Omasal Flow of Soluble Proteins, Peptides, and Free Amino Acids in Dairy Cows Fed Diets Supplemented with Proteins of Varying Ruminal Degradabilities¹

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ABSTRACT

Three ruminally and duodenally cannulated cows were assigned to an incomplete 4 × 4 Latin square with four 14-d periods and were fed diets supplemented with urea, solvent soybean meal, xylose-treated soybean meal (XSBM), or corn gluten meal to study the effects of crude protein source on omasal canal flows of soluble AA. Soluble AA in omasal digesta were fractionated by ultrafiltration into soluble proteins greater than 10 kDa (10K), oligopeptides between 3 and 10 kDa (3–10K), peptides smaller than 3 kDa (small peptides), and free AA (FAA). Omasal flow of total soluble AA ranged from 254 to 377 g/d and accounted for 9.2 to 15.9% of total AA flow. Averaged across diets, flows of AA in 10K, 3–10K, small peptides, and FAA were 29, 217, 50, and 5 g/d, respectively, and accounted for 10.3, 71.0, 17.5, and 1.6% of the total soluble AA flow. Cows with diets supplemented with solvent soybean meal had higher flows of Met, Val, and total AA associated with small peptides than those whose diets were supplemented with XSBM, whereas supplementation with corn gluten meal resulted in higher total small peptide-AA flows than did XSBM. Averaged across diets, 27, 75, and 93% of soluble AA in 10K, 3–10K, and peptides plus FAA flowing out of the rumen were of dietary origin. On average, 10% of

the total AA flow from the rumen was soluble AA from dietary origin, indicating a substantial escape of dietary soluble N from ruminal degradation. Omasal concentrations and flows of soluble small peptides isolated by ultrafiltration were substantially smaller than most published ruminal small peptide concentrations and outflows measured in acid-deproteinized supernatants of digesta.

Key words: dairy cow, omasal flow, soluble amino acid, peptide

INTRODUCTION

Protein degradation by ruminal microbes results in the formation of ammonia, and peptides and AA are intermediates in this process. Ammonia that accumulates in excess of microbial requirements is converted to urea in the liver and excreted in the urine, resulting in inefficient N utilization by the animal (Broderick et al., 1991). In vitro and in vivo evidence suggests that the rate-limiting step in ruminal protein degradation may be peptide hydrolysis (Russell et al., 1983; Chen et al., 1987b), and the degradation rate may depend on the degradability (Broderick and Wallace, 1988) and AA profile of the protein (Yang and Russell, 1992). Mounting evidence indicates that peptides and free AA (FAA) stimulate microbial yield and fermentation (Argyle and Baldwin, 1989; Chikunya et al., 1996; Carro et al., 1999). Furthermore, peptides (mainly di- and tripeptides) may contribute significantly to AA absorption from the intestines of ruminants (Remond et al., 2000) and may have nutritional benefits for the host animal (Webb and Matthews, 1998). Therefore, alteration of proteolysis and peptide formation in the rumen by dietary manipulation may have nutritional benefits for both ruminal microbes and the host animal, improving utilization of dietary N for productive purposes.

New systems of ruminant ration formulation (AFRC, 1992; Sniffen et al., 1992; NRC, 2001) rely on rate constants for ruminal protein degradation and

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passage. Most commonly, the in situ method has been used to assess the kinetics of protein degradation in the rumen. However, this technique has theoretical limitations that call its accuracy into question (Broderick et al., 1991). Two major concerns are 1) that the rates of ruminal degradation of the soluble N fraction (fraction A) from all feeds incubated in situ are assumed to be infinite and 2) that the portion of dietary protein that is degraded in the rumen (fraction B) is assumed to be entirely used for microbial protein synthesis or production of ammonia and carbon skeletons, or both. However, if a portion of these fractions (i.e., A and B) escapes ruminal degradation as soluble proteins, peptides, and free AA, the validity of these assumptions may not hold true.

Despite the potential nutritional and metabolic importance of the ruminal peptide pool, most of the information available on degradation and metabolism of soluble N fractions in ruminants is based on methodologies that may be unreliable. Peptide AA are usually determined by quantitation of free AA concentrations before and after hydrolysis of samples previously deproteinized by acid precipitation. However, a substantial proportion of the soluble proteins may remain in solution after acid deproteinization (Moughan et al., 1990) and the use of strong acids may result in partial hydrolysis of proteins and release of peptides, leading to an overestimation of the peptide N fraction (Seal and Parker, 1998; Choi et al., 2002a). Moreover, the extent of this chemical deproteinization depends on the type and concentration of the acid used (Greenberg and Shipe, 1979; Moughan et al., 1990; Butts et al., 1991) and on the structure of the proteins being precipitated (Bhatti, 1972; Greenberg and Shipe, 1979). Because the acid type (e.g., perchloric, trichloroacetic, sulfosalicylic), concentration, and times and temperatures of incubation vary widely among published methodologies, interpretation and comparison of data obtained using these techniques should be done with caution. Therefore, a more reliable and reproducible method is needed to study the nutritional and metabolic significance of the flow of soluble AA from the rumen and to test the validity of the in situ technique for estimating rates of protein degradation in the rumen.

The objective of this experiment was to study the effect of feeding diets differing in concentration, ruminal degradability, and source of protein supplement on flows of soluble proteins, peptides, and FAA at the omasal canal with special emphasis on the methodology used for soluble N fractionation.

Table 1. Ingredient and chemical composition of experimental diets¹

Item	Diet			
	A	B	C	D
	(urea)	(SSBM)	(XSBM)	(CGM)
	(% of DM)			
Ingredients				
Alfalfa silage	25.0	25.0	25.0	25.0
Corn silage	35.0	35.0	35.0	35.0
Shelled corn, ground	34.8	32.1	32.7	28.6
Urea	2.3	—	—	—
Solvent soybean meal	—	5.2	—	—
Xylose-treated soybean meal	—	—	4.6	—
Corn gluten meal	—	—	—	8.5
Salt	0.14	0.14	0.14	0.14
Sodium bicarbonate	1.0	1.0	1.0	1.0
Calcium diphosphate	0.60	0.41	0.42	0.45
Limestone	0.60	0.60	0.65	0.65
Sodium sulfate	0.14	0.14	0.14	0.14
Magnesium oxide	0.14	0.14	0.14	0.18
Mineral and vitamin mix ²	0.25	0.25	0.25	0.25
Chemical composition				
DM, %	50.4	50.4	50.7	49.6
CP, % of DM	19.7	15.8	15.3	18.2
NDF, % of DM	31.8	28.6	30.0	28.1
ADF, % of DM	20.0	17.8	17.5	17.6
Starch, % of DM	28.1	28.1	27.2	26.1
TAAN, % of total N	35.5	68.8	64.4	82.3
NPN, % of TN	64.5	31.2	35.6	17.7
NFC, % of DM	41.2	44.8	45.5	42.5
NE _L , ³ Mcal/kg	1.57	1.59	1.56	1.58

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal; TAAN = total AA-N.

²Contained 5.0% Mg, 7.5% K, 10.0% S, 3.0% Zn, 3.0% Mn, 2.0% Fe, 0.5% Cu, 0.025% I, 0.015% Se, 0.004% Co, 2,200 IU of vitamin A/g, 660 IU of vitamin D₃/g, and 8 IU of vitamin E/g.

³Estimated using the NRC (2001) model.

MATERIALS AND METHODS

Animal Management, Experimental Design, and Sampling

Three ruminally and duodenally cannulated lactating Holstein cows averaging 154 (±92) DIM at the beginning of the trial were assigned to an incomplete 4 × 4 Latin square with four 14-d periods and were fed one of 4 diets differing in the proportion and source of the protein supplement. Each cow was randomly assigned to 1 of 3 treatment sequences in which a treatment never followed the same treatment more than once. The TMR contained (DM basis) 35% corn silage, 25% alfalfa silage, 2.7% vitamins and minerals, and 37% concentrate [CP supplement plus ground shelled corn (**GSC**)]. Diets (Table 1) differed in the proportion and source of CP supplement: diet A (urea; 34.8% GSC, 2.3% urea); diet B [32.1% GSC, 5.2% solvent soybean meal (**SSBM**)]; diet C [32.7% GSC, 4.6% xylose-treated SBM (**XSBM**; SoyPass, LignoTech USA, Inc., Rothschild, WI)]; or diet D [28.6% GSC, 8.5% corn gluten meal (**CGM**)]. Details of the surgical

procedures, animal management, and sampling and analyses of silages, concentrates, TMR, and Orts are described by Ipharraguerre et al. (2007).

Milk weights were recorded at each milking throughout the trial. Milk samples were taken at each milking during the last 3 d of each period, preserved with 2-bromo-2-nitropropane-1,3-diol, and stored at 4°C. Samples were sent to Dairy One Cooperative Inc. (Ithaca, NY) for analyses of fat, true protein, and MUN by infrared procedures (Foss 4000; Foss North America, Eden Prairie, MN). Samples of ruminal fluid were collected at 1800, 1900, 2000, and 2200 h on d 8 and at 0100, 0500, 0600, 0700, 0800, 1000, and 1300 h on d 9 of each period. Ruminal samples were collected from multiple sites in the rumen and pH of the ruminal fluid was measured immediately by a glass electrode (Advanced Portable pH/mV/Ion Meter, model AP25; Denver Instrument, Göttingen, Germany); 10-mL subsamples were then preserved by addition of 0.2 mL of 50% (vol/vol) H₂SO₄ and stored at -20°C. Just before analysis, samples were thawed, centrifuged (15,000 × g, 4°C, 15 min), and analyzed for ammonia and total FAA (Broderick and Kang, 1980) using flow-injection analysis (Dual-Channel QuikChem 8000 FIA; Lachat Instruments, Milwaukee, WI), and for VFA (Brotz and Schaefer, 1987).

Digesta flow leaving the rumen was quantified using the omasal sampling technique developed by Huhtanen et al. (1997) as modified by Ahvenjärvi et al. (2000). Indigestible NDF, CoEDTA, and YbCl₃ were used as markers to assess digesta flows at the omasal canal, and the external microbial marker ¹⁵N was used to measure microbial N flows from the rumen. Details on marker preparation, infusion, and analyses are reported in Ipharraguerre et al. (2007). Omasal spot samples of 355 mL were collected from the omasal canal on d 12, 13, and 14 of each period such that the 12 samples represented a 24-h feeding cycle over 3 d. Each spot sample was swirled vigorously and poured into 3 containers to obtain subsamples of 200, 125, and 30 mL. The 125-mL subsamples were pooled over the 4 daily sampling times to yield one 500-mL composite from each cow on each sampling day, and the composite sample was processed later that day to isolate bacteria. The 200-mL subsamples were pooled and stored at -20°C as they were collected over all 12 sampling times, to yield one 2.4-L omasal composite from each cow in each period. The 30-mL subsamples were squeezed through 1 layer of 0.45-μm Dacron mesh, the filtrate centrifuged at 50,000 × g for 15 min at 5°C to precipitate particulate matter, and the supernatant was held on ice until stored at -80°C within 30 min of collection for later determination of soluble N flow at the omasal canal.

The 2.4-L pooled omasal composites were separated into 3 digesta phases: fluid phase (**FP**), large particle phase (**LP**), and small particle phase (**SP**). Concentrations of Co, Yb, and indigestible NDF in LP and SP and of Co and Yb in FP were used to mix DM from freeze-dried FP, SP, and LP in the correct proportions to reconstitute the omasal true digesta (**OTD**) flowing out of the rumen based on the triple-marker method of France and Siddons (1986). Digesta phase separation and marker analyses are described in Ipharraguerre et al. (2007). Aliquots of the SP and LP phases were mixed in the correct proportions based on the markers to yield a 2-g sample that was ground through a 0.5-mm screen with a Udy Cyclone Sample mill (Udy Corporation, Fort Collins, CO) and defined as small plus large particles (**SP + LP**). Feed and OTD samples were sequentially ground through 2- and 1-mm screens using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and analyzed for DM at 105°C; ash and OM (AOAC, 1980); and total N using a combustion assay (Leco FP-2000 N Analyzer; Leco Instruments, Inc., St. Joseph, MI).

At the end of each sampling day, the 500-mL omasal composites were separated to yield fluid and particle phases that were equivalent to the FP and the SP + LP phases from the 2.4-L composites, respectively. Fluid-associated bacteria (**FAB**) and particle-associated bacteria (**PAB**) were isolated from these fluid and particle phases, respectively, and pooled over the 3 sampling days of each period to yield one composite each of FAB and PAB per cow per period. The OTD, FAB, PAB, and individual feeds were analyzed for 16 AA after hydrolysis in 6 N HCl (Nagel and Broderick, 1992), using norleucine as the internal standard and ion-exchange chromatography with ninhydrin detection (Beckman 6300 Amino Acid Analyzer; Beckman Instruments, Inc., Palo Alto, CA). Samples of ¹⁵N natural background, FAB, PAB, FP, and SP + LP (100 μg of N) were weighed into tin cups (5 × 9 mm; Costech Analytical Technologies, Valencia, CA) and, after addition of 50 μL of 72-mM K₂CO₃ solution, were placed in a 60°C oven to volatilize ammonia. Samples were analyzed for NAN and ¹⁵N enrichment of NAN by isotope ratio mass spectrometry (Stable Isotope Facility, Department of Agronomy and Range Science, University of California-Davis, Davis, CA). Other details about collection, processing, and analysis of samples of OTD, FAB, and PAB are reported in Ipharraguerre et al. (2007).

Fractionation of Soluble N Fractions

At the end of the experiment, the 30-mL omasal subsamples were fractionated as outlined in Figure 1.

Figure 1. Fractionation and analysis of soluble proteins, peptides, and free AA in omasal digesta. PVDF = polyvinylidene fluoride; HArg = homoarginine (internal standard); MW = molecular weight; 3–10K = 3 to 10 kDa; 10K = 10 kDa.

Samples were slowly thawed in an ice bath and a 13.5-mL aliquot was centrifuged at $50,000 \times g$ for 15 min at 0°C . The supernatant was filtered through a 0.1- μm filter (Millex-VV, no. SLVV033RB, PVDF filter, Millipore, Billerica, MA) and 5 mL of filtrate was saved

for later analysis of FAA. A 5.4-mL aliquot of filtrate was pipetted into a tared 10-kDa molecular weight (MW) cut-off ultracentrifugation filter with a cellulose membrane (Amicon-Ultra, no. UFC901024, Millipore) and 0.6 mL of 2 mM homoarginine solution was added

as an internal standard for determination of FAA in retentates. This filtration device was centrifuged at $5,000 \times g$ for 30 min at 5°C . Then, 6 mL of McDougall's buffer (McDougall, 1948) was added to the retentate and the device was centrifuged again at $5,000 \times g$ for 30 min at 5°C . The vials from the filtration device containing filtrate and retentate solutions were weighed and their weights recorded for recovery calculations. A 0.5-mL aliquot of retentate was transferred to a glass culture tube for later hydrolysis and determination of AA in soluble proteins greater than 10 kDa (**10K**) in omasal digesta. The remaining retentate solution was stored at -80°C for later analysis. An 8-mL aliquot of filtrate was transferred to a tared 3-kDa MW cut-off ultracentrifugation filter with a cellulose membrane (Centriprep-3, no. 4302, Millipore) and centrifuged once at $3,000 \times g$ at 5°C for 65 min and then 4 more times for 20 min at the same speed and temperature. Filtrate and retentate vials containing their corresponding solutions were weighed and their weights recorded for recovery calculations. A 0.25-mL aliquot of retentate and a 1-mL aliquot of filtrate were transferred to glass culture tubes for later hydrolysis and determination of individual AA in soluble oligopeptides between 3 and 10 kDa (**3–10K**), and peptides of less than 3 kDa (called small peptides hereafter) plus FAA. The remaining retentates and filtrates were stored at -80°C for later analysis. To the tubes containing 0.5 mL of 10K retentate and 0.5 mL of small peptides plus FAA filtrate, 0.625 mL of 12 N HCl with 1 g/L of phenol and 0.125 mL of 1 mM norleucine were added. To tubes containing 0.25 mL of 3–10K retentate, 312.5 μL of 12 N HCl with 1 g/L of phenol and 62.5 μL of 1 mM norleucine were added. All tubes were flushed with N_2 gas for 20 s, sealed with Teflon tape and capped tightly, and heated at 110°C for 24 h (Gehrke et al., 1985; Nagel and Broderick, 1992). Hydrolysates were evaporated to moistness using a concentrator (SpeedVac Concentrator SVC-200H, Savant Instruments, Inc., Farmingdale, NY), redissolved in 1 mL of distilled water, evaporated again, dissolved in 1 mL of pH 2.2 sodium citrate buffer (Na-S, Beckman Instruments, Inc., Palo Alto, CA), and analyzed for individual AA by ion-exchange chromatography as described earlier. The 5-mL filtrates saved for FAA analysis were evaporated to moistness and dissolved in 2 mL of pH 2.2 sodium citrate buffer. An attempt was made to analyze FAA on this nonhydrolyzed sample containing peptide- and protein-bound AA. However, ammonia and peptide-bound AA appeared to saturate the binding sites of the ion-exchange column, hindering chromatographic separation. Therefore, 1 mL of 2 M K_2CO_3 was added to 1 mL of evaporated and buffer-dissolved sample, heated at 60°C for 24 h

to volatilize ammonia, the pH adjusted to 2.2 by adding 2 mL of 0.16 M sodium citrate and 2.2 M HCl, and filtered through a 3-kDa MW cut-off ultracentrifugation filter before analysis of individual AA by ion-exchange chromatography as described earlier. The concentration of homoarginine in each of the 4 soluble N fractions was used to compute the content of each AA in its free form in the 10K, 3–10K, and small peptide fractions as follows:

$$\begin{aligned} \text{AA}_i \text{ from FAA in bound AA fraction} = \\ (\text{AA}_i \text{ in FAA/HArg in FAA}) \\ \times \text{HArg in bound AA fraction} \end{aligned}$$

where bound AA fraction is either 10K, 3–10K, or small peptide.

Free AA remaining in bound AA fractions were subtracted from those fractions and added to the FAA fraction. Remaining retentates from the 10-kDa membrane and both retentates and filtrates from the 3-kDa membranes were thawed and an equal volume of 2 M K_2CO_3 was added. Samples were evaporated using a SpeedVac concentrator to a volume of approximately 200 μL . Concentrated samples were transferred to tin cups (5×9 mm; Costech Analytical Technologies, Valencia, CA) and placed in a 60°C oven for 24 h to volatilize ammonia. Samples were analyzed for ^{15}N enrichment by isotope ratio mass spectrometry as described earlier. The ^{15}N enrichments of FAB and PAB and of each soluble N fraction were used to calculate the proportion of soluble N of microbial origin in each fraction.

Statistical Analyses

Data were analyzed using PROC MIXED in SAS (SAS Institute, 1999). The following model was fitted to all variables that did not have repeated measurements over time:

$$Y_{jkl} = \mu + P_j + C_k + T_l + \varepsilon_{jkl},$$

where Y_{jkl} is the dependent variable, μ is the overall mean, P_j is the effect of period j , C_k is the effect of cow k , T_l is the effect of treatment l , and ε_{jkl} is the residual error. The degrees of freedom for treatment were partitioned into 3 single-degree-of-freedom orthogonal contrasts: urea vs. true protein (urea vs. CGM), high vs. low predicted protein degradability (SSBM vs. XSBM), and soybean vs. corn protein (XSBM vs. CGM). All terms were considered fixed except for C_k , which was considered random.

The following model was used for ruminal variables for which there were repeated measurements over time (pH, NH_3 , and total FAA):

$$Y_{jklm} = \mu + P_j + C_k + T_l + \varepsilon_{jkl} + Z_m + ZT_{ml} + \omega_{jklm}$$

where Y_{jklm} is the dependent variable, μ is the overall mean, P_j is the effect of period j , C_k is the effect of cow k , T_l is the effect of treatment l , ε_{jkl} is the whole plot error, Z_m is the effect of time m , ZT_{ml} is the interaction between time m and treatment l , and ω_{jklm} is the subplot error. Based on the largest value for Akaike's information criterion (Littell et al., 1996), the heterogeneous autoregressive structure ARH(1) was selected as the appropriate covariance structure. The subject of the repeated measurements was defined as cow (square $\times$ period $\times$ treatment). All terms were considered fixed, except for C_k , which was considered random. Because the ZT_{ml} interaction was not significant at $\alpha = 0.05$ for any of the ruminal variables measured, treatment means were compared across sampling times using the contrasts described above. Differences between least squares means were considered significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Diet Composition, Animal Production, and Ruminal Digestion

Diets were formulated to be isoenergetic (mean = 1.58 Mcal/kg of NE_L) and to provide 2 levels of CP: high (19.7 and 18.2% for diets A and D) and low (15.8 and 15.3% for diets B and C; Table 1). Within each CP level, diets differed in the N form (urea vs. true protein for diets A and D), the predicted ruminal degradability of the soybean protein (nontreated vs. treated SBM for diets B and C), and the source of the protein (soybean vs. corn for diets C and D). Dietary concentrations (% of dietary DM) of NDF, ADF, starch, and NFC ranged from 28.1 to 31.8%, from 17.5 to 20%, from 26.1 to 28.1%, and from 41.2 to 45.5%, respectively. Diets differed substantially in the proportion of the total N in AA, with diet D being highest (82.3%), diets B and C intermediate (68.8 and 64.4%, respectively), and diet A the lowest (35.5%). The chemical composition of the silages and concentrate mixes is presented in Table 2.

Because of the small number of experimental units, the milk production and composition data reported herein are least squares means averaged across diets. Yields of milk, milk fat, and milk true protein averaged 31.2, 1.39, and 0.91 kg/d across diets. Concentrations of fat, true protein, and urea N in milk averaged 4.49%, 2.98%, and 10.6 mg/dL across diets.

When fed high-CP diets, cows whose diets were supplemented with urea had similar DMI, ruminal pH, and FAA concentrations but a higher ruminal ammonia concentration (Table 3) than cows whose diets were supplemented with CGM. Previous reports indicate that intakes of DM by lactating cows fed diets supplemented with urea were depressed, either significantly (Reynal and Broderick, 2003) or not significantly (Wohlt et al., 1991), by 2 kg/d compared with cows whose diets were supplemented with CGM. However, urea-supplemented diets contained less CP than did CGM diets, confounding the effects of source and level of dietary protein (Wohlt et al., 1991; Reynal and Broderick, 2003). Within the low-CP diets, there was no difference between SSBM and XSBM on DMI or any of the ruminal variables measured. Replacing XSBM with CGM resulted in a significant decrease in DMI and ruminal FAA concentration. These results are in agreement with data from a meta-analytic review of the literature (Ipharraguerre and Clark, 2005) showing that the replacement of SSBM with corn by-products frequently depresses DMI by lactating dairy cows. It should be noted, however, that in this study the effects of dietary protein source (soybean vs. corn) and concentration (15.3 vs. 18.2% CP) are confounded. Within CP level, neither urea vs. true protein nor SSBM vs. XSBM had significant effects on omasal flows and ruminal digestibilities of DM and OM (Table 3). Higher DMI for cows fed XSBM resulted in higher flows of DM and OM at the omasal canal, compared with cows fed CGM. However, apparent ruminal digestibility of DM and true ruminal OM digestibility were not affected by dietary treatment. As expected, cows fed diets supplemented with urea had smaller flows of total NAN and nonammonia nonmicrobial N (NANMN) at the omasal canal but a higher true ruminal digestibility of N than cows fed CGM (Table 4). Cows fed XSBM had a greater flow of NANMN (222 vs. 177 g of N/d) but a similar flow of microbial NAN compared with cows fed SSBM, resulting in a trend ($P < 0.09$) for a greater flow of NAN. Previous work showed similar changes in the ruminal outflow of N fractions when SSBM was replaced with XSBM (Ipharraguerre et al., 2005) and other rumen-protected soy products (Ipharraguerre and Clark, 2005). Replacing XSBM with CGM depressed the omasal flows of total NAN (from 622 to 538 g/d) and NANMN (from 222 to 163 g/d), at least in part because of the associated effects on DMI. Microbial efficiencies were not significantly affected by dietary treatments (Table 4).

As expected from the AA concentrations of the concentrate mixes (Table 2), the intakes of all AA, except Lys, were significantly higher for cows fed CGM compared with urea, and intakes of individual AA were

Table 2. Nutrient composition of silages and concentrate mixes¹

Item ²	Alfalfa silage	Corn silage	Urea mix	SSBM mix	XSBM mix	CGM mix
OM, % of DM	88.0	95.0	90.3	89.6	89.6	91.1
CP, % of DM	22.5	8.8	29.3	16.8	17.1	26.0
NDF, % of DM	39.0	33.5	9.0	9.0	12.4	6.6
ADF, % of DM	30.0	18.7	2.1	2.9	3.4	1.7
Starch, % of DM	0.46	31.6	45.2	42.4	40.8	33.7
TAAN, % of TN	51.2	55.5	20.6	93.8	77.0	100.0
NPN, % of TN	48.8	44.5	79.4	6.2	23.0	0.0
AA, % of total AA						
Essential						
Arg	2.29	2.56	5.42	5.87	6.10	3.19
His	1.85	1.97	2.52	2.46	2.49	1.78
Ile	4.67	4.39	2.77	3.29	3.55	2.89
Leu	8.24	11.2	10.7	8.47	8.93	14.5
Lys	4.89	2.87	3.21	5.39	4.32	1.73
Met	1.36	1.65	1.72	1.30	1.37	1.93
Phe	5.07	4.02	4.17	4.57	4.43	4.96
Thr	5.08	4.86	3.99	3.99	4.21	3.40
Val	6.17	6.23	3.94	4.00	3.87	3.85
Nonessential						
Ala	8.57	12.6	7.19	5.03	5.43	8.35
Asp	17.4	7.41	8.02	11.0	10.8	6.34
Cys	1.56	2.06	3.49	2.73	2.56	2.64
Glu	10.5	16.6	19.8	21.4	20.9	23.3
Gly	5.47	5.68	4.63	4.09	4.61	2.92
Pro	7.45	8.48	8.87	7.25	6.88	8.23
Ser	5.62	4.03	5.34	5.51	5.60	5.31
Tyr	3.86	3.33	4.27	3.58	3.91	4.67

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

²TAAN = total AA-N (total N determined as N in 17 AA in protein from acid hydrolysates of each silage and concentrate mix); NPN = total N – total AA-N.

not significantly different for cows fed the SSBM and XSBM diets (Table 5). Although cows fed CGM consumed 4.5 kg less DM than cows fed XSBM, the higher total AA concentration in the CGM diet (82.3 vs. 64.4% of total N; Table 1) resulted in intakes of Leu, Met, Phe, Ala, Cys, Glu, Pro, and Tyr that were significantly higher than those for cows fed XSBM (Table 5). Con-

versely, the higher Lys content in soybean meal (Table 2) and the greater DMI of cows fed XSBM resulted in a higher Lys intake for cows fed XSBM compared with those fed CGM.

Except for Arg, His, Lys, Met, Thr, Val, and Gly, omasal flows of individual and total AA were significantly higher for cows fed CGM compared with urea

Table 3. Effect of dietary treatment on DMI, milk production, ruminal metabolism, omasal flows, and ruminal digestibilities

Item	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	A (urea)	B (SSBM)	C (XSBM)	D (CGM)		A vs. D	B vs. C	C vs. D
DM intake, kg/d	18.1	20.3	22.5	18.0	2.2	0.93	0.16	0.03
Rumen								
pH	6.06	6.27	6.07	6.15	0.14	0.58	0.20	0.62
Free AA, mM	2.76	3.36	3.79	2.53	0.29	0.56	0.25	<0.01
NH ₃ -N, mg/dL	22.2	13.3	10.6	12.3	1.4	<0.01	0.14	0.35
Flow at the omasal canal, kg/d								
DM	11.3	13.8	14.0	11.6	1.1	0.53	0.66	<0.01
OM	8.97	11.27	11.28	9.11	0.91	0.72	0.97	<0.01
Ruminal digestibility, %								
DM, apparent	37.9	30.3	38.2	33.6	3.4	0.40	0.17	0.38
OM, true	60.7	53.6	59.2	59.0	1.9	0.57	0.12	0.93

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

Table 4. Effect of dietary treatment on intake, flow at the omasal canal, and ruminal digestibility of N fractions

Item ¹	Diet ²				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Total N intake, g/d	575	500	572	519	63	0.24	0.15	0.25
Omasal flows, g/d								
Total NAN	456	567	622	538	36	0.03	0.09	0.03
LAB-NAN	102	132	134	120	7	0.17	0.84	0.25
PAB-NAN	259	258	266	255	26	0.90	0.75	0.68
Total microbial NAN	361	389	400	375	28	0.57	0.66	0.34
NANMN	95	177	222	163	14	<0.01	0.01	<0.01
Microbial efficiency, g/kg	35.8	40.5	32.9	40.1	3.5	0.36	0.15	0.17
True ruminal N digestibility, %	84.3	63.7	60.3	66.0	4.9	0.03	0.59	0.84

¹LAB-NAN = liquid-associated bacteria NAN, microbial efficiency = g of microbial NAN/kg of OM truly digested in the rumen; PAB-NAN = particle-associated bacteria NAN; NANMN = nonammonia nonmicrobial N.

²SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

(Table 6). Replacing SSBM with XSBM resulted in a significant increase in the flows of Phe, Glu, and Ser, and a trend for a significant increase in total AA flow ($P = 0.10$), which is in general agreement with data reported elsewhere (Ipharraguerre and Clark, 2005; Ipharraguerre et al., 2005). In the present trial, flows of Arg, Ile, and Lys were significantly reduced when XSBM was replaced with CGM in the diets. Similarly, replacement of SSBM with CGM significantly reduced the flow of Lys to the intestines of cows fed a corn silage-based diet (Klusmeyer et al., 1990). However, when compared with XSBM, the higher intake of Leu

by cows fed CGM (457 vs. 252 g of AA/d; Table 5) resulted in a significantly higher omasal flow of this essential AA (265 vs. 241 g of AA/d; Table 6).

There were no dietary effects on flows of individual AA of microbial origin (Table 7). However, flows of AA of dietary origin were significantly affected by diets (Table 8). Cows fed diets supplemented with urea had significantly lower flows of 3 essential AA (Leu, Phe, and Val), 6 nonessential AA (Ala, Cys, Glu, Pro, Ser, and Tyr), and total AA of dietary origin, compared with cows fed CGM diets. The flow of Leu was significantly higher for cows fed CGM, compared with those fed

Table 5. Effect of dietary treatment on intake of AA (g/d)

AA	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Essential								
Arg	55	118	116	103	11.8	0.01	0.9	0.26
His	33.8	59.5	59.8	63.5	6.6	<0.01	0.94	0.5
Ile	63	102	109	119	12	<0.01	0.5	0.36
Leu	160	229	252	457	40	<0.01	0.58	0.01
Lys	61	132	113	85	11.5	0.07	0.13	0.05
Met	25.2	35.8	39.1	63.3	5.6	<0.01	0.55	0.02
Phe	73	121	123	171	16	<0.01	0.88	0.04
Thr	74	118	124	136	13	<0.01	0.58	0.34
Val	86	131	135	159	15	<0.01	0.76	0.13
Nonessential								
Ala	146	188	210	311	28	<0.01	0.41	0.02
Asp	188	328	329	298	34	0.02	0.96	0.3
Cys	37.8	61.0	59.4	83.5	7.8	<0.01	0.81	0.03
Glu	249	465	465	711	65	<0.01	0.99	0.03
Gly	83.1	126.5	136.7	129.8	13.9	0.02	0.36	0.5
Pro	132	198	201	285	26	<0.01	0.9	0.03
Ser	83.3	140.3	144.8	183.8	18.0	<0.01	0.78	0.07
Tyr	62.7	94.7	103.9	153.9	14.1	<0.01	0.5	0.03
Total	1,616	2,649	2,724	3,516	334	0.01	0.83	0.09

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

Table 6. Effect of dietary treatments on flows of individual AA (g/d) at the omasal canal

AA	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Essential								
Arg	84	124	136	107	9	0.10	0.18	0.04
His	29.4	47.6	48.4	36.4	4.1	0.32	0.95	0.14
Ile	96	126	131	112	9	0.03	0.24	0.02
Leu	178	225	241	265	16	<0.01	0.11	0.05
Lys	113	152	150	114	8	0.83	0.75	<0.01
Met	60.1	71.3	78.1	70.8	13.3	0.65	0.76	0.68
Phe	99	123	144	137	11	<0.01	0.03	0.27
Thr	106	136	145	125	9	0.1	0.37	0.09
Val	93	120	129	114	9	0.06	0.27	0.12
Nonessential								
Ala	143	180	186	190	11	<0.01	0.48	0.65
Asp	234	316	348	277	20	0.03	0.07	<0.01
Cys	39.5	49.0	52.9	50.5	3.2	<0.01	0.11	0.27
Glu	309	414	476	452	26	<0.01	0.01	0.13
Gly	109	140	150	125	9	0.09	0.23	0.03
Pro	94	122	131	141	7	<0.01	0.07	0.09
Ser	101	134	146	134	8	<0.01	0.03	0.03
Tyr	97	126	131	126	8	0.01	0.43	0.43
Total	1,985	2,604	2,822	2,576	158	0.04	0.08	0.10

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

XSBM. Replacement of SSBM with XSBM did not affect the flows of individual AA of dietary origin.

Soluble NAN Fractionation and Analysis: Methodological Considerations

Despite the potential benefits of small peptides on ruminant nutrition, most studies on digestion and me-

tabolism of peptides have relied on techniques that may result in biased estimates of peptide concentration. Most commonly, samples are first chemically deproteinized by acid precipitation, and peptide concentration in the supernatant is measured by the “difference” method, in which peptide AA are calculated as the difference between total AA in hydrolyzed supernatant minus free AA before hydrolysis. However,

Table 7. Effect of dietary treatments on flows of microbial AA (g/d) at the omasal canal

AA	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Essential								
Arg	74.4	98.3	94.6	89.8	6.6	0.17	0.59	0.50
His	27.7	31.5	29.9	27.1	3.6	0.90	0.75	0.56
Ile	84.6	96.4	96.4	85.9	10.4	0.93	0.99	0.48
Leu	137	155	158	141	14	0.79	0.85	0.38
Lys	103	131	136	106	11	0.84	0.75	0.14
Met	42.0	45.9	56.2	60.2	12.6	0.47	0.65	0.82
Phe	81.1	96.8	96.9	87.9	8.5	0.57	0.99	0.46
Thr	94.5	108.8	110.9	99.0	8.9	0.73	0.87	0.38
Val	86.8	100.7	102.0	90.2	9.0	0.78	0.91	0.36
Nonessential								
Ala	129	148	150	133	11	0.76	0.89	0.30
Asp	205	268	257	215	24	0.79	0.77	0.31
Cys	27.4	31.6	31.8	28.1	2.6	0.85	0.95	0.40
Glu	248	287	291	259	23	0.75	0.91	0.36
Gly	95.3	109.1	110.8	98.9	8.6	0.75	0.88	0.34
Pro	65.5	73.4	74.9	67.5	6.1	0.81	0.86	0.40
Ser	80.0	91.8	94.2	83.0	7.5	0.77	0.82	0.32
Tyr	86.3	100.5	99.1	89.4	8.4	0.78	0.90	0.41
Total	1,668	1,974	1,990	1,762	125	0.25	0.93	0.27

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

Table 8. Effect of dietary treatments on flows of nonammonia nonmicrobial AA (g/d) at the omasal canal

AA	Diet ¹				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Essential								
Arg	9.5	26.1	39.9	18.4	7.7	0.47	0.25	0.13
His	1.7	16.4	18.4	9.3	5.0	0.37	0.80	0.30
Ile	12.2	29.2	35.0	26.3	6.8	0.25	0.60	0.45
Leu	42	70	83	123	9	<0.01	0.31	0.03
Lys	9.9	20.9	13.8	8.0	6.5	0.85	0.50	0.58
Met	19.0	22.0	22.4	11.0	11.9	0.70	0.99	0.55
Phe	18.0	25.2	47.3	48.9	6.3	0.02	0.06	0.83
Thr	11.7	27.2	33.5	25.7	4.4	0.08	0.32	0.24
Val	6.4	18.7	26.8	23.5	3.9	0.04	0.20	0.55
Nonessential								
Ala	14.7	31.7	35.3	56.0	5.0	0.01	0.65	0.06
Asp	28	48	91	61	25	0.40	0.30	0.46
Cys	12.0	17.5	21.2	22.4	2.7	0.04	0.31	0.71
Glu	60	127	185	193	22	0.02	0.14	0.80
Gly	13.8	31.2	39.2	25.8	3.5	0.08	0.18	0.06
Pro	28.6	48.5	56.9	73.2	4.7	<0.01	0.22	0.06
Ser	21.1	42.2	52.1	51.0	6.2	0.04	0.32	0.90
Tyr	10.5	25.2	31.4	36.2	3.9	0.02	0.34	0.45
Total	319	627	832	814	95	0.04	0.17	0.61

¹SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

agents and conditions used for deproteinization vary substantially among published methodologies. Trichloroacetic, perchloric, and sulfosalicylic acids have been widely used to deproteinize samples of whole blood, blood plasma, and ruminal and intestinal contents. Concentrations of acid, temperatures, and times of incubation have varied. Trichloroacetic acid has been used at a final concentration of 8.3% (wt/vol) followed by incubation at room temperature for 1 h (Volden et al., 2002); at 5% (wt/vol) and samples held on ice for either 30 min (Broderick and Wallace, 1988; Choi et al., 2002a) or overnight (Choi et al., 2002a); and at 10% (wt/vol) and samples incubated at 4°C for 17 h (Moughan et al., 1990; Butts et al., 1991). Perchloric acid has been used at 5% (wt/vol) followed by immediate centrifugation (Chen et al., 1987a; Wallace and McKain, 1990; Backwell et al., 1997), and at 7% (wt/vol) followed by incubation at 22°C for 10 to 15 min (Moughan et al., 1990). Finally, deproteinization conditions used with sulfosalicylic acid have been 3.6% (wt/vol) followed by storage at -80°C (Remond et al., 2000); 2.5% (wt/vol) at 4°C for 16 h (Galibois et al., 1991); and 4% (wt/vol) at room temperature for 15 min (Bernard et al., 2001).

The cut-offs of tungstic and trichloroacetic acids are considered to be approximately at peptide sizes of 3 and 10 AA, respectively (Licitra et al., 1996). Assuming an average MW of 127 per AA (based on the average occurrence of individual AA in over 200 proteins; Klappper, 1977), 3 and 10 AA would correspond to average

MW of 381 and 1,270, respectively. However, the extent of deproteinization and the MW of the proteins being precipitated are dependent on the type and concentration of the acid used. Trichloroacetic (10% wt/vol) and perchloric acids (7% wt/vol) precipitated, respectively, 26 and 70% of the total soluble N in ileal digesta of rats (Butts et al., 1991). Greenberg and Shipe (1979) reported that complete precipitation of β -LG was achieved at about 15, 20, and 0.5% (wt/vol) of trichloroacetic, sulfosalicylic, and tungstic acids, respectively. In addition, more than one-half of the soluble N from ileal digesta of rats remained in solution after deproteinization with perchloric (7% wt/vol) and trichloroacetic acids (10% wt/vol) with, respectively, 35 and 69% of the supernatant N being in the form of soluble protein (Ohara and Ariyoshi, 1979; Moughan et al., 1990). Additionally, the extent of precipitation also depends on protein structure. When TCA was used at 5% (wt/vol) to precipitate β -LG, BSA, keratin, hemoglobin, myosin, and actomyosin, 12, 0, 14, 4, 1, and 21% of the protein remained in the supernatant, respectively (Greenberg and Shipe, 1979). Even at a 10% (wt/vol) concentration of TCA, 11% of keratin remained in solution (Greenberg and Shipe, 1979). In the study of Bhatti (1972), complete precipitation of soluble proteins from rapeseed meal required a 6-fold increase in TCA concentration compared with that required to precipitate CN and hemoglobin. Furthermore, as suggested by Choi et al. (2002a) and Seal and Parker (1998), the use of strong acids may result in

partial hydrolysis of proteins, release of peptides, and overestimation of the peptide-N fraction. Therefore, interpretation and comparison of data obtained using these techniques should be done with caution.

Alternatively, soluble N fractions can be separated using UF with membranes of different MW cut-offs without addition of acids. When filtered through a 10-kDa cut-off membrane, from 88 to 94% (mean 91%) of the N in purified proteins of MW between 14,300 and 669,000 Da was recovered in the retentate, whereas from 90 to 100% (mean 95%) of the N in peptides between 146 and 6,000 Da was recovered in the filtrate solution (Butts et al., 1991). When peptide concentrations in sheep blood and plasma measured in the filtrate from a 3-kDa cut-off filter were compared with those measured in the supernatant after deproteinization with sulfosalicylic acid, chemical deproteinization overestimated peptide concentrations in blood and plasma samples by 160 and 56%, respectively (Bernard et al., 2001). Backwell (1998) reported that oligopeptides of MW as high as 9.3 kDa remained in the supernatant after deproteinization of sheep blood samples with sulfosalicylic acid (final concentration of 13% wt/vol). When plasma samples from sheep that were deproteinized with sulfosalicylic acid (final acid concentration of 3.3% wt/vol) were filtered through a 3-kDa cut-off membrane, peptide AA concentrations were less than half of those in deproteinized samples without filtration (Remond et al., 2000). Ultrafiltration has also been used as a pretreatment before separating peptide AA by reverse-phase chromatography (Seal and Parker, 1996). Because UF, alone or followed by chromatographic separation, should not affect the structure and solubility of proteins and peptides, its use may result in more accurate estimates of soluble N fractions compared with chemical deproteinization. However, the selectivity of the membranes used in UF depends on the size and shape of the proteins and decreases as the MW approaches the cut-off value of the membrane (Butts et al., 1991; Millipore Technical Guide, Millipore Corp.). Moreover, a small proportion of the total volume filtered containing molecules smaller than the MW cut-off will remain in the retentate. Therefore, the MW cut-offs of the membranes are approximate values and some degree of cross-contamination among fractions should be expected. Caution is needed when handling the samples during collection and analysis to avoid protein degradation and contamination. Bacterial and protozoal cells should be immediately removed after sampling to avoid cell lysis and overestimation of soluble N fractions. Functional proteases and peptidases will result in inaccurate estimates of soluble N fractions if supernatants are not immediately processed or stored, preferably at -80°C ,

until processed. These are some disadvantages compared with chemical deproteinization, where enzyme activity is immediately stopped by acidification, facilitating sample handling. Nonetheless, immediate high-speed centrifugation followed by freezing in liquid N should minimize degradation and contamination of soluble N fractions separated by UF. Therefore, UF of properly processed and stored samples is a preferred method of separating soluble N fractions than chemical deproteinization.

Omasal Flows of Total AA in Each Soluble N Fraction

Omasal flows of AA in proteins greater than 10K averaged 29 g/d across diets ($P > 0.10$), accounting for 1.1 to 1.6% of total AA flow and from 7 to 13% of the total soluble AA flow (Table 9). Flows of AA in soluble 3–10K oligopeptides averaged 217 g/d across diets ($P > 0.10$) and accounted for 6.4 to 12.5% of total AA flows at the omasal canal and for most of the soluble AA flow (65 to 79%; Table 10). Cows supplemented with urea tended ($P = 0.09$) to have lower proportions of soluble AA as 3–10K oligopeptides than cows fed true protein from CGM (65.4 vs. 79.3%). Soluble AA fractions containing bound AA of MW above 3 kDa accounted for 78 to 86% of the soluble AA flow and 7.5 to 13.6% of the total AA flow at the omasal canal. On the contrary, soluble protein (defined as NAN in a 10% TCA precipitate) accounted for only 0.9 to 1.6% (Choi et al., 2002b), 21 to 23% (Choi et al., 2003), and 20 to 26% (Choi et al., 2002a) of the total omasal flow of soluble NAN (SNAN). However, incomplete precipitation of proteins by acid deproteinization might have resulted in underestimation of soluble proteins in these studies.

Flows of small peptides were significantly affected by the protein supplement (Table 11). Cows whose diets were supplemented with SSBM had higher flows of small peptide-AA than those whose diets were supplemented with XSBM (59.6 vs. 45.5 g of AA/d), whereas supplementation with CGM resulted in higher small peptide-AA flows than did XSBM (49.1 vs. 45.5 g of AA/d). However, small peptide-AA accounted for only 1.63 to 2.80% of the total AA flow and 12.7 to 20.3% of the total soluble AA flow. Similarly, Broderick and Wallace (1988) estimated that between 1.2 and 2.4% of the ruminal NAN flow in sheep fed ryegrass-based diets was contributed by FAA and peptides, determined by trinitrobenzene sulfonic acid and fluorescamine reactions, respectively, on TCA supernatants. However, peptide-AA concentrations in the perchloric acid supernatant of the rumen fluid of sheep assayed by fluorescamine reaction were, on av-

Table 9. Effect of dietary treatment on omasal canal flows of individual AA (g/d) in soluble proteins of molecular weights greater than 10 kDa

AA ¹	Diet ²				SEM	Contrast (<i>P</i> -value)		
	A (urea)	B (SSBM)	C (XSBM)	D (CGM)		A vs. D	B vs. C	C vs. D
Essential								
Arg	0.96	0.87	0.90	0.72	0.19	0.32	0.91	0.4
His	0.63	0.59	0.59	0.52	0.11	0.42	0.97	0.49
Ile	1.12	1.22	1.22	1.12	0.19	0.95	0.85	0.56
Leu	1.87	1.69	1.69	1.41	0.37	0.38	1	0.55
Lys	2.04	1.93	2.04	1.62	0.37	0.38	0.79	0.39
Met	0.34	0.34	0.24	0.32	0.06	0.76	0.29	0.33
Phe	0.78	0.77	0.79	0.64	0.21	0.62	0.92	0.57
Thr	2.47	2.55	2.55	2.21	0.34	0.57	0.85	0.41
Val	1.84	1.84	1.84	1.50	0.33	0.4	0.95	0.43
Nonessential								
Ala	2.54	2.80	2.80	2.29	0.38	0.54	0.78	0.36
Asp	4.09	4.37	4.47	3.71	0.57	0.58	0.86	0.33
Cys	0.94	0.77	0.86	0.86	0.26	0.84	0.83	0.92
Glu	4.20	4.31	4.41	3.78	0.74	0.64	0.95	0.52
Gly	1.66	1.77	1.77	1.55	0.21	0.7	0.99	0.51
Pro	1.23	1.23	1.23	1.15	0.16	0.72	0.92	0.66
Ser	1.88	1.88	1.88	1.58	0.30	0.55	0.96	0.62
Tyr	1.28	1.36	1.33	1.13	0.21	0.55	0.92	0.45
Total								
g/d	29.9	30.3	30.6	26.1	4.8	0.53	0.95	0.48
% of TSAA flow	13.2	10.0	11.0	7.0	2.4	0.13	0.74	0.27
% of TAA flow	1.57	1.15	1.07	1.06	0.17	0.12	0.76	0.98
Microbial								
g/d	21.0	22.4	21.9	20.0	3.7	0.94	0.94	0.78
% of total SP flow	70.2	74.1	71.5	76.6	5.8	0.46	0.75	0.55
Dietary								
g/d	8.9	7.8	8.7	6.1	2.1	0.2	0.8	0.32
% of total SP flow	29.8	25.9	28.5	23.4	5.8	0.46	0.75	0.55

¹TSAA = total soluble AA; SP = soluble proteins of molecular weight >10 kDa; TAA = total AA.²SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

erage, 50% of the concentration determined by ion-exchange chromatography of individual peptide-AA in hydrolyzed supernatant (Wallace and McKain, 1990). Therefore, it is possible that the peptide concentrations reported by Broderick and Wallace (1988) were also underestimated by fluorescamine. On the contrary, peptides accounted for an average of 63 (Choi et al., 2002a) and 60% (Choi et al., 2002b) of the SNAN flowing at the omasal canal, and for an average of 76% of the soluble N in ruminal digesta (Chen et al., 1987b). However, as discussed earlier, incomplete acid precipitation of proteins in these studies might have resulted in an overestimation of peptide fractions.

Omasal flows of FAA ranged between 3.73 and 5.68 g/d across diets ($P > 0.10$) and accounted for only 0.14 to 0.34% of the total AA flow and for 1.04 to 1.87% of the soluble AA flow (Table 12). When measured on solvent-extracted and ultrafiltered (1,000 MW cut-off filter) samples of duodenal digesta, FAA accounted for 0.34% of the total AA flowing at the duodenum of sheep (Peterson et al., 1971). Similarly, Volden et al. (2001) reported that ruminal outflow of FAA (13.0 to 34.8 g

of AA/d) contributed a small proportion (between 1.3 and 1.8%) of the total duodenal AA flow in dairy cows fed grass silage-diets supplemented with proteins of differing ruminal degradabilities. However, omasal FAA-N flow in cows fed grass silage-diets supplemented with barley plus either skimmed-milk powder, wet distillers solubles, or rapeseed meal accounted for between 24 and 44% of the total SNAN flow (Choi et al., 2002b). In a companion study, Choi et al. (2002a) reported that FAA-N flows in cows fed grass silage diets supplemented with barley, rapeseed meal, or a combination of the two contributed between 11.3 and 13.2% of total SNAN.

The omasal flows of total soluble AA ranged from 254 to 377 g/d, and from 9.2 to 15.9% of total AA flow, but were not significantly affected by dietary treatment (Table 13). Similarly, omasal flows of SNAN in cows fed grass silage-based diets supplemented with barley and proteins of differing degradabilities in 3 experiments contributed between 5.7 and 8.9% of the total NAN flow (Choi et al., 2002a,b; Choi et al., 2003).

Table 10. Effect of dietary treatment on omasal canal flows of individual AA (g/d) in soluble oligopeptides of molecular weights between 3 and 10 kDa

AA ¹	Diet ²				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Essential								
Arg	4.20	4.97	4.85	8.86	1.80	0.13	0.96	0.17
His	5.54	6.50	6.21	8.46	0.63	0.05	0.77	0.09
Ile	6.74	9.09	7.31	12.93	1.78	0.1	0.55	0.12
Leu	9.37	12.08	8.52	21.26	4.87	0.15	0.6	0.13
Lys	11.8	11.7	12.1	18.2	2.6	0.17	0.92	0.18
Met	2.56	3.62	2.34	4.69	1.81	0.46	0.65	0.43
Phe	3.66	7.19	6.25	7.55	1.77	0.21	0.73	0.63
Thr	10.9	11.6	10.3	17.3	2.7	0.18	0.73	0.15
Val	9.70	11.54	9.70	15.97	2.09	0.1	0.54	0.1
Nonessential								
Ala	13.7	20.2	14.2	24.4	4.3	0.17	0.39	0.18
Asp	23.4	26.8	27.8	37.3	5.6	0.18	0.9	0.32
Cys	6.7	17.2	11.6	15.0	3.1	0.12	0.24	0.44
Glu	25.3	27.6	25.4	43.9	8.9	0.19	0.85	0.19
Gly	13.9	16.5	15.4	20.2	1.7	0.08	0.69	0.15
Pro	9.1	10.8	10.2	16.6	2.9	0.13	0.88	0.17
Ser	8.9	9.6	8.9	15.1	2.6	0.16	0.85	0.16
Tyr	5.69	5.69	4.53	9.57	2.07	0.23	0.71	0.16
Total								
g/d	171	213	186	297	43	0.13	0.7	0.16
% of TSAA flow	65.4	67.9	69.9	79.3	3.9	0.09	0.74	0.19
% of TAA flow	10.79	7.93	6.40	12.54	3.05	0.68	0.72	0.21
Microbial								
g/d	61.1	40.2	34.7	80.8	19.2	0.33	0.78	0.21
% of total OP flow	35.7	18.9	18.7	27.2	5.8	0.07	0.9	0.08
Dietary								
g/d	110.1	172.5	150.9	216.3	19.9	0.02	0.13	0.05
% of total OP flow	64.3	81.1	81.3	72.8	5.8	0.07	0.9	0.08

¹TSAA = total soluble AA; TAA = total AA; OP = soluble oligopeptides of molecular weight between 3 and 10 kDa.

²SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

Soluble bound AA and FAA contributed a substantial proportion (an average of 13% across diets) of the total AA flowing out of the rumen. However, small peptides accounted for only 2% of the total AA flow at the omasal canal. This finding suggests that published ruminal concentrations and outflows of peptides might have been overestimated when measured in a chemically deproteinized supernatant.

Omasal Flows of Individual AA Associated with Each Soluble N Fraction

Only 7 out of 18 AA assayed by ion-exchange chromatography (Arg, His, Ile, Leu, Lys, Phe, and Tyr) were found at detectable concentrations in their free form (Table 12). Concentrations of total FAA (sum of 7) in omasal digesta averaged 26 mg of AA/L across diets. Flows of individual AA with soluble proteins greater than 10K were not significantly affected by dietary treatment (Table 9). When compared with urea, supplementation with CGM resulted in higher

flows of His (8.5 vs. 5.5 g of AA/d) and a trend for significantly higher flows of Ile ($P < 0.10$) and Val ($P < 0.10$) associated with soluble oligopeptides of 3–10K (Table 10). Cows fed CGM tended ($P < 0.10$) to have higher flows of His and Val in 3–10K oligopeptides than cows fed XSBM.

There was no difference between urea and CGM or XSBM and CGM on flows of individual small peptide-AA (Table 11). However, flows of Met, Val, Ala, Cys, Gly, and total AA in peptides of less than 3 kDa were significantly higher in cows fed SSBM than XSBM. Yang and Russell (1992) reported that dipeptides containing Pro and either Met, Gly, or Val were degraded by ruminal bacteria *in vitro* at rates that were more than 2-fold slower than those of dipeptides containing Met, Gly, and Val in combination with AA other than Pro. Although in the present study peptide-bound Pro was not significantly affected by diet, flows of small peptide-bound Met, Gly, Val, and total AA were 460, 25, 44, and 29% higher, respectively, in cows fed SSBM than XSBM. Therefore, feeding SSBM might have re-

Table 11. Effect of dietary treatment on omasal canal flows of individual AA (g/d) in soluble peptides of molecular weights smaller than 3 kDa

AA ¹	Diet ²				SEM	Contrast (<i>P</i> -value)		
	A (urea)	B (SSBM)	C (XSBM)	D (CGM)		A vs. D	B vs. C	C vs. D
Essential								
Arg	0.93	0.81	1.12	1.27	0.22	0.18	0.21	0.53
His	1.44	1.96	1.66	1.55	0.15	0.52	0.13	0.60
Ile	1.59	1.78	1.97	1.97	0.19	0.2	0.51	1.00
Leu	2.06	2.34	2.34	2.62	0.37	0.21	0.97	0.55
Lys	1.83	1.93	2.09	1.93	0.26	0.65	0.62	0.54
Met	0.56	1.12	0.20	0.53	0.18	0.91	0.03	0.27
Phe	0.26	0.79	0.38	0.39	0.17	0.62	0.17	0.96
Thr	2.81	2.81	2.47	2.81	0.43	0.88	0.24	0.31
Val	3.76	4.93	3.43	3.51	0.50	0.36	<0.01	0.79
Nonessential								
Ala	6.55	10.88	6.04	6.87	1.65	0.81	0.03	0.58
Asp	5.23	5.80	5.61	5.32	0.76	0.92	0.81	0.66
Cys	4.55	8.15	4.03	3.77	0.94	0.36	<0.01	0.81
Glu	5.99	5.67	4.94	6.30	1.05	0.86	0.54	0.32
Gly	4.29	5.09	4.07	4.13	0.27	0.49	<0.01	0.21
Pro	2.55	2.47	2.47	2.88	0.41	0.37	0.91	0.24
Ser	2.03	2.10	1.95	2.25	0.38	0.57	0.68	0.45
Tyr	0.85	0.97	0.79	0.98	0.17	0.49	0.37	0.33
Total								
g/d	47.3	59.6	45.5	49.1	5.6	0.12	<0.01	0.05
% of TSAA flow	19.5	20.3	17.5	12.7	2.1	0.11	0.42	0.21
% of TAA flow	2.80	2.34	1.63	2.13	0.47	0.20	0.18	0.30
Microbial								
g/d	3.56	2.99	2.49	4.63	0.81	0.15	0.16	0.01
% of total SPt flow	7.53	5.01	5.47	9.43	5.80	0.27	0.77	0.07
Dietary								
g/d	43.7	56.6	43.0	44.5	4.8	0.44	<0.01	0.64
% of total SPt flow	92.5	95	94.5	90.6	5.8	0.27	0.77	0.07

¹TSAA = total soluble AA; TAA = total AA; SPt = soluble peptides of molecular weight <3 kDa.²SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.**Table 12.** Effect of dietary treatment on omasal canal flows of individual free AA (g/d)

AA ¹	Diet ²				SEM	Contrast (<i>P</i> -value)		
	A (urea)	B (SSBM)	C (XSBM)	D (CGM)		A vs. D	B vs. C	C vs. D
Essential								
Arg	0.2	0.2	0.2	0.4	0.2	0.49	0.69	0.53
His	0.2	0.2	0.2	0.1	0.0	0.52	0.1	0.83
Ile	0.8	0.8	0.6	0.5	0.2	0.02	0.02	0.29
Leu	2	2	1	2	1	0.99	0.13	0.26
Lys	1.2	1.1	0.8	0.8	0.3	0.03	0.08	0.78
Phe	0.7	0.8	0.4	0.7	0.2	0.99	0.09	0.21
Nonessential								
Tyr	0.7	0.7	0.4	0.6	0.3	0.79	0.25	0.31
Total								
g/d	5.31	5.68	3.73	4.69	1.80	0.66	0.24	0.45
% of TSAA flow	1.87	1.83	1.59	1.04	0.44	0.08	0.49	0.18
% of TAA flow	0.34	0.21	0.14	0.22	0.11	0.25	0.50	0.44
Microbial								
g/d	0.40	0.28	0.20	0.44	0.13	0.91	0.4	0.06
% of total FAA flow	7.53	5.01	5.47	9.43	5.8	0.15	0.38	0.17

¹The following AA were in nondetectable concentrations (detection limit: 0.05 mg of AA-N per L of omasal fluid phase): Met, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro, and Ser. TSAA = total soluble AA; TAA = total AA; FAA = free AA.²SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

Table 13. Effect of dietary treatments on the omasal flows of total soluble AA

Soluble AA flow ¹	Diet ²				SEM	Contrast (<i>P</i> -value)		
	A	B	C	D		A vs. D	B vs. C	C vs. D
	(urea)	(SSBM)	(XSBM)	(CGM)				
Total								
g/d	254	308	266	377	48	0.14	0.56	0.17
% of TAA flow	15.5	11.6	9.2	15.9	3.7	0.93	0.63	0.23
Microbial								
g/d	90.3	70.7	62.4	109.5	21.8	0.29	0.57	0.2
% of TSAA flow	33.2	20.6	21.7	28.8	2.6	0.29	0.74	0.22
% of TAA flow	5.40	1.77	2.10	5.34	2.10	0.98	0.92	0.45
Dietary								
g/d	168	242	206	271	25	<0.01	0.03	<0.01
% of TSAA flow	66.8	79.4	78.3	71.2	2.7	0.29	0.74	0.22
% of TAA flow	10.7	8.0	8.2	13.1	2.8	0.56	0.97	0.4

¹TAA = total AA; TSAA = total soluble AA.²SSBM = solvent soybean meal; XSBM = xylose-treated soybean meal; CGM = corn gluten meal.

sulted in greater formation and accumulation of peptides containing Pro in combination with Met, Gly, and Val, compared with XSBM. Similarly, passage of ruminal peptides (determined in the chemically deproteinized supernatant of rumen fluid) increased with increasing intakes of degradable protein by cows in diets supplemented with either SSBM, extruded SBM, or fish meal (Chen et al., 1987b).

Flows of Ile and Lys in their free form were significantly higher for cows whose diets were supplemented with urea, compared with CGM (Table 12). Cows fed SSBM had higher flows of Ile ($P = 0.02$) and a trend for significantly higher flows of His, Lys, and Phe compared with cows whose diets were supplemented with XSBM.

Despite the alteration of the individual soluble AA flow from the rumen of cows by dietary manipulation observed in the present study, the extent of these changes was relatively small compared with the total flow of each AA. Although the flow of Met in small peptides was 5.6 times higher for cows fed SSBM than for cows fed XSBM (1.12 vs. 0.2 g/d; Table 11), the 0.92 g/d difference represented only 1.3% of the total flow of Met at the omasal canal (mean 70 g/d; Table 6).

Omasal Flows of Soluble AA from Microbial and Dietary Origin

The microbial contribution to total SNAN flows at the omasal canal of cows fed diets containing grass silage, barley, and rapeseed meal averaged 61 (Choi et al., 2002a) and 71% (Choi et al., 2003) across diets. However, microbial SNAN flows were estimated based on ¹⁵N enrichment of the supernatant of omasal samples that were acidified with H₂SO₄ before centrifugation. The addition of a strong acid to digesta samples

before removal of microbial cells by centrifugation could have resulted in cell lysis, release of cytoplasmic soluble N, and overestimation of microbial contributions to SNAN flows. On the contrary, in the present study only 25% of oligopeptides and 7% of peptides plus FAA flowing at the omasal canal were of microbial origin (Tables 10–12). Although most of the soluble proteins greater than 10K were of microbial origin (mean 73%), this fraction constituted only 10% of the total soluble AA flowing at the omasal canal (Table 9). Dietary soluble AA contributed, on average, 73% of total soluble AA and 10% of total AA flows (Table 13), indicating a substantial escape of dietary soluble AA from ruminal degradation. These results call into question the validity of assuming that ruminal degradation of the soluble N fraction (fraction A) of feeds incubated in situ occurs at an infinite rate and that the portion of protein that is degraded in the rumen (fraction B) is solely used for microbial protein synthesis, production of ammonia and carbon skeletons, or both.

Soluble AA flows of dietary origin were significantly affected by dietary treatment. Replacing XSBM with CGM resulted in higher flows of total (206 vs. 271 g/d) and oligopeptide-associated (151 vs. 216 g/d) soluble AA of dietary origin, whereas replacing urea with CGM increased oligopeptide-associated (110 vs. 216 g/d) and total (168 vs. 271 g/d) dietary soluble AA flows (Tables 10 and 13). The flow of soluble peptides of dietary origin in cows fed SSBM was significantly higher than for cows fed XSBM (57 vs. 43 g/d; Table 11). Differences in DMI among treatments might have accounted for part of these findings.

CONCLUSIONS

The study of the metabolic and nutritional significance of ruminal peptide and FAA pools to both mi-

crobes and the host animal depend on accurate and reproducible techniques for isolating and analyzing these fractions. Based on data reported in this study and in the literature, it would seem reasonable to suggest that published contributions of peptides to the ruminal soluble N pool might have been overestimated because of incomplete precipitation or partial hydrolysis of soluble proteins by acid treatments.

In the present study, small peptides isolated by ultrafiltration contributed between 13 and 20% of the total AA in the soluble fraction and less than 3% of the total AA flowing at the omasal canal of cows fed diets differing in the concentration and source of supplemental protein. On the other hand, AA in soluble 3–10K oligopeptides averaged 71%, whereas FAA accounted for less than 2% of total soluble AA flows across diets. Therefore, our results suggest that hydrolysis of oligopeptides (3–10K) was the rate-limiting step during microbial degradation of soluble proteins and that small peptides and FAA were rapidly utilized by ruminal microbes and did not accumulate in the rumen. On average, 73% of total soluble AA and 10% of total AA flows were of dietary origin, indicating a substantial escape of dietary soluble AA from ruminal degradation and calling into question the use of in situ estimations of protein degradation to predict the flow of RUP. Although the omasal flow of some individual AA associated with peptides and oligopeptides was significantly affected by dietary treatment, these changes were relatively small compared with the total flow of AA in their soluble and insoluble forms. The methodology described in the present paper may allow for more accurate quantitation of the ruminal peptide and FAA pools, their variations caused by the diet, and their metabolic and nutritional significance to the ruminal microbes and the host animal.

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