Asian-Aust. J. Anim. Sci.
Vol. 21, No. 3 : 392 - 403
March 2008

www.ajas.info

Effects of Level and Degradability of Dietary Protein on Ruminal Fermentation and Concentrations of Soluble Non-ammonia Nitrogen in Ruminal and Omasal Digesta of Hanwoo Steers

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ABSTRACT : Four ruminally fistulated Hanwoo steers were used to determine the effects of level and degradability of dietary protein on ruminal fermentation, blood metabolites and concentration of soluble non-ammonia nitrogen (SNAN) in ruminal (RD) and omasal digesta (OD). Experiments were conducted in a 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Factors were protein supplements with two ruminal crude protein (CP) degradabilities, corn gluten meal (CGM) that was low in degradability (rumen-degraded protein (RDP), 23.4% CP) or soybean meal (SBM) that was high in degradability (RDP, 62.1% CP), and two feeding levels of CP (12.2 or 15.9% dry matter). Ruminal fermentation rates and plasma metabolite concentrations were determined from the RD collected at 2-h intervals and from the blood taken by jugular puncture, respectively. The SNAN fractions (free amino acid, peptide and soluble protein) in RD and OD collected at 2-h intervals were assessed by ninhydrin assay. Mean ruminal ammonia concentrations were 40.5, 74.8, 103.4 and 127.0 mg/L for low CGM, high CGM, low SBM and high SBM, respectively, with statistically significant differences ($p<0.01$ for CP level and $p<0.001$ for CP degradability). Blood urea nitrogen concentrations were increased by high CP level ($p<0.001$) but unaffected by CP degradability. There was a significant ($p<0.05$) interaction between level and degradability of CP on blood albumin concentrations. Albumin was decreased to a greater extent by increasing degradability of low CP diets (0.26 g/dl) compared with high CP diets (0.02 g/dl). Concentrations of each SNAN fraction in RD ($p<0.01$) and OD ($p<0.05$) for high CP diets were higher than those for low CP diets, except for peptides but concentrations of the sum of peptide and free amino acid in RD and OD were significantly higher ($p<0.05$) for high CP diets than for low CP diets. Soybean meal diets increased free amino acid and peptide concentrations in both RD ($p<0.01$) and OD ($p<0.05$) compared to CGM diets. High level and greater degradability of CP increased ($p<0.001$) mean concentrations of total SNAN in RD and OD. These results suggest that RDP contents, increased by higher level and degradability of dietary protein, may increase release of free amino acids, peptides and soluble proteins in the rumen and omasum from ruminal degradation and solubilization of dietary proteins. Because SNAN in OD indicates the terminal product of ruminal metabolism, increasing CP level and degradability appears to increase the amount of intestine-available nitrogen in the liquid phase. (**Key Words :** Soluble Non-ammonia Nitrogen, Ruminal Digesta, Omasal Digesta, Dietary Protein, Ruminal Fermentation)

INTRODUCTION

Once dietary crude protein (CP) enters the rumen, it is subjected to microbial attack in the rumen and extensive degradation into peptides and, subsequently, amino acids and ammonia (Tamminga, 1979) that are mainly used for microbial protein synthesis. If protein degradation is rapid, more feed nitrogen (N) is degraded to ammonia than the quantity required for optimum microbial protein synthesis. As a result, ruminal concentrations of free amino acid would be expected to be low (Wright and Hungate, 1967; Chen et al., 1987a). However, Chen et al. (1987b) and Robinson and McQueen (1994) reported that peptides accumulate in the rumen, and Choi et al. (2002b) observed that a significant amount of soluble non-ammonia N (SNAN), composed of amino acids, peptides and soluble protein, was present in the rumen. In addition, a substantial amount of SNAN can escape ruminal degradation and be

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Received June 19, 2007; Accepted November 11, 2007

potentially absorbed as amino acids and peptides in the small intestine. This is supported by observations that a portion of intraruminally administered free amino acid escaped the rumen (Volden et al., 1998) and a considerable proportion of SNAN has been measured in the liquid phase of digesta flowing from the rumen (Choi et al., 2002a; 2002b; 2002c; 2003).

Previous studies have reported no difference in concentrations of SNAN when comparing different types of protein supplement in the rumen (Chen et al., 1987b; Robinson and McQueen, 1994; Robinson et al., 1998; Choi et al., 2002b) and omasum (Choi et al., 2002b; 2002c). However, the degradability of protein supplements used by Choi et al. (2002c) and Robinson et al. (1998) was not measured. In addition, differences in CP degradability among protein supplements used by Choi et al. (2002b), Robinson and McQueen (1994) and Chen et al. (1987b) were small. Thus, the effect of degradability of dietary protein on SNAN still remains unclear. Furthermore, little is known about the concentration response of SNAN in the rumen and omasum caused by varying the dietary level of CP. The objectives of the present study were to investigate the effects of level (CP, 12.2 vs. 15.9%) and degradability (rumen-degraded protein (RDP), 23.4 vs. 62.1% CP) of dietary protein on ruminal fermentation, blood parameters and concentrations of SNAN in the ruminal (RD) and omasal digesta (OD) of Hanwoo steers.

MATERIALS AND METHODS

Animals, treatments and management

Four Korean Hanwoo beef steers, (mean 697.25±18.05 kg) fitted with permanent ruminal cannulae, were used in a 4×4 Latin square experiment with four 14-day experimental periods. The treatments were arranged in a 2×2 factorial experiment with steers offered concentrates supplemented with two types of protein supplement differing in ruminal CP degradability, corn gluten meal (CGM) that is low in degradability (RDP, 23.4% CP) or soybean meal (SBM) that is high in degradability (RDP, 62.1% CP), at two feeding levels of CP, 12.2 (L) or 15.9% (H) dry matter (DM). This factorial arrangement of treatments resulted in four dietary treatments: LCGM, HCGM, LSBM and HSBM. Steers were adapted to experimental stalls for 10 days prior to commencement of the experimental period and fed 9.0 kg of each diet on an as fed basis per day. Ingredients and chemical composition of the experimental diets are shown in Table 1. Diets were offered twice daily at 09:00 and 17:00 h. Each steer had free access to water and free-choice minerals throughout the experiment. Each experimental period consisted of an adaptation period of 12 days and a collection period of 2 days.

Measurements and sample collection

Feed intake was recorded daily but, due to feed restrictions of 9.0 kg per day, intake was fairly similar among treatments. Representative samples of the experimental diets were collected daily and composited at the end of each period for analysis. For *in situ* determination of feeds, three additional Korean Hanwoo steers (mean 545.33±31.47 kg) with permanent ruminal cannulae were used. They were fed a diet (% DM basis; CP 10.0, ether extract (EE) 2.3, ash 7.1, neutral detergent-insoluble fiber (NDF) 33.6 and acid detergent-insoluble fiber (ADF) 18.8) of orchardgrass (45.6) and concentrate (54.4), composed of ground corn (56.0), soyhulls (11.2), wheat hulls (19.7), calcium phosphate (0.4), limestone (1.1) and vitamin-mineral mix (0.4; see Table 1 for ingredients). Test feeds, experimental diets and rice straw were milled through a 2-mm screen with a Wiley mill (Thomas Scientific, Model4, NJ, USA) and 5 g of each feed was placed in Nylon bags (NL 130-030/330PW, NBC Inc., Tokyo, Japan) of approximately 10×15 cm (47 μm pore size; sample size: surface area = 16.67 mg/cm^2). Bags were incubated in triplicate in the rumen of steers for 3, 6, 9, 12, 24, 48, 72 and 96 h. The 0 h time point represented bags that were not incubated but were treated in the same manner as other bags upon removal from the rumen. All bags were placed in the rumen at the same time and removed at the determined intervals thereafter. Upon removal from the rumen, the bags were rinsed with cold tap water for 0.5 h and then dried in a forced draft oven (60°C for 48 h). Each bag was weighed and feed residues were analyzed for CP according to procedure 976.05 of the Association of Official Analytical Chemists (AOAC, 1990). Disappearance of nutrients at each incubation time was expressed relative to the original nutrient content of the feed which was not incubated and washed. Degradation values of CP in the rumen were calculated as described by Ørskov and McDonald (1979) using the NLIN procedure in the Statistical Analysis Systems (SAS, 2002; version 9.1).

On day 13 of each period, blood was collected from the jugular vein of steers before the morning feeding and at 15:00 h into two 7-ml vacuum tubes (BD-vacutainer, Becton & Dickinson, NJ, USA) containing ethylenediaminetetraacetic acid. Once collected, samples were immediately placed on ice and later centrifuged at 2,000×g for 15 min at 4°C to collect plasma. Plasma was stored in plastic vials at -20°C until analysis.

Digesta was sampled from the rumen via a ruminal fistula and from the omasal canal using a system of alternating vacuum and pressure. On day 13 of each period, approximately 80 ml of RD was collected from the rumen before morning feeding and at 1, 3, 5 and 7 h post-feeding. On the following day, 80 ml of OD was collected using a tube (14 mm i.d.) that was passed through the ruminal

Table 1. Ingredients and chemical composition of experimental diets[1]

Items	Diets[2]			
	LCGM	HCGM	LSBM	HSBM
Ingredients (% dry matter (DM))				
Ground corn	50.5	48.7	51.4	46.7
Cottonseed hull	17.8	17.2	18.1	16.5
Skimmed rice bran	6.0	5.8	6.1	5.6
Calcium phosphate	0.6	0.6	0.6	0.6
Vitamin-mineral additive[3]	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Corn gluten meal	5.6	11.6	-	-
Soybean meal	-	-	8.7	17.7
Rice straw	19.0	15.6	14.6	12.4
Total	100.0	100.0	100.0	100.0
Composition (% DM)				
Dry matter (%)	87.7	87.4	87.6	87.7
Crude protein (CP)	12.0	16.2	12.5	15.7
Ether extract	3.1	3.0	3.1	3.1
Ash	5.8	6.0	5.4	5.1
Neutral detergent fiber	40.5	37.9	32.8	33.6
Acid detergent fiber	19.9	16.0	17.7	16.2
Calorie (gross energy, Mcal/kg DM)	4,328.1	4,443.7	4,363.8	4,387.3

[1] Each steer had free access to water and a free choice mineral (Rincal block, Daehan New Pham, Seoul, Korea; provided the following nutrients per kg: I, 150 mg; Mn, 200 mg; S, 4,000 mg; Co, 100 mg; Fe, 2,000 mg; Zn, 100 mg; Ni, 50 mg; Cu, 100 mg; Mg, 3,000 mg; Ca, 2,000 mg; Se, 40 μg; NaCl, 380 g) throughout the experiment.

[2] LCGM = Low level (CP, 12.2% DM) of corn gluten meal (ruminally degradable protein, 23.4% CP); HCGM = High level (CP, 15.9% DM) of corn gluten meal; LSBM = Low level of soybean meal (ruminally degradable protein, 62.1% CP); HSBM = High level of soybean meal.

[3] Provided the following nutrients per kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany): Vit. A, 2,650,000 IU; Vit. D_3, 530,000 IU; Vit. E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

cannula and positioned in the omasal canal according to procedures outlined by Huhtanen et al. (1997), with exceptions described by Ahvenjärvi et al. (2000) as follows: 1) larger sampling tube (14 vs. 9.5 mm i.d.), 2) solenoid valves instead of a three way ball valve to control vacuum and pressure phases in a pump and 3) a 0.5 kg weight inserted into the abomasum for securing the sampling device in the omasum. Omasal digesta was collected at the same times as RD. After collection, pH of RD was immediately measured (Pinnacle M530, Corning, NY, USA) and it was then filtered through four layers of cheesecloth and 1% of saturated $HgCl_2$ added to stop microbial activity. The sample of RD filtrate was divided into three sub-samples for measurement of ammonia N, volatile fatty acids (VFA) and SNAN. The sample of OD obtained at each sampling interval was filtered through four layers of cheesecloth, 1% of saturated $HgCl_2$ added and the filtrate frozen at -20°C for SNAN analysis.

Chemical analyses

Feed samples were analyzed for moisture, CP, EE, and ash according to AOAC procedures 934.01, 976.05, 920.39, and 927.02, respectively (AOAC, 1990). The concentration of NDF corrected for residual ash (aNDFom) was determined with heat stable amylase and sodium sulphite according to methods of Van Soest et al. (1991), while the content of ADF corrected for residual ash (ADFom) was determined according to procedure 973.18 of AOAC (1990). Gross energy concentration was measured by a bomb calorimeter (CA-3, Shimadzu corporation, Kyoto, Japan). Soluble N was calculated as the sum of A fraction (non-protein N) and B_1 fraction (buffer-soluble true protein) of CP determined according to the Cornell net carbohydrate and protein system (Licitra et al., 1996).

Plasma was analyzed for total protein (TP), blood urea nitrogen (BUN), albumin and creatinine using an automated blood analyzer (Express Plus, Ciba-Corning, CA, USA) according to the biuret method of Flack and Woollen (1984), the urease method of Roch-Ramel (1967), the bromocresol green method of Doumas et al. (1971) and the picric acid method of Husdan and Rapoport (1968), respectively.

To determine ruminal ammonia N concentrations, samples were centrifuged at 2,000×g for 15 min at 4°C and the supernatant was analyzed as described by Chaney and Marbach (1962). To determine ruminal VFA concentrations, samples were mixed with 1 ml of metaphosphoric acid/water (25:75, w/v) and 0.2 ml of pivalic acid/water (10:90, w/v) for use as an internal standard (98% purity) according to Erwin et al. (1961). After standing for 30 min, samples were centrifuged at 2,000×g for 15 min at 4°C. The supernatant was analyzed with a wall-coated open tubular-fused silica capillary column (CP-7485, Varian, CA, USA) using a gas chromatograph (CP-3800, Varian, CA, USA). A column temperature of 150°C was used with helium carrier

gas at a flow rate of 100 ml/min. Temperature of both injector and detector was 130°C. The hydrogen flow to the flame jet and air flow to the detector chamber were 40 and 400 ml/min, respectively.

To determine SNAN concentrations, samples were prepared according to Choi and Choi (2003) as follows. Ruminal digesta and OD were centrifuged at 1,000×g for 10 min at 4°C to eliminate small particles and ruminal protozoa followed by high-speed centrifugation (10,000×g for 60 min at 4°C) to eliminate rumen bacteria. Supernatant was mixed with trichloroacetic acid (TCA) to a final concentration of 5%, w/v, stored in ice overnight and centrifuged at 10,000×g for 60 min at 4°C. The supernatant was assumed to contain free amino acids, peptides and ammonia N while the TCA-precipitated pellet was comprised of protein. Each fraction of SNAN in the sample was estimated as follows: 1) free amino acid as N from the supernatant without acid-hydrolysis, 2) peptide as N from the difference between hydrolyzed supernatant (6 M HCl at 110°C for 24 h) and free amino-acid N and 3) protein as N from the hydrolysis of TCA-precipitate. The pellet was carefully washed with TCA/water (5:95, w/v) to remove attached residues. Once rinsed, the pellet and supernatant were mixed with 6 M HCl that was flushed with N_2 gas for 45 min. Each sample in 6 M HCl was flushed with N_2 gas for 20-30 s prior to acid hydrolysis. Tubes containing either the pellet or supernatant were tightly capped and hydrolyzed at 110°C for 24 h. Hydrolyzed and non-hydrolyzed samples were evaporated to dryness under vacuum at 45°C. Dried samples were dissolved in distilled water and alpha-amino N was measured using the ninhydrin assay according to the method of Lie (1973).

Statistical analyses

Data obtained from the analysis of blood metabolites were subjected to statistical analysis using the GLM procedure of SAS (2002; version 9.1) according to the following statistical model:

$$Y_{ijkl} = \mu+A_i+P_j+L_k+D_l+(L\times D)_{kl}+e_{ijkl}$$

where A, P, L, D and L×D are animal, period, level and degradability effects and interaction between level and degradability, respectively.

Data obtained from pH, ammonia N and profiles of VFA and SNAN determined at each sampling interval were analyzed with the MIXED procedure of SAS (2002) for repeated measures (Littell et al., 1998) according to the following statistical model:

$$Y_{ijklm} = \mu+A_i+P_j+L_k+D_l+(L\times D)_{kl}+e_{ijkl}+T_m+(A\times T)_{im} + (P\times T)_{jm}+(L\times T)_{km}+(D\times T)_{lm}+(L\times D\times T)_{klm}+e_{ijklm}$$

where T is time effect, and A×T, P×T, L×T, D×T and L×D×T are animal by time, period by time, level by time, degradability by time and level by degradability by time interactions, respectively. Animal effect, animal by time interaction and error terms (e_{ijkl} defined as between unit error and e_{ijklm} as within unit error) are multivariate normally distributed random effects with AR (1) covariance structure. Orthogonal contrasts used in post-ANOVA comparisons were as follows; 1) effect of protein level (low level vs. high level of CP), 2) effect of degradability of protein source (CGM vs. SBM) and 3) the interaction between level and degradability of CP.

RESULTS AND DISCUSSIONS

Nutrient composition and *in situ* degradation of feeds

The ingredients and chemical composition of diets are presented in Table 1. Diets were formulated to be isoenergetic, with 4,400 Mcal of gross energy/kg DM. Although diets were originally formulated to contain 12% CP in the low protein diet and 16% CP in the high protein diet, CP contents of the experimental diets actually fed in the present experiment averaged 12.0, 16.2, 12.5 and 15.7% DM for LCGM, HCGM, LSBM and HSBM, respectively.

Nitrogen composition and *in situ* CP degradation of diets are presented in Table 2. Soluble proteins calculated as the sum of the A and B_1 fractions were 5.4 and 11.4% CP for CGM and SBM, respectively. Similarly, RDP calculated as the effective protein degradability obtained from *in situ* data were 23.4 and 62.1% CP for CGM and SBM, respectively.

Ruminal metabolism

Effects of level and degradability of dietary protein on ruminal pH and concentrations of ammonia N and VFA in the rumen are shown in Table 3. Neither CP level nor degradability of protein sources had an effect on average ruminal pH. Within 7 h after feeding, the ruminal pH of all treatments was greater than 6.3 (Figure 1A), which is the pH identified as critical for maintaining ruminal fiber digestion (Stewart, 1977; Hiltner and Dehority, 1983).

The concentrations of ammonia N increased with increasing level ($p<0.01$) and degradability ($p<0.001$) of CP. These results agree with Klusmeyer et al. (1990) who observed ruminal ammonia N concentrations of 19 and 25 mg/L for CGM diets containing CP levels of 11.0 and 14.5%, respectively, compared to 54 and 105 mg/L for SBM diets containing the same CP levels, respectively. Feeding higher levels of CP increased ammonia N in the rumen because more dietary protein was available for microbial degradation. CGM had lower production of ammonia N in the rumen than SBM and the concentration was numerically lower even when more CGM was added to

Table 2. Nitrogen composition and *in situ* crude protein degradation of experimental diets

Items[1]	Protein supplement[2]		Experimental diet[3]			
	CGM	SBM	LCGM	HCGM	LSBM	HSBM
Composition (% DM)						
CP	63.0	51.5	12.0	16.2	12.5	15.7
Soluble protein[4]	3.4	5.9	2.1	2.7	2.6	3.6
Soluble protein (% CP)	5.4	11.4	16.1	16.6	18.5	20.6
RDP[5]	14.7	32.0	6.2	6.9	8.1	10.6
RDP (% CP)	23.4	62.1	47.6	41.7	59.1	60.1
In situ CP degradability (% CP)						
Rapidly degradable fraction	1.5	7.6	24.8	20.4	27.3	21.6
Slowly degradable fraction	59.3	95.3	54.0	55.4	66.2	74.7
Potential degradability[6]	60.8	102.9	78.8	75.8	93.5	96.3
Rate of degradation (h^{-1})	0.023	0.054	0.024	0.024	0.027	0.030
Effective protein degradability[7]	23.4	62.1	47.6	41.7	59.1	60.1

[1] DM = Dry matter; CP = Crude protein; RDP = Rumen-degraded protein.
[2] CGM = Corn gluten meal; SBM = Soybean meal.
[3] LCGM = Low level (CP, 12.2% DM) of corn gluten meal (RDP, 23.4% CP); HCGM = High level (CP, 15.9% DM) of corn gluten meal; LSBM = Low level of soybean meal (RDP, 62.1% CP); HSBM = High level of soybean meal.
[4] Calculated as the sum of A and B_1 fractions according to Cornell net carbohydrate and protein system (Licitra et al., 1996).
[5] Calculated as the effective protein degradability obtained from *in situ* degradability data.
[6] Sum of rapidly and slowly degradable fractions.
[7] According to Ørskov and McDonald (1979); using a rumen outflow rate of 0.02 and 0.04 h^{-1} for rice straw and other feeds, respectively.

Table 3. Effects of level and degradability of dietary protein on rumen pH, concentrations of ammonia and volatile fatty acid (VFA) in the rumen

Items	Diets[1]				SEM[2]	Statistical significance of (p<)[3]	
	LCGM	HCGM	LSBM	HSBM		Level	Degradability
pH	6.63	6.55	6.66	6.62	0.05	0.234	0.263
Ammonia (mg/L)	40.48	74.84	103.40	127.00	10.69	0.007	0.001
VFA (mM)							
Acetate	60.66	61.64	62.39	61.22	2.23	0.966	0.777
Propionate	17.12	17.02	17.20	17.71	1.01	0.846	0.698
A:P ratio[4]	3.57	3.64	3.70	3.49	0.15	0.556	0.946
Isobutyrate	1.26	1.46	1.35	1.57	0.12	0.104	0.425
Butyrate	9.71	10.54	10.90	10.22	0.87	0.914	0.545
Isovalerate	2.89	3.43	2.58	3.01	0.24	0.034	0.084
Valerate	0.90	1.01	0.90	0.98	0.06	0.123	0.867
Total	92.54	95.09	96.12	94.70	3.84	0.886	0.690

[1] LCGM = Low level (crude protein (CP), 12.2% dry matter (DM)) of corn gluten meal (rumen-degraded protein, 23.4% CP); HCGM = High level (CP, 15.9% DM) of corn gluten meal; LSBM = Low level of soybean meal (rumen-degraded protein, 62.1% CP); HSBM = High level of soybean meal.
[2] Standard error of mean; n = 80.
[3] The interaction between level and degradability of protein source was not significant (p>0.05).
[4] Acetate:propionate ratio.

elevate the CP content of the diet (74.84 vs. 103.40 mg/L for HCGM and LSBM, respectively). This result arises due to the fact that CGM is less degradable in the rumen than SBM. Increasing level or degradability of protein usually results in increased ammonia N concentration in the rumen (Chanjula et al., 2004; Promkot and Wanapat, 2005; Olmos Colmenero and Broderick, 2006a; Wanapat and Khampa, 2007). A ruminal ammonia N level of 50 mg/L is widely accepted as the minimum concentration at which maximum microbial growth and activity would occur *in vitro* (Satter and Slyter, 1974). According to Kang-Meznarich and Broderick (1980), ranges of ammonia N for maximal microbial growth were between 33 and 85 mg/L *in vivo*. Thus, although not determined in the present study, microbial growth for LCGM may be limited due to ammonia N concentration being less than 33 mg/L at 5 h post-feeding (Figure 1B). This possibility is supported by the observations of Olmos Colmenero and Broderick (2006b) in which microbial protein flow was decreased when ruminal ammonia N levels were lower than 30 mg/L from 4 to 24 h after feeding.

Acetate:propionate ratio and concentrations of total VFA, acetate, propionate, butyrate and valerate were unaffected by treatments. Isobutyrate, however, tended ($p = 0.10$) to increase with increasing CP level while isovalerate was increased at the high CP level ($p<0.05$) and tended ($p = 0.08$) to be higher for CGM than for SBM. In the rumen, branched-chain VFA are derived from catabolism of

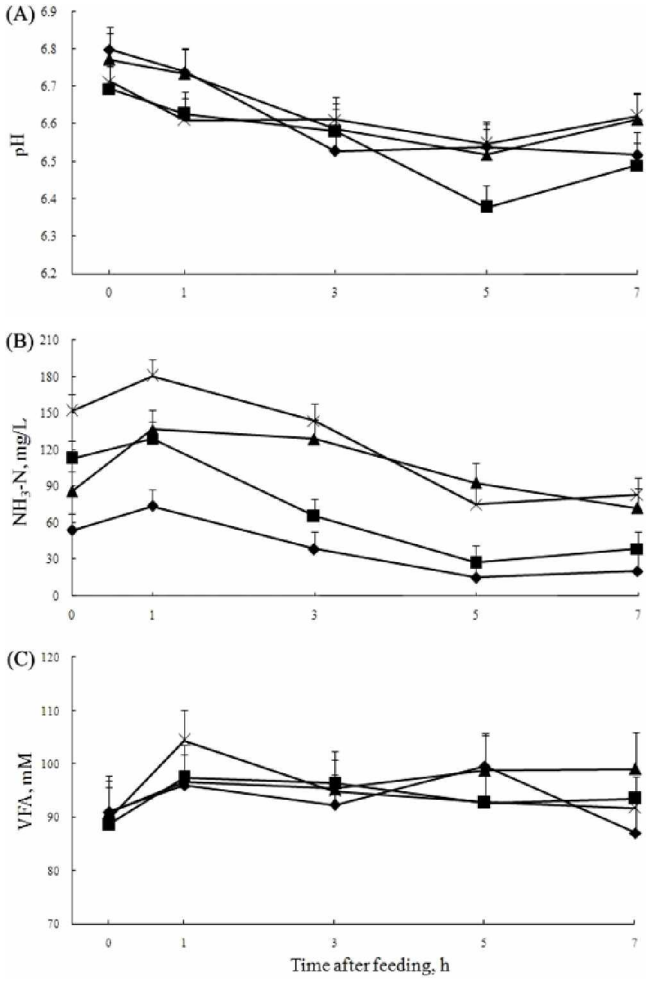

Figure 1. Temporal changes after feeding in pH (A) and concentrations of ammonia nitrogen (NH_3-N; B) and total volatile fatty acids (VFA; C) in the rumen of steers fed low level (crude protein (CP), 12.2% dry matter (DM)) of corn gluten meal (♦) (rumen-degraded protein, 23.4% CP), high level (CP, 15.9% DM) of corn gluten meal (■), low level of soybean meal (▲) (rumen-degraded protein, 62.1% CP) or high level of soybean meal (×). Bars indicate standard error of the mean (n = 16). Zero (0) time means sampling before feeding at 09:00 h and diets were fed immediately after 0 time sampling.

branched-chain amino acid (Wolin et al., 1997). Thus, increasing dietary protein level and (or) ruminal degradability of the feed would increase concentration of branched-chain amino acids within ruminal contents. However, overall fermentation pattern was similar among treatments in the present study, considering that changes observed for isobutyrate and isovalerate were small. In addition, there were no differences in the concentration of the three main VFA (acetate, propionate and butyrate) and also of total ruminal VFA 7 h post-feeding (Figure 1C).

Blood plasma metabolism

Effects of level and degradability of dietary protein on concentrations of BUN, TP, albumin and creatinine in plasma are shown in Table 4. CP level and degradability did not affect concentrations of TP and creatinine in plasma. High CP level increased ($p<0.001$) BUN concentrations but CP degradability had no affect on this parameter. Promkot and Wanapat (2005) also observed a linear increase in BUN with increasing dietary CP levels. The increased BUN concentration observed at higher levels of dietary protein was probably a result of increased absorption of ruminal ammonia N across the rumen wall that was not incorporated into microbial protein. This resulted in greater quantities of ammonia being converted to urea in the liver for either recycling to the rumen through saliva or excretion in urine (Moscardini et al., 1998).

It is interesting that BUN was unaffected by degradability of CP considering that BUN is highly related to ruminal ammonia N. As degradability of CP is increased, ruminal protein hydrolysis is also increased, thereby elevating ruminal ammonia N concentration. In the present study, differences in ruminal ammonia N between CGM and SBM diets did not result in differences in BUN. Although ammonia N for LSBM was numerically higher than that for HCGM (103.40 vs. 74.84 mg/L), BUN for LSBM was numerically lower than that for HCGM (11.86 vs. 16.08 mg/dl). Blood urea N is also increased by the increased supply of protein to the intestine (Broderick et al., 1974). Thus, it might be hypothesized that significant amounts of RUP and microbial protein were absorbed in the small intestine and contributed to an increased level of BUN for HCGM. In fact, postruminal digestibility of N and concentration of BUN were increased as rumen-undegraded protein supplement was elevated (Wright et al., 1998).

A significant ($p<0.05$) interaction between level and

Table 4. Effects of level and degradability of dietary protein on concentrations of blood urea nitrogen (BUN), total protein (TP), albumin and creatinine in plasma

Items	Diets[1]				SEM[2]	Statistical significance of (p<)		
	LCGM	HCGM	LSBM	HSBM		Level	Degradability	Interaction
BUN (mg/dl)	10.33	16.08	11.86	15.89	0.72	0.002	0.423	0.280
TP (g/dl)	7.05	7.00	6.99	6.98	0.08	0.718	0.693	0.824
Albumin (g/dl)	4.00	3.90	3.74	3.88	0.03	0.590	0.017	0.021
Creatinine (mg/dl)	1.40	1.38	1.46	1.46	0.03	0.710	0.194	0.709

[1] LCGM = Low level (crude protein (CP), 12.2% dry matter (DM)) of corn gluten meal (rumen-degraded protein, 23.4% CP); HCGM = High level (CP, 15.9% DM) of corn gluten meal; LSBM = Low level of soybean meal (rumen-degraded protein, 62.1% CP); HSBM = High level of soybean meal.

[2] Standard error of mean; n = 32.

Table 5. Effects of level and degradability of dietary protein on the concentrations (mg N/L) of nitrogenous fractions of soluble non-ammonia nitrogen (SNAN) either in the rumen or entering the omasal canal

Items	Diets[1]				Site[2]	SEM[3]	Statistical significance of (p<)[4]	
	LCGM	HCGM	LSBM	HSBM			Level	Degradability
FAA[5]								
Ruminal	21.38	29.17	32.24	50.50	33.37[a]	3.55	0.009	0.004
Omasal	40.38	52.88	57.76	73.51	56.23[b]	4.18	0.016	0.005
Peptide								
Ruminal	56.78	62.55	91.46	95.69	76.44[a]	5.75	0.255	0.001
Omasal	94.34	93.62	116.98	122.22	106.74[b]	9.21	0.811	0.023
FAA+peptide								
Ruminal	77.35	91.73	123.70	146.20	109.65[a]	8.37	0.025	0.001
Omasal	134.21	146.50	174.73	195.73	162.80[b]	6.80	0.026	0.001
Soluble protein								
Ruminal	10.80	31.69	28.44	43.78	28.75[a]	2.33	0.001	0.001
Omasal	13.57	52.04	44.30	52.67	40.66[b]	7.20	0.017	0.066
SNAN[6]								
Ruminal	87.93	123.42	152.14	189.98	138.37[a]	6.68	0.001	0.001
Omasal	146.17	198.54	219.03	248.40	203.85[b]	7.26	0.001	0.001

[1] LCGM = Low level (crude protein (CP), 12.2% dry matter (DM)) of corn gluten meal (rumen-degraded protein, 23.4% CP); HCGM = high level (CP, 15.9% DM) of corn gluten meal; LSBM = Low level of soybean meal (rumen-degraded protein, 62.1% CP); HSBM = High level of soybean meal.
[2] Different letters express a statistical significance for the mean of each fraction between sampling sites (at least $p<0.05$).
[3] Standard error of mean; n = 80.
[4] The interaction between level and degradability of protein sources was not significant ($p>0.05$).
[5] Free amino acid.
[6] Calculated as the sum of free amino acid, peptide and soluble protein.

degradability of CP was observed for albumin. This suggests that albumin concentrations in blood were decreased to a greater extent by increasing degradability of low CP diets (0.26 g/dl) compared with high CP diets (0.02 g/dl). Because plasma albumin adheres chemically to various substances such as amino acids in the blood, playing a role in their transport (Rivera et al., 2005), increased albumin concentration could be explained by more absorption of true protein in the small intestine when rumen-undegraded protein was fed compared to RDP. However, no difference in TP concentration was observed despite there being a higher albumin concentration. As albumin normally constitutes about 60% of the plasma protein in the blood of ruminants (Nikokyris and Kandylis, 1991) and total protein in blood reflects availability of protein (Lohakare et al., 2006), the reason for this apparent discrepancy is unclear.

Soluble non-ammonia nitrogen in the ruminal and omasal digesta

Dietary responses of soluble non-ammonia nitrogen : Effects of level and degradability of dietary protein on concentrations of amino acid, peptide, soluble protein and total SNAN in the RD and OD are shown in Table 5. Concentrations of each SNAN fraction and total SNAN in RD (at least $p<0.01$) and OD (at least $p<0.05$) for high CP diets were greater than those for low CP diets, with the exception of the peptide fraction. However, concentrations of the sum of free amino acids and peptides in RD and OD were significantly higher ($p<0.05$) for high CP diets than for low CP diets (Table 5). The lack of differences in peptide concentrations between the two CP levels in both RD and OD may be related to the method employed to analyze SNAN fractions. Ninhydrin generally reacts with an amino group of amino acids. Thus, ninhydrin also reacts with terminal amino groups of peptides as well as those of free amino acids (Rosen, 1957). As a result, peptide concentration could be somewhat underestimated and consequently free amino acid concentration may be overestimated to the same extent. As the amount of peptides in blood are increased, this degree of underestimation will be increased, a relationship that would reduce the difference in peptide concentration among treatments. However, the procedure used to estimate the sum of free amino acid and peptide concentrations does not have this analytical error because all peptides are hydrolyzed to ninhydrin-reactive amino acids.

The level of protein in the diet positively affected the concentration of SNAN in both RD and OD. This result is consistent with report from Chen et al. (1987b) that concentrations in the rumen and estimated flow to the omasum of peptide (including free amino acid) for low CP (14.5% DM) diets were lower than those for high CP (20.6% DM) diets (106 vs. 150 mg/L and 22 vs. 34 g/d for concentration and flow, respectively) when soybean meal was fed. Using autoclaved SBM, Chen et al. (1987b) also observed lower concentrations in the rumen (84 vs. 128 mg/L and 27 vs. 36 mg/L for peptide and soluble protein,

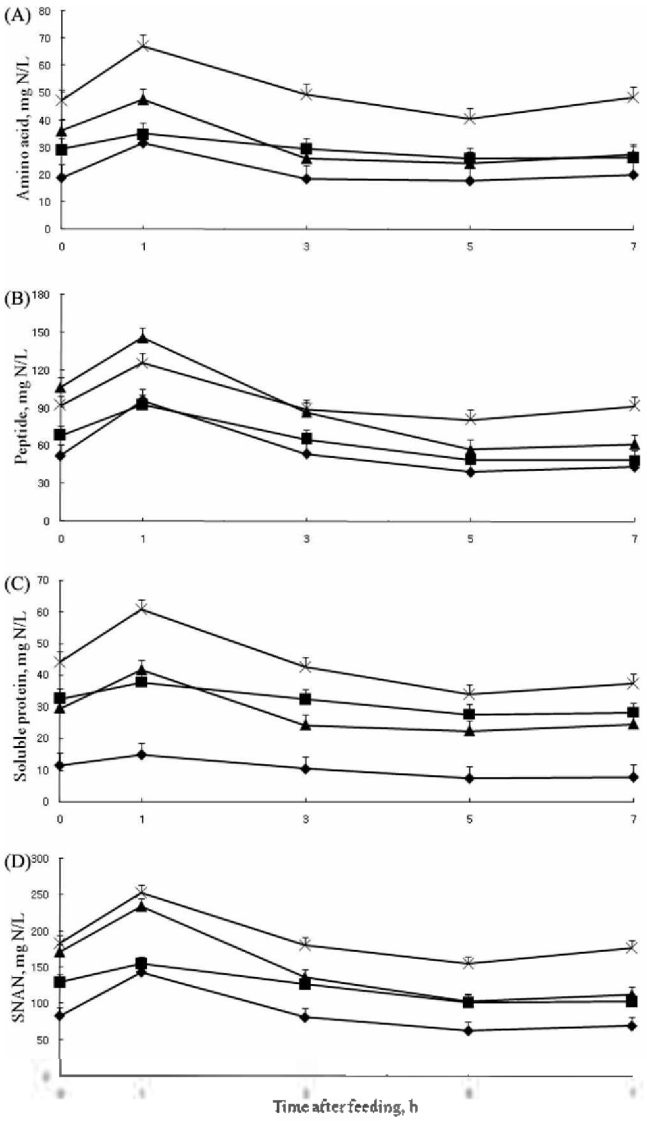

Figure 2. Temporal changes after feeding in concentrations of free amino acid (A), peptide (B), soluble protein (C) and total soluble non-ammonia nitrogen (SNAN; D) in the ruminal digesta of steers fed low level (crude protein (CP), 12.2% dry matter (DM)) of corn gluten meal (♦) (rumen-degraded protein, 23.4% CP), high level (CP, 15.9% DM) of corn gluten meal (■), low level of soybean meal (▲) (rumen-degraded protein, 62.1% CP) or high level of soybean meal (×). Bars indicate standard error of the mean (n = 16). Zero (0) time means sampling before feeding at 09:00 h and diets were fed.immediately after 0 time sampling.

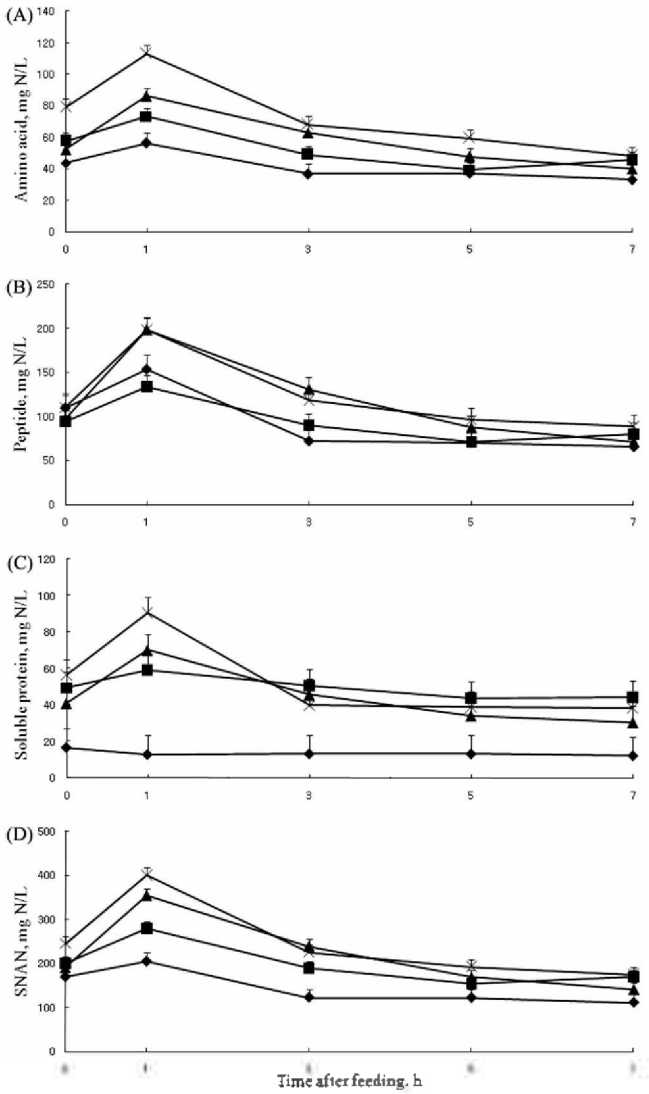

Figure 3. Temporal changes after feeding in concentrations of free amino acid (A), peptide (B), soluble protein (C) and total soluble non-ammonia nitrogen (SNAN; D) in the omasal digesta of steers fed low level (crude protein (CP), 12.2% dry matter (DM)) of corn gluten meal (♦) (rumen-degraded protein, 23.4% CP), high level (CP, 15.9% DM) of corn gluten meal (■), low level of soybean meal (▲) (rumen-degraded protein, 62.1% CP) or high level of soybean meal (×). Bars indicate standard error of the mean (n = 16). Zero (0) time means sampling before feeding at 09:00 h and diets were fed immediately after 0 time sampling.

respectively) and lower estimated flow to the omasum (19 vs. 31 g/d and 6 vs. 9 g/d for peptide and soluble protein, respectively) of peptide (including free amino acid) and soluble protein for low CP (14.4% DM) than for high CP diets (19.3% DM). Increasing protein level with supplemented protein enhances the amount of CP degraded in the rumen. As a result, both HCGM and HSBM supplied more RDP than LCGM and LSBM, respectively (Table 2). Increased concentrations of SNAN by increasing CP supply might have been caused by proteolysis of the increased RDP fraction.

Soybean meal diets increased concentrations of individual SNAN fractions and total SNAN in both RD ($p<0.01$) and OD ($p<0.05$) compared with CGM diets, with the exception of soluble protein in OD which tended ($p = 0.066$) to increase. These results are inconsistent with previous studies showing that there was no difference in the concentration of peptide including free amino acids between diets supplemented with different types of protein in RD (Chen et al., 1987b; Robinson and McQueen, 1994; Robinson et al., 1998; Choi et al., 2002b) and OD (Choi et

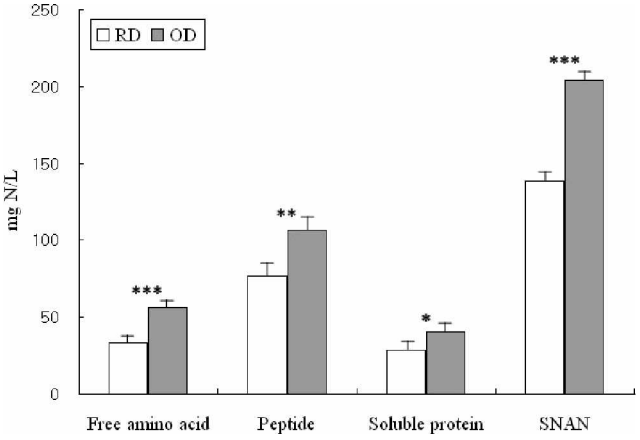

Figure 4. Comparison of free amino acid, peptide, soluble protein and total soluble non-ammonia nitrogen (SNAN) between ruminal (RD) and omasal digesta (OD). *, ** and *** indicate a difference between RD and OD within each fraction and total SNAN ($p< 0.05$, $p<0.01$ and $p<0.001$, respectively). Bars indicate standard error of the mean (n = 16).

al., 2002b; 2002c). However, degradability of protein supplements, fish meal and soybean meal used by Choi et al. (2002c) and blood meal and soybean meal used by Robinson et al. (1998), was not measured in these studies. In addition, the differences in CP degradability (RDP, % CP) among protein supplements used in these studies were small (Chen et al., 1987b; Robinson and McQueen, 1994; Choi et al., 2002b). Thus, these studies did not show the effect of degradability of dietary protein on concentrations of SNAN.

In the present study, difference in protein degradability resulted in a RDP supply remarkably higher for SBM diets than that for CGM diets (Table 2). It is expected that high RDP would be accompanied by relatively high ruminal protein degradation and in many cases high solubilization of protein. Solubilized protein may be hydrolyzed and release free amino acids and peptides in the rumen. In addition, increasing RDP supply in the rumen probably results in enhanced microbial protein synthesis because microbes can benefit from amino-N for growth. Zerbini et al. (1988) reported that feeding soybean meal to cows increased efficiency of microbial protein synthesis compared with feeding fish meal. In the study of Choi et al. (2002a), in which flow of microbial non-ammonia N into the omasal canal was increased from 159 to 241 g N/d when RDP supply was increased by supplementing barley and rapeseed meal, concentrations of total SNAN in OD and soluble N originated from microbial protein were also increased from 97 to 173 mg N/L and from 67 to 106 mg N/L, respectively. Therefore, increasing RDP supply may result in increased SNAN fractions coming from microbial cells as well as solubilized dietary protein.

While SNAN in RD may be further metabolized before flowing out from the rumen, SNAN in OD indicates the terminal product of ruminal metabolism. Thus, SNAN concentration in OD rather than in RD represents a more reliable estimate of intestine-available nitrogen in the liquid phase (Ahvenjärvi et al., 2000). The current findings indicate that increasing CP level and degradability increases concentration of SNAN that is produced from ruminal metabolism and, subsequently, is provided to the intestine.

Temporal changes of soluble non-ammonia nitrogen after feeding : Temporal changes in concentrations of amino acid, peptide, soluble protein and total SNAN in the RD and OD after feeding are depicted in Figure 2 and 3, respectively. Concentration of each SNAN fraction increased rapidly after feeding and reached a maximum after 1 h. After reaching maximum levels, concentrations declined thereafter and returned to near pre-feeding levels within a 3-h period while concentrations of soluble protein in RD (Figure 2C) and OD (Figure 3C) for LCGM remained relatively constant throughout the feeding period and those for HCGM did not show a clear peak at 1 h post-feeding. Conversely, peptide concentrations in RD (Figure 2B) and OD (Figure 3B) for LCGM and HCGM increased after feeding, suggesting that soluble proteins for CGM were rapidly degraded to peptides shortly after consumption. Temporal changes in the concentration of total SNAN in RD (Figure 2D) and OD (Figure 3D) after feeding were similar to that of peptides because it was the largest proportion of total SNAN. Average proportions of peptides in total SNAN concentration were 55 and 52% for RD and OD, respectively, suggesting that soluble proteins were rapidly degraded to peptides which appeared to accumulate in the rumen. Temporal changes of total SNAN in OD after feeding were also similar to those in RD. Total SNAN concentrations in RD and OD were greater on SBM than on CGM diets during the feeding period.

Metabolism of soluble non-ammonia nitrogen : Our results showed that significant amounts of SNAN accumulated in the rumen. Accumulation of SNAN may indicate that fractions of SNAN have an opportunity to escape the rumen (Volden et al., 2002), but actual concentrations of total SNAN escaping the rumen were observed to be higher than those in RD due to higher concentrations of each SNAN fraction in the present study (Table 5; Figure 4). Choi et al. (2002b) observed a similar pattern when dairy cows were fed grass silage and rolled barley grain supplemented with protein sources (skimmed milk powder, wet distiller's solubles and rapeseed meal), as they reported a significantly higher concentration of SNAN in OD compared with RD (96 vs. 73 mg/L). These authors speculated that more concentrate particles entered and stayed in the reticulum than the rumen because concentrate has higher functional specific gravity than that of roughage. This leads to greater concentration of SNAN fractions in

OD than RD. A relatively higher dietary ratio (84:16) of concentrate:roughage in the present study than that (50:50) in the study of Choi et al. (2002b) may also have resulted in a greater difference (138.37 vs. 203.85 mg N/L) in total SNAN concentrations between RD and OD in the present study.

Pooled across treatments, mean concentrations of free amino acid in RD (33.37 mg N/L) and OD (56.23 mg N/L) were higher than the values that have been reported in a number of studies (Wright and Hungate 1967; Chen et al., 1987a; Broderick and Wallace, 1988; Nolan, 1993; Choi et al., 2002a; 2002b; 2002c; 2003). Similarly, mean concentration of peptide in OD (106.74 mg N/L) was somewhat higher than reported values (58-91 mg N/L; Choi et al., 2002a; 2002b; 2002c; 2003), although that in RD (76.44 mg N/L) in our study agrees with published values (Chen et al., 1987b; Robinson and McQueen, 1994; Robinson et al., 1998). The higher concentration of the sum of free amino acids and peptides in OD than reported values may be attributed to a high dietary ratio (84:16) of concentrate:roughage in the present study. Because concentrate contributes to soluble fractions more than roughage, one might expect SNAN concentration in RD to increase when a dietary ratio of concentrate:roughage increases. However, soluble substances may remain densely in the reticulum, due to high functional specific gravity of concentrate, and enter the omasal canal without completely mixing with rumen contents. Thus, a high dietary ratio of concentrate:roughage might influence SNAN concentration in OD more than in RD. The highest contribution of peptide to total SNAN agrees with Choi et al. (2002a) who observed that peptide rather than free amino acid or soluble protein was quantitatively the most important SNAN fraction. Mean concentrations of soluble protein pooled across treatments were 28.75 and 40.66 mg N/L in RD and OD, respectively. The concentrations of soluble protein have been shown to vary between 1-38 mg N/L in RD (Chen et al., 1987b; Robinson and McQueen 1994; Robinson et al., 1998; Choi et al., 2002b) and between 0.4-23 mg N/L in OD (Choi et al., 2002a; 2002b; 2002c; 2003). Mean concentration of soluble protein was the lowest among the fractions of SNAN (Figure 4). The lower concentrations of soluble protein in RD or OD compared with peptide concentration suggest that soluble true protein may be rapidly degraded to peptides in the rumen.

CONCLUSIONS

Results obtained in the present study showed that concentrations of SNAN in RD and OD were increased by higher level of dietary protein. In addition, SBM diets increased concentrations of SNAN in RD and OD compared to CGM diets, indicating that protein supplement with higher ruminal CP degradability results in increased SNAN. As dietary level and ruminal degradability of CP increased, RDP supply increased in the rumen, resulting in enhanced ruminal protein degradation. Consequently, increased proteolysis by increasing CP level and degradability may contribute to increased components of SNAN in the rumen and, subsequently, in the omasum. Because SNAN in OD indicates the terminal product of ruminal metabolism, increasing CP level and degradability has been shown to increase the amount of intestine-available nitrogen in the liquid phase.

ACKNOWLEDGMENTS

This study was supported by Technology Development Program of the Ministry of Agriculture and Forestry, Republic of Korea. The authors acknowledge the financial support of the Ministry of Agriculture and Forestry.

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