improved (Guinard and Rulquin, 1995; Schwab et al 1976,1992; NRC, 2001; Pisulewski et al, 1996). Yet, supplementing apparent Met-deficient diets with HMB did not increase milk protein concentrations (Ellis, 1986; Ellis et al., 1986; Johnson et al., 1999; Rode et al., 1998). Moreover, supplementing diets with HMB had either little or no effect on blood Met concentrations (Johnson et al., 1999; Papas et al., 1974; Polan et al., 1970; Robert et al., 1997). These observations question the use of HMB as a substitute for RPMet that are fed to achieve a desired concentration of Met in MP and to increase supplies of Met to the mammary gland.

More recently, it has been demonstrated that the isopropyl ester of HMB (HMBi) is considerably more effective than HMB as a source of MP-Met for lactating cows (Robert et al., 2001a,b). The researchers reported that HMBi was 56% as effective as Smartamine M™ in increasing blood Met concentrations. The two sources of metabolizable Met were given as a pulse-dose in equimolar amounts (49 g of Met equivalents) to non-lactating cows fed 8.5 kg/d of DM. In previous work using a similar blood response technique, the same workers reported that HMB was only 3% as effective as Smartamine M™ in increasing blood Met concentrations (Robert et al., 1997).

Similar results were obtained in a recent study conducted at the University of New Hampshire. Schwab et al. (2001) estimated that HMBi was 53% as effective as Smartamine M™ in increasing milk protein percentages. In order to

estimate the "Met bioavailability" of HMB and HMBi for lactating cows, dose response titrations were carried out using milk true protein content as the response criteria. Four Met sources were used: 1) Smartamine M™, 2) HMB, 3) HMBi, and 4) a combination of 1/3 HMB and 2/3 HMBi (HMB/HMBi). Treatment levels were (g Met equivalents/d per 25 kg of DMI): Smartamine M™ (0, 10, 15, 20, and 25), HMB and HMBi (0, 15, 20, 25, and 30), and HMB/HMBi (0/0, 5/10, 8.3/16.7, 11.7/23.3, and 15/30). Milk protein concentrations increased with all treatments except HMB. The corrected milk protein percentages were: Smartamine M™ (2.99, 3.08, 3.15, 3.15, and 3.13; quadratic effect), HMB (3.04, 3.02, 3.03, 3.06, and 3.03), HMBi (3.05, 3.11, 3.16, 3.17, and 3.19; linear effect), and HMB/HMBi (3.07, 3.13, 3.12, 3.16, and 3.18; linear effect). Based on differences of slope, it was calculated that HMB was 53% as effective as Smartamine M™ and that the HMB/HMBi combination was 43% as effective as Smartamine M™. The results of the experiment indicated that HMB provided little or no Met for milk protein synthesis. However, both Smartamine M™ and HMBi were effective at providing post-ruminal Met as evidenced by the increase in milk protein concentration.

Commercial Products

Alimet® (Novus International, Inc. St. Louis, MO, USA) and **Rhodimet™ AT88** (Adisseo, Inc., Antony France). Both are liquid sources of HMB. Chemically, the compounds are the same and both are used extensively as a substitute for methionine in the poultry and swine industry. Novus International also has Alimet® patented for use in dairy cows and recommends its use as a source of RPMet.

Efficacy of Methionine Products

Responsible use of RPMet products and Met analogs as Met supplements requires estimates of their ability to provide (or spare) absorbed Met. Insofar as possible, estimates of "Met bioavailability" must be accurate and reliable under the conditions in which they are fed.

Approaches

Unfortunately, there is no universally accepted, standardized procedure(s) for obtaining estimates of Met bioavailability. These are needed to bring uniformity to estimates of Met bioavailability and to more accurately compare the efficacy of different products. Current approaches can be categorized as factorial approaches, blood response approaches, and production response approaches.

The factorial approach involves independent measurements of ruminal escape, intestinal disappearance (digestibility), and in the case of free or protected forms of HMB, metabolic conversion to Met. Animals with cannulae in the rumen, duodenum, and preferably also in the ileum, are required. Estimates of

ruminal escape and intestinal disappearance of Met from RPMet products have been obtained using both the in situ, nylon-bag procedure and the cannulated cow in vivo procedure. Use of the in situ procedure requires measurements of rate of passage of RPMet products from the rumen. The in situ procedure is not suitable for soluble products such as HMB and HMBi.

Blood response approaches are an attractive alternative to factorial approaches because they are easier to conduct and they allow liquid and pulverulent products to be evaluated. Studies with cattle have shown that a linear relationship exists between increasing supplies of absorbable Met and plasma Met concentrations. Two variations of the approach have been used. The first is the dose-response approach that involves determination of differences in slope of measured blood Met concentrations between graded dietary doses of the product and graded intestinal doses of infused methionine. The second is the "area under the curve" (AUC) approach. This approach involves the ruminal administration of a pulse-dose of equimolar amounts of different Met sources and comparing the AUC of the plasma Met response curves that result. To obtain estimates of Met bioavailability for Met products, Smartamine™ M has been used as the positive control treatment with the assumption that it has a Met bioavailability value of 80%.

A production response approach has recently been used to obtain estimates of the Met bioavailability values for free HMB and HMBi (Schwab et al., 2001). This approach, as previously described, involved determination of differences in slope of measured milk protein concentrations between graded dietary doses of the HMB products and Smartamine™ M when cows were fed a Met-deficient diet. Numerous studies with lactating cows indicate that content of milk protein in milk is more responsive than milk yield to small changes in concentrations of Met in MP.

A Comparison of Some Available Products

Of the products described in this paper, Smartamine™ M appears to have the greatest efficacy as a source of absorbable Met. Nylon bag studies indicate ruminal stability exceeding 90% at 24 h and intestinal release values approximating 90% (determined either by the amount released after 1 h in a pH 2.0 HCL-pepsin solution or by the mobile bag technique after exposure to the HCL-pepsin solution). A cannulated cow in vivo experiment involving early lactation cows indicated ruminal escape values that averaged 90% across four different diets and an average intestinal disappearance value of 98%, resulting in an average Met bioavailability value of 88% (Robert and Williams, 1997).

Several studies using the different approaches mentioned above, plus the use of in vitro studies, indicate that the efficacy of Mepron® M85 as a source of absorbable Met is intermediate between that of Smartamine™ M and lipid protected products. Mepron® M85 has been shown to be degraded faster in the

rumen than Smartamine™ M and result in significantly smaller increases in plasma sulfur AA concentrations than Smartamine™ M. A summary of in situ nylon experiments indicates ruminal protection values that approximate 90% at 2 h, 85% at 6 h, 70% at 12 h, 60% at 24 h, and 15% at 96 h. Estimates of passage rate from the rumen, which have not been reported, are needed to calculate ruminal escape. Mepron® M85 also is less digestible in the small intestine than Smartamine™ M. Use of the mobile bag technique has indicated that 25-70% of the Mepron® M85 entering the small intestine is excreted in the feces, with larger amounts being excreted with higher feed intakes. It appears questionable as to whether the mobile bag technique is an appropriate method for measuring the intestinal release of Met from a RPMet product such as Mepron® M85 that relies on abrasion and physical forces for its degradation.

Of the Met analogs, as already discussed, only HMBi appears to provide significant quantities of Met to the lactating dairy cow.

Summary and Conclusions

Until HMBi becomes available, the Met-source options for increasing supplies of metabolizable Met to lactating cows are the three described RPMet products. The use of these products along with selective use of protein supplements, give dairy nutritionists the opportunity to more adequately balance diets for Lys and Met.

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