MAXIMIZING MILK COMPONENTS AND METABOLIZABLE PROTEIN UTILIZATION THROUGH AMINO ACID FORMULATION

C.G. Schwab¹ and G.N. Foster² ¹Department of Biological Sciences University of New Hampshire and ²Central Valley Nutritional Associates, LLC Tulare County, California

Heger and Frydrych (1989) concluded, largely from research with non-ruminant animals, that when the essential amino acids (EAA) are absorbed in the profile as required by the animal, the requirements for total EAA is reduced and their efficiency of use for protein synthesis is maximized. Research with lactating dairy cows has been shown many times that increasing predicted concentrations of Lys and Met in metabolizable protein (MP) to recommended levels increases efficiency of use of MP for milk protein synthesis. This should not be a surprising observation in feeding situations where Lys and Met are the first two limiting AA. Therefore, it is reasonable to conclude that maximizing milk components and MP utilization in lactating dairy cows requires providing a profile of AA in MP that matches the profile required for the combined functions of maintenance, reproduction, and milk production, and that the MP is provided in amounts that meets but doesn't exceed requirements for optimal health, reproduction and milk production.

Research to date indicates that of the twenty AA that occur in proteins, only the amount and profile of EAA in MP are of concern. Providing a mixture of nonessential AA (NEAA) to post-weaned dairy calves (Schwab et al., 1982), or lactating dairy cows (Oldham et al., 1979; Schwab et al., 1976; Whyte et al., 2006), where one or more EAA were shown to be limiting, were without benefit. It was also observed that infusing the 10 EAA into the abomasum of lactating cows fed protein deficient diets resulted in increases in yields of milk protein that were similar to the yields that were obtained when casein was infused. Collectively, these observations indicate that when AA supplies approach requirements for total absorbable AA, requirements for total NEAA are met before the requirements for the most limiting EAA.

In recognition of these observations, and because Lys and Met had been shown to be the first two limiting EAA for lactating dairy cows fed diets common to North America, NRC (2001) published dose-response plots that related changes in measured percentages and yields of milk protein to model-predicted changes in Lys and Met concentrations in MP. By using a rectilinear model to describe the dose-response relationships, breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk protein were determined to be 7.2 and 2.4%, respectively; corresponding values for maximal protein yield were 7.1 and 2.4%. These were the first estimates ever presented by a Dairy NRC Committee to evaluate a diet for adequacy of AA concentrations in MP, and have proven exceptionally useful for routine users of the

NRC (2001) model in their quest to increase milk component yields with similar or lower predicted flows of MP. Because they can be rather easily achieved, target levels for Lys and Met in MP have typically been 6.6 and 2.2%, respectively. Both values approximate 96% of the concentrations needed, according to NRC (2001), for maximal content and yield of milk protein.

It is recognized that histidine (His) has been identified as the first limiting AA when grass silage and barley and oat diets are fed, with or without feather meal as a sole or primary source of supplemental RUP (Kim et al., 1999, 2000, 2001a, 2001b; Huhtanen et al., 2002; Korhonen et al., 2000; Vanhatalo et al., 1999). Based on NRC (2001) predicted concentrations of Lys, Met, and His in MP for the diets fed in these experiments, coupled with similar evaluations of diets where cows have (or have not) responded to increased levels of Lys and Met in MP, leads us to speculate that His may become the third limiting AA rather quickly in some diets, particularly where barley and wheat products replace significant amounts of corn in the diet.

The purpose of this paper is 3-fold: 1) to share the results of a recent re-evaluation of the Lys and Met dose-response plots using the "final version" of the NRC (2001) model, 2) to share the results of a recent effort to develop the same dose-response plots using CPM-Dairy (v.3.0.10) and AMTS.Cattle (v.2.1.), and 3) to review our approach for maximizing milk components and MP utilization.

RECENT RE-EVALUATION OF THE LYS AND MET DOSE-RESPONSE PLOTS USING THE "FINAL VERSION" OF NRC (2001)

To determine what the "requirements" for Lys and Met in MP are when the NRC (2001) model is used to evaluate diets, the NRC committee used the indirect dose-response approach described by Rulquin et al. (1993). This approach has the "unique benefit" of allowing requirement values to be estimated using the same model as that used to predict concentrations of EAA in MP.

Because the AA submodel [AA equations (see pages 74-81 in NRC 2001)] had to be developed before the final version of the NRC model was available, a beta version of the model was used to predict the concentrations of Lys and Met in MP in the studies used to develop the dose-response plots. Because of this, and concerned that changes may have been made to the rest of the model, as part of model validation, that may have changed predicted concentrations of Lys and Met in MP, Schwab et al. (2009) re-evaluated the Lys and Met dose-response plots using the final version of the model.

All steps, as stated in NRC (2001), were repeated. In brief, generating the doseresponse plots involves 5 steps: 1) predicting concentrations of Lys and Met in MP for control and treatment groups in experiments in which postruminal supplies of Lys, Met, or both, were increased and production responses measured, 2) identifying "fixed" concentrations of Lys and Met in MP that are intermediate to the lowest and highest values in the greatest number of Lys and Met experiments, 3) calculating, by linear regression, a "reference production value" for each production parameter in each Lys experiment that corresponds to the "fixed" level of Lys in MP and in each Met experiment that corresponds to the "fixed" level of Met in MP, 4) calculating "production responses" (plus and minus values) for control and treatment groups relative to the "reference production values", and 5) regressing the production responses on the predicted concentrations of Lys and Met in MP.

The "revised" dose-response plots that relate changes in milk protein concentrations to changes in predicted concentrations of Lys and Met in MP for the NRC (2001) model are presented in Figure 1. While the plots are strikingly similar to those published in NRC (2001), there are differences between the "published" and "revised" breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk of milk protein. The breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content of milk of milk protein.

Met in MP for maximal content of milk protein were 6.80 and 2.29%, respectively (see figure legends), slightly lower than the values of 7.24 and 2.38% reported in NRC (2001). The breakpoint estimates for the required concentrations of Lys and Met in MP for maximal yield of milk protein were 7.10 and 2.52%, respectively (plots not shown). These values are also different from the NRC (2001) values of 7.08 and 2.38%. It was concluded by Schwab et al. (2009), from a comparison of the predicted flows of microbial MP and feed MP with the beta and final versions of the two models, along with a re-examination of feed inputs, that the primary reason for the differences in breakpoint estimates was differences in feed inputs for some of the studies. It is suggested that the new values be used as the reference values when using the NRC (2001) model to optimize Lys and Met concentrations in MP for lactating cows.

DEVELOPMENT OF LYS AND MET DOSE-RESPONSE PLOTS FOR CPM-DAIRY (V.3.0.10) AND AMTS.CATTLE (V.2.1.1)

Recognizing that the indirect dose-response approach for identifying optimal concentrations of Lys and Met in MP had not been extended to other models, Whitehouse et al. (2009) repeated the same steps, using the same studies as used for NRC (2001), to generate Lys and Met dose-response plots for CPM-Dairy and AMTS.Cattle. Because ration formulation and diet evaluation programs differ in the approach and assumptions taken to estimate AA supply, it was expected that estimated "requirements" for Lys and Met in MP would be different from those for NRC (2001).

The resulting dose-response plots that relate changes in milk protein concentrations to changes in predicted concentrations of Lys and Met in MP for CPM-Dairy and AMTS.Cattle are presented in Figures 2 and 3, respectively. As noted in Figures 1-3 and Table 1, differences existed among the three models in the breakpoint estimates for the required concentrations of Lys and Met in MP for maximal content and yield of milk protein. This was expected, as models differ in the approach for predicting supplies of AA. These differences lead to differences in predicted supplies of RDP, RUP, MP and MP-AA (Whitehouse et al., 2009). The AA prediction model in NRC (2001) is semi-factorial in nature, where some of the parameters are determined by regression. In contrast, CPM-Dairy and AMTS.Cattle use factorial approaches for predicting AA flows



Figure 1. Revised NRC (2001) Lys and Met plots for milk protein concentrations. Regression analysis for Lys was limited to data where Met was 2.07 % or greater of MP. For the linear part of the model y = -0.818 + 0.125x and for the plateau $y = -0.818 + 0.125 \times 6.80$. Regression analysis for Met was limited to data where Lys was 6.16 % or greater of MP. For the linear part of the model y = -0.560 + 0.271x and for the plateau y = -0.560 + 0.271x and for the plateau $y = -0.560 + 0.271 \times 2.29$.



Figure 2. Lys and Met plots for milk protein concentrations for CPM-Dairy. Regression analysis for Lys was limited to data where Met was 2.17 % or greater of MP. For the linear part of the model y = -0.763 + 0.107x and for the plateau y = -0.763 + 0.107 x**7.46**. Regression analysis for Met was limited to data where Lys was 6.65 % or greater of MP. For the linear part of the model y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x and for the plateau y = -0.576 + 0.259x.



Figure 3. Lys and Met plots for milk protein concentrations for AMTS.Cattle. Regression analysis for Lys was limited to data where Met was 1.94 % or greater of MP. For the linear part of the model y = -0.795 + 0.124x and for the plateau $y = -0.795 + 0.124 \times 6.68$. Regression analysis for Met was limited to data where Lys was 6.09 % or greater of MP. For the linear part of the model y = -0.506 + 0.242x and for the plateau $y = -0.506 + 0.242 \times 2.40$.

Table 1. Breakpoint estimates for required concentrations of Lys and Met in MP for maximal content and yield of milk protein for the NRC, CPM, and AMTS models.

	NRC Model				
Item	Optimal Lys	Optimal Met	Lys r ²	Met r ²	Optimal Lys/Met
Content of milk protein	6.80	2.29	.82	.75	2.97
Yield of milk protein	7.10	2.52	.65	.36	2.82
	CPM Model				
Content of milk protein	7.46	2.57	.83	.73	2.90
Yield of milk protein	7.51	2.50	.53	.46	3.00
	AMTS Model				
Content of milk protein	6.68	2.40	.83	.76	2.78
Yield of milk protein	6.74	2.31	.65	.38	2.92

to the small intestine (O'Connor et al., 1993). Prediction models based on the factorial method require the assignment of AA values to model-predicted supplies of ruminally synthesized microbial protein, RUP, and if predicted, endogenous protein. CPM-Dairy (v.3.0.10) uses CNCPSv.5 and AMTS.Cattle (v.2.1.1) uses CNCPSv.6. The latest version of CNCPS has expanded CHO pools, modified CHO A1-B1 degradation rates, the soluble fractions (e.g., sugar, NPN) flow with the liquid phase instead of the solid phase, and the passage rate equations have been updated. The result of these and other changes have led to reductions in ruminal CHO degradation, higher RUP and lower microbial protein flows, and lower predicted flows of Lys and Met to the small intestine, as compared to CPM-Dairy.

MAXIMIZING MILK COMPONENTS AND MP UTILIZATION THROUGH AA FORMULATION

We consider the following 5 steps as being important to maximizing milk components and MP utilization through AA formulation. A brief discussion of each step follows.

Step #1: Feed a blend of high quality forages, processed grains, and byproduct feeds to provide a blend of fermentable carbohydrates and physically effective fiber that maximizes feed intake, milk production, and yield of microbial protein

Microbial protein, based on research to date, has an excellent AA composition for lactating dairy cows. The average reported concentrations of Lys and Met in bacterial true protein approximate 7.9% and 2.6%, respectively; values that exceed the concentrations in nearly all feed proteins (NRC, 2001), and values that exceed the optimal concentrations in MP as estimated by the NRC (2001), CPM-Dairy (v.3.0.10) and AMTS.Cattle (v.2.1.1) models (Table 1). Realizing maximal benefits of feeding a balanced supply of fermentable carbohydrates on feed intake, milk production, and yields of microbial protein requires use of high quality feeds, adequate intakes of physically effective fiber, well-balanced and consistent diets, unlimited supplies of fresh water, and superior bunk management.

Step #2: Feed adequate but not excessive levels of RDP to meet rumen bacterial requirements for AA and ammonia

Realizing the benefits of feeding a balances supply of fermentable carbohydrates on maximizing yields of microbial protein also requires balancing diets for RDP. Rumen degraded feed protein is the second largest requirement for rumen microorganisms. It supplies the microorganisms with peptides, AA, and ammonia that are needed for microbial protein synthesis. The amount of RDP required in the diet is determined by the amount of fermentable carbohydrates in the diet. Diet evaluation models differ slightly in their estimates of RDP in feeds. The NRC (2001) model typically predicts RDP requirements of 10 to 11% of diet DM. Monitor feed intake, fecal consistency, milk/feed ratios, and MUN to make the final decision. A common target value for MUN is 10-12 mg/dl. Don't short-change the cows on RDP...carbohydrate balancing can be negated with an inadequate supply of RDP. A deficiency of RDP will suppress the ability of the microorganisms to reproduce, but they can continue to ferment carbohydrates. This results in higher feed intake, but milk/feed ratios will be low because of lower than expected synthesis of microbial protein.

Avoid over-feeding feeding RDP to the point that rumen ammonia concentrations markedly exceed bacterial requirements. Not only does it result in wastage of RDP, but research (e.g., Boucher et al., 2007), as well as a summary of N passage studies where rumen ammonia concentrations were also measured (Peter Robinson, personal communication), indicate that rumen ammonia concentrations in excess of bacterial requirements decreases flows of microbial protein to the small intestine.

Step #3: Feed high-Lys protein supplements to achieve a level of Lys in MP that comes as close as possible to meeting the optimal concentration (see Table 1)

If protein supplementation is required, select high quality, high-Lys protein supplements (e.g., soybean and canola meals, blood meal, and fishmeal). Feeding low-Lys feeds such as distiller's grains or corn gluten meal as sources of additional protein is not consistent with balancing for AA. Purposely selecting high-Lys protein supplements has been the only option, at least until the recent release of the first rumen-protected Lys sources on the market, to at least partially compensate for the low content of Lys in the RUP fractions from forages, grains and distiller's grains. Achieving target formulation levels for Lys in MP will become easier, and the value of lower Lys protein supplements extended, if these rumen-protected Lys products can be demonstrated to be cost effective sources of MP-Lys.

Step #4: Feed a "rumen-protected" Met supplement in the amounts needed to achieve the optimal ratio of Lys and Met in MP (see Table 1)

Feeding a rumen-protected Met supplement, in conjunction with one or more of the aforementioned high-Lys protein supplements, is almost always necessary to achieve the correct Lys/Met ratio in MP (see Table 1). We continue to be surprised with first time evaluation of diets how often we see Lys to Met ratios in MP of 3.3 or

higher...values as high as 3.5 and 3.6 are not uncommon. "Out of balance" Lys to Met ratios lowers the efficiency of use of MP for protein synthesis and the more "out of balance" the ratios, the less efficient the use.

To achieve the desired predicted ratio of Lys to Met in MP, and to ensure full use of the available MP-Lys for protein synthesis, also requires that a realistic estimate of efficacy be used for the Met product that one elects to feed. Over-estimating the bioavailability of some of the Met supplements has been way too common. Doing so leads to disappointing production outcomes, and the nutritionist and dairy producer believing that balancing for Lys and Met has minimal impacts on animal performance.

Step #5: Don't overfeed RUP

Three factors determine the cows' requirement for RUP. These are: 1) supply of microbial protein, 2) RUP digestibility, and 3) AA composition of RUP. Given the rather high probability that a nutritional model will not be very accurate in predicting the cows' exact requirement for RUP, because of the multitude of factors that affect the requirement for RUP, we suggest that you let your cows tell you how much they need. Routinely used models don't adjust MP requirements, and thus RUP requirements, for changes in AA balance, so let the let models be initial guides, not the final answer. Don't be surprised, as a result of balancing for Lys and Met in MP, how little RUP is actually needed in the diet.

Field experience indicates that cows are more responsive to changes in diet RUP content when RUP has a good AA balance vs. when the balance is not good. This makes sense because the nutritional potency of the RUP is greater when it has a good AA balance vs. a poor AA balance.

Balancing for Lys and Met in MP, using the steps as outlined, has led to many important benefits, both in research and on-farm implementation. A summary of published studies and reported observations include: 1) reduced need for supplemental RUP for a given level of milk and milk production, or increased milk and milk protein production with the same intake of RUP, 2) reduced N excretion per unit of milk or milk protein produced, 3) more predictable changes in milk and milk protein production to changes in RUP supply, 4) improved herd health and reproduction, and 5) increased herd profitability. There are many good reviews in the literature summarizing the benefits of enriching rations in metabolizable Lys and Met (e.g., Garthwaite et al., 1999; NRC, 2001; Rulquin and Verite, 1993; Schwab et al., 2007, and Sloan, 1997). More recent experiments highlight the value of increasing concentrations of Lys and Met in MP on increasing the efficiency of use of MP for milk and milk protein production (e.g., Noftsger and St-Pierre, 2003; Chen et al., 2009).

As expected, the responses that one achieves in balancing diets for Lys and Met in MP depends on ones "starting point". It should also be noted that where it is possible, field nutritionists with experience in balancing for Lys and Met will also lower dietary RDP and/or RUP if the previous diets allow. This has the benefit of often reducing the usual

added expense of replacing low Lys protein supplements with high Lys protein supplements and the cost of adding one or more ruminant protected Met sources to the diet. In reducing dietary CP, it is important, for the reasons previously stated, not to cause a deficiency of RDP. When employing these feeding strategies, the results of a 10-herd field study in 2006 indicated a return on investment (ROI) ranging from 1.1 - 5.5; the average ROI was 3.35:1 (Driver, 2007).

We also typically observe ROI of 2.5 or higher. Increases in butterfat content and milk yields are common and contribute to the favorable ROI. Balancing diets for Lys and Met, because of the stated benefits, is an attractive option for increasing dairy herd profitability, even with current low milk prices and high feed costs. It is no longer uncommon to hear reports of increases in milk protein and milk fat concentrations of 0.10 - 0.25 and 0.10 - 0.15 percentage units, respectively, and 2 - 4 lb more milk as a result of balancing for Lys and Met, with little or no effect on feed costs.

The second author of this paper has also had excellent success in balancing diets for Lys and Met. He uses Formulate2, a commercially available ration formulation model that he has developed that uses NRC (2001) as its operating platform. The model not only implements the NRC (2001) model, but fully integrates the model's equations with its optimization processes. Thus, it provides the ability to set and accurately meet MP-AA constraints at the duodenal level while minimizing the cost of doing so.

In addition, the work reported by Schwab et al. (2003, 2004) to extend the NRC model to predicting changes in lactation from changes in supplies of MP-Lys and MP-Met has been incorporated into Formulate2 as a pop-up calculator. The calculator permits acquisition of milk flow and milk composition inputs from the background diet record and utilizes the equations developed from this work to generate target values for grams of MP-Lys and MP-Met. The calculator also permits re-calculation of the MP requirement based on a user stipulated concentration of the selected MP-AA (MP-Lys or MP-Met) in MP. As a result, diets are routinely balanced with Formulate2/NRC 2001 for RDP, MP-Lys and MP-Met without regard to CP%.

Using these tools it has been possible to improve concentrations of MP-Lys and MP-Met in the diets of fresh and early lactation animals allowing the reduction of dietary CP levels to a range of approximately 16.0% to 16.5% via reduced feed RUP while maintaining RDP at acceptable levels. As a result, significant improvements in milk protein percent, total milk solids percent and butterfat percentages have been achieved as well as improved yields of these components. Additionally, because of the reduction of dietary RUP achieved by this approach, a frequent result has been lower total concentrate costs by allowing the inclusion of lower cost feeds that can contribute well to total dietary NFC. This approach has been implemented with herds at all levels of milk production with equal effect.

The primary basis for realizing these responses has been the excellent predictive reliability exhibited by the NRC (2001) model in commercial production environments in terms of NE(I) allowable and MP allowable milk. In all situations where previous diets

have been reviewed and revised a significant gap between energy allowable milk and MP allowable milk has been observed. This phenomenon has been evident regardless of which other model was used to formulate the previous diets. In these diets, MP allowable milk has been found to be the fundamental limiting factor in production of milk and milk components and thus the primary factor predicting animal response. Simply closing this gap, even without optimizing MP-Lys and MP-Met in MP, has produced significant results. The additional responses and benefits described previously are obtained when AA balance and reduction of RUP/MP are addressed. It is important to note that what has been described has been achieved by using Formulate2/NRC 2001 "out-of-the-box" without the need to make adjustments to the rates and constants of various model predictive mechanisms.

One example of a farm situation follows. The graphs below illustrate changes in milk protein percentage and milk protein yield when balancing for MP-Lys and MP-Met in a herd of approximately 1700 cows with significant, ongoing management issues and less than average milk production. The two periods shown are the same weeks of the year in different years.



Herd average milk flow was 63.2 lbs for the 2007 period and 70.0 lbs for the 2008 period. The 2007 diets were balanced for MP only with dietary CP at 17.9% which did yield improvement over historical milk protein production. The MP supply targets for the high cows for both years were very similar; 2846 g in 2007 and 2868 g in 2008 on equal DMI.

Though diets during the second period were balanced for MP-Lys and MP-Met, no direct attempt was made to minimize RUP/MP. Consequently, CP in the diet of high producing animals was 17.3%. Subsequently, CP in such diets has been reduced to 16.5% with no apparent negative effects.







CONCLUSIONS

The adoption of the concept of balancing diets for AA continues to increase. Balancing diets to come as close as possible to meeting model determined optimal concentrations of Lys and Met in MP is the first step to balancing diets for AA. Benefits include: 1) increased yield of milk and milk components, 2) reduced N excretion per unit of milk or milk protein produced, 3) more predictable changes in milk and milk protein production to changes in RUP supply, 4) improved herd health and reproduction, and 5) increased herd profitability. Increases in milk protein and fat concentrations of 0.1-0.25 percentage units for protein and 0.1-0.15 for fat and returns on investment of 2.0 to 3.5 are typical. Increases in milk yield are more common in early lactation cows than late lactation cows, and can be rather significant if balancing for Lys and Met is started before calving. With high feed costs and low milk prices, an important benefit of AA balancing has been the opportunity to increase milk and milk component yields with less RUP supplementation and similar or lower feed costs.

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