In Situ Ruminal Disappearance of Essential Amino Acids in Protein Feedstuffs[1]

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ABSTRACT

Four protein sources were incubated in situ to estimate AA disappearance. Bags containing either soybean meal, corn gluten meal, herring meal, or meat meal were washed in water or suspended in the rumen of two Holstein cows for 8, 12, 16, 24, 48, 72, and 120 h. Cytosine, a bacterial marker for microbial contamination, was used to correct the essential AA profile for microbial contribution to determine the residual essential AA composition of the protein sources after incubation. Ruminal disappearance of individual essential AA was different among feedstuffs. Relative to original feed protein, soybean meal and corn gluten meal decreased the concentration of specific essential AA in the RUP. Concentration of all essential AA, except Arg and His, increased in undegraded meat meal protein. The difference between original and residual AA concentrations in herring meal approached statistical significance. Use of the original AA profile of the feed protein to predict essential AA available for absorption is not accurate because accuracy differs with sources.

(Key words: essential amino acids, ruminal disappearance in situ, protein supplements)

Abbreviation key: CGM = corn gluten meal, EAA = essential AA, EUEAA = estimated undegraded EAA, HM = herring meal, MM = meat meal, PUEAA = predicted undegraded EAA, SBM = soybean meal.

INTRODUCTION

Diet formulation to meet the AA requirements for ruminants is a challenging goal in animal nutrition (14). Current limitations of this approach are the lack of information regarding the efficiency of absorption and utilization of individual AA and the inability to estimate accurately the individual AA supply at the duodenum (19).

The amount of AA that is available for absorption in the small intestine is a combination of microbial protein that is synthesized in the rumen and the dietary protein that survives ruminal degradation. Several studies have been conducted to define the ruminal fate of dietary AA. Differences in degradation rate for specific AA within feed protein have been observed with in vivo (21, 23) and in vitro methods (5, 8), indicating selective removal of certain AA by the ruminal microorganisms.

Analyses of AA composition of feed residues after incubation in situ are not always consistent with this result. In some studies (27, 28), the AA profile of the residues in bags after ruminal exposure resembled closely that of the original feedstuff. Other research (6, 16), which evaluated the AA composition of RUP after correction for microbial contamination, found differences in the AA profile between ingested protein and RUP. Thus, prediction of feed AA supply at the duodenum by applying the origi-

nal AA composition of the feed to the protein fraction that escapes ruminal degradation is possibly inaccurate.

Therefore, this study was designed with two objectives: 1) to compare the essential AA (EAA) composition of four protein feedstuffs with their residues after ruminal suspension in situ for varying intervals and 2) to estimate the quantity of individual feed EAA available for absorption in the small intestine after correcting for the EAA composition of microbial protein in the bag.

MATERIALS AND METHODS

Soybean meal (SBM), corn gluten meal (CGM), herring meal (HM), and meat meal (MM) were used in the study. All feedstuffs were ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) with a 2-mm mesh screen. The DM, CP, ether extract, and ash content of the feeds was determined according to AOAC procedures (3); NDF content was estimated by the procedure of Van Soest et al. (25). Geometric mean diameter of the feed particles was measured according to the classification of particle size in feedstuffs (7).

Ruminal degradation in situ of feed CP was determined by the nylon bag technique (12). Spun polyester dacron bags (10 × 15 cm) with pore size of 40 μm were filled with 6 ± .6 g of each protein source (20 mg/cm^2 of surface) (10). Four bags for each feedstuff were suspended via ruminal cannula for 8, 12, 16, 24, 48, 72, and 120 h in each of two dry Holstein cows (mean BW of 500 kg). Cows were fed a standard ration (13.9% CP and 57.7% NDF on a DM basis) of 6 kg/d of mixed grass hay (60% Italian ryegrass, 30% orchardgrass, and 10% other grasses) and 2 kg/d of a mixture of equal amounts of corn, barley, sunflower meal, and SBM in two equal meals at 0900 and 1700 h. Bags for all incubation intervals were inserted into the rumen before the morning meal to provide a similar starting environment (2). Upon removal from the rumen, the bags were rinsed immediately and mildly agitated in cold tap water for 15 min and then oven-dried at 60°C to a constant weight. The same washing procedure was applied to four bags of each protein source without incubation (0 time) to estimate water-soluble losses of CP. The residual content of the four bags was combined, ground through a 1-mm screen, and analyzed for total N by the Kjeldahl procedure (3) using Tecator Kjeltec (Tecator, Inc., Herndon, VA).

Contamination of residues remaining in the bag by ruminal microorganisms was estimated using cytosine as a marker (4). To obtain the cytosine content of the microbial fraction, ruminal fluid was collected from both cows via cannula by a vacuum pump and combined into one container. After fluid was strained through cheese cloth, separation was carried out according to Robinson and Sniffen (15). Filtered ruminal fluid was centrifuged at 1000 × g for 10 min to remove feed particles and protozoa. The bacterial pellet was isolated from the supernatant, centrifuged at 11,000 × g for 20 min, resuspended in saline solution (.9% NaCl), recentrifuged, and finally transferred in saline solution prior to freeze-drying.

AA Analysis

Samples of the original feedstuffs, the residues of incubation, and four subsamples of the ruminal bacteria were analyzed for AA composition using an automated AA analyzer (Beckman 118 CL; Beckman Instruments, Fullerton, CA). Prior to analysis, samples of HM and MM were defatted (3) to avoid interference during ion-exchange separation. All samples were hydrolyzed in 6N HCl at 105°C for 21 h in test tubes sealed under continuous N$_2$ flow and filtered through Whatman paper number 1 (Whatman International Ltd., Maidstone, England) (21). Cystine and Trp are oxidized by acid hydrolysis (22) and could not be measured.

The AA profile of the ruminal bacteria (Table 1) was multiplied by the residual microbial protein on the digested residues, estimated by cytosine assay, and then subtracted from the bag residue to determine the residual EAA composition of the feeds after incubation. This procedure allowed the calculation of EAA degradation in feed at the different times and the difference between the EAA profile of the original feed protein and the residual protein in the bags.

Estimation of Degradability

Degradabilities of CP and individual EAA for the four protein sources were computed

TABLE 1. The AA composition of ruminal bacteria.

AA	$\overline{X}$	SD
	(g/100 g of AA)	
Arg	4.5	.1
His	1.5	.1
Ile	5.0	.3
Leu	7.4	.2
Lys	8.2	.1
Met	1.8	.5
Phe	5.0	.2
Thr	6.1	.1
Val	5.6	.1
Ala	8.0	.1
Asp	12.3	.3
Glu	14.0	.2
Gly	5.7	.1
Pro	3.7	.4
Ser	5.2	.1
Tyr	5.3	.2

using the Marquardt iterative method in the nonlinear regression procedure of SAS (18). The equation was the first-order kinetics model with one component as described by Ørskov and McDonald (13):

$$p = a + b(1 - e^{-kt})$$

where

p = potential degradability (percentage),
a = readily degraded fraction (percentage),
b = fraction degraded at measurable rate (percentage),
k = rate of disappearance of b fraction (percentage per hour), and
t = time (hours).

Overall degradabilities for CP and individual EAA were estimated with the equation proposed by Ørskov and McDonald (13):

$$P = a + b \, k(k + k_p)^{-1}$$

where

P = overall degradability (percentage), and
k_p = fractional passage rate (percentage per hour).

Fractional passage rates were 5 and 8%/h as proposed by Agricultural Research Council (1)

for medium (<15 kg/d) and high (>15 kg/d) milk production. The RUP and the estimated undegraded EAA (EUEAA) content of the feeds were then calculated as 100 − P. The original EAA composition of the protein sources was applied to RUP to calculate the predicted undegraded EAA (PUEAA) of the feeds.

Statistical Analysis

Two sets of statistical analyses were conducted for each feedstuff. Individual EAA contents of the original feed protein and of the residual protein in the bags after incubation were compared using the general linear models procedure of SAS (18). The data set included the incubations for 0, 8, 12, 16, and 24 h based on the assumption that the mean ruminal retention time of the protein sources would not exceed 24 h in a lactating dairy cow. Differences between EUEAA and PUEAA were analyzed by ANOVA using ruminally undegraded EAA and cow as independent variables.

RESULTS AND DISCUSSION

The protein sources analyzed in the study represented a wide range of protein supplements included in dairy rations. Chemical composition and mean geometric diameter of the feedstuffs are in Table 2. All feeds had a CP content >50% of the total DM. The low ash and the high CP of MM indicated that it was derived from tissue sources. Both animal feedstuffs contained >10% ether extract, which may have increased the mean geometric diameter of their ground particles because lipid tends to promote feed particle adhesion. Particle size distribution of the feedstuffs indicated no fraction <38 μm in diameter. Particles with smaller diameter would filter out of the bags (40-μm pore size).

Table 3 presents CP disappearance of the protein sources after correction for microbial contamination and the contribution of microbial CP after incubation for different times in the rumen. No appreciable microbial contamination was detected in HM residues. The SBM and MM exhibited appreciable contamination only within the 16 h of ruminal exposure with peak contamination at 8 h. Colonization of CGM by ruminal microbes

TABLE 2. Chemical composition and mean geometric diameter of the feedstuffs.[1]

Item	SBM		CGM		HM		MM	
	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
DM, %	89.5	.9	90.0	.2	87.1	.4	87.7	.1
CP, % of DM	51.3	.3	67.7	.4	74.3	.1	84.1	1
Ash, % of DM	7.0	.1	2.3	.3	15.0	.1	4.3	.1
Ether extract, % of DM	1.4	.1	2.1	.1	10.7	.2	11.6	.1
NDF, % of DM	18.5	.3	13.9	.4	. . .	. . .	. . .	. . .
Geometric diameter, mm	.23	.03	.39	.01	.43	.02	.52	.01

[1]SBM = Soybean meal, CGM = corn gluten meal, HM = herring meal, and MM = meat meal.

was prolonged and peaked by 16 h of incubation. The delay in peak for CGM might be influenced by the hydrophobicity of gluten protein. The CGM forms a gelatinous mass in the bag when it is moistened by ruminal fluid, reducing total surface area for bacteria to adhere (4).

In spite of the higher ether extract content, CP in HM and MM exhibited greater water solubility (41.4 and 34.9%, respectively) than other protein sources. Hydrophobic properties of CGM protein reduced water-soluble losses (12.3%), and SBM was intermediate in solubility (20.6%).

Protein degradability of all the feedstuffs approached 100% by 120 h, but differences among feedstuffs in rate of disappearance were large (Table 3). The SBM showed the highest rate of CP degradation (11.69%/h) and agrees with the mean rate of degradation (10.2%/h) reported for SBM by Nocek and Russell (11). Estimated RUP for SBM were 25.7 and 31.4% for fractional passage rates of 5 and 8%/h, respectively, and were within the range of RUP (35 ± 12%; n = 39) reported by the NRC (9). Regardless of passage rate, RUP values for CGM were higher than reported (55%) by NRC (9). The in situ method probably underestimates CP degradation of CGM (4, 21). The CP of HM had a low rate of disappearance (2.14%/h) despite its high water solubility, resulting in RUP of about 40%. Conversely, MM protein had a modest degradation rate (4.36%/h), but more than one-third was soluble, resulting in RUP <20% at both rates of passage (Table 3).

Essential AA composition of original SBM and residues after ruminal incubation for different times is reported in Table 4. Water-soluble losses were significant for all EAA,

compared with the original EAA profile. Ruminal exposure consistently lowered concentrations for Arg, His, and Lys.

Residual CP of CGM (Table 5), in agreement with that of SBM, significantly decreased for content of Arg, His, and Lys at all incubation times except 24 h, when the EAA composition of the residual CP resembled that of the original feed (Table 5). Therefore, the viscous-like conformation that occurs when hydrophobic polypeptides are moistened by the ruminal fluid (24) reduces CP disappearance of CGM in situ but permits selective removal of EAA by the ruminal microbes, although to a lesser extent than observed with SBM.

The contents of all EAA of HM were reduced by water solubility (Table 6). Ruminal incubation of HM induced significant changes only for specific EAA: Arg, His, Lys, and Met decreased in concentration in the residues, but Phe and, to some extent, the branched-chain AA content were increased relative to the original feed.

The pattern of EAA disappearance of MM (Table 7) was remarkably different from that of the other protein sources. Relative to the original EAA profile, only Arg and His were lost significantly by washing in water. All of the other EAA increased in concentration in the residue for several intervals. This effect could have been due to processing of the supplement, which may have caused a partial denaturation of the original protein conformation. At least two hypotheses are possible explanations for this degradation pattern: 1) degradation of EAA is slow compared with that of the nonessential AA fraction of the supplement or 2) most EAA may escape degradation because they are a part of slowly degradable polypeptides. The former has been reported by Varvikko (26) for CP of barley and barley straw.

TABLE 3. In situ CP disappearance at different incubation times, CP degradation rate, and RUP of the feedstuffs[1] after correction for microbial contamination.

Item	SBM			CGM			HM			MM		
	$\overline{X}$	SD	RMN[2]	$\overline{X}$	SD	RMN	$\overline{X}$	SD	RMN	$\overline{X}$	SD	RMN
						(% of CP)						
Incubation time, h												
0[3]	20.6	. . .	. . .	12.3	. . .	. . .	41.1	. . .	. . .	34.9	. . .	. . .
8	71.0	13.5	5.3	26.6	9.5	4.5	57.9	2.6	0	79.0	10.5	10.4
12	79.2	15.1	4.6	30.6	10.7	6.7	59.2	4.0	0	84.4	5.9	8.5
16	80.6	18.1	4.1	33.1	1.5	9.3	61.0	4.4	0	84.3	6.0	5.1
24	97.7	1.9	0	50.7	23.8	2.3	67.0	5.3	0	89.3	3.4	0
48	99.6	.1	0	77.4	13.6	.2	81.8	6.7	0	95.2	.7	0
72	99.6	.1	0	96.3	5.1	0	89.7	5.0	0	97.4	1.2	0
120	99.2	.3	0	99.6	.2	0	94.5	3.6	0	98.2	.1	0
Rate of disappearance, %/h	11.69	6.17		2.87	.96		2.14	.57		4.36	1.29	
RUP, % at												
k_p[4] = 5%/h	25.7	9.8		61.8	7.9		36.9	3.8		15.8	7.0	
k_p = 8%/h	31.4	11.4		71.3	6.7		41.5	3.2		18.9	8.9	

[1]SBM = Soybean meal, CGM = corn gluten meal, HM = herring meal, and MM = meat meal.

[2]Residual microbial N (percentage of total residual N).

[3]Zero time incubation represents CP water soluble losses.

[4]Fractional passage rate.

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TABLE 4. Essential AA composition of soybean meal CP and its residues after intervals of ruminal exposure in situ.

AA	Original feed	0 h[1]	8 h	12 h	16 h	24 h	SE
			(g/100 g of CP)				
Arg	8.8	5.8**	6.5**	6.0**	5.8**	5.2**	.46
His	3.0	1.9**	2.5*	2.4*	2.2**	2.2*	.14
Ile	4.4	3.1**	4.5	4.3	4.1	4.0	.27
Leu	8.7	6.2**	9.0	8.4	8.0	8.4	.55
Lys	7.0	4.8**	5.0**	5.3**	5.2**	5.6**	.07
Met	1.8	1.5[†]	2.0	1.8	1.7	1.9	.14
Phe	5.7	4.2**	5.5	5.2[†]	4.9*	4.8[†]	.28
Thr	4.6	3.2**	4.8	4.4	4.0[†]	4.1	.30
Val	4.6	3.3**	5.0	4.7	4.3	4.6	.23
BCAA[2]	17.7	12.7**	18.5	17.4	16.4	17.0	1.05
EAA[3]	48.7	34.2**	44.8[†]	42.6*	40.4*	41.0[†]	2.33

[1]Zero-time incubation represents CP water-soluble losses.

[2]Branched-chain AA.

[3]Essential AA (except Trp).

[†]$P < .10$.

*$P < .05$.

**$P < .01$.

The pattern of disappearance in situ of EAA was different across feedstuffs. Application of original EAA composition of feed CP to RUP to predict PUEAA may be questionable. To test this hypothesis, PUEAA and EUEAA were compared (Tables 8 to 11). The quantity of Arg and His from SBM available for absorption in the small intestine at the lower fractional passage rate was reduced when it was estimated on the basis of EAA disappearance in situ (Table 8). Predicted amount of ruminally undegraded Arg from SBM was 20.9 g/kg of feed

TABLE 5. Essential AA composition of corn gluten meal CP and its residues after intervals of ruminal exposure in situ.

AA	Original feed	0 h[1]	8 h	12 h	16 h	24 h	SE
			(g/100 g of CP)				
Arg	3.7	3.0[†]	3.1	3.1[†]	3.0[†]	3.7	.41
His	2.4	2.0[†]	2.0[†]	1.8*	1.7*	2.2	.26
Ile	3.7	3.1	3.2	3.2	3.3	3.9	.44
Leu	22.0	18.4	20.2	18.2	19.8	23.4	3.35
Lys	1.9	1.5[†]	1.4*	1.3*	1.2*	1.7	.03
Met	2.6	2.1	2.1	2.1[†]	2.3	2.7	.33
Phe	7.6	6.4	6.9	7.0	6.7	8.3	.95
Thr	4.4	3.5[†]	3.6	3.6	3.6	4.5	.59
Val	5.2	4.2[†]	4.5	4.6	4.2[†]	5.4	.64
BCAA[2]	30.9	25.8	27.8	26.0	27.3	32.7	4.38
EAA[3]	53.4	44.2	47.0	44.8	45.8	55.8	7.00

[1]Zero-time incubation represents CP water-soluble losses.

[2]Branched-chain AA.

[3]Essential AA (except Trp).

[†]$P < .10$.

*$P < .05$.

TABLE 6. Essential AA composition of herring meal CP and its residues after intervals of ruminal exposure in situ.

AA	Original feed	0 h[1]	8 h	12 h	16 h	24 h	SE
			(g/100 g of CP)				
Arg	7.0	5.8**	6.3**	6.6*	6.1**	6.5*	.18
His	2.3	1.5**	1.7**	1.6**	1.7**	1.8**	.06
Ile	4.3	3.4*	4.7	5.1*	4.6	5.0*	.11
Leu	9.0	6.8**	8.9	9.4	8.6	9.1	42
Lys	10.5	7.3**	8.9**	9.4**	8.6**	9.0**	.28
Met	3.6	2.3**	3.2**	3.2**	3.1**	3.1**	.12
Phe	4.5	3.8**	4.9*	4.9*	4.8	5.2**	.19
Thr	5.7	4.2**	5.8	6.2	5.5	5.9	.39
Val	5.1	4.2*	5.2	5.6†	5.1	5.5	.31
BCAA[2]	18.3	14.4**	18.8	20.0†	18.2	19.5	1.04
EAA[3]	52.0	39.4**	49.5	52.0	48.0†	51.2	2.08

[1]Zero-time incubation represents CP water-soluble losses.

[2]Branched-chain AA.

[3]Essential AA (except Trp).

†$P < .10$.

*$P < .05$.

**$P < .01$.

CP when the AA content in the original feed was used and dropped to 15.5 g/kg of feed CP when the disappearance in situ of the AA was considered. At the higher fractional passage rate, only Arg was different, but Lys approached statistical significance at both turnover rates (Table 8). Predicted undegraded Lys and Met for a fractional passage rate of 8%/h were 22.0 and 5.7 g/kg of feed CP, respectively, and were similar to those reported by

TABLE 7. Essential AA composition of meat meal CP and its residues after intervals of ruminal exposure in situ.

AA	Original feed	0 h[1]	8 h	12 h	16 h	24 h	SE
			(g/100 g of CP)				
Arg	9.9	7.8**	7.9**	7.4**	6.9**	6.5**	.32
His	1.9	1.5**	1.9	2.0	1.9	1.8	.07
Ile	2.2	2.1	3.7**	3.9**	3.7**	4.0**	.39
Leu	5.0	5.0	8.6**	9.2**	8.6**	8.8**	.70
Lys	5.5	5.3	7.6**	8.2**	7.5**	7.6**	.49
Met	1.3	1.3	1.9†	2.1*	1.7	2.0†	.38
Phe	3.1	3.1	4.3**	4.6**	4.4**	4.4**	.26
Thr	2.8	2.9	4.5**	5.3**	5.0**	5.1**	.21
Val	3.3	3.2	5.2**	5.4**	5.3**	5.5**	.49
BCAA[2]	10.6	10.5	17.6**	18.7**	17.7**	18.4**	1.58
EAA[3]	35.0	32.3	45.6**	48.1**	45.1**	45.8**	2.82

[1]Zero-time incubation represents CP water-soluble losses.

[2]Branched-chain AA.

[3]Essential AA (except Trp).

†$P < .10$.

*$P < .05$.

**$P < .01$.

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TABLE 8. Predicted (PUEAA) and estimated (EUEAA) undegraded AA content of soybean meal at two fractional passage rates.

AA	k_p^1 = 5%/h				k_p = 8%/h			
	PUEAA[2]	EUEAA[3]	P <	SE	PUEAA	EUEAA	P <	SE
	— (g/kg of feed CP) —				— (g/kg of feed CP) —			
Arg	20.9	15.5	.04	.30	27.6	20.7	.02	.25
His	7.1	5.9	.03	.05	9.4	7.9	.11	.25
Ile	10.5	10.6	.89	.85	13.8	14.2	.84	1.35
Leu	20.7	21.4	.71	1.55	27.3	28.7	.67	2.35
Lys	16.6	12.0	.14	.95	22.0	15.8	.12	1.15
Met	4.3	4.6	.40	.25	5.7	6.2	.43	.40
Phe	13.6	13.1	.56	.60	17.6	17.9	.78	.95
Thr	10.9	11.4	.68	.90	14.4	15.4	.61	1.35
Val	10.9	11.8	.46	.75	14.4	15.8	45	1.15
BCAA[4]	42.0	43.8	.68	3.20	55.5	58.6	.64	4.75
EAA[5]	115.4	106.2	.24	3.70	152.8	141.9	.35	6.70

[1]Fractional passage rate.

[2]PUEAA accounts for AA based on feed AA content only.

[3]EUEAA accounts for differential AA losses of protein.

[4]Branched-chain AA.

[5]Essential AA (except Trp).

Schwab (19) using the same mathematical procedure (22.6 and 5.1 g/kg of feed CP).

Differences were large between individual PUEAA and EUEAA for CGM, particularly at the higher fractional passage rate (Table 9): Arg, His, Lys, and Met of CGM disappeared more rapidly in situ than other EAA based on the undegraded EAA of the feed CP. Therefore, application of feed EAA composition to RUP would overestimate the undegraded quantity of several EAA of this supplement.

The degradation patterns of CP and individual EAA were similar between PUEAA and EUEAA in HM (Table 10). These data

TABLE 9. Predicted (PUEAA) and estimated (EUEAA) undegraded AA content of corn gluten meal at two fractional passage rates.

AA	k_p^1 = 5%/h				k_p = 8%/h			
	PUEAA[2]	EUEAA[3]	P <	SE	PUEAA	EUEAA	P <	SE
	— (g/kg of feed CP) —				— (g/kg of feed CP) —			
Arg	22.9	20.1	.09	.35	26.4	23.0	.03	.15
His	14.9	12.2	.03	.10	17.1	13.0	.02	.05
Ile	22.9	21.3	.12	.30	26.4	24.1	.11	.40
Leu	136.0	127.1	.22	3.20	156.9	144.1	.15	3.10
Lys	11.8	8.9	.02	.10	13.6	10.1	.01	.05
Met	16.1	14.3	.10	.25	18.6	16.2	.06	.20
Phe	46.9	44.9	.39	1.45	54.2	51.2	.28	1.45
Thr	27.2	23.9	.11	.55	31.4	27.1	.07	.45
Val	32.1	29.2	.11	.50	37.1	33.2	.08	.45
BCAA[4]	190.9	177.5	.19	4.00	220.4	201.5	.14	3.95
EAA[5]	330.0	301.2	.15	6.80	380.8	342.3	.11	6.15

[1]Fractional passage rate.

[2]PUEAA accounts for AA based on feed AA content only.

[3]EUEAA accounts for differential AA losses of protein.

[4]Branched-chain AA.

[5]Essential AA (except Trp).

TABLE 10. Predicted (PUEAA) and estimated (EUEAA) undegraded AA content of herring meal at two fractional passage rates.

AA	k_p^1 = 5%/h				k_p = 8%/h			
	PUEAA[2]	EUEAA[3]	P <	SE	PUEAA	EUEAA	P <	SE
	— (g/kg of feed CP) —				— (g/kg of feed CP) —			
Arg	25.9	23.8	.28	.95	29.0	26.4	.19	.80
His	8.5	6.4	.16	.55	9.6	7.0	.14	.55
Ile	15.9	18.4	.22	.90	17.9	20.3	.20	.80
Leu	33.2	33.7	.81	1.60	37.4	37.4	.98	1.45
Lys	38.7	33.5	.17	1.35	43.5	37.4	.12	1.20
Met	13.1	11.6	.14	.35	14.9	13.1	.14	.40
Phe	16.6	18.6	.23	.75	18.7	20.4	.23	.65
Thr	21.0	21.9	.54	1.00	23.6	24.3	.62	.95
Val	18.9	20.2	.45	1.10	21.2	22.3	.47	1.00
BCAA[4]	67.6	71.7	.46	3.55	75.9	79.4	.48	3.30
EAA[5]	191.9	187.6	.71	8.70	215.6	208.1	.52	7.80

[1]Fractional passage rate.

[2]PUEAA accounts for AA based on feed AA content only.

[3]EUEAA accounts for differential AA losses of protein.

[4]Branched-chain AA.

[5]Essential AA (except Trp).

disagree with data of Table 6 in which several EAA significantly decreased in concentration after water wash and ruminal exposure. However, the relative amounts of several AA did not change appreciably after they were washed in water (Table 6).

The inaccuracy of PUEAA estimates was evident for MM (Table 11). Except for Arg and His, the quantity of all measured EAA available for absorption at the duodenum was significantly lower than that of the original EAA profile of the supplement. Therefore, concen-

TABLE 11. Predicted (PUEAA) and estimated (EUEAA) undegraded AA content of meat meal at two fractional passage rates.

AA	k_p^1 = 5%/h				k_p = 8%/h			
	PUEAA[2]	EUEAA[3]	P <	SE	PUEAA	EUEAA	P <	SE
	— (g/kg of feed CP) —				— (g/kg of feed CP) —			
Arg	15.7	11.8	.28	1.80	18.7	14.6	.33	2.30
His	3.0	3.0	.80	.15	3.6	3.6	.91	.35
Ile	3.5	5.8	.04	.06	4.2	6.9	.05	.20
Leu	7.9	13.6	.04	.35	9.5	16.2	.01	.10
Lys	8.7	11.9	.02	.10	10.5	14.3	.08	.45
Met	2.1	3.0	.04	.05	2.5	3.7	.06	.10
Phe	4.9	6.9	.02	.05	5.9	8.2	.04	.15
Thr	4.4	7.6	.08	.40	5.3	9.0	.06	.30
Val	5.3	8.1	.02	.05	6.3	9.6	.06	.30
BCAA[4]	16.8	27.8	.03	.40	20.1	33.0	.03	.50
EAA[5]	55.4	71.4	.07	1.60	66.3	85.4	.13	3.90

[1]Fractional passage rate.

[2]PUEAA accounts for AA based on feed AA content only.

[3]EUEAA accounts for differential AA losses of protein.

[4]Branched-chain AA.

[5]Essential AA (except Trp).

tration in the EAA profile of the RUP was increased relative to the original CP of the feedstuff.

Both Lys and Met are frequently considered to be first-limiting EAA in most of the rations fed to high producing dairy cows (17, 20). Both PUEAA and EUEAA of these two EAA at 8%/h fractional passage rate are described in Figure 1. Estimated undegraded Lys was less than predicted for SBM (P < .12), CGM (P < .01), and HM (P < .12), but was greater for MM (P < .08). Undegraded Met in SBM was unaffected by calculation procedure. Relative to the predicted values, estimated undegraded Met using the original AA content of the feed was lower for CGM (P < .06) and HM (P < .14) but higher for MM (P < .06).

On the basis of the ruminal disappearance in situ of the EAA in this study, a substitution of CGM for SBM in the ration fed to a high

producing dairy cow would decrease the amount of undegraded Lys by 36% and increase undegraded Met by 261%. Equal replacement of SBM protein with HM should increase undegraded Lys and Met by 237 and 211%, respectively. Conversely, the substitution of MM for SBM should decrease undegraded Lys and Met by 10 and 40%, respectively.

CONCLUSIONS

Application of the original EAA profile of the feed CP to RUP to predict the quantity of individual dietary EAA reaching the small intestine contributed to inaccuracies. Incubation in situ of four different protein sources exhibited selective disappearance of EAA in the rumen. Different rates of disappearance for individual EAA modified the concentration of EAA in the RUP relative to the original CP.

The different affinity to water and the peculiar ruminal disappearance of EAA, across very different feedstuffs, reduce the possibility of accurate prediction of EAA for absorption from RUP.
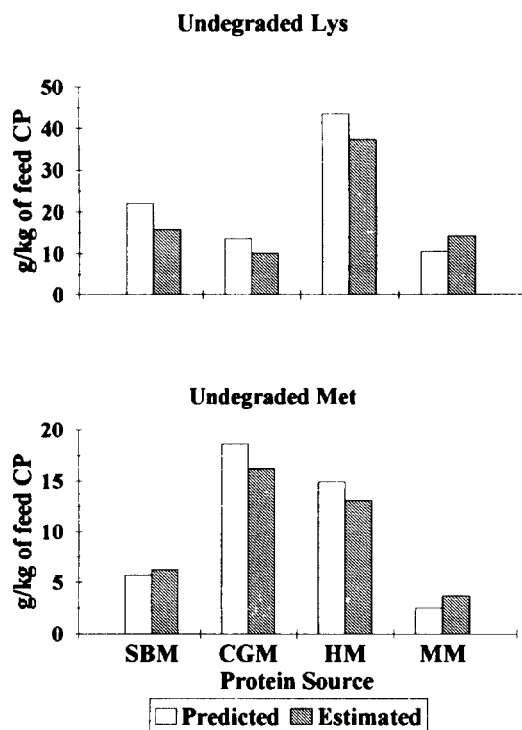
Figure 1. Predicted and estimated ruminally undegraded Lys and Met for soybean meal (SBM), corn gluten meal (CGM), herring meal (HM), and meat meal (MM) at the fractional passage rate of 8%/h.

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