

METABOLIZABLE PROTEIN AND AMINO ACID NUTRITION OF THE COW: WHERE ARE WE IN 2007

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INTRODUCTION

Considerable progress has been made over the last 30 years in the United States to develop systems that describe protein requirements and protein adequacy of diets for dairy cows. Ration formulation systems have moved from balancing diets for crude protein (CP) and digestible protein to the use of nutritional models (e.g., NRC, 2001; CNCPS, CPM-Dairy, and Amino Cow) that recognize that the cow has two sets of dietary protein requirements (RDP and RUP) and that metabolically, she has requirements for individual amino acids (AA) rather than metabolizable protein (MP) per se. While current models and feed inputs are less than perfect, and our understanding of the N requirements of rumen bacteria and the AA requirements of the cow at different physiological states and levels of milk production are not clearly defined, current knowledge and consistent use of a model allows for more precise formulation of diets for RDP, RUP and AA.

This paper is an attempt to summarize the current “state of the art” of balancing diets for protein and AA as well as to highlight one area out of several that need further research.

THE BENEFITS OF BALANCING DIETS FOR RDP AND RUP

A discussion of the benefits of balancing dairy cow rations for RDP, RUP and AA first begs the question of what happens when these dietary components are in short supply. There are three goals in balancing dairy rations for protein and AA. The first goal is to meet the RDP (ammonia, AA and peptides) requirements for maximum carbohydrate digestion and synthesis of microbial protein. Research and field experience has shown that not meeting these requirements decreases microbial digestion of carbohydrates, decreases microbial digestion of protein, decreases synthesis of microbial protein, decreases feed intake and milk yield, and may decrease content of milk protein. The second goal is to meet the MP requirements of the cow for maintenance, growth, optimum health and reproduction, and desired levels of milk and milk protein production with minimal intake of RUP. A shortage of MP has been shown to decrease milk yields and content of milk protein, weight gains, and in a few studies, decreased reproductive efficiency, possibly through effects on endocrine function. The final goal in balancing diets for protein and AA is to meet the protein (RDP and RUP) and AA requirements of the cow for a desired milk yield and content of protein and fat with a minimum amount of dietary CP. Clearly, overfeeding dietary CP (RDP or RUP) is wasteful and often decreases animal performance.

Matching “actual” supplies of RDP and RUP with “actual” requirements is clearly a challenge for nutritionists. However, experience has shown that consistent use of a model, understanding its strengths and weaknesses and learning to use it more as a guide than for its absolute recommendations, and having high quality feeds and protein supplements to work with often allows for feeding diets that are lower in content of CP. Additionally, one or more of the following benefits can be realized: increased milk and milk protein production, lower feed costs, improved reproduction, and increased herd profitability.

THE LIMITING AMINO ACID THEORY

Twenty AA are needed for protein synthesis. Absorbed AA are provided to ruminants by microbial protein, RUP, and endogenous protein. Ten AA are classified as essential and 10 as nonessential. Essential AA (EAA) refers to those AA that cannot be synthesized in animal tissues, or at least not at rates sufficient to meet requirements for protein synthesis. Therefore, they must be absorbed. When absorbed in the profile as required by the animal, the requirement for total EAA is reduced and their efficiency of use for protein synthesis is maximized. The nonessential AA (NEAA) are readily synthesized in animal tissues from each other, or from metabolites of intermediary metabolism as well as from surplus EAA. Unlike the EAA, there remains little evidence that the profile of absorbed NEAA is important for efficiency of use of absorbed AA for protein synthesis. Moreover, several experiments have demonstrated that NEAA as a group of AA do not become more limiting than EAA when dairy cattle are fed conventional diets (e.g., Schwab et al., 1977; Whyte et al., 2006). However, it is acknowledged that research is currently too limited to rule out the fact that a selected NEAA, if provided in amounts greater than provided by the diet, may have some benefit to the animal in certain situations.

The term limiting AA has traditionally been used to identify the EAA that are in shortest supply relative to requirements. For example, the first limiting AA is that EAA supplied in the smallest amount relative to requirements. In like fashion, the second limiting AA is that EAA supplied in the second smallest amount relative to requirements.

The limiting AA theory has been adopted as a central dogma of animal protein nutrition. The theory is perhaps described best by the barrel and stave example. If the staves of a barrel are of different heights, relative to the full length of the barrel, then the volume of liquid that the barrel can hold will be determined by the length of the shortest stave. The shortest stave might be regarded as being the most limiting, because it determines the capacity or volume of the barrel. In similar fashion, the efficiency of use of absorbed AA is determined by the supply of the first limiting AA.



In the barrel that is shown, Met is the first limiting AA. If the supply of Met (i.e., length of the stave), relative to requirements, is increased such that it is co-limiting with Lys, then Met and Lys will be co-limiting AA. Increasing the supply of Met such that it is equally limiting with Lys will increase the efficiency of use of absorbed AA (i.e., the barrel can hold more liquid).

Research and field experience indicate that Met is most often the first limiting AA for milk protein production, and that Lys is most often the second limiting AA. Attempts to demonstrate that other EAA are either more limiting than Met or Lys for improved efficiency of use of MP for milk protein production in North America have been difficult. Therefore, an important goal in balancing diets for AA is to feed a complementary source of dietary proteins and rumen-protected forms of Met to ensure that the Met and Lys staves in the barrel are as long as possible, and of the same length (their supply relative to requirements are the same).

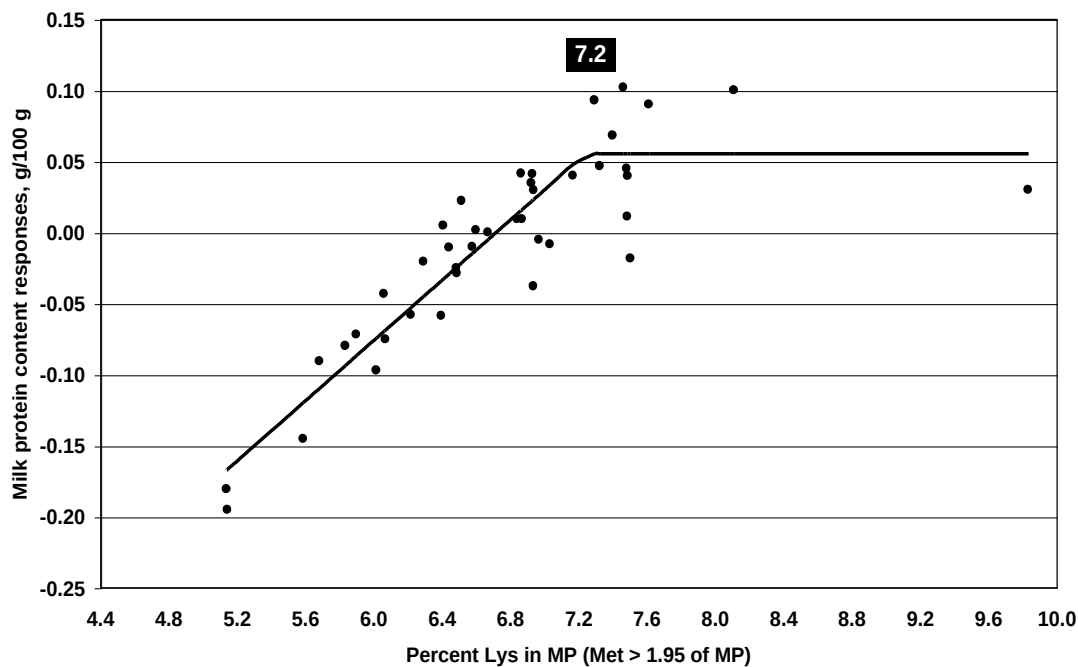
Histidine has been identified in a number of studies as first limiting when grass silage and barley and oat diets are fed, with or without feather meal as a sole or primary source of supplemental RUP (Kim et al., 1999, 2000, 2001a, 2001b; Huhtanen et al., 2002; Korhonen et al., 2000; Vanhatalo et al., 1999). We speculate that histidine may be the third limiting AA in some corn-based rations, particularly where no blood meal is being fed. However, the practical significance of being able to determine the subsequent limiting AA remains a relatively academic question in today's environment. It is still a major challenge even to achieve 90% of estimated requirements for Lys and Met with the ingredients currently available. Until these levels can be pushed higher,

there is unlikely to be any issues of responses to Lys and Met being inhibited by the limitation of other AA.

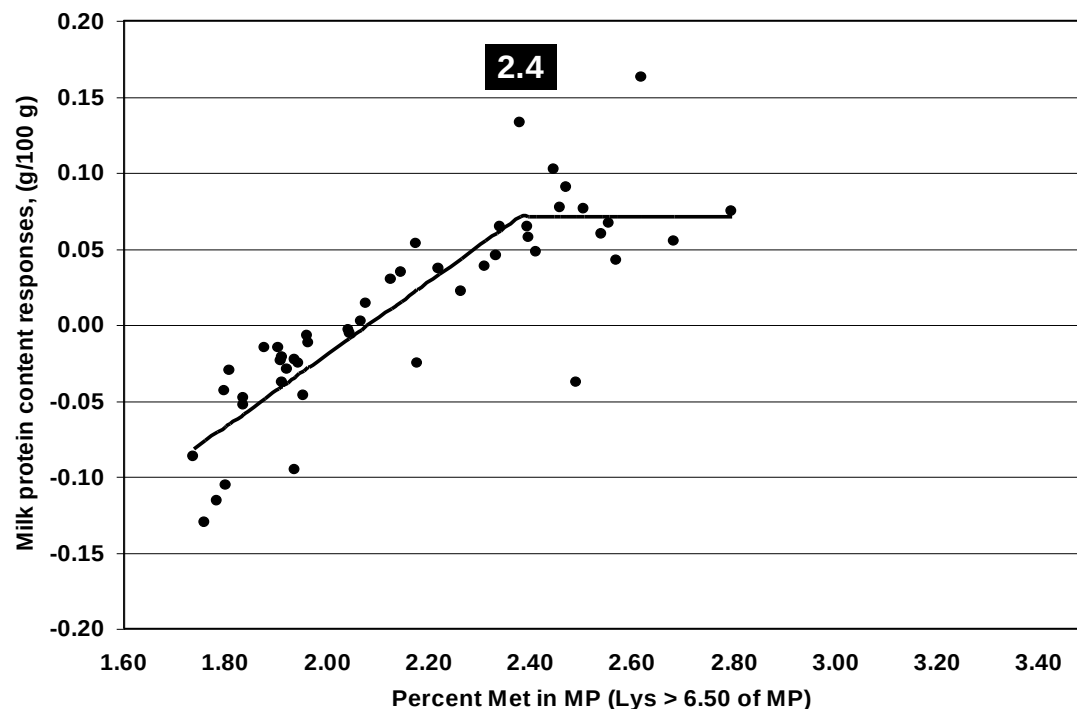
TARGET FORMULATION LEVELS FOR LYS AND MET IN MP

At present, our knowledge is not sophisticated enough to accurately determine individual AA requirements using the traditional factorial approach of estimating a requirement for each physiological function (e.g., maintenance, growth, lactation, and pregnancy). The currently accepted and more robust approach is the indirect response curve method proposed first by Rulquin and Verite (1993). This approach was subsequently used in NRC (2001). The advantage of this method is that the determination of supplies and requirements of AA are interdependent. Requirements are estimated as a dose response function using the approach established to estimate the supplies of metabolizable AA. Therefore, requirements are dependent on and can vary between different formulation systems. However, to the purist, there can be only one requirement for an animal at a defined physiological status and determined level of production. Therefore, a more correct terminology to use would be “target formulation levels or recommendations”, rather than requirements.

Below you will see the representation of the dose response curves used to establish the levels of Lys and Met that are needed in MP to optimize milk protein concentration in NRC (2001). Optimums were established at 7.2 and 2.4% of MP for Lys and Met, respectively. However, these levels usually cannot be achieved in practice. This is particularly true on corn based rations where it is difficult to achieve Lys levels higher than 6.7% of MP. Thus, practical target formulation levels of 6.66 Lys and 2.22 Met as a % of MP have been suggested with respect to the NRC (2001) formulation approach.



It is important to note that the desired Met levels in MP will depend on the level of Lys that can be achieved. The first step is to maximize Lys as a % of MP, then balance the Met to keep a 3.04:1.00 ratio to maximize efficiency of utilization of MP and prevent the unnecessary overfeeding of Met. It should be noted that these target formulation levels need to be defined for each formulation system.



ACHIEVING THE TARGET LEVELS FOR LYS AND MET IN MP

First and foremost, ingredients should be selected to maximize microbial protein synthesis. Microbial protein has an excellent profile of AA and high concentrations of both Lys and Met. Fermentable carbohydrates are the drivers to maximize microbial protein synthesis. Thus, feeding a good balance of readily fermentable carbohydrates with highly digestible NDF sources is the first priority. Obviously an adequate quantity of RDP needs to be fed to ensure the rumen fermentable carbohydrate is effectively transformed into microbial protein. Skimping on RDP is not recommended. It is suggested that microbial protein should represent at least 50% of MP. The remaining MP will have to come from RUP and endogenous sources. All RUP sources have lower concentrations of either Lys or Met and more often both compared to microbial protein. The success of employing AA formulation principles resides in careful selection of raw materials that can truly help increase Lys and Met supplies. Raw materials and protected AA products with ‘WISHFUL THINKING’ values for Met and Lys should not be used – they only discredit the use of the sound principles of AA formulation.

Blood meal has the greatest potential to elevate Lys levels when included in a ration due to its high CP, RUP and Lys content such that with a 1lb inclusion, daily Lys supply can be improved by more than 20 g. However, care should be taken when sourcing blood meal and blended

products to ensure the product is consistent and meets expectations. Fishmeal, although not as high in Lys as blood meal, is richer in Met and provides a balanced source of both AA, but the same precautions should be taken when sourcing fishmeal as for blood meal. Soybean meal and protected soya products also have higher than average Lys concentrations (~6.2% of CP) and their incorporation in the ration can be very helpful in meeting target Lys concentrations in MP. The inclusion of corn distillers and brewers grains should be minimized as they are low in Lys and make reaching target Lys levels extremely challenging.

Rumen protected Met products are essential if the goal is to achieve the practical recommendations for Lys and Met in MP. Rumen protected Met products are feed ingredients and should be formulated into the diet accordingly, not fed at a single dosage rate irrespective of ration composition. They are concentrated sources of metabolizable Met and should be offered along with the conventional feed ingredients available on farm to “best cost” rations to meet target ration metabolizable Lys and Met levels. Because they are concentrated sources of Met, an accurate assessment of the real Met contributions of the technologies available commercially is needed so that these products can be used appropriately and to maximum advantage. The most efficacious products for increasing concentrations of Met in MP are Smartamine® M, Mepron M85®, and MetaSmart®. MetaSmart® is the isopropyl ester of hydroxymethyl butanoic acid (HMB). Esterifying HMB with isopropanol (MetaSmart®) slows the normal rapid degradation of HMB by rumen microflora and facilitates absorption across the rumen and epithelial walls. MetaSmart® has the big advantage of being pelletable, which is not feasible with any of the encapsulated Met technologies (Smartamine® M and Mepron M85®). Alimet® and Rhodimet® AT 88, both sources of HMB, have been shown to have negligible effects on improving the Met status of cows.

THE BENEFITS OF BALANCING DIETS FOR LYSINE AND METHIONINE

There are many good reviews in the literature summarizing the benefits of enriching rations in metabolizable Lys and Met (e.g., NRC, 2001; Rulquin and Verite, 1993; and Sloan, 1997). The following discussion highlights the primary benefits.

Enhanced milk and milk component yields

Garthwaite et al. (1998) summarized 12 published feeding trials concerning the effects of enriching rations in metabolizable Lys and Met. For seven trials commencing immediately post calving or within the first 2 or 3 weeks of lactation and continuing to at least 120 days in lactation, daily milk yield was increased an average of 1.5 lb, milk protein by 80 g, and milk protein percentage increased by 0.16 percentage units. In five similar studies where the rations were enriched in Lys and Met in the close-up ration as well as for the first third of lactation, daily milk yield was increased an average of 5 lb, milk protein by 112 g, and milk protein percentage increased by 0.09 percentage units. In these five trials, daily milk fat yield was also increased by 115 g and milk fat percentage by 0.10 percentage units. In all cases, the “AA balanced” diets had either the same or lower levels of dietary CP than the “basal” diets. This summary of experiments not only showed the importance of enriching diets with Lys and Met on milk performance, but it also showed that the principles of balancing rations for Met and Lys should also be applied in the close-up rations to extract maximum benefit during lactation.

We have both collected and seen several data sets of production responses to increasing Lys and Met concentrations in MP with high Lys protein supplements and rumen protected Met products. 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 are less frequent and are more commonly observed in early lactation cows. Although the increase in the rate of implementation of AA balancing has been primarily in geographical areas where milk protein is well remunerated within the milk pricing scheme, closer analysis of the biology behind AA balancing encourages the use, irrespective of whether there is a premium for milk protein or not.

Improved efficiency of use of MP

This is the factor that is fundamental to achieving the benefit of balancing rations for Lys and Met. In essence, when these AA are limiting, the dairy cow has an oversupply of all other AA, and when they are supplied in more adequate amounts, the missing links are provided (the short staves in the barrel are lengthened) and more milk protein molecules can be synthesized. This reduces the surplus of the AA and unless there is a considerable surplus of MP because of overfeeding RUP, the efficiency of utilization of MP is increased.

Of interest was the observation that when only relying on MP to estimate AA requirements, retrospective calculations showed that actual milk yield falls short of MP allowable milk in 90% of situations (NRC 2001). In a more recent analysis, Schwab et al. (2004) showed the overall efficiency of utilization of MP for milk protein secretion to be only of the order of 0.64 compared to the NRC book value of 0.67, whereas MP utilization was calculated to be greater than 0.67 when balancing for Lys and Met was integrated into the formulation approach.

It would seem essential to at least pay a minimum attention to the content of Lys and Met of MP if you wish to continue to rely on a factor of 0.67 for the conversion of MP to milk protein. As an example, let us consider the impact of a lower efficiency of utilization of MP. For a cow producing 40 kg of milk at 3.0% milk protein, if the overall efficiency of MP utilization falls from 0.67 to 0.60, milk protein yield would be predicted to drop by 10% (120 g). A 120 g loss in milk protein yield equates to 2 kg less milk with a lower milk protein concentration (-0.15%). The studies of Piepenbrink et al. (1999) and McLaughlin et al. (2002) demonstrate this important facet of balancing rations for AA. Piepenbrink et al. (1999) fed a Met enriched ration, and studied in a dose response manner using a replicated Latin square design, the response to increasing supplies of Lys. Milk protein secretion increased in a linear fashion. The optimum response was an extra 173 g of milk protein (2.7 kg milk, +0.2% milk protein) to increasing daily MP-Lys up to an addition of 34 g. The efficiency of utilization of MP for milk protein synthesis was only 0.53 for the imbalanced ration without any supplemental Lys supplementation. Intakes of DM did not change. At the optimum level of Lys supplementation, the efficiency of utilization of MP was improved to 0.67. Likewise, McLaughlin et al. (2002) performed a very similar experiment, increasing milk protein output by 217 g/day (2 kg more milk, +0.27% milk protein) by increasing MP-Lys supply by 49.5 g.

These results indicate that when only MP is considered as the entity defining AA supplies, there is no estimation of likely limiting AA. Therefore, milk performance is likely to be less predictable because of this. Schwab et al. (2004) presented an update, which compared MP, Lys, and Met supplies as predictors of milk volume and milk protein yield. MP supply does an adequate job (r^2 of 0.65) of predicting milk volume and a slightly better job of predicting milk protein yield (r^2 of 0.74). One would expect the latter to be more closely correlated as both the input and outputs are in units of protein. Compared to MP, Met supply was a better predictor of both milk volume (r^2 of 0.76) and milk protein yield (r^2 of 0.81). However, when studies were limited to those in which the Lys:Met ratio in MP was less than 3.25:1.00, Lys supply proved to be the best predictor of both milk volume and milk protein yield with r^2 of over 0.90. This analysis shows that predictability of milk performance is improved by paying attention to at least the first two limiting AA. By moving in this direction with our formulation approaches, we will be reducing the variation in predicting milk performance not increasing it. By continuing to formulate rations uniquely on a MP basis with no consideration for metabolizable Lys and Met, performance will be depressed and less predictable, and milk protein and milk fat yields will not be optimized, reducing net returns from the sale of milk.

Rather than continuing the traditional approach of formulating diets for 18% CP or more without consideration of Lys and Met levels in MP, integrating a formulation approach to increase levels of Lys and Met in MP allows rations to be formulated at 16.5 to 17.5% CP while maintaining or increasing milk yield and increasing yields of milk components.

Reduction in metabolic disorders

High feed efficiency in itself may not be a good indicator of a healthy ration if it is at the expense of mobilizing energy reserves too rapidly, which could lead to metabolic disorders and delayed or impaired reproduction. Nevertheless when rations are balanced for Lys and Met, due to the improved efficiency of use of MP, less ‘energy’ is needed to eliminate surplus amino acid N as urea, allowing energy to be put to a more productive use. A further reason that could help explain the improvement in feed efficiency and in particular energy status may be associated with the other roles of Met in metabolism, rather than simply as a building block for milk protein synthesis.

Met has long been advocated as having a favorable role on hepatic metabolism through its capacity as a methyl donor. A series of trials (Bauchart et al., 1998) illustrate more clearly the roles that Met plays in hepatic metabolism. Met plays a key role in assuring the synthesis of apoprotein B, an essential component in the formation of the very low density lipoprotein (VLDL) complex which is responsible for evacuating triglycerides from the liver to peripheral tissues. One study that illustrates this mode of action of Met and Lys was realized by Durand et al. (1992). They measured across the liver the net appearance or disappearance of VLDL, before, after and during portal infusion of extra Lys and Met. Before and after the infusions there was a negative balance whereas during the infusion a positive balance was obtained. It is hypothesized that this may be due to Met acting at three different levels to predispose these effects. Firstly, Met is an essential building block for the formation of apoprotein B. Secondly, Met appears to be involved in the gene transcription and or translation of mRNA for apoprotein B synthesis. Thirdly, Met may also act as a methyl donor to favor lecithin synthesis which is

essential for the elaboration of the hydrophilic envelope of hepatic VLDL. The net effect would be a reduction in the risks of fat infiltration of the liver which predispose problems such as fatty liver and ketosis.

Two lactation studies were subsequently carried out over the first 4 to 6 wk of lactation. Cows were fed to be fat at calving and then fed an energy restricted diet at the beginning of lactation. Half the cows were fed supplementary Lys and/or Met. The improvements in performance were dramatic - an extra 2.5 kg of milk and an increase of 0.25 percentage units of milk protein – the combined increase in milk component yield was over 250 g/day. In the second trial the milk performance improvements were also associated with a large reduction in circulating ketone body levels in the second week of lactation, confirming that enhancing the supply of Met and Lys can help reduce metabolic disorders.

Improved reproduction

Conventional wisdom would indicate that any ration manipulation that can help minimize metabolic disorders and improve energy status of cows in early lactation should also have a potential to positively influence reproductive parameters (Santos et al., 2005). Robert et al. (1996) observed a better uterine involution (% of animals whose uterus has regressed to normal size at 45 days post calving). This was associated with a reduced number of inseminations needed per conception but neither effect was significant. They also measured milk progesterone levels every 3 days for the first 112 days of lactation to follow the cyclicity. They were able to show that the cows receiving a ration balanced for Lys and Met had higher progesterone levels pre successful ovulation than control animals. This is considered to potentiate a strong ovulation. Also during the 5 days after insemination, progesterone levels were also higher which is often regarded as a positive factor for the embryo to successfully implant. Thiaucourt (1996) was able to demonstrate in field trials (53 farms, 2000 cows) that feeding rations formulated to be rich in Lys and Met improved timing to first insemination and calving interval by 5 days ($P < 0.1$).

The other avenue through which ration amino acid balancing should be able to positively influence reproductive function is by facilitating a reduction in high circulating levels of blood urea through the lowering of ration CP content without hurting milk performance. There is a generally accepted negative association between plasma, serum, and milk urea N and conception rates in high producing lactating cows (Butler et al., 1996, Ferguson et al., 1989, Santos, 2005). Elrod et al. (1993) found that by overfeeding RUP or RDP in the diet, uterine pH was reduced on day 7 of the estrous cycle of heifers and in the case of overfeeding RDP this was associated with a much lower conception rate.

A role in immune response?

The role of Met and Lys in immune function is still somewhat speculative in dairy cows. It has been shown in chicks that sulphur AA status is an important determinant of the immune response to a Sheep Red Blood Cell challenge. Similarly, in newly arrived, “stressed” feedlot steers, Spears et al. (1996) showed that fortifying the diets with Lys and Met reduced rectal temperatures compared to Controls after inoculation with IBR intranasally followed 7 days later

with an injection of pig red blood cells. This was accompanied by an improved humoral response as indicated by a higher IgM titer.

In dairy cows, there is only some indirect evidence that balancing rations for Lys and Met may be positively impacting the immune system. In the field study involving 2000 cows across 53 farms, Thiaucourt (1996) observed the classical improvements in milk protein % (+0.13) and milk production in early lactation (+3.5 lb/day) when feeding rations balanced for Lys and Met. Because it was available, somatic cell count was also tracked and was found to be reduced by 50,000/ml. Thiaucourt (1996) speculated that one or more of three different factors could have contributed to this phenomenon. First, the general immune response of animals is improved when their energy status is improved. Second, the extra supply of Met is known to increase circulating levels of taurine, an AA thought to be important in maintaining the stability of cell membranes and in anti-oxidant reactions. And finally, the synthesis of the keratin ring, a protein rich in cysteine, at the extremity of the teat duct may be improved. This would enhance protection against intra-mammary infection.

WHAT'S NEXT?

There are several areas where more research is needed to more accurately balance diets for protein and AA. These include a better understanding of the absorption and metabolism of AA by the liver and extra-hepatic tissues, better definition of AA requirements for maintenance, growth, pregnancy and postnatal, and milk protein synthesis, more accurate prediction of RDP and RUP supplies and synthesis of microbial protein, more accurate prediction of AA availability from microbial protein, RUP, and endogenous secretions, and improved feed analysis and diet evaluation models. While space does not allow further discussion of each of these, we have elected to highlight the issue of digestibility of RUP and its constituent AA, an important factor for the accurate prediction of MP-AA supplies.

Intestinal digestibility of RUP does vary among and within feedstuffs (Stern and Bach, 1996); therefore, several techniques have been developed to measure the digestibility of RUP in a variety of feedstuffs (NRC, 2001). The most common of these techniques include the mobile bag technique (generally considered the gold standard) and an *in situ/in vitro* enzymatic procedure (Calsamiglia and Stern, 1995). The first step of both procedures is to incubate the feeds *in situ* in the rumen of cattle to obtain the rumen undegraded residue of the feeds. With the mobile bag technique, a small amount of the rumen undegraded feed residue is then weighed into bags and soaked in a pepsin/HCl solution for typically one hour. The bags are then inserted into the duodenum of cattle via duodenal cannulas and collected either at the ileum (via ileal cannulas), or more commonly in the feces. After the bags are collected, they are rinsed to remove contaminating proteins (i.e., endogenous protein) and analyzed for N content. Digestibility of RUP is calculated based on the disappearance of N from the bag. Although the mobile bag technique is generally considered the gold standard, concerns regarding the digestibility estimates obtained with this procedure do exist. Some of the major concerns are errors associated with cannula placement and digesta flow markers and the assumption that the N that disappears from the bag is absorbed by the animal (NRC, 2001). In addition, collecting the bags from the feces, which represents the majority of the cases, is not ideal because microbial degradation and synthesis of protein in the large intestine can influence digestibility

measurements. Although more costly and difficult, determining ileal digestibility of RUP is more accurate. However, regardless of the specific procedures, the mobile bag technique is still not practical for routine analysis of RUP digestibility; therefore, an *in vitro* procedure to rapidly estimate RUP digestibility is desired.

The *in situ/in vitro* procedure of Calsamiglia and Stern (1995) provides a rapid, more practical alternative to estimate RUP digestibility. With this procedure, after the rumen undegraded feed residues are obtained, the samples are incubated in reaction tubes with a pepsin/HCl solution for one hour followed by a pancreatin incubation for 24 h. This procedure has thus been termed the “three-step procedure”. At the end of the pancreatin incubation step, trichloroacetic acid is added to the tubes to precipitate undigested protein. The tubes are then centrifuged, and the supernatant is analyzed for N content. Digestibility of RUP is calculated as TCA soluble N/amount of N in the initial sample. Estimates of small intestinal digestibility of RUP of 34 duodenal digesta samples obtained *in vivo* and with the pancreatin incubation step of the three-step procedure were strongly correlated ($R^2 = 0.91$).

In the current dairy NRC (2001) publication, RUP digestibility estimates were determined by summarizing 48 studies in which the mobile bag technique was employed and 6 studies in which the three-step procedure was used. The average RUP digestibility values were then rounded to the nearest 5 percentage units to emphasize the lack of precision in arriving at mean values. For those feeds in which there was limited or no data, the values reported in the French PDI system (determined via digestibility experiments with sheep; Jarrige, 1989) were used. Because of this approach, the RUP digestibility values in the NRC (2001) feed library are closer at attempting to account for the variability in the digestibility of RUP among feeds than the previous NRC (1989) publication, which assumed that the RUP digestibility of all feeds was 80%. However, relying on mean values of RUP digestibility when formulating diets is still not ideal because the RUP digestibility coefficients within feedstuffs that have been reported in the literature vary widely. For example, in a recent review of the literature, we found reported RUP digestibility values for corn gluten feed from 25 (Kononoff et al., 2007) to 84% (Van Straalen et al., 1997) and RUP digestibility values for soybean hulls from 20 (Kononoff et al., 2007) to 72% (Masoero et al., 1994). The NRC (2001) RUP digestibility values for corn gluten feed and soybean hulls are 85 and 70%, respectively. Based on these reported observations, when relying on NRC (2001) model default values for RUP digestibility, the actual supply of MP to the animal may be over or underestimated when evaluating diets with the model. However, due largely to a lack of a standardized and commercially accepted completely *in vitro* method for estimating RUP digestibility, feeds are not routinely analyzed for digestible RUP content.

Another issue with NRC (2001) RUP digestibility estimates is that it is assumed in the model that the digestibility of each individual AA in RUP (RUP-AA) is the same as total RUP. Data from non-ruminant animals indicates that the digestibility of individual AA in feed protein also varies (Muley et al., 2007; Stein et al., 2006). Obtaining accurate estimates of digestibility of RUP-AA is especially concerning for Lys when feeds are heat processed. The ϵ -amino group present on the side chain of Lys readily participates in the Maillard reaction in the presence of reducing sugars and heat. During the Maillard reaction, Lys containing compounds form which are unavailable for absorption by animals. Therefore, in order to advance nutritional models, efforts need to be made to determine the differences in digestibility of individual AA in the RUP

fraction of feeds, particularly for Lys, and also, to identify rapid, reliable *in vitro* methods that can be used by commercial laboratories for routine analysis of RUP and RUP-AA digestibility.

The three-step procedure mentioned previously is more practical for routine analysis of RUP digestibility than the mobile bag technique, but one of its limitations is that it does not allow for analysis of digestibility of individual AA. Because trichloroacetic acid is added to the incubation tubes to precipitate undigested protein, the digested samples cannot be analyzed for AA content. The three-step procedure also requires feeds to be ruminally incubated *in situ* which hinders the wide spread use of the procedure by commercial feed testing laboratories. Recently, Irshaid and Suedekum (2007) modified the three-step procedure of Calsamiglia and Stern (1995) to eliminate the *in situ* ruminal incubation step. In lieu of a ruminal *in situ* incubation, the authors digested feed samples with a protease from *Streptomyces griseus*, and then digested the remaining residue with pepsin and pancreatin enzymes. There was a strong correlation between RUP digestibility determined with the completely *in vitro* procedure of Irshaid and Suedekum (2007) and the original three-step procedure ($R^2 = 0.99$). It appears that this completely *in vitro* procedure may be useful for routine analysis of feeds for RUP digestibility.

Gargallo et al. (2006) also made modifications to the original three-step procedure of Calsamiglia and Stern (1995) but in a different way. These authors modified the procedure so that trichloroacetic acid is no longer utilized and digestibility of individual AA can be determined. In their modifications, the feeds are still ruminally incubated *in situ*, but after the ruminal incubation step, the residues are analyzed for AA content and placed in smaller nylon bags. These smaller bags are incubated in a pepsin solution for 1 h followed by incubation in a pancreatin solution for 24 h in a DaisyII incubator (ANKOM Technologies) in constant rotation at 39°C. The residue that remains in the bag after the pancreatin incubation step can then be analyzed for protein and amino acid content. Intestinal digestibility of RUP and RUP-AA can be calculated based on disappearance of total protein and individual AA from the bags. The authors observed a strong correlation between estimates of RUP digestibility obtained using their modified three-step procedure and those obtained using the original three-step procedure ($R^2 = 0.84$), but the authors did not validate their modifications to the procedure with *in vivo* measurements.

Recently in our lab, we analyzed 3 samples of soybean meal (SBM), 3 samples of SoyPlus®, 5 samples of dried distillers grains with solubles (DDGS), and 5 samples of fishmeal using the modified three-step procedure of Gargallo et al. (2006) and the precision-fed cecectomized rooster assay. The cecectomized roosters were tube-fed the rumen undegraded feed residue of the samples mentioned. Titgemeyer et al. (1990) evaluated the use of the precision-fed cecectomized rooster assay as a technique for estimating small intestinal digestibility of AA in cattle. The authors tube-fed freeze-dried duodenal digesta to cecectomized roosters and measured intestinal AA digestibility. The small intestinal digestibility of the samples was previously determined in cattle, and the correlation between the mean digestibility values obtained in cattle and roosters was strong ($R^2 = 0.94$). The authors concluded that the precision-fed cecectomized rooster assay is an appropriate technique for estimating small intestinal digestibility of AA in cattle. We observed a strong correlation between RUP-AA digestibility estimated using the procedure of Gargallo et al. (2006) and RUP-AA digestibility measured in cecectomized roosters (R^2 for total AA = 0.93; R^2 for RUP-Lys = 0.94). However, although the

values were highly correlated, the procedure of Gargallo et al. (2006) tends to over predict *in vivo* Lys digestibility estimates by about 10%.

In addition to the procedure of Gargallo et al. (2006), we also evaluated the use of the immobilized digestive enzyme assay (IDEA™) for estimating digestibility of RUP-AA (Boucher et al. 2007b). The IDEA method was pioneered to estimate intestinal digestibility of protein in human foodstuffs and is a rather lengthy and complicated procedure (Church et al., 1984). However, Novus International, Inc. has recently developed IDEA™ kit assays (specific for each feed) that are more rapid and easier to perform than the original IDEA assay. With the IDEA assay, the feed sample is reacted with a variety of proteolytic digestive enzymes that are immobilized to glass beads. After digestion of the sample with the enzymes, the hydrolysis of the peptide bonds is quantified by reaction with o-phthaldialdehyde (OPA). The IDEA value is then calculated based on the reaction of the sample with OPA before and after digestion with the enzymes. The IDEA™ kit assays contain digester tubes with specific concentrations of enzymes that yielded the strongest correlation between the calculated IDEA value of the samples and *in vivo* digestibility measurements. Novus also provides prediction equations with the kits, so that the IDEA value can be used to predict digestibility of individual amino acids in feed protein. The prediction equations were developed based on regression analysis of IDEA values with true digestibility measurements obtained with the precision-fed cecectomized rooster assay. For the SBM kit, the regression coefficients between the IDEA values and true AA digestibility in the roosters ranged from 0.73 to 0.91 (Schasteen et al., 2007). We wanted to determine if the IDEA™ kits could be used to estimate digestibility of amino acids in the RUP fraction of feeds. The IDEA values were obtained using the samples described above and were then correlated to true AA digestibility values obtained in the roosters. There was a strong correlation between the IDEA value and AA digestibility for the SBM ($R^2 = 0.82$ and 0.91 for total AA and Lys digestibility, respectively) and DDGS kits ($R^2 = 0.95$ and 0.94 for total AA and Lys digestibility, respectively; Boucher et al., 2007b). However, the IDEA™ kit assay did not seem to be a good predictor of RUP-AA digestibility in fishmeal ($R^2 = 0.47$ and 0.53 for total AA and Lys digestibility, respectively). Our data set is limited, but the IDEA™ assay may be a useful procedure for estimating digestibility of RUP-AA in certain feedstuffs once a bigger database is established.

Even with the promising procedure of Gargallo et al. (2006) and the IDEA™ kits, estimating Lys digestibility is still of concern. As mentioned previously, compounds can form with Lys during the Maillard reaction that may be digested by proteolytic enzymes, but are not available for absorption and utilization by animals. With other AA, obtaining estimates of digestion with proteolytic enzymes is likely sufficient since most other AA are generally present in forms that the animal can absorb. However, because of the unique aspects of Lys, it appears that to accurately estimate digestion and absorption of Lys *in vitro*, the amount of Lys that is available for absorption and utilization also needs to be quantified. The guanidination assay has been used in swine nutrition to estimate the availability of Lys in feed proteins (Moughan and Rutherford, 1996). In the guanidination procedure, homoarginine forms by the reaction of O-methylisourea with available Lys (Lys in which the ϵ -amino group is not bound to another compound). In our lab, using the same samples as above, we analyzed the residues using the guanidination assay (Boucher et al., 2007a). The amount of Lys that was converted to homoarginine was strongly correlated to true Lys digestibility ($R^2 = 0.90$), and the percent Lys converted to homoarginine

was on average a more accurate predictor of true Lys digestibility than the method of Gargallo et al. (2006). Homoarginine analysis perhaps gives a more accurate description of the Lys that will not only be digested, but also absorbed in a form that the animal can utilize. The information that can be obtained from the guanidination procedure is useful, but the procedure takes 4 days to complete, which is a drawback for widespread use of the procedure by commercial laboratories.

Results of the procedures evaluated in our lab and by others are promising for the development of an in vitro procedure that can be utilized for routine analysis of feeds for RUP and RUP-AA digestibility. However, in order to make advancements in more accurately meeting the MP requirements of lactating cows, more research in this area is needed. Some key points regarding analytical procedures for estimating RUP digestibility that will need to be considered are: the procedure should be done completely in vitro, the procedure should estimate digestibility of individual AA, and the procedure should accurately estimate the availability of Lys. As far as we know, there is not a single valid, accepted procedure that meets all of these criteria. Development of such a procedure, or combination of procedures, will be critical for advancement of nutritional models.

TAKE-HOME MESSAGE

The dairy cow has two sets of N requirements: the N requirements of rumen fermentation and the AA requirements of the cow. Considerable progress has been made in meeting these requirements with more accuracy. Experience has shown that consistent use of a nutritional model such as NRC (2001) or CPM-Dairy, while understanding their strengths and weaknesses, and learning to use them more as a guide than for their absolute recommendations, allows for more precise formulation of diets for RDP, RUP and AA. The adoption of the concept of balancing diets for AA continues to increase. Balancing diets to meet the targeted levels of 6.6-6.8% Lys and 2.2% Met in MP is the first step to balancing diets for AA. 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 are less frequent and are more commonly observed in early lactation cows. Although the increase in the rate of implementation of AA balancing has been primarily in geographical areas where milk protein is well remunerated within the milk pricing scheme, closer analysis of the biology behind AA balancing encourages the use, irrespective of whether there is a premium for milk protein or not.

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