FEED INGREDIENT FACTORS INFLUENCING INTESTINAL AMINO ACID SUPPLY IN RUMINANTS

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Introduction

Because of the extensive bacterial fermentation occurring in the rumen, the amino acid (AA) composition of protein reaching the small intestine usually has little resemblance to that of the original feed protein. This microbial fermentation has advantages and disadvantages for the host animal. It allows ruminants to subsist and produce due to the synthesis of microbial protein from the products of breakdown of different nitrogen (N) sources, including nonprotein N. In contrast, because of the bacterial breakdown, ruminants often utilize high-quality protein sources less efficiently than would a nonruminant. It also increases the difficulty of predicting the supply and profile of AA reaching the small intestine (Robinson, 1996).

Current requirements for ruminants are largely expressed as total crude protein (CP) and sometimes as ruminal degradable protein (DIP) and undegradable protein (UIP). However, like all animals, ruminants actually require AA absorbed from the small intestine as precursors for protein synthesis and it is becoming clear that the pattern of AA absorbed from the small intestine can be a determinant of growth and milk production as well as of efficiency of protein utilization (Rulquin et al., 1993; Schwab, 1996). Recent interest has been focused on formulation of diets and protein supplements for ruminants to provide a specific profile or supply of AA to the small intestine. Alteration of the intestinal AA profile by means of selecting protein sources that provide complementary profiles of absorbable AA is feasible (Erasmus et al., 1994). This discussion will focus on several factors associated with individual protein sources that influence the quantity and profile of AA that those protein sources provide to the small intestine.

Limiting Amino Acids for Growth and Lactation in Cattle

Improvement of our knowledge of the AA requirements for different stages and levels of production, coupled with an accurate system for predicting AA flow to the duodenum would permit combining supplemental sources of UIP in a way that total diet UIP, ruminally synthesized microbial protein, and supplemental rumen protected AA could be manipulated to optimize the balance of intestinal AA in a consistent manner. The logical, and probably most economical approach to optimizing AA balance for the ruminant would be: 1) maximizing the amount of bacterial protein synthesized in the rumen, 2) increasing the amount of UIP to supplement or complement the bacterial AA profile reaching the duodenum, and 3) feeding rumen protected AA that may be limiting production.

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There is probably sufficient information available to allow reliable formulation of protein supplements for ruminants that provide given quantities and profiles of EAA. For this approach to be useful, the appropriate array of AA that are deficient and must be supplemented must first be identified. A comparison of EAA profiles of mixed ruminal bacteria, duodenal digesta collected from animals fed different basal diets (corn silage, alfalfa hay or silage. concentrate, or fresh alfalfa) containing no supplemental protein or only nonprotein N, and some estimated AA requirements is presented in Table 1.

To predict how supplemental protein must affect the EAA supply to optimize AA utilization by the host, comparisons of the EAA profiles of the supply in duodenal digesta of animals fed the various basal diets can be made to the estimated EAA requirements. This must be done with some reservation because of the tenuous reliability of the estimated requirements. There are still very few direct determinations of the AA requirements of beef cattle growing at rates representative of those observed in practice or of lactating cows at representative levels of production. Estimated AA requirements in Table 1 are metabolizable AA requirements for maintenance and 1 kg/d gain of a 250-kg steer (NRC, 1996) and for maintenance and milk production of a 650-kg Holstein cow producing 45 kg milk (3.1% protein, 3.5% fat) daily (Barry et al., 1994: CNCPS). Bearing in mind the limitations inherent in making such estimates, it seems that undegraded supplemental protein must provide increased proportions of histidine and lysine in growing cattle fed any of the basal diets and greater proportions of threonine in those fed concentrate diets and total sulfur AA in those consuming fresh alfalfa. However, several other AA are consistently only slightly less limiting; e.g., total sulfur AA, leucine, threonine, arginine, and total aromatic AA in most cases. These AA should not be overlooked in formulation of UIP supplements as they may become limiting.

For lactating cows, there is somewhat less consistency in the specific AA that might be predicted to be limiting in animals fed different basal diets. It appears that histidine would be one of the most limiting AA in cows fed most of these basal diets. Cows fed corn silage diets are also likely to be limited by supply of lysine and arginine, those fed higher levels of concentrates by isoleucine and lysine, and those grazing on high-quality alfalfa by total sulfur AA and isoleucine. Again, several other AA (valine, total aromatic AA, lysine in most cases) are only slightly less limiting. In both growing and lactating animals, it seems that a well-balanced supply of most of the EAA is more likely to meet the needs of the host than is supplementation that targets only one or two of the EAA thought to be most limiting. The reason for such observations is the balance and constancy of EAA supply imposed by ruminal microbial protein synthesis. The implication of these observations is the probability that provision of UIP with an appropriate EAA profile is more likely to maximize production responses than is supplementation with a single EAA, even if it is ascertained that that EAA is most limiting.

Thus, it is important that we understand and are able to quantify the nature of the EAA supply that is provided from supplemental UIP sources. The remainder of this paper will discuss some factors that clearly influence the supply of intestinally absorbable EAA that are provided by individual protein sources.

Factors that Influence Intestinal AA Supply from Individual Protein Sources

Heat Treatment of Whole Soybeans

Intact whole soybeans may provide high quality protein and energy supplements for ruminants. Whole soybeans do not require processing before feeding; however, it is apparent that heat and (or) physical treatment may improve the nutritive value (particularly of the protein) of the whole soybeans. Because of its ease and lower costs, heat processing is the most practical and economical way of decreasing ruminal protein degradation and increasing supply of AA to the small intestine (Kung and Rode, 1996). Identification of the optimal heating regime for whole soybeans has been elusive. This is due to 1) interactions between physical form (whole, ground, etc.) and heat that have been inadequately described, 2) transformations of dietary protein by pre-gastric fermentation, and 3) undefined effects of anti-nutritional factors (e.g., trypsin inhibitors) in ruminants.

We have conducted several studies in our laboratory that have focused on the impact of these factors on the supply of AA that is provided by heat processed soybeans. In one of these studies (Aldrich et al., 1995), steers with ruminal, duodenal, and ileal cannulas were fed diets supplemented with raw whole soybeans or whole soybeans roasted at various temperatures. The results of this work were used to calculate the supply of AA that entered and disappeared from the small intestine from the soybean sources themselves. Values for those calculated supplies of individual and total EAA and of total AA from raw soybeans and for one of the roasted soybean treatments are presented in Table 2.

When the roasted soybeans were fed, the quantities of total EAA and total AA entering the small intestine increased by 59 and 70%, respectively, compared to when the raw soybeans were fed. If expressed as ruminal escape values, about 26 and 44% of the dietary AA reached the duodenum for raw and roasted soybeans, respectively. However, the most striking observation that was made in this experiment was the very low quantity of AA that disappeared from the small intestine when raw soybeans were fed. Only about 8% of the total AA that entered the postruminal portion of the GI tract of the steers fed raw soybeans disappeared across the small intestine. This contrasts with an intestinal disappearance coefficient of about 73% for the total AA reaching the small intestine for the roasted soybeans. Thus, roasting soybeans improved the small intestinal digestibility of the soybean AA. This observation would implicate a role for heating soybeans for ruminants similar to that for nonruminants; namely, that heating deactivates the trypsin inhibitors in soybeans and improves their utilization.

This last observation compelled us to take a closer look at the potential role the trypsin inhibitors might have when whole soybeans are fed to ruminants. It has been perceived that trypsin inhibitors are degraded or inactivated by microbial activity in the rumen and thus do not interfere with intestinal digestion. This seemed not to be the case, especially when whole soybeans are fed. In a subsequent experiment (Aldrich et al., 1997), a different approach was taken to evaluating this problem. Raw or roasted soybeans were incubated in ruminal fluid for 16 hrs and then freeze-dried. Activity of trypsin inhibitors was measured both before and after ruminal incubation. The soybeans that had been incubated in ruminal fluid as well as raw soybeans and roasted soybeans that had not been incubated were crop-intubated to roosters and digestibility of AA measured. The precision-fed rooster assay is a good indicator of intestinal digestibility of AA (Titgemeyer et al., 1990).

In vitro incubation in ruminal fluid did not decrease the activity of trypsin inhibitors in raw soybeans nor did it improve digestibility of AA in raw whole soybeans by roosters. It appears that ruminal fermentation may not reduce the effects of trypsin inhibitors on subsequent intestinal digestion. Roasting drastically reduced trypsin inhibitor activity of soybeans and improved AA digestibility. It would seem that heating whole soybeans not only increases escape of protein from ruminal degradation but also probably enhances postruminal digestion of the escaped protein. These two factors work synergistically to dramatically increase the supply of intestinally absorbed AA to the host.

Variation Among Protein Sources in Supplying Intestinally Available Amino Acids

Other studies in our laboratory have evaluated several feedstuffs as sources of ruminally undegraded AA that disappear in the small intestine. Data are reported in Table 3 that summarize the quantities of EAA that disappeared from the small intestine from soybean meal (SBM), corn gluten meal (CGM), blood meal (BM), fish meal (FM), and high quality (23% crude protein) alfalfa hay.

Soybean meal and alfalfa were least effective in providing absorbable AA to the small intestine. Corn gluten meal and BM supplied the most, providing 747 and 635 g total AA per kg of protein: FM was intermediate (400 g per kg FM protein). In addition to these large differences among protein sources in quantity of total AA disappearing in the intestine, large differences can be observed in the ability of different proteins to provide important individual AA to the animal. For example, while CGM provided more than 8 times as much total AA as did SBM, both protein sources provided similar quantities of lysine (about 11.5 g/ kg of protein) to the host animal. Therefore, if lysine were one of the most limiting AA for production, supplementation of similar quantities of CGM or SBM protein would be expected to provide for similar responses despite the fact that CGM provides much more undegraded protein. Generally, the supply of absorbable individual EAA from the five protein sources was similar to that of total EAA (i.e., CGM = BM > FM > SBM = alfalfa) with several exceptions; 1) CGM, as noted, was a poor source of lysine and an excellent source of sulfur AA (methionine + cysteine) and leucine, 2) BM was a poor source of methionine, isoleucine, and tyrosine and an excellent source of lysine and histidine, and 3) FM was a poor cysteine source.

Three factors contribute to the quantity and profile of AA disappearing in the small intestine from each protein source. First, profile is determined largely by the AA composition of the original protein source. Protein sources, especially those resistant to ruminal degradation, generally provide excess or deficient quantities of individual AA relative to the proportion in the original protein. Second, the fraction of dietary protein which reaches the small intestine as AA (e.g., ruminal escape) is the major determinant of the quantity of AA delivered to the animal. In these evaluations, quantities of total AA N entering the small intestine were equal to approximately 15, 72, 80, 50, and 19% of N consumed from SBM, CGM, BM, FM, and alfalfa, respectively. These values correspond well to previously reported ruminal escape values summarized by NRC (1985) for these protein sources. Third, some variation occurs in the proportion of AA entering the small intestine that disappear at that site (e.g., intestinal availability). This factor accounts for less difference among protein sources than either of the first two factors but is still important. Disappearance coefficients of undegraded total AA from SBM, CGM, BM, FM, and alfalfa were 55, 80, 71, 70, and 80 %, respectively.

When total absorbable AA limit production, any of the protein sources high in undegradable protein may suffice to improve performance. However, when specific EAA limit production, certain protein sources may be superior in their ability to supply these AA. Similarly, feeding proteins that escape ruminal degradation may be of little benefit if limiting AA are deficient in the supplemental protein fed. As these data demonstrate, protein sources vary greatly in the quantities of individual AA that they supply for absorption from the small intestine. It is especially noteworthy that each of the protein sources evaluated is a poor source of at least one EAA. Differences among protein sources will be important as nutritionists attempt to define and meet AA needs of ruminants; combinations of protein sources may be best able to supply these AA to the ruminant in optimal proportions.

Variation Within Protein Sources in Supplying Intestinally Available Amino Acids

Virtually all protein concentrates used in ruminant diets are derived from processing of materials from a variety of sources. Oilseed meals, grain processing byproducts, and byproducts of the distilling and brewing industries are all commonly utilized in diets for beef and dairy cattle. All of these plant protein sources are subjected to a sequence of processing factors that can influence their composition and, in some cases, their feeding value. Another group of protein supplements that are used increasingly in ruminant feeding are animal proteins that are derived from the packing and rendering industries. Feedstuffs such as poultry byproduct meal, feather meal, blood meal, meat meal, and meat and bone meal (MBM: currently only MBM of nonruminant origin can b used in ruminant diets). Even more than the plant protein sources previously mentioned, these products are subject to great variation in factors such as the species from which they are derived, the nature of the raw materials found in a given product (i.e., soft tissue vs connective tissue vs carcass trimmings vs bone, etc.), and processing variables (time, temperature, pressure, etc. applied during cooking and drying). All feedstuffs will vary somewhat in compositional variables; this may or may not have much impact on nutritional value. In the case of animal byproducts, this degree of variation might be anticipated to be quite a bit greater than for many other feeds.

We investigated this issue in our laboratory with respect to the composition and value of meat and bone meal (MBM) in providing AA for disappearance from the small intestine of cattle. This project was undertaken several years ago; since then, the use of MBM of ruminant origin in ruminant diets has been disallowed by FDA. However, this work was conducted before this restriction was imposed; therefore, some of the samples discussed here included materials obtained from cattle. In Table 4, some selected compositional variables are summarized for 15 samples of MBM that were analyzed.

Concentration of crude protein in the 15 samples averaged 53.2% and had a coefficient of variation of 5.8%. Collagen concentration averaged 25.2% of dry matter and had a CV of 18.4%, indicating a substantial range of difference in type of raw materials in the MBM sources. Sources of MBM that had been characterized by distributors as high in connective tissue and bone had high collagen concentrations. Ash concentration averaged 30.9% but also showed great variation (CV = 18.4%). Ash and collagen are both high in bone and were positively correlated (r = +.72) among these samples.

Concentrations of several individual AA that may be important for ruminants varied greatly among MBM samples. The CV for threonine, methionine, isoleucine, leucine, histidine, and lysine were 8.3, 14.9, 11.1, 9.3, 10.4, and 8.2%, respectively. These are greater CV than was noted for the crude protein content of these samples (5.8%) which indicates that the samples vary more in concentrations of individual AA that they do for total protein; this probably indicates different proportions of raw materials among the MBM samples.

Four of the MBM products analyzed in the preceding survey were included as the principal protein supplement in diets fed to steers. The MBM sources provided about 40% of the total protein in the diet and included sources that represented the range of values observed for the analytical portion of this project. Estimates of in vivo ruminal protein escape values and quantities of AA disappearing across the small intestine of cattle fed the different MBM sources are reported in Table 5. Estimates of ruminal escape of protein for the four MBM sources were 31% for source A, 55% for source B, 83% for source C, and 24% for source D. Thus, estimates of "bypass" vary tremendously for different MBM sources. Because of the limited number of MBM sources that could be tested in vivo, there are great limitations to our ability to develop relationships between compositional data and these measurements. However, apparent ruminal protein escape for these samples was negatively related to crude protein concentration (r = -.70) and positively related to collagen (r = +.56) and ash (r = +.48) concentrations. This suggests that samples higher in collagen and ash (which are often also low in crude protein) may have higher ruminal escape values; again, some caution is needed in making such interpretations because of the limited data available.

The quantities of individual and total AA disappearing from the small intestine of the steers were not impacted by source of MBM as greatly as we had hypothesized that they would be. This lack of impact is due in part to the supply of AA that arise from microbial protein and non-MBM ingredients in the diets; these contributions dilute the impact of the MBM protein. Also, the large differences in apparent ruminal protein escape tend to eliminate some of the anticipated differences in AA supply because the lower quality MBM sources were less extensively degraded in the rumen.

Summary

Numerous factors impact the ability of individual feed ingredients to provide AA to the ruminant. Several examples of the effects of several of these factors have been discussed. The ability of feedstuffs to provide specific AA for absorption from the small intestine of the ruminant is an increasingly important consideration in diet formulation for high-producing animals. Large differences between feeds due to inherent characteristics of those feeds and to differences in processing exist. In addition, considerable variation exists within some feedstuffs because of differences in processing and(or) chemical composition.

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Table 1. Essential amino acid (EAA) profiles of ruminal microbial protein (MCP), duodenal digesta of ruminants fed different basal diets and some estimated EAA requirements for growing steers and lactating cows.

	Source of AA						
		Duodenal digesta				Requirements	
Amino acid	MCP[a]	Corn silage based diets[a]	Alfalfa-based diets[a]	Concentrate diets[a]	Fresh alfalfa[b]	Growing steer[c]	Lactating cow[c]
Threonine	10.13	9.97	11.04	8.88	10.67	9.74	7.91
Valine	10.05	10.97	11.14	11.56	11.08	9.99	11.13
Total sulfur (Met + Cys)	7.78	7.45	6.72	7.26	5.60	7.13	6.61
Isoleucine	9.46	10.79	11.21	9.94	9.86	6.99	10.79
Leucine	14.30	17.76	17.58	19.99	15.67	16.73	15.71
Histidine	4.10	3.92	3.88	4.51	4.19	6.24	5.26
Lysine	15.29	13.39	13.80	12.10	14.03	15.98	14.64
Arginine	8.47	8.51	8.97	8.46	8.67	8.24	9.23
Total aromatic (Phe + Tyr)	17.47	17.25	19.02	17.32	20.22	17.48	16.26
Tryptophan	2.93	ND[d]	ND	ND	ND	1.50	2.45

[a] Merchen and Titgemeyer, 1992.

[b] Elizalde et al., 1998.

[c] Requirements were for metabolizable EAA as calculated for a 250 kg Angus × Simmental steer gaining 1 kg BW/d (NRC, 1996) or for a 650 kg mature Holstein cow producing 45 kg of milk (3.5% fat, 3.1% protein) per d. (CNCPS; Barry et al.,1994)

[d] Not determined

Table 2. Effect of roasting whole soybeans on EAA entering and disappearing from the small intestine of cattle. Values are expressed as g AA/kg CP consumed from the protein source

	Entering SI		Disappearing from SI	
Amino acid	Raw	Roasted[a]	Raw	Roasted[a]
	---------------------g/kg CP---------------------			
Threonine	11.1	20.6	-4.9	13.2
Valine	13.6	24.9	-1.5	17.6
Methionine	0	1.7	-1.2	0.9
Isoleucine	12.8	22.1	0.5	17.1
Leucine	22.7	38.5	6.9	31.1
Histidine	7.6	12.3	1.2	9.0
Lysine	14.8	26.4	0.5	19.5
Arginine	18.2	29.2	5.2	22.3
Total aromatic (Phe + Tyr)	23.2	42.0	1.5	30.9
Total EAA	136.8	217.7	8.2	161.3
Total AA	255.9	434.4	19.7	319.1

[a] Roasted at 300°F; Aldrich et al., 1995.

Table 3. Quantities of individual and total EAA disappearing from the small intestine of cattle from different protein sources.

	Protein source[a]				
Amino acid	SBM[b]	CGM[b]	BM[b]	FM[b]	Alfalfa[c]
	g/kg CP				
Threonine	3.4	19.8	22.4	15.5	7.0
Valine	10.2	46.6	61.8	26.6	11.0
Total sulfur (Met + Cys)	0	17.6	6.9	8.5	3.7 (met only)
Isoleucine	9.9	28.8	5.6	18.1	9.4
Leucine	5.8	127.7	84.0	29.8	19.8
Histidine	1.4	11.8	41.6	7.2	1.9
Lysine	11.8	11.5	60.2	35.7	7.5
Arginine	9.3	24.5	33.1	32.5	9.6
Total aromatic (Phe + Tyr)	15.5	84.9	59.5	40.3	20.7
Total EAA	64.2	373.1	374.7	213.9	90.6
Total AA	89.9	746.6	634.6	400.2	156.8

[a] SBM = soybean meal; CGM = corn gluten meal; BM = blood meal; FM = fish meal

[b] Titgemeyer et al., 1989.

[c] High-quality alfalfa hay (23% CP); Atwell et al., 1990.

Table 4. Variation in concentrations of some selected nutrients in 15 samples of meat and bone meal obtained from various distributors.

Constituent	Mean (n=15)	Range	SD	CV,%
	% DM			
Crude protein	53.2	46 to 60	3.1	5.8
Collagen	25.2	21 to 39	4.6	18.3
Ash	30.9	22 to 36	5.8	18.8
Threonine	1.80	1.38 to 1.95	.15	8.3
Methionine	.74	.51 to .98	.11	14.9
Isoleucine	1.44	1.03 to 1.65	.16	11.1
Leucine	3.22	2.46 to 3.78	.30	9.3
Histidine	.98	.68 to 1.52	.19	19.4
Lysine	2.80	2.15 to 3.06	.23	8.2

Table 5. Ruminal protein escape values and quantities of some AA disappearing in the small intestine of cattle fed different sources of meat and bone meal (MBM).

Item	Source of MBM			
	A	B	C	D
Ruminal escape, %	31	55	83	24
	g AA disappearing from SI			
Threonine	51	45	43	46
Methionine	20	16	17	18
Isoleucine	37	32	31	33
Leucine	86	79	75	81
Histidine	24	17	21	21
Lysine	62	53	53	57
Total EAA	419	368	363	391
Total NEAA[a]	533	501	541	509
Total AA	953	869	904	900

[a] Non-essential AA.