

AMINO ACIDS AND THEIR APPLICATION IN FORMULATING DIETS FOR CATTLE

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Introduction

There is growing interest to balance cattle rations for absorbable amino acids (AA). This interest is out of the realization: 1) that content and yield of milk protein is influenced by intestinal AA balance, 2) that efficiency of use of ruminally undegraded dietary protein (RUP) for growth and milk protein production is influenced by AA composition, and 3) that the balance of AA in RUP generally is not adequate to maximize efficiency of use of total digestible protein for protein synthesis. These observations are of more interest than in the past because of the increasing attention that must be given to ration formulation to support higher levels of production, the growing emphasis on lean tissue growth and milk protein production, and the increasing desire to minimize waste of dietary protein in relation to protein production.

The purpose of this paper is to review the importance of intestinal AA balance, the nutritive value of sources of absorbable AA, production responses of cattle to improvements in intestinal AA balance, progress towards establishing AA requirements, and guidelines for ration formulation to improve intestinal AA balance.

The Importance of Intestinal Amino Acid Balance

Ruminants, like poultry and swine, have metabolic requirements for AA rather than protein per se. Amino acids are provided by ruminally synthesized microbial protein, RUP, and endogenous secretions. Proteins from these sources must be digested in the small intestine to release the AA for absorption. Protein digestion starts in the abomasum with acid-pepsin digestion and is completed in the small intestine with pancreatic and intestinal proteases.

Amino acids absorbed from the small intestine are the building blocks for the synthesis of tissue and milk proteins. Amino acids are joined together according to a predetermined genetic code during *de novo* synthesis of proteins; i.e., the AA composition of a protein is the same every time it is synthesized. Of the approximately twenty AA found in animal tissue and milk proteins, 9-10 are considered to be "essential". Essential AA (EAA), unlike "nonessential" AA (NEAA), cannot be synthesized by animal tissues. Moreover, each EAA is required in different amounts. Because of these characteristics, EAA are required in proportion to one another. When they are absorbed in the correct balance (i.e., all are equally limiting), their efficiency of use for protein synthesis is maximized and urinary output of urea per unit of lean tissue gain or per unit of milk protein produced is reduced. In contrast, their efficiency of use for protein synthesis is less than

maximum when they are absorbed in a balance that is less than ideal. In this case, it will be the quantity of the first limiting AA (the EAA in shortest supply relative to requirements) that will determine the extent of protein synthesis and thus the rate of lean tissue gain or the amount of milk protein produced.

In some cases, improving intestinal AA balance provides an opportunity to achieve levels of animal productivity not otherwise possible. In other cases, it provides the opportunity to reduce the amount of RUP that must be fed to achieve a given level of growth or milk protein production. Reducing ration RUP has the advantage of creating more "room" in the diet to meet other critical needs of ruminal fermentation or of the host animal. Indeed, that in itself may increase milk yield, milk protein content, and feed intake.

Unlike the EAA, individual NEAA do not have to be absorbed in specific amounts; they can be synthesized from one another or, if the total amount of NEAA absorbed is less than the requirement, from one or more of the EAA. In short, the nutritive value of absorbed AA is determined by the balance of EAA and the contribution of total EAA to total AA.

Sources of Absorbable Amino Acids and Their Nutritive Value

Ruminally synthesized microbial protein supplies 50% or more of the absorbable AA when rations are balanced properly. Microbial protein is the cellular protein of the bacteria, protozoa, and fungi that multiply in the rumen and pass along with unfermented feed to the small intestine. Over 200 species of bacteria, more than 20 species of protozoa, and at least 12 species of fungi have been isolated from ruminal contents. Bacteria provide the majority of the total microbial protein leaving the rumen of high producing ruminants.

Microbial protein is considered to be a consistently high quality source of absorbable AA. It has an apparent intestinal digestibility of about 85%, an EAA pattern that is similar to that of lean body tissue and milk (Table 1), and an EAA pattern that is assumed to be fairly constant and not influenced markedly by changes in diet. Although similar in EAA composition to lean body tissue and milk, ruminally synthesized microbial protein does not appear to possess an ideal or perfect EAA balance. For example, methionine (Met), lysine (Lys), and threonine (Thr) have been identified as first, second, and third limiting, respectively, both for growing sheep (Nimrick *et al.*, 1970; Storm and Orskov, 1984) and cattle (Richardson and Hatfield, 1978) when semi-purified diets were fed and microbial and endogenous proteins were the only sources of absorbable AA.

The assumption that the EAA pattern of microbial protein is fairly constant is based on three observations: (1) a multitude of different microorganisms inhabit the rumen; (2) the variation in EAA profiles between major groups of microorganisms, as well as among the predominant strains within each group, are small to moderate; and (3) protozoa are retained selectively in the rumen and do not contribute to postruminal protein supply in proportion to their contribution to the total microbial biomass in the rumen. Therefore, it is not surprising that the EAA profiles of protein in duodenal digesta were unaffected when the distribution of major morphological groups of ruminal microorganisms in early lactation dairy cows were altered by

feeding a yeast culture (Putnam, 1994) or when sheep were defaunated (Merchen and Titgemeyer, 1992).

In contrast to ruminally synthesized microbial protein, there are large apparent differences in the nutritive value of RUP from different protein supplements. First, there are differences in intestinal digestibility, both among and within feedstuffs (Table 2). A summary of estimates obtained by using the mobile bag technique (Schwab, 1995a) and estimates obtained using a recently developed *in vitro* approach (Stern *et al.*, 1994) indicate that the RUP-digestibilities of most feed proteins are similar (80 to 90%); however, there are exceptions. Digestibility is often the lowest and generally the most variable for meat and bone meal, batch-dried blood meal, and hydrolyzed feather meal (Table 2). These same animal proteins also exhibited large variations in the amount of RUP that they contained (40 to 88%, 78 to 98%, and 50 to 88%, respectively) (Stern *et al.*, 1994). Because of these two potential sources of variation, a large difference may exist between the amount of "digestible" RUP that one assumes a protein supplement is providing and what actually is being provided.

Feed proteins also vary greatly in EAA balance (Table 1). Fortunately, from the standpoint of formulating diets for a specific pattern of absorbable AA, there seems to be little difference between the EAA composition of a feed protein and the EAA composition of the RUP fraction of the same feed. This tentative conclusion is based on limited research using the Dacron bag technique and correcting the AA composition of feed residues for bacterial contamination (Bozak *et al.*, 1986; Crooker *et al.*, 1986; Crooker and Fahey, 1987; Schwab *et al.*, 1986; Schwab *et al.*, unpublished). Although it is expected that the EAA profile of the digestible RUP fraction may be different from the EAA profile of the intact feed protein, the author agrees with Rulquin and Vèrité (1993) that the difference for most feeds appears to be small in comparison with the difference that probably exists between the estimated and actual content of digestible RUP.

Limiting Amino Acids

Direct evidence as provided by abomasal or duodenal infusion studies, or by feeding high quality supplements of rumen-protected Met (RPMet) or rumen-protected lysine (RPLys), indicates that Lys and Met are generally the two most limiting AA for lactating dairy cows and growing cattle. This should be expected because: 1) Met and Lys are first and second limiting in ruminally synthesized microbial protein for growing cattle (Richardson and Hatfield, 1978); 2) Lys and Met are the first two limiting AA for lactating dairy cows fed conventional forages and energy feeds but without protein supplements (Schwab *et al.*, 1976); 3) most feed proteins have lower amounts of Lys and Met than ruminally synthesized bacterial protein (Table 1); 4) the contribution of Lys to total EAA in RUP often is slightly lower than in the same feeds before exposure to ruminal fermentation (Bozak *et al.*, 1986; Crooker *et al.*, 1986; Crooker and Fahey, 1987; Schwab *et al.*, 1986; Schwab *et al.*, unpublished); and 5) Lys and cysteine, the latter of which can be synthesized in the body from Met, are more susceptible to heat processing and may have lower intestinal digestibilities than other EAA in RUP. There is no definitive evidence that NEAA become limiting before any of the EAA, particularly before Lys or Met, when ruminants are fed conventional diets (Rulquin *et al.*, 1995).

Production Responses of Lactating Dairy Cows to Improved Lysine and Methionine Nutrition

Production responses include variable increases in content and yield of milk protein, milk production, and feed intake. As summarized by Rulquin and Vèritè (1993), Rulquin *et al.* (1995), and Schwab (1995b), the experiments that have been conducted confirm the expected. First, the sequence of Lys and Met limitation is determined by their relative concentrations in RUP. For example, Lys is first limiting when corn and corn by-product feeds provide all or most of the RUP, whereas Met is first-limiting when smaller amounts of corn are fed or when most of the RUP is provided by oilseed or animal-derived proteins. Second, content of milk protein is more responsive than milk yield to supplemental Lys and Met, particularly in post-peak lactation cows. In regard to milk protein content, it is noteworthy that responses occur within 36 to 48 h, that responses remain similar or become greater after peak lactation, that responses are independent of level of milk yield or the genetic potential for milk protein content as reflected by breed differences, and that casein is the milk protein fraction that is most affected and not the whey or NPN fractions. Third, milk protein responses generally are greater when Lys and Met are supplied together rather than when either AA is supplied alone. Fourth, milk protein responses to Lys plus Met are greater when levels of either or both in RUP are low rather than high and often greater when intake of CP is high rather than lower. Greater responses to limiting AA with higher intakes of CP probably occur because with increasing levels of dietary CP (particularly RUP), AA passage to the small intestine is increased and up to a point, any "proportional deficiency" of an AA becomes a larger "quantitative deficiency". This phenomenon will occur with increasing levels of ration CP until total AA passage is sufficiently high such that the quantitative deficiency becomes less. Fifth, increasing duodenal concentrations of Lys and Met increases the content of milk protein more than would be expected by increasing ration CP. And sixth, milk yield responses to Lys and Met are limited generally to cows in early lactation when the need for absorbable AA, relative to absorbable energy, is the highest.

In most of the studies referred to above, a Latin square was used as the experimental design and in none of the experiments did cows receive supplemental AA before or at time of calving. Three experiments were reported recently in which cows were assigned to AA treatments prior to or at calving and in which they remained on their initial AA treatments for the duration of the experiment. Robert *et al.* (1994) evaluated the effects of feeding 15.0 g/d of Smartamine™ M (Rhône-Poulenc Animal Nutrition, Atlanta, GA), which supplied 10.5 g of Met, from 2 wk before calving to 12 wk post-calving. The ration was ad libitum corn silage, 1 kg/d of hay, and soybean meal, formaldehyde-treated soybean meal, and a production concentrate containing 12.7% each of the two soybean meals according to milk production. Methionine supplementation: 1) had no effect on DM intake, 2) tended to increase milk yield during the first 6 wk of lactation (32.5 vs. 31.5 kg/d) with the difference being more evident for multiparous cows (38.8 vs. 37.0 kg/d), and 3) increased milk concentrations of both total protein and casein; respective increases were greater during the first 6 wk of lactation (+ .14 and + .15 % units) than the second 6 wk (+ .10 and + .12 % units).

Socha *et al.* (1994c) fed RPMet and RPMet plus Lys from 2 wk before expected calving through the first 15 wk of lactation. Cows received the same basal diet prior to calving either with: (1) no AA; (2) 15 g/d of Smartamine™ M, which supplied 10.5 g of Met; or (3) 6 g/d of Smartamine™ M plus 40 g/d of Smartamine™ ML, which together supplied 10.2 g of Met and 16.0 g of Lys. The prepartum basal diet contained (% of DM): 31.1 corn silage, 16.7 haycrop silage, 7.2 alfalfa hay, 32.0 corn meal, 6.9 solvent-extracted soybean meal, 2.8 raw soybeans, and .7 blood meal. At parturition, cows continued to receive the assigned AA treatments but were switched to one of two diets consisting of (% of DM): 22.3 corn silage, 12.6 haycrop silage, 9.7 alfalfa hay, 6.1 raw soybeans, 1.4 blood meal, and either 37.1 corn meal and 5.3 expeller soybean meal (16 % CP diet), or 31.8 corn meal and 11.5 solvent-extracted soybean meal (18.5% CP diet). There were no significant ($P > .05$) interactions between ration CP and AA treatments for intake and production traits. There were several noteworthy observations. First, DM intake tended to be higher for cows receiving RPLys plus Met as compared to cows receiving the other two treatments. Second, milk yield tended to be higher with RPLys and Met, particularly during peak production (Table 3). Third, milk true protein concentrations were elevated slightly, particularly when RPLys and Met were added to the 18.5% CP ration (2.93 vs. 2.83 %). And last, RPLys plus Met increased yields of milk CP (1399 vs. 1302 and 1300 g/d) and true protein (1311 vs. 1218 and 1218 g/d) (Table 3) over basal and RPMet treatments.

Wu *et al.* (1995) evaluated the effect of increasing Met from 4.3 to 5.0 % and Lys from 14.4 to 15.0 % of estimated absorbable EAA (using the Cornell Net Carbohydrate and Protein Model) on lactational performance of multiparous Holstein cows from 5 to 75 DIM. The supplemental Lys (15.2 g/d) and Met (10.6 g/d) were provided by a combination of Smartamine™ ML (38 g/d) and Smartamine™ M (7 g/d). Amino acid supplementation: (1) tended to increase milk yield (41.8 vs. 40.1 kg/d), (2) increased milk protein content (2.92 vs. 2.83) and milk protein yield (1210 vs 1125 g/d), and (3) tended to increase DMI (23.8 vs. 23.1 kg/d).

More experiments like these need to be conducted. It is becoming increasingly clear that production studies designed to determine the value of improving intestinal AA balance must be initiated at or before parturition. Only in this way can the full effects on herd health and lactational performance be realized.

Responses of Growing Cattle to Improved Lysine and Methionine Nutrition

There are considerably less studies than for lactating dairy cows. However they confirm the expected. First, Met is first limiting when small amounts of RUP are consumed and ruminally synthesized microbial protein supplies nearly all of the absorbed AA. Titgemeyer and Merchen (1990) observed a 17% increase in nitrogen retention with abomasally infused Met when 680-lb steers gaining .9 kg/day were fed a semi-purified diet based on ammoniated corn cobs, corn starch, molasses, and urea; a small amount of casein was included in the diet to provide ruminal microorganisms with a supply of AA and peptides. Oklahoma workers (Lubsy, 1993) observed a 9% increase in weight gains of lightweight calves grazing native pasture when the diet was supplemented with 5 g/day of Smartamine™

M. Second, the sequence of Lys and Met limitation is determined by their relative concentrations in RUP. For example, when rations contained large amounts of corn with most of the supplemental nitrogen provided by urea, Lys clearly was first-limiting (Burris *et al.*, 1976; Hill *et al.*, 1980). In contrast, Met was first-limiting when steers were fed a diet of sorghum silage, corn cobs, and urea, and meat and bone meal provided the supplemental RUP (Klemesrud and Klopfenstein, 1994). And last, weight gain responses to improved AA nutrition are the greatest when feeding and husbandry practices are followed that support high rates of growth. For example, feeding 10 g/day of Smartamine™ ML increased weight gains 8.5% when 150-kg calves were fed a growing ration of (% of DM) 43 prairie hay, 35 corn, 14 alfalfa pellets, and 6 soybean meal and weight gains averaged 1.6 lb/day (Brazle and Stokka, 1994). In contrast, feeding 10 g/day of Smartamine™ ML to 157-kg Holstein steers increased weight gains 19.3% when weight gains averaged 3.5 lb/day (Van Amburgh *et al.*, 1993); steers were fed a diet of (% of DM) 76 whole dry corn grain, 15 corn silage, and 9 solvent extracted soybean meal.

Amino Acid Requirements of Lactating Dairy Cows

Three approaches have been used to estimate the EAA requirements of lactating dairy cows; "factorial" (mathematical), "direct-dose response", and "indirect dose-response". Requirements for AA can be expressed either in daily amounts (g/d) or on the basis of profiles or patterns. The author prefers the latter because: (1) they can be determined more accurately, (2) it is easier to formulate a diet for a desired pattern of absorbable AA than a given quantity of an AA, (3) the field nutritionist is in a better position than the researcher to fine-tune on-farm diets for amounts of RUP and rumen degradable protein, and (4) the approach is consistent with the concept of "ideal protein" as proposed and used in poultry and swine nutrition.

The factorial approach. Scientists from several countries have proposed mathematical models to quantify AA requirements of lactating cows (Evans and Patterson, 1985; Mantysaari *et al.*, 1989; O'Connor *et al.*, 1993; Oldham, 1980; Rohr and Lebzein, 1991). The Cornell Net Carbohydrate and Protein System (CNCPS) for evaluating cattle diets and associated AA submodel is the most dynamic of the factorial models described to date (O'Connor *et al.*, 1993). The EAA requirements of Holstein cows for three levels of milk production as determined by using the CNCPS are presented in Table 4. The requirements are expressed on the basis of both daily amounts (g/d) and as profiles (each EAA as a % of total EAA). Of particular interest is the lack of influence that level of milk production has on the "predicted" proportional requirements of most EAA, including Lys and Met; estimates of the latter are 16.3 and 5.2% of total EAA, respectively. Requirements as determined by a factorial approach should be confirmed in production experiments using the dose-response approach.

The direct dose-response approach. Use of this approach to determine AA requirements of lactating cows is extremely limited and currently restricted to Lys and Met. For such studies, postruminal supplies of Lys or Met are increased in graded fashion via abomasal or duodenal infusion while production responses and AA flows to the small intestine are measured. Rulquin *et al.* (1990) conducted two experiments and Schwab *et al.* (1992) conducted four experiments to determine the required contribution of Lys to total EAA in duodenal digesta for maximum

synthesis of milk protein. In all six experiments, duodenally cannulated Holstein cows were infused with graded levels of Lys; a constant amount of Met also was infused to ensure that Met was not limiting. In a similar fashion, Rulquin *et al.* (unpublished) conducted one experiment and Socha *et al.*, (1994a,b,c) conducted three experiments to determine the Met requirement.

An overall summary of the experiments is shown in Table 5. The six estimates for the required content of Lys in total EAA flowing to the small intestine average 14.7%. Although the results of the six Lys experiments are similar, it is emphasized that only one experiment was conducted with cows during the first 14 wk of lactation; in that experiment, it was concluded that Lys needed to constitute 15.2% of total EAA in duodenal digesta. In contrast to the Lys experiments in which milk protein responses plateaued and a requirement could be determined, this was not the case for most of the Met experiments. The infusion of incremental amounts of Met caused linear increases of milk protein content in three experiments (Rulquin *et al.*, unpublished; Socha *et al.*, 1994b,c) with a quadratic response observed in one experiment (Socha *et al.*, 1994a); linear increases of protein yield occurred for two of the four experiments (Rulquin *et al.*, unpublished; Socha *et al.*, 1994a). This approach to determining requirements indicates that **Lys should contribute about 15.0% of total EAA in duodenal digesta for maximum content and yield of milk protein and Met should contribute about 5.3% of total EAA** when, and only when, levels of Lys approximate 15.0% of total EAA. These values (i.e., requirements) are higher than the measured values for duodenal Lys and Met when early-lactation cows are fed conventional diets (Table 6).

The indirect dose-response approach. This approach involves 3 steps: (1) calculating levels of Lys and Met (% of total AA or % of total EAA) in duodenal digesta for control and treatment groups in experiments in which postruminal supplies of Lys, Met, or both were increased (either by intestinal infusion or by feeding in ruminally protected form) and production responses were measured, (2) calculating (by extrapolation) "reference production values" in each experiment for fixed levels of Lys and Met in duodenal digesta that are intermediate between the low and high levels as calculated for most of the experiments, and (3) calculating production responses (plus and minus values) for control and treatment groups relative to the "reference production values".

This approach has been used by Rulquin *et al.* (1993) and Socha and Schwab (1994). Rulquin *et al.* (1993) estimated duodenal concentrations of digestible Lys (LysDI) and Met (MetDI), each expressed as a percentage of total digestible protein (PDI) using the newly revised French PDI system; PDI is assumed to represent the sum of the 18 standard AA. Socha and Schwab (1994) estimated duodenal concentrations of Lys and Met by using the regression equations presented in Figure 1. The dose-response curves resulting from these efforts for milk protein content are presented in Figures 2 and 3. There are four noteworthy observations. First, there is a better relationship between milk protein content responses and duodenal levels of Lys than with duodenal levels of Met. Second, when intestinal levels of Lys were low (< 6.5 LysDI or <14.0% of total EAA), increasing intestinal levels of Met **decreased** content of milk protein. And third, a comparison of the **apparent requirements for intestinal Lys (15.0-16.0% of EAA) and Met (5.0-5.5% of EAA)** (Figure 3) with the contributions of Lys and Met to total EAA in feeds (Table 1) and with the calculated levels of Lys and Met in duodenal digesta of high-

producing, early lactation cows (Table 7) indicates the difficulty of meeting simultaneously the required contributions of both Lys and Met for maximum content of milk protein.

Methods to Balance Rations for Amino Acids

Several computer models have been developed which predict AA passage to the small intestine of cattle. Some provide print-outs of delivery of individual absorbable AA, and of their requirements, to the nearest .01 gram. Clearly, this implies a level of accuracy that simply does not exist. While some models are better than others, most, as expected, appear to predict the balance of AA in duodenal digesta more accurately than absolute flows of individual AA to the duodenum. The precision by which computer models predict passage of absorbable AA to the small intestine will improve as more research data becomes available.

Of greater concern is our limited knowledge of AA requirements (i.e., ideal balance of absorbable AA). Progress has been made for Lys and Met for lactating dairy cows but similar efforts are needed for growing cattle. Moreover, further refinement of their requirements probably should await estimates of requirements of other potentially limiting AA. Finally, it is important to remember that the requirement for an AA is not derived from a factorial approach but from the relationship between intestinal concentration and the productive response of the animal. In short, a calculated requirement should be confirmed in production experiments using the dose-response approach.

Guidelines for Ration Formulation

Clearly, more research is needed and ration formulation programs must become more sophisticated before cattle rations can be balanced for AA with the precision possible for poultry and swine. Nevertheless, sufficient progress has been made to improve intestinal AA balance in a predictable fashion and allow for improved conversion of diet crude protein to tissue growth and milk production. However, it should be noted that because the typical production response to graded levels of AA is one of diminishing returns, the "practical" requirements to which one formulates will be governed by economic considerations.

Using conventional feed proteins. There are four ways to improve the balance of Lys and Met in absorbable protein. **First**, follow feeding recommendations to maximize ruminal fermentation and thus, synthesis of microbial protein. Microbial protein has an apparent excellent pattern of AA for cattle. Feeding for maximal ruminal fermentation not only increases feed intake and production but it allows for greater use of rumen-degradable feed protein, thereby reducing the need for RUP. Increasing absorbable AA from microbial protein and decreasing the need for AA from RUP are both "win-win" changes for improving intestinal AA balance. The reader is referred to the recent review by Erdman (1995) for factors that affect flow of microbial protein from the rumen.

Second, consider differences in intestinal digestibility of RUP sources. Undigested RUP

simply occupies diet space that could be filled with feedstuffs of nutritional value. For example, consider a 19.0% CP diet in which 40% of the CP is RUP and RUP digestibility is 72%. The RDP content of this diet is 11.4 % of DM (19.0×0.60), RUP is 7.6% (19.0×0.40) and the digestible RUP content is 5.5% (7.6×0.72). However, by careful selection of RUP supplements, lets assume that diet RUP digestibility is 84% rather than 72%. This change in diet RUP digestibility lowers RUP from 7.6% to 6.5% of diet DM ($5.5\% \text{ digestible RUP} \div 0.84$). Assuming the types and amounts of rumen fermentable carbohydrate remain similar, then the RDP content of the diet should remain at 11.4% of DM. The "improved" diet contains 11.4% RDP, 6.5% RUP (instead of 7.6%), 17.9% CP (instead of 19.0%) and RUP is 36% of CP (instead of 40%). This example serves to remind us that the correct amounts of RDP and RUP in a diet are, at least in large part, a function of unrelated factors, and that there is little basis for expressing RUP as a percentage of CP.

Third, do not over-feed RUP. This practice not only increases feed cost but it may decrease synthesis of microbial protein if it replaces needed RDP or fermentable carbohydrate. Moreover, as indicated by the negative coefficients for RUP in the two equations presented in Figure 1, feeding more RUP would almost always decrease the content of Lys, Met, or both in absorbable protein.

DIP needed at ~10.5-11.0% of DM

And **fourth**, manipulate the proportions of supplemental protein in the diet to achieve a predicted Lys/Met ratio that approximates 2.8-3.0/1.0 in absorbable protein. Field nutritionists in the Northeast are reporting improvements in milk protein, milk yield, or both by using this approach (C.J. Canale, personal communication). Achieving the correct balance between the first two limiting AA is the first step in balancing for AA. Selecting "bypass" protein supplements to achieve the "required" level of one of the two AA, but not the other, is of no benefit and in the case of Met, could be counter-productive by decreasing milk yield and milk protein content. These four approaches have the advantage of generally decreasing or not changing the cost per pound of feed.

Using rumen-protected AA. After nearly three decades of research, an option that is becoming available to increase Lys and Met in absorbable protein is the use of RPLys and RPMet supplements. These concentrated sources of Lys and Met have the advantage of allowing nutritionists to extend the use of low Lys and Met feeds and to raise intestinal levels higher than what could be accomplished with conventional feedstuffs. The latter advantage is particularly important for early lactation cows that usually are fed higher levels of RUP.

Several factors have to be considered before RPLys and RPMet supplements are fed. These include: 1) predicted contributions of Lys and Met to other AA in duodenal digesta, 2) level of management, 3) price received for milk protein, 4) cost of RUP-supplements, and 5) efficacy and cost of RPLys and Met supplements. As with many new technologies, evidence suggests that the best managed herds will benefit the most. Moreover, it will be with these herds that improvement in production will be most easily measured. These products should not be used unless diets have been evaluated appropriately and production responses can be predicted. Moreover, like "bypass protein" supplements, RPAA supplements are not created equal. They differ in bioavailability; i.e, ruminal stability and intestinal release (Schwab, 1995a). They also

differ in structural integrity and thus in their ability to withstand mixing and handling. Their cost, relative to anticipated benefits, will be the deciding factor determining the extent of their use.

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Table 1. A comparison of the EAA profiles of body tissue and milk with that of ruminal bacteria and protozoa and common feeds.

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
	----- (% of total EAA) -----									
Animal products										
Lean tissue ¹	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1
Milk ²	7.2	5.5	11.4	19.5	16.0	5.5	10.0	8.9	3.0	13.0
Rumen microbes										
Bacteria ³	10.2	4.0	11.5	16.3	15.8	5.2	10.2	11.7	2.7	12.5
Bacteria ⁴	10.6	4.3	11.6	15.5	17.3	4.9	10.0	11.0	2.6	12.2
Protozoa ⁵	9.3	3.6	12.7	15.8	20.6	4.2	10.7	10.5	2.8	9.7
Forages ⁶										
Alfalfa	10.9	5.2	10.9	18.4	11.1	3.8	12.2	10.6	3.4	13.5
Corn silage	6.4	5.5	10.3	27.8	7.5	4.8	12.0	10.1	1.4	14.1
Haycrop silage	8.9	5.3	11.0	18.9	10.3	3.8	13.5	10.3	3.3	14.7
Grains ⁶										
Barley	12.8	5.9	9.6	18.4	9.6	4.5	13.3	9.1	3.1	13.6
Corn, yellow	10.8	7.0	8.2	29.1	7.0	5.0	11.3	8.4	1.7	11.5
Corn gluten feed	12.0	7.9	8.5	24.6	8.2	4.6	10.1	9.6	1.6	12.8
Oats	15.6	5.4	9.5	18.1	10.0	4.3	11.5	9.2	3.2	13.3
Sorghum	9.4	5.8	9.4	30.9	5.6	4.3	12.6	8.0	2.2	11.8
Wheat	15.2	6.6	9.7	18.9	8.0	4.6	12.6	8.3	3.4	12.6
Plant proteins ⁶										
Brewer's grain	8.9	6.4	10.6	17.6	11.4	4.8	10.3	11.4	3.0	15.6
Corn gluten meal	6.9	4.7	9.3	36.4	3.8	5.5	13.8	7.5	1.5	10.7
Corn DDG w/ solubles	7.7	7.2	9.8	26.3	6.2	5.2	11.1	10.3	2.7	13.4
Cottonseed meal	25.4	6.0	7.7	13.9	9.6	3.8	12.2	7.7	2.9	10.8
DDG w/ solubles	19.9	6.5	15.4	18.7	6.5	3.7	15.4	8.9	1.6	14.6
Linseed meal	25.7	5.2	13.3	14.8	8.1	3.5	11.1	8.9	3.5	11.8
Peanut meal	13.5	5.4	9.9	15.2	10.0	2.4	11.5	6.5	2.8	10.6
Rapeseed meal	14.0	6.7	9.3	16.9	13.1	4.8	9.5	10.5	3.0	12.4
Safflower meal	22.3	6.5	8.8	15.1	7.9	3.7	11.4	7.4	4.6	12.3
Soybean meal	16.3	5.7	10.8	17.0	13.7	3.1	11.0	8.6	3.0	10.6
Sunflower meal	19.4	5.9	10.1	15.5	8.6	5.4	11.0	9.1	2.8	12.3
Animal proteins ⁶										
Blood meal	7.6	11.2	2.1	22.8	15.7	2.1	12.3	8.1	2.7	15.4
Feather meal	14.7	1.1	10.0	29.3	3.9	2.1	10.0	10.5	1.5	17.1
Fish meal (menhaden)	13.1	5.7	9.3	16.5	17.0	6.3	8.8	9.5	2.4	11.3
Meat meal	18.8	5.2	8.0	17.1	14.1	3.7	9.7	9.1	2.0	12.4
Meat & bone meal	20.5	5.5	7.8	16.2	14.2	3.6	9.2	9.0	1.8	12.1
Tankage	18.8	5.2	8.0	17.1	14.1	3.7	9.7	9.1	2.0	12.4
Whey, dry	5.6	3.7	12.4	20.1	17.5	4.3	7.4	13.2	3.8	11.9

¹ From Ainslie *et al.* (1993); average values of empty, whole body carcasses as reported in 3 studies.

² Each value is an average of 3 observations from Jacobson *et al.* (1970), McCance and Widdowson (1978), and Waghorn and Baldwin (1984).

³ From Clark *et al.* (1992); average values from 61 dietary treatments.

⁴ From Storm and Orskov (1983); average values from 62 literature reports.

⁵ From Storm and Orskov (1983); average values from 15 literature reports.

⁶ Calculated from values presented in "European Amino Acid Table: first edition 1992" except for DDG w/ solubles, linseed meal, peanut meal, and feather meal that were calculated from values presented in "Feedstuff Ingredient Analysis Table: 1991 edition".

Table 2. Estimates of intestinal digestion of the RUP fraction of various protein supplements.¹

	n	Range	Average
High digestibility			
Soybean meal, expeller	3	98-100	99
Soybean meal, solvent	5	86-93	90
Corn gluten meal	2	86-91	89
Soybean meal, lignosulfonate	6	82-92	88
Medium digestibility			
Blood meal, ring-dried	10	72-90	81
Distiller's grains, dried	5	72-85	81
Fish meal, menhaden	13	73-88	80
Cottonseed meal, mechanical	1	---	80
Brewer's grains, dried	5	73-79	77
Cottonseed meal, solvent	1	---	71
Low digestibility			
Feather meal, hydrolyzed	12	58-75	67
Blood meal, batch-dried	12	29-86	63
Meat and bone meal	11	41-70	55

¹ From Stern *et al.* (1994). Measurements were made by incubating the feedstuffs in the rumen by using the dacron bag technique and then subjecting the residue (which would include the RUP) to a two-step *in vitro* assay that simulates intestinal protein digestion. Studies have indicated that results obtained with this technique are highly correlated with estimates of intestinal digestion obtained in the cow.

Table 3. Effect of feeding rumen-protected methionine (Met) and lysine plus methionine (Lys/Met) from 2 weeks prepartum through 15 week on milk and milk protein production of multiparous Holstein cows^{1,2}

Week of lactation	Milk (kg/d)			True protein (g/d)		
	Basal	Met	Lys/Met	Basal	Met	Lys/Met
1-3	36.7	34.8	38.0	1176	1121	1246
4-6	45.1	44.6	48.2	1220	1241	1342
7-9	46.0	44.4	48.4	1240	1226	1344
10-12	44.8	43.7	46.6	1253	1252	1331
13-15	42.3	42.5	43.4	1208	1240	1283
Average	43.0	42.5	44.9	1218 ^b	1218 ^b	1311 ^a

¹ From Socha *et al.* (1994c).

² Treatments were no rumen-protected amino acids (basal); 15 g/d of Smartamine™ M, which supplied 10.5 g of methionine (Met); or 6 g/d of Smartamine™ M plus 40 g/d of Smartamine™ ML, which supplied 10.2 g of methionine and 16.0g of lysine (Lys/Met) (Rhône-Poulenc Animal Nutrition).

^{a,b} (P < .05).

Table 4. Requirements of Holstein cows for absorbed EAA at three levels of milk production as determined by using the Cornell Net Carbohydrate and Protein System.¹

EAA	60 lb/d		100 lb/d		140 lb/d	
	g/d	(% of EAA)	g/d	(% of EAA)	g/d	(% of EAA)
Arg	67	(10.5)	88	(9.6)	111	(9.1)
His	37	(5.9)	54	(5.8)	70	(5.8)
Ile	76	(11.8)	116	(12.5)	156	(12.8)
Leu	112	(17.5)	162	(17.5)	212	(17.5)
Lys	104	(16.3)	151	(16.3)	198	(16.3)
Met	33	(5.1)	48	(5.2)	63	(5.2)
Phe	58	(9.0)	84	(9.0)	110	(9.1)
Thr	56	(8.8)	80	(8.7)	104	(8.6)
Trp	17	(2.7)	27	(2.9)	36	(3.0)
Val	79	(12.3)	117	(12.6)	154	(12.7)
Total EAA	638		926		1214	

¹ The following animal factors were kept constant: age, 42 mo.; frame size, 5; BW, 1300 lb; flesh condition, 3; days pregnant, 0; DIM, 80; lactation no., 2; butter fat, 3.5%; and milk true protein, 3.0%.

Table 5. Determination of the required contributions (%) of Lys and Met to total EAA¹ in duodenal digesta for milk protein production of lactating dairy cows consuming conventional diets.²

Reference	Lys	Reference	Met
Rulquin <i>et al.</i> , 1990	14.9 14.8	Rulquin (unpublished)	≥ 5.1
Schwab <i>et al.</i> , 1992	15.2 14.0 14.5 14.7	Socha <i>et al.</i> , 1994b Socha <i>et al.</i> , 1994c Socha <i>et al.</i> , 1994a	≥ 5.5 5.3 ?
Average	14.7		≥ 5.3

¹ Includes Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val.

² Involved graded infusions of Lys (in the presence of constant supplemental Met) and Met (in the presence of constant supplemental Lys) into the duodenum of cannulated Holstein cows with simultaneous measurement of milk and milk protein production and AA flows to the small intestine.

Table 6. Effect of ration composition (% of DM, excluding fat, mineral and vitamin supplements) on measured passage of lysine (Lys) and methionine (Met) to the duodenum of Holstein cows during the first 150 d of lactation.

Item	References ¹									
	1	3	2	3	4	4	5	1	5	5
Alfalfa hay				10.0			45.4		13.3	26.0
Alfalfa silage	25.0				30.0	30.0		25.0		
Corn silage	25.0	29.0	26.2	28.7	20.0	20.0		25.0	28.5	5.0
Grass-legume silage		17.0	14.2							
Corn	40.9	23.8	24.7	25.5	40.0	34.0	34.6	36.7	27.1	30.2
Wheat byproducts		8.0	9.4							
Beet pulp										
Soyhulls				12.5			8.3		4.0	12.3
Soybean meal		7.0	9.0	16.1	4.0	11.0			8.5	4.0
Roasted soybeans							6.0		4.8	
Whole cottonseed									9.0	9.0
Distiller's grains		9.0	9.8	4.7			1.8		1.8	
Brewer's grains										
Corn gluten meal	.9									8.0
Blood meal	2.1							1.5		
Feather meal	.3							3.5		
								.6		
Fish meal	1.5				4.0					
Meat meal	.6							2.5		
Animal/fish blend		3.0						1.0		
Urea	.2	.1								1.5
								.3		
Diet CP, % DM	16.2	16.8	17.3	17.3	18.1	18.1	19.0	19.2	19.6	19.7
Flow to duodenum, g/d										
Total EAA	1619	1330	1353	1384	1395	1650	1924	1970	1840	1836
Lys	217	183	179	194	164	235	246	264	249	235
Met	61	56	55	61	52	61	63	73	64	64
Flow to duodenum, % EAA										
Lys	13.4	13.7	13.2	14.0	11.8	14.2	12.9	13.4	13.5	12.8
Met	3.8	4.2	4.1	4.4	3.7	3.7	3.3	3.7	3.5	3.5

¹ (1)Christensen *et al.*, 1993; (2)Schwab *et al.*, 1992b; (3)Cunningham *et al.*, 1993; (4)Klusmeyer *et al.*, 1991; and (5)Cunningham *et al.*, 1991.

Table 7. Calculated contributions of lysine (Lys) and methionine (Met) to total EAA in duodenal digesta during early lactation of some high-producing commercial dairy herds.¹

Item	Herd No.								
	1	2	3	4	5	6	7	8	9
Milk production									
Milk, lb	27028	28814	28887	28259	25881	27378	29110	29098	2592
Milk fat, %	3.72	3.43	3.71	3.58	3.87	3.66	3.37	3.62	7
Milk protein, %	3.06	2.97	3.08	2.99	3.14	3.09	3.09	3.08	3.54
Rations, % of DM									3.03
Alfalfa hay	5.2	-	-	-	7.1	21.6	-	-	3.5
Alfalfa silage	44.0	42.0	32.0	36.5	38.2	17.3	16.5	31.0	31.7
Corn silage	-	10.1	17.8	-	5.5	4.4	13.2	14.4	8.8
Dry shelled corn	-	26.1	-	-	-	32.9	-	-	7.0
High moisture shelled corn	29.6	-	28.6	-	-	-	-	-	25.6
High moisture ear corn	-	-	-	34.9	29.5	-	-	29.2	-
Whole cottonseed	9.0	6.5	-	9.4	-	-	37.4	-	8.8
Soy hulls	-	-	4.1	-	-	-	-	10.0	-
Soybean meal	2.7	-	4.8	6.6	.8	13.9	11.9	2.9	-
Raw soybeans	-	4.0	-	-	9.1	-	-	-	6.1
Roasted soybeans	-	-	6.6	-	-	-	-	-	-
Distiller's dried grains	-	-	-	-	1.0	4.7	11.4	6.8	-
Corn gluten meal	-	1.4	-	-	-	-	-	-	-
Meat and bone meal	4.4	4.8	2.5	3.5	4.7	-	1.6	-	-
Blood meal	1.0	1.2	.9	1.7	1.2	-	3.4	2.6	-
Urea	-	-	-	.2	-	-	.6	.2	4.5
CP, % of DM	19.4	20.0	18.6	19.6	19.0	19.4	22.1	18.8	19.1
RUP, % of CP	36	37	37	37	34	35	38	38	39
Flow to duodenum, % EAA									
Lys	13.6	13.3	13.8	13.6	13.9	12.9	12.0	13.5	13.4
Met	4.2	4.5	4.3	4.4	5.0	4.6	4.6	4.2	4.2
Lys/Met ratio	3.2	3.0	3.2	3.1	2.8	2.8	2.6	3.2	3.2

¹ Calculated using the regression equations shown in Figure 1.

Lysine equation:

$$Y = 14.43 - .04X_1 - .29X_2 + .54X_3 + C \quad (R^2 = .82)$$

Y = Lys in duodenal digesta, % of EAA
X₁ = Ration RUP, % of ration CP
X₂ = Ration CP, % of ration DM
X₃ = Ration RUP-Lys, % of total RUP-EAA
C = Constants for stage of lactation: 1st 100 d, -.13,
2nd 100 d, .80; and >200 d, 0.0

Methionine equation:

$$Y = 5.36 - .08X_1 + 3.94X_2 + C \quad (R^2 = .55)$$

Y = Met in duodenal digesta, % of EAA
X₁ = Ration RUP, % of ration CP
X₂ = Ration RUP-Met, % of ration CP
C = Constants for stage of lactation: 1st 100 d, -.15;
2nd 100 d, .34; and >200 d, 0.0

Figure 1. Equations developed by Socha and Schwab (1994) to predict the contributions of lysine (Lys) and methionine (Met) to total EAA in duodenal digesta of lactating dairy cows. The data base used to develop the Lys equation was 29 studies (78 diets) in which amino acid passage to the small intestine was measured; the Met equation was developed from 28 studies involving 75 observations.

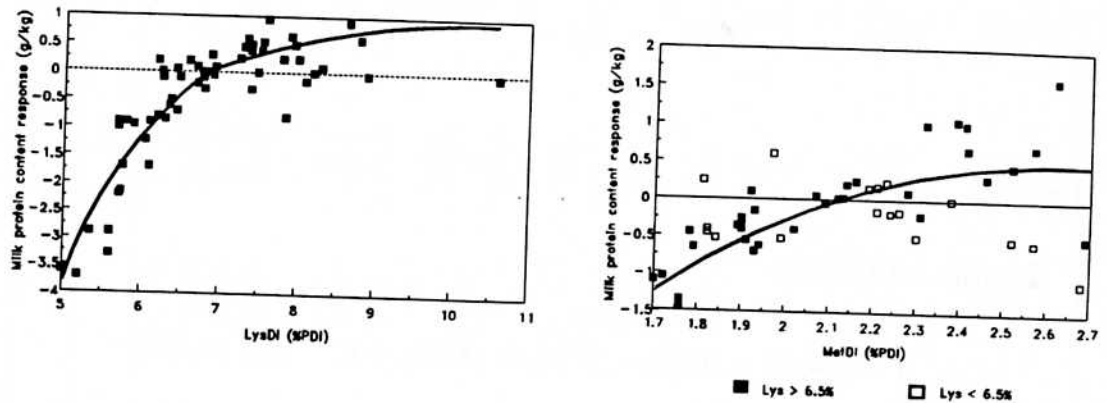


Figure 2. Milk protein content responses as a function of calculated duodenal contributions of digestible lysine (LysDI) and digestible methionine (MetDI) to total digestible protein (PDI). The dose-response line for Met is from studies with calculated duodenal concentrations of LysDI greater than 6.5% of PDI (Rulquin *et al.*, 1993).

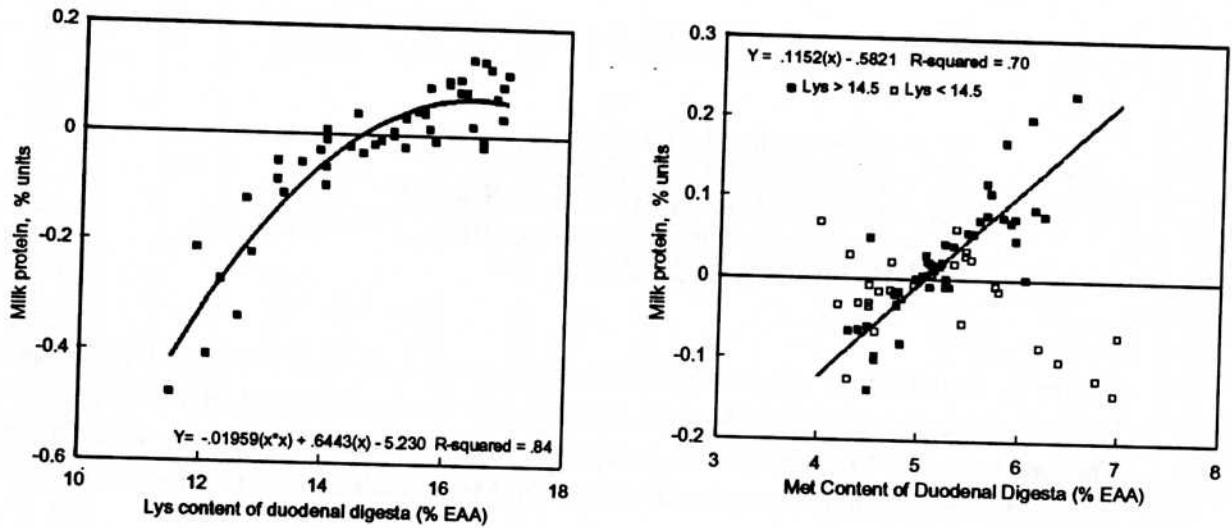


Figure 3. Milk protein content responses as a function of calculated contributions of lysine (Lys) and methionine (Met) to total essential amino acids (EAA) in duodenal digesta. The dose-response line for Met is from studies with calculated duodenal concentrations of Lys greater than 14.5% EAA. Lysine and Met in duodenal digesta were estimated by using linear regression (Socha and Schwab, 1994).