AMINO ACIDS AND THEIR APPLICATION IN FORMULATING DIETS FOR CATTLE

Charles G. Schwab
Department of Animal and Nutritional Sciences
University of New Hampshire

INTRODUCTION

There is growing interest in balancing cattle rations for amino acids (**AA**). This interest originates from the realization that content and yield of milk protein is influenced by profile of absorbed AA, as well as total AA supply, and that efficiency of use of ruminally undegraded feed protein (**RUP**) for growth and milk protein production is influenced by intestinal digestibility and AA composition. 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 crude protein (**CP**) and to maximize its conversion to tissue and milk proteins.

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

THE IMPORTANCE OF INTESTINAL AMINO ACID PROFILES

Cattle, like poultry and swine, have metabolic requirements for AA rather than protein per se. In fact, other than during the first 24 to 36 h of life when colostral immunoglobulins (antibodies) are absorbed, cattle do not absorb intact proteins. Instead, they absorb the individual AA, the building blocks of protein. Of the approximate 20 standard AA found in plant and animal proteins, 9-10 are considered generally to be *essential*. Essential AA (**EAA**), unlike *nonessential* AA (**NEAA**), either cannot be synthesized by animal tissues or if they can, not in amounts sufficient to meet metabolic needs. An animal has a different requirement for each of the EAA. When EAA are absorbed in the correct profile (i.e., all are equally limiting), efficiency of use of AA for protein synthesis is maximized and urinary excretion of endogenously synthesized urea (from *left-over* AA) per unit of protein accretion or milk protein produced is reduced. In contrast, efficiency of use of absorbed AA for protein synthesis is less than maximum when they are absorbed in a profile that is less than ideal. In this case, it is the quantity of the first limiting AA (the EAA in shortest supply relative to requirements) that will determine the extent of protein synthesis and the rate of protein accretion or the amount of milk protein produced.

SOURCES OF ABSORBABLE AMINO ACIDS AND THEIR NUTRITIVE VALUE

Ruminally synthesized microbial protein supplies 50% or more of 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. Bacteria provide the majority of the total microbial protein leaving the rumen of high producing ruminants.

Microbial protein is considered to be a consistent, high quality source of absorbable AA. It has an apparent intestinal digestibility of about 85%, an EAA profile 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 may not possess an ideal or perfect EAA balance. For example, methionine (**Met**), lysine (**Lys**), and threonine have been identified as first, second, and third limiting AA, respectively, for nitrogen retention of growing sheep (Nimrick et al., 1970; Storm and Orskov, 1984) and cattle (Richardson and Hatfield, 1978) when semi-purified diets devoid of RUP were fed and microbial and endogenous proteins were the only sources of absorbed AA.

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

	(% of total EAA)										EAA
Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	(%CP)
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	38.5
Corn, yellow	10.8	7.0	8.2	29.1	7.0	5.0	11.3	8.4	1.7	11.5	42.3
Corn gluten feed	12.0	7.9	8.5	24.6	8.2	4.6	10.1	9.6	1.6	12.8	38.8
Oats	15.6	5.4	9.5	18.1	10.0	4.3	11.5	9.2	3.2	13.3	42.8
Sorghum	9.4	5.8	9.4	30.9	5.6	4.3	12.6	8.0	2.2	11.8	39.8
Wheat	15.2	6.6	9.7	18.9	8.0	4.6	12.6	8.3	3.4	12.6	31.9
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	46.3
Corn gluten meal	6.9	4.7	9.3	36.4	3.8	5.5	13.8	7.5	1.5	10.7	44.2
Corn DDG w/ solubles	7.7	7.2	9.8	26.3	6.2	5.2	11.1	10.3	2.7	13.4	37.7
Cottonseed meal	25.4	6.0	7.7	13.9	9.6	3.8	12.2	7.7	2.9	10.8	43.1
DDG w/ solubles	19.9	6.5	15.4	18.7	6.5	3.7	15.4	8.9	1.6	14.6	43.3
Linseed meal	25.7	5.2	13.3	14.8	8.1	3.5	11.1	8.9	3.5	11.8	41.1
Peanut meal	13.5	5.4	9.9	15.2	10.0	2.4	11.5	6.5	2.8	10.6	36.9
Rapeseed meal	14.0	6.7	9.3	16.9	13.1	4.8	9.5	10.5	3.0	12.4	41.9
Safflower meal	22.3	6.5	8.8	15.1	7.9	3.7	11.4	7.4	4.6	12.3	40.5
Soybean meal	16.3	5.7	10.8	17.0	13.7	3.1	11.0	8.6	3.0	10.6	47.6
Sunflower meal	19.4	5.9	10.1	15.5	8.6	5.4	11.0	9.1	2.8	12.3	45.0
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	49.4
Feather meal	14.7	1.1	10.0	29.3	3.9	2.1	10.0	10.5	1.5	17.1	31.4
Fish meal (menhaden)	13.1	5.7	9.3	16.5	17.0	6.3	8.8	9.5	2.4	11.3	44.8
Meat & bone meal	20.5	5.5	7.8	16.2	14.2	3.6	9.2	9.0	1.8	12.1	38.0
Whey, dry	5.6	3.7	12.4	20.1	17.5	4.3	7.4	13.2	3.8	11.9	50.8

[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 using a 2-step *in vitro* assay.[1]

Protein supplements	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
Distillers grains, dried	5	72-85	81
Fish meal, menhaden	13	73-88	80
Cottonseed meal, mechanical	1	---	80
Brewers 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 RUP) to a two-step *in vitro* assay that simulates intestinal protein digestion.

The second major source of absorbed AA is RUP. All feedstuffs, other than nonprotein nitrogen supplements, contain some RUP. In contrast to ruminally synthesized microbial protein, there are large differences in the quality of RUP from different feeds. There are differences in intestinal digestibility, both among and within feeds. Estimates obtained using the mobile bag technique (Schwab, 1995a) and a recently developed *in vitro* approach (Table 2) indicate that the RUP-digestibilities of most feed proteins are similar (80 to 90%). However, as noted in Table 2, there are exceptions. Estimates of intestinal digestibility were lowest and most variable for meat and bone meal, batch-dried blood meal, and hydrolyzed feather meal. These same animal proteins also varied considerably in the amount of RUP that they contained (Stern et al., 1994). Because of variations in both amounts and digestibility of RUP, a large difference may exist between the amount of RUP that one assumes a protein supplement is providing and what is actually being provided as *digestible protein* (RUP x intestinal digestion coefficient for RUP).

In addition to differences in intestinal digestibility, feed proteins vary greatly in pattern of EAA (Table 1). Because of this, and because most feed proteins also differ from microbial protein in EAA composition (Table 1), most of the variation in profile of EAA leaving the rumen is accounted for by the amount of RUP in the diet, and the EAA composition of diet RUP. 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 RUP fraction of the same feed. This conclusion is based on 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 Vèritè (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 cows and growing cattle. This should not be too surprising given that: 1) Met and Lys are first and second limiting AA in ruminally synthesized microbial protein for growing cattle; 2) most feed proteins have lower amounts of Lys and Met, relative to total EAA, than ruminally synthesized bacterial protein (Table 1); 3) 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 4) Lys and cystine (Cys), the latter of which can be synthesized in the body from Met, probably have lower intestinal digestibilities than other AA in RUP. Regarding the last point, intestinal digestibilities of Lys and Cys usually are lower than other AA for swine and poultry (Parsons, 1994). The digestibility of Lys is particularly low in many cereal byproduct feeds and the digestibility of Cys is low in several animal protein meals.

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). Therefore, the nutritive value of absorbed AA for cattle appears to be determined by the profile of EAA and the contribution of total EAA to total AA.

PRODUCTION RESPONSES OF LACTATING DAIRY COWS TO INCREASED SUPPLIES OF LYSINE AND METHIONINE

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), research has confirmed 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 proteins, animal-derived proteins, or a combination of the two. 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 the first couple of days, 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 basal levels of either or both in RUP are low rather than high and often greater when intake of CP is higher rather than lower. Greater intakes of RUP generally elicit greater responses to Lys and Met because most feed proteins have lower amounts of Lys, Met, or both, relative to total EAA, than ruminally synthesized microbial protein (Table 1). Fifth, increasing duodenal concentrations of Lys and Met increases 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 calving. Three experiments were reported recently in which cows were assigned to AA treatments prior to or at calving and in which cows remained on their initial AA treatments following calving. Robert et al. (1994) evaluated the effects of feeding 15.0 g/d of a RPMet product (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, 2 lb/d of hay, and soybean meal, formaldehyde-treated soybean meal, and a production concentrate containing 12.7% each of the two soybean meals; the latter was fed 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 (71.5 vs. 69.3 lb/d) with the difference being more evident for multiparous cows (85.4 vs. 81.4 lb/d), and 3) increased milk concentrations of both total protein (+.12% units) and casein (+.14% units).

Socha et al. (1994) 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 Smartamine M, which supplied 10.5 g of Met; or (3) 6 g/d of Smartamine M plus 40 g/d of a RPMet + Lys product (Smartamine ML, Rhône-Poulenc Animal Nutrition, Atlanta, GA), 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

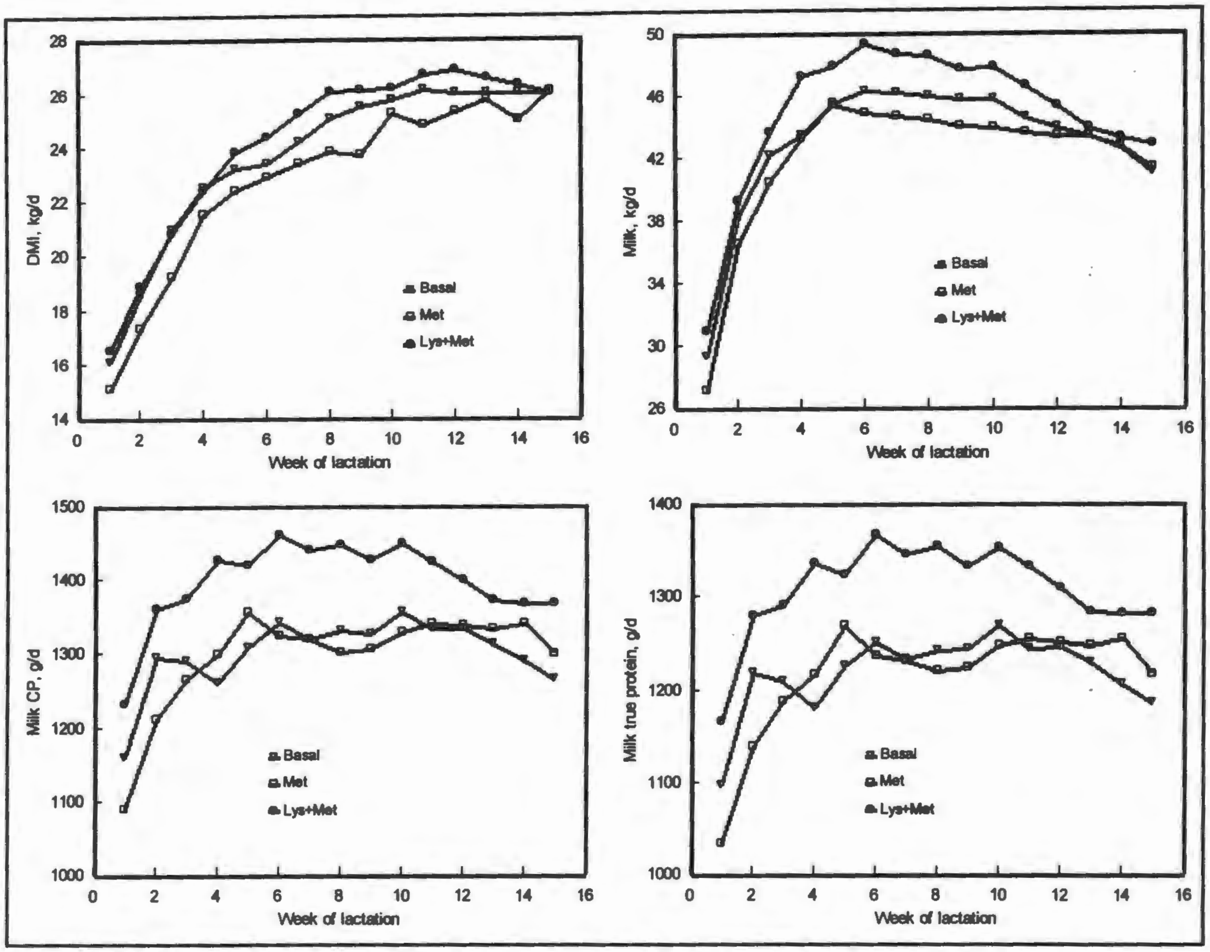


Figure 1. Dry matter intake, and yield of milk, milk CP, and milk true protein by week of lactation for early lactation multiparous Holstein cows fed no rumen-stable AA (▿), 15 g/d of a rumen-stable Met product, which supplied 10.5 g/d Met (□), or 6 g/d of rumen-stable Met product plus 40 g/d of a rumen-stable Lys plus Met product, which together supplied 10.2 g of Met and 16 g of Lys (●) (Socha *et al.*, 1994).

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 (Figure 1). 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. Relative to feeding no RPAA, supplementing the 16.0% CP diet with RPLys and Met increased milk yield 2.0 lb/d for the 15-wk period whereas supplementing the 18.5% CP diet with RPLys and Met increased milk yield 7.0 lb/d. Third, milk protein concentrations were elevated, particularly when RPLys and Met were added to the 18.5% CP ration (3.13 vs. 3.03 %). And last, RPLys plus Met increased yields of milk CP and true protein 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 [CNPCS]) on lactational performance of multiparous Holstein cows for the first 75d of lactation. 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 (92.0 vs. 88.2 lb/d), (2) increased content (2.92 vs. 2.83%) and yield (1210 vs. 1125 g/d) of milk protein, and (3) tended to increase DM intake (52.4 vs. 50.8 lb/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 profiles must be initiated at or before calving. Only in this way can the full effects on herd health and lactational performance be realized.

RESPONSES OF GROWING CATTLE TO INCREASED SUPPLIES OF LYSINE AND METHIONINE

In comparison to lactating dairy cows, considerably fewer studies have been conducted with growing cattle. However, the studies 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 2.0 lb/d 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 (Lusby, 1993) observed a 9% increase in weight gains of lightweight calves grazing native pasture when the diet was supplemented with 5 g/d 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 increased supplies of Lys and Met are greater when cattle have higher vs. lower rates of growth. For example, feeding 10 g/d of Smartamine ML (supplied 1.5 g/d Met and 4.0 g/d Lys) increased weight gains 8.5% when newly arrived 330-lb calves were fed a ration of (% of DM) 43 prairie hay, 35 corn, 14 alfalfa pellets, and 6 soybean meal, and weight gains averaged 1.6 lb/d (Brazle and Stokka, 1994). The calves fed RPLys plus Met had fewer (P<.01) repulls for sickness (16 vs. 34%) during the last 28 d of the 56-d experiment. In contrast, feeding 10 g/d of Smartamine ML to 345-lb Holstein steers increased weight gains 19.3% when weight gains averaged 3.5 lb/d (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. In a similar fashion, feeding 8.0 g/d of Smartamine ML to finishing crossbred steers (533 lb at start of experiment) gaining over 4.5 lb/d increased weight gains 13-14% (Klemesrud et al., 1995); cattle were fed a basal diet of (% of DM) 45 wet corn gluten feed, 22.5 high moisture corn, 20 dry rolled corn, 5 corn silage, 5 alfalfa hay, and 2.5 dry supplement.

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 profile 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 ruminally degraded feed protein **(RDP)**, and (4) the approach is consistent with the concept of *ideal protein*, as used in poultry and swine nutrition. Regarding point # 3, the concept of balancing rations for RDP and RUP is well-established. Recognizing the differences between the RUP fraction of feed proteins in regard to postruminal intestinal digestibility (Stern et al., 1994) and AA composition and formulating diets for a particular profile of absorbable EAA increases considerably one's ability to fine-tune diets for amounts of RUP.

The factorial approach. Scientists from several countries have proposed mathematical models to quantify AA requirements of lactating dairy cows. The 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 3. 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 of level of milk production 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.

As recognized by the authors (O'Connor et al., 1993), the CNCPS probably can be improved in its ability to predict requirements for absorbable AA (and in its ability to predict passage of absorbable

Table 3. 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]

	60 lb/d		100 lb/d		140 lb/d	
EAA	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%.

AA to the small intestine). Continued research and aggressive field evaluation are both important to the eventual refinement of the model. Calculated proportional requirements of Lys, Met, and other potentially limiting EAA as determined by any 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 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 4. 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 for maximum yield of milk protein. 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., 1994a,c) with a quadratic response observed in one experiment (Socha et al., 1994b); linear increases of protein yield occurred for two of the four experiments (Rulquin et al., unpublished; Socha et al., 1994b). The use of this approach to determine 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 in duodenal digesta approximate 15.0% of total EAA. These values are higher than the measured values of 13.7-14.1% for duodenal Lys and 4.1-4.6% for duodenal Met when early-lactation cows are fed conventional diets (Cunningham et al., 1991; 1993; Schwab et al., 1992).

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 simple regression) *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

Lysine equation:

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

Y = **Lys** in duodenal digesta, % of EAA
X_1 = Ration RUP, % of ration CP
X_2 = Ration CP, % of ration DM
X_3 = 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
X_1 = Ration RUP, % of ration CP
X_2 = 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 2. 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.

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 2. The dose-response curves resulting from these efforts for milk protein content are presented in Figures 3 and 4. There are three 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, increasing intestinal levels of Met when Lys levels were low (< 6.5 LysDI or <1450% of total EAA), ***decreased*** content of milk protein in most cases. For this reason, the dose-response relationships for Met that are presented were derived from the portion of the data in which duodenal Lys concentrations were calculated to exceed 6.5% of total AA (Figure 3) or 14.5% of total EAA (Figure 4). Unlike Rulquin et al. (1993), Socha and Schwab (1994) did not obtain the expected dose-response curve of diminishing increments for Met. 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)** with the contributions of Lys and Met to total EAA in feeds (Table 1) indicates the difficulty of meeting simultaneously the required contributions of both Lys and Met for maximum content and yield of milk protein.

A comparison of the approaches. The different approaches for estimating the required contributions of Lys and Met to total EAA in duodenal digesta have provided remarkably similar results. The dose-response approaches are expected to provide somewhat lower estimates than a factorial approach. The extent to which this occurs is a function of the *match* between the required profile of absorbed EAA and the profile as presented to the animal. Unless the match is perfect, EAA other than the most limiting ones will be supplied in excess of need. For example, if it is assumed that the *overage* of non-limiting EAA is only 10%, then the mean requirement of 14.7% for Lys as determined by Rulquin et al. (1990) and Schwab et al. (1992) with typical diets using the direct dose-response approach (Table 4) would be 16.3% under the rare situation in which all absorbed EAA are in perfect balance. Incidentally, this is the requirement for Lys as

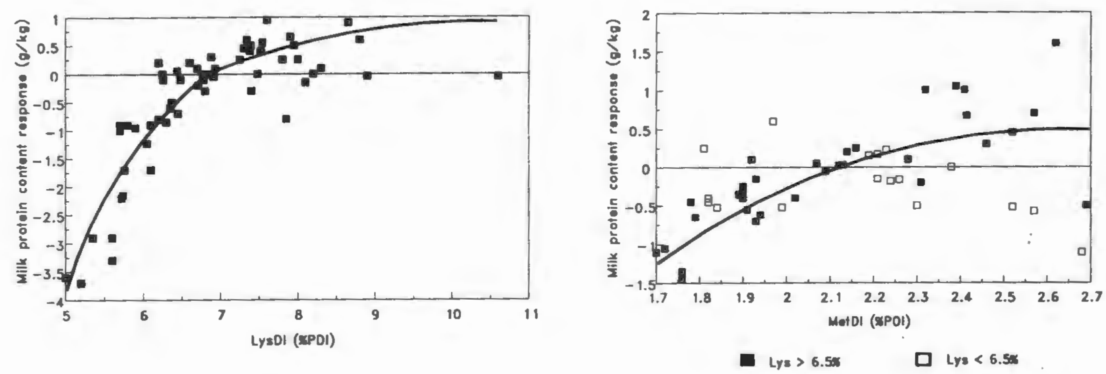
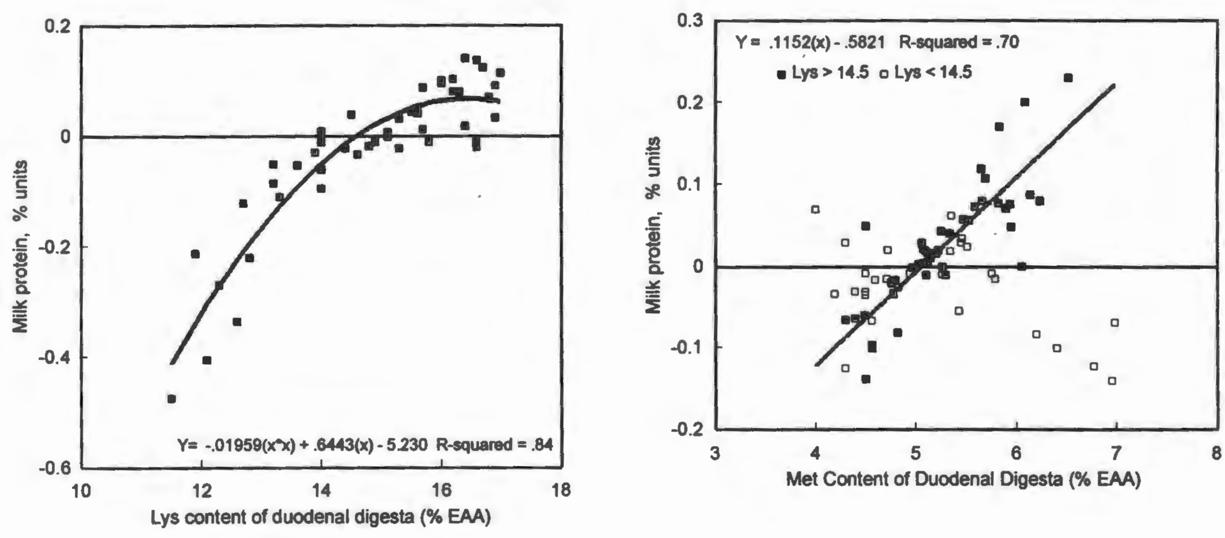


Figure 3. Milk protein content responses as a function of calculated duodenal contributions of digestible lysine (LysDI) and digestible methionine (MetDI) to total digestible protein (PDI). The dose-response line for Met is from studies with calculated duodenal concentrations of LysDI greater than 6.5% of PDI (Rulquin *et al.*,1993).

Figure 4. 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).



estimated by the CNCPS (Table 3). That the requirement for Lys as determined by the indirect dose-response approach (Figure 4) is closer to 16.3% than 14.7% may reflect the manner in which certain calculations were made. In particular, the use of simple regression to calculate the *reference production values* implies a linear relationship, rather than a curvilinear or quadratic relationship, between changes in intestinal concentrations of Lys or Met and milk protein content. The result would be an under-estimation of changes in milk protein to a change in duodenal concentrations of Lys at lower Lys concentrations and an over-estimation of changes in milk protein at higher Lys concentrations, thereby flattening the dose-response curve and moving the point at which the curve plateaus to the right. Nevertheless, until more research is conducted, **it seems reasonable to conclude that the required percentages of Lys and Met in duodenal digesta for maximum content and yield of milk protein approximates 15 and 5.3% or more of total EAA, respectively, when conventional diets are fed.**

METHODS TO BALANCE RATIONS FOR AMINO ACIDS

Several computer models have been developed that 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 does not exist. Whereas some models are better than others, most, as expected, appear to predict the profile 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 profile of absorbable AA). Indeed, many factorial models have been developed that estimate AA requirements. However, calculated requirements should be confirmed in growth or production experiments using the dose-response approach. Progress has been made for Lys and Met for lactating dairy cows but similar efforts are needed for growing cattle. Moreover, further refinement of Lys and Met requirements of lactating dairy cows probably should await knowledge of the dose-response relationships of other potentially limiting AA.

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. However, sufficient progress has been made to improve intestinal AA profiles in a predictable fashion and allow for improved conversion of diet CP to lean tissue growth and milk protein production. Moreover, it should be noted that because the typical production response to graded levels of EAA is one of diminishing returns, the *practical* requirements to which one formulates will be governed by economic considerations. Five general guidelines follow.

Table 4. Determination of the required contributions (%) of Lys and Met to total EAA[1] in duodenal digesta for milk protein production of lactating dairy cows consuming conventional diets.[2]

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

Follow feeding recommendations to maximize ruminal fermentation and 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 RDP, thereby reducing the need for more costly 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 recent reviews (e.g., Clark, 1995 and Erdman, 1995) for factors that affect flow of microbial protein from the rumen. Effects of forage quality and grain processing cannot be over-emphasized.

Consider differences in intestinal digestibility of RUP sources. Undigested RUP 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 x 0.60), RUP is 7.6% of DM (19.0 x 0.40), and the digestible RUP content is 5.5% of DM (7.6 x 0.72). By careful selection of RUP supplements, let's assume that diet RUP digestibility is 84% rather than 72%. This change in diet RUP digestibility lowers the requirement for RUP from 7.6% to 6.5% of diet DM (5.5% digestible RUP ÷ 0.84 = 6.5%). Assuming the types and amounts of rumen fermentable carbohydrates in the diet remain similar, then the RDP content of the diet should remain at 11.4% of DM. The *improved* diet contains 11.4% RDP (the same as the original diet), 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 of three important points: (1) the correct amounts of RDP and RUP in a diet are, at least in large part, a function of unrelated factors; (2) RUP may not be supplying the quantity of absorbable AA that we assume; and, (3) there is little basis for expressing RUP as a percentage of total diet CP.

Do not over-feed RUP. Feeding too much not only increases feed cost, it may reduce the efficiency of use of absorbed AA because of a less desirable profile of AA. Because most feed proteins have lower amounts of Lys and Met, relative to total EAA, than ruminally synthesized microbial protein (Table 1), feeding more RUP would be expected to decrease the content of Lys, Met, or both in absorbed EAA. Moreover, feeding too much RUP may decrease ruminal fermentation and synthesis of microbial protein if it replaces fermentable carbohydrate or needed RDP. If this occurs, the content of Lys, Met, or both in absorbed EAA will decrease even further.

Select protein supplements with the goal of maximizing Lys and Met in RUP and manipulate the proportions of feed proteins to achieve a predicted Lys/Met ratio in absorbable AA that approximates 2.8-3.0/1.0. Whether the correct ratio is 2.8/1.0, 2.9/1.0, 3.0/1.0, or 3.1/1.0 is not known. Factors such as age and growth rates of growing cattle, milk yield and stage of lactation of lactating cows, as well as the computer model used, are some factors that may affect the ideal ratio of these two EAA. 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 animal performance.

Use rumen-protected AA, in conjunction with protein supplements, to achieve desired levels of Lys and Met in absorbable EAA. After nearly three decades of research, an option that is becoming available to increase Met and Lys in absorbed AA is the use of RPMet and Lys supplements. These concentrated sources of Met and Lys allow nutritionists to raise intestinal levels of Met and Lys higher than what can be accomplished with conventional feed proteins. This is important particularly when greater amounts of RUP must be fed. In other cases, their use can extend the use of protein supplements with low concentrations of Met, Lys, or both.

Several factors have to be considered before RPMet and Lys supplements are fed. These include: 1) predicted contributions of Met and Lys to total EAA in duodenal digesta, 2) level of management and current animal performance, 3) price received for milk protein in the case of lactating cows, 4) cost of RUP-supplements, and 5) efficacy and cost of RPMet and Lys supplements. As with many new technologies, evidence suggests that the best managed animals will benefit the most. Moreover, it will be with these animals that improvements in performance will be measured most easily. These products ***should not*** be fed unless diets have been evaluated appropriately, and animal responses can be predicted and measured. Moreover, like bypass protein supplements, RPAA supplements are not created equal. They differ in AA 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. The challenge of protecting AA has been to identify a combination of process and coating materials that will provide a consistent product with both high ruminal escape and intestinal release, a high payload of AA, and resistance to the mechanical and thermal stresses of storage and handling. The cost of RPAA supplements, relative to anticipated benefits, will be the deciding factor determining the extent of their use.

SUMMARY

There are several reasons for considering intestinal AA profiles when formulating diets. First, it may allow for a higher level of animal productivity than otherwise possible. This appears to be particularly true for milk yield and content of milk protein of early lactation cows where both absorbed energy and AA are likely to be limiting. In this case, feeding more RUP (without a change in the amount of RDP) to increase the supply of a limiting AA will likely be without benefit because of the decrease it would cause in the amount of fermentable carbohydrate in the diet and thus, in the supply of absorbed or metabolizable energy (**ME**). Second, improving the profile of absorbable AA in situations where one or more of the EAA are indeed first-limiting nutrients will increase the use of absorbed AA for protein synthesis and therefore, reduce the quantity of those that are not needed for protein synthesis or other essential functions. Reducing this *overage* of AA will reduce AA deamination and the amount of ME required for urea synthesis, thereby sparing ME for milk production. And last, improving the profile of absorbable AA provides an opportunity to reduce the amount of RUP that must be fed to achieve a given level of growth or milk protein production. Reducing RUP has the advantage of creating *space* 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.

REFERENCES

Ainslie, S. J., D. G. Fox, T. C. Perry, D. J. Ketchen, and M. C. Barry. 1993. Predicting amino acid adequacy of diets fed to Holstein steers. J. Anim. Sci. 71:1312.

Bozak, C. K., C. G. Schwab, and J. E. Nocek. 1986. Changes in amino acid pattern of feed proteins upon exposure to rumen fermentation using the *in situ* bag technique. J. Dairy Sci. 69(Suppl. 1):108 (Abstr.)

Brazle, F. K., and G. L. Stokka. 1994. The effect of protected lysine*methionine amino acids on gain and health of newly arrived calves. J. Anim. Sci. 72(Suppl. 1):292 (Abstr.)

Burris, W. R., J. A. Boling, N. W. Bradley, and A. W. Young. 1976. Abomasal lysine infusion in steers fed a urea supplemented diet. J. Anim. Sci. 42:699.

Clark, J. H. 1995. Ruminal aspects of protein nutrition of lactating dairy cows. *In* Proc. of California Animal Nutrition Conference, May 3-4, Fresno, CA, p. 95-104.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304.

Crooker, B. A., J. H. Clark, R. D. Shank, and E. E. Hatfield. 1986. Effect of ruminal exposure on the amino acid profile of heated and formaldehyde-treated soybean meal. J. Dairy Sci. 69:2648.

Crooker, B. A., and C. G. Fahey, Jr. 1987. Effects of ruminal exposure upon the amino acid profile of feeds. Can. J. Anim. Sci. 67:1143.

Cunningham, K. D., M. J. Cecava, and T. T. Johnson. 1993. Nutrient digestion, nitrogen, and amino acid flows in lactating cows fed soybean hulls in place of forage or concentrate. J. Dairy Sci. 76:3523.

Cunningham, K. D., T. L. Lykos, N. L. Whitehouse, and C. G. Schwab. 1991. Effect of ration on amino acid flow to the small intestine in early lactation Holstein cows. J. Dairy Sci. 74(Suppl. 1):179.(Abstr.)

Erdman, R. 1995. Factors affecting flow of microbial protein from the rumen. *In* Proc. of 4-State Applied Nutrition and Management Conference, Aug 2-3, La Crosse, WI, p. 34-47.

Hill, G. M., J. A. Boling, and N. W. Bradley. 1980. Postruminal lysine and methionine infusion in steers fed a urea-supplemented diet adequate in sulfur. J. Dairy Sci. 63:1242.

Jacobson, D. R., H. H Van Horn, and C. J. Sniffen. 1970. Lactating ruminants. Fed. Proc. 29:35.

Klemesrud, M. J., and T. J. Klopfenstein. 1994. Addition of ruminal escape methionine and lysine to meat and bone meal. J. Anim. Sci. 72(Suppl. 1):94 (Abstr.)

Klemesrud, M.., T. Kloppenstein, and R. Stock. 1995. Rumen-stable lysine appears to improve value of corn gluten feed for finishing calves. Feedstuffs 67:14

Lusby, K. S. 1993. Rumen-stable methionine improves gain of lightweight cattle. Feedstuffs 65:14.

McCance, R. A., and E. Widdowson. 1978. *In*: The Composition of Foods, 4th ed. D.A.T. Southgate and A.A. Paul, eds. HMSO, London, Engl.

Nimrick, K., E. E. Hatfield, J. Kaminski, and F. N. Owens. 1970. Qualitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutr. 100:1293.

O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets. IV. Predicting amino acid adequacy. J. Anim. Sci. 71:1298.

Parsons, C. M. 1994. Application of amino acid digestibilty in feed formulation. Pages 261-268 *in* Proc. 55th Minnesota Nutr. Conf. & Roche Technical Symposium, Bloomington, MN.

Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acid in growing cattle. J. Anim. Sci. 46:740.

Robert, J. C., B. K. Sloan, and S. Bourdeau. 1994. The effects of supplementation of corn silage plus soybean meal diets with rumen protected methionine on the lactational performance of dairy cows in early lactation. J. Dairy Sci. 77(Suppl. 1):92 (Abstr.)

Rulquin, H., and R. Vérité. 1993. Amino acid nutrition of dairy cows: production effects and animal requirements. Pages 55-77 *in* Recent Advances in Animal Nutrition. P.C. Garnsworthy and D.J. A. Cole, eds. Nottingham University Press.

Rulquin, H., L. Le Henaff, and R. Vérité. 1990. Effects on milk protein yield of graded levels of lysine infused into the duodenum of dairy cows fed diets with two levels of protein. Reprod. Nutr. Dev. (Suppl 2):238S.

Rulquin, H., P. M. Pisulewski, R. Vérité, and J. Guinard. 1993. Milk production and composition as a function of postruminal lysine and methionine supply: a nutrient-response approach. Livest. Prod. Sci. 37:69.

Rulquin, H., R.Vérité, J. Guinard, and P. M. Pisulewski. 1995. Dairy cows requirements for amino acids. Pages 143-160 *in* M. Ivan, ed., Animal Science Research and Development: Moving Toward a New Century. ISBN 0-662-23589-4, Centre for Food and Animal Reseach Contribution 2321, Ottawa, Ontario, Canada.

Schwab, C.G. 1995a. Protected proteins and amino acids for ruminants. Pages 115-141 *in* R.J. Wallace and A. Chesson, eds. Biotechnology in Animal Feeds and Animal Feeding. V.C.H. Press, Weinheim, Germany.

Schwab, C.G. 1995b. Rumen-protected amino acids-their role in nutrition of high producing ruminants. Pages 161-175 *in* M. Ivan, ed., Animal Science Research and Development: Moving Toward a New Century. ISBN 0-662-23589-4, Centre for Food and Animal Reseach Contribution 2321, Ottawa, Ontario, Canada.

Schwab, C. G., C. K. Bozak, and J. E. Nocek. 1986. Change in amino acid pattern of soybean meal and corn gluten meal upon exposure to rumen fermentation. J. Anim. Sci. 63(Suppl. 1):158 (Abstr.)

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992. Amino acid limitation and flow to the duodenum at four stages of lactation. II. Extent of lysine limitation. J. Dairy Sci. 75:3503.

Socha, M. T., and C. G., Schwab. 1994. Developing dose-response relationships for absorbable lysine and methionine supplies in relation to milk and milk protein production from published data using the University of New Hampshire equations. J. Anim. Sci. 72(Suppl 2):124. (Abstr.)

Socha, M. T., C. G. Schwab, D. E. Putman, N. A. Kierstead, N. L. Whitehouse, B. D. Garthwaite, and G. A. Ducharme. 1994a. Determining methionine requirements of dairy cows during midlactation by postruminally infusing incremental amounts of methionine. J. Dairy Sci. 77(Suppl. 1):93. (Abstr.)

Socha, M. T., C. G. Schwab, D. E. Putman, N. L. Whitehouse, N. A. Kierstead, B. D. Garthwaite, and G. A. Ducharme. 1994b. Determining methionine requirements of dairy cows during early by postruminally infusing incremental amounts of methionine. J. Dairy Sci. 77(Suppl. 1):65. (Abstr.)

Socha, M. T., C. G. Schwab, D. E. Putman, N. L. Whitehouse, N. A. Kierstead, B. D. Garthwaite, and G. A. Ducharme. 1994c. Determining methionine requirements of dairy cows during peak lactation by postruminally infusing incremental amounts of methionine. J. Dairy Sci. 77(Suppl. 1):92. (Abstr.)

Socha, M. T., C. G. Schwab, D. E. Putnam, N. A. Kierstead, N. L. Whitehouse, B. D. Garthwaite, G. A. Ducharme, and J. C. Robert. 1994d. Production responses of early lactation cows fed rumen-stable methionine or rumen-stable lysine plus methionine at two levels of dietary crude protein. J. Dairy Sci. 77(Suppl. 1):93 (Abstr.)

Stern, M. D., S. Calsamiglia, and M. I. Endres. 1994. Dynamics of ruminal metabolism and their impact on intestinal protein supply. Pages 105-116 *in* Proc. Cornell Nutr. Conf. Feed Manuf., Cornell Univ., Ithaca, NY.

Storm, E., and E. R. Orskov. 1983. The nutritive value of rumen micro-organisms in ruminants. 1. Large scale isolation and chemical composition of rumen micro-organisms. Br. J. Nutr. 50:463.

Storm, E., and E. R. Orskov. 1984. The nutritive value of rumen micro-organisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. Br. J. Nutr. 52:613.

Titgemeyer, E. C., and N. R. Merchen. 1990. The effect of abomasal methionine supplementation of nitrogen retention of growing steers postruminally infused with casein or nonsulfur-containing amino acids. J. Anim. Sci. 68:750.

Van Amburgh, M., T. Perry, D. Fox, and G. Ducharme. 1993. Growth response of Holstein steers supplemented with rumen protected lysine and methionine. J. Anim. Sci. 71(Suppl. 1):260 (Abstr.)

Waghorn, G. C., and R. L. Baldwin. 1984. Model of metabolite flux within mammary gland of the lactating cow. J. Dairy Sci. 67:531.

Wu, Z., R. Fisher, and C. E. Polan. 1995. Lactational performance of cows fed low or high RUP during late gestation and Met and Lys in subsequent lactation. J. Dairy Sci. 78(Suppl. 1):266. (Abstr.)