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Effects of Protein Supply from Soyhulls and Wheat Bran on Ruminal Metabolism, Nutrient Digestion and Ruminal and Omasal Concentrations of Soluble Non-ammonia Nitrogen of Steers

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ABSTRACT : Three beef steers fitted with permanent cannulae in the rumen and duodenum were used to determine the effects of protein supply from soyhulls (SH) and wheat bran (WB) on ruminal metabolism, blood metabolites, nitrogen metabolism, nutrient digestion and concentrations of soluble non-ammonia nitrogen (SNAN) in ruminal (RD) and omasal digesta (OD). In a 3×3 Latin square design, steers were offered rice straw and concentrates formulated either without (control) or with two brans to increase crude protein (CP) level (9 vs. 11% dietary DM for control and bran-based diets, respectively). The brans used were SH and WB that had similar CP contents but different ruminal CP degradability (52 vs. 80% CP for SH and WB, respectively) for evaluating the effects of protein degradability. Ruminal ammonia concentrations were higher for bran diets ($p<0.01$) than for the control, and for WB ($p<0.001$) compared to the SH diet. Similarly, microbial nitrogen and blood urea nitrogen were significantly increased ($p<0.05$) by bran and WB diets, respectively. Retained nitrogen tended ($p<0.082$) to be increased by SH compared with the WB diet. Intestinal and total tract CP digestion was enhanced by bran diets. In addition, bran diets tended ($p<0.085$) to increase intestinal starch digestion. Concentrations of SNAN fractions in RD and OD were higher ($p<0.05$) for bran diets than for the control, and for WB than for the SH diet. More rumen-degraded protein supply resulting from a higher level and degradability of CP released from SH and WB enhanced ruminal microbial nitrogen synthesis and ruminal protein degradation. Thus, free amino acids, peptides and soluble proteins from microbial cells as well as degraded dietary protein may have contributed to increased SNAN concentrations in the rumen and, consequently, the omasum. These results indicate that protein supply from SH and WB, having a low level of protein (13 and 16%, respectively), could affect ruminal metabolism and nutrient digestion if inclusion level is relatively high (>20%). (**Key Words :** Soyhulls, Wheat Bran, Soluble non-ammonia Nitrogen, Omasal Digesta, Nutrient Digestion)

INTRODUCTION

Dietary protein available for absorption in the intestine is often supplemented to improve the performance of cattle. The common practice for increasing metabolizable protein supply is to feed protein supplements that are low in ruminal degradability (NRC, 2001). However, typical rumen-undegraded protein (RUP) supplements such as corn gluten meal (CGM) and palm meal are usually the most expensive ingredients in cattle diets. Brans have varying ruminal crude protein (CP) degradabilities despite lower CP levels compared to that of typical protein sources; rumen-degraded protein (RDP) contents of rice hull, soyhulls (SH) and wheat bran (WB) are estimated to be 52.3, 55.4 and 79.3%, respectively (NRC, 2001). We thought it might be interesting to find out if rice hull or SH having relatively low ruminal degradability could play a role of RUP in diets. If so, manipulation of ruminal protein release using brans could be beneficial due to their lower prices than conventional protein supplements. However, lack of data on metabolic or digestive responses to increasing dietary CP contents and RUP contents with brans makes it difficult to

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use them as protein sources.

A substantial amount of soluble non-ammonia nitrogen (SNAN), composed of amino acids, peptides and soluble protein, can escape ruminal degradation (Choi et al., 2002a; 2002b; 2002c; 2003) and, thus, be potentially absorbed as amino acids and peptides in the small intestine. A rise in concentrations of SNAN in the omasum by increasing CP level with protein supplements has been reported using skimmed milk powder, wet distiller's solubles and rapeseed meal (Choi et al., 2002b), and CGM, soybean meal (SBM) and fish meal (Choi et al., 2002c). In addition, comparing SBM and CGM, Oh et al. (2008) reported that protein supplement with higher ruminal CP degradability increases SNAN in the rumen and omasum. Although responses of SNAN to manipulation of ruminal protein release with brans have not been studied, we hypothesized that increasing level or ruminal degradability of dietary CP with brans would enhance SNAN concentrations in the rumen, and, subsequently, in the omasum if protein released from brans could influence ruminal nitrogen (N) metabolism in the same manner as protein supplements. Therefore, the present study was conducted to investigate the effects of increasing CP level (9 vs. 11%) with SH and WB and ruminal protein degradability of SH and WB (RDP, 52 vs. 80% of CP, respectively) on ruminal metabolism, blood metabolites, N metabolism, nutrient digestion and SNAN concentrations in the ruminal (RD) and omasal digesta (OD) of beef steers.

MATERIALS AND METHODS

Animals, treatments and management

Three Korean Hanwoo beef steers (mean 525.5±2.8 kg) fitted with permanent cannulae in both the rumen and duodenum were used in a 3×3 Latin square experiment with three 14-day experimental periods. Steers were offered concentrates formulated either without (control) or with two brans to increase CP level (9 and 11% of dietary dry matter (DM) for control and bran-based diets, respectively). The brans used were SH and WB that had similar CP contents but different ruminal CP degradabilities (RDP, 52 vs. 80% of CP for SH and WB, respectively; Table 1) for evaluating the effect of protein degradability. Steers were adapted to experimental stalls for 10 days prior to commencement of the experimental period. During the experimental period, 5.6 kg/d of concentrate and 1.4 kg/d of rice straw (concentrate:roughage ratio of 80:20, as fed basis) were fed to the steers to ensure that effects of feed intake were excluded. Ingredients and chemical composition of the experimental diets are shown in Table 1. Diets were offered twice daily at 09:00 and 21:00 h in equal amounts. Each steer had free access to water and free choice mineral throughout the experiment. Each experimental period consisted of an adaptation period of 11 days and a collection period of 3 days.

Measurements and sample collection

Feed intake was recorded daily but steers consumed each meal completely because intake was restricted (7 kg/d). Representative samples of the experimental diets were collected daily, composited at the end of each period, dried and ground through a 1-mm screen with a Wiley mill (Thomas Scientific, Model 4, NJ, USA) for analysis. For *in situ* determination of diets, three other Korean Hanwoo steers (mean 569.3±1.8 kg) with permanent ruminal cannulae were used. They were fed the control diet (see Table 1 for ingredients and chemical composition). Test feeds, control, SH-based and WB-based diets, were milled through a 2-mm screen and 5 g of each feed was placed in a nylon bag (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. Upon removal from the rumen at the determined intervals, 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 CP at each incubation time was expressed relative to the original CP content of the feed, which was not incubated and washed. Then, degradation values of DM and 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) assuming a rumen outflow rate of 0.02 and 0.04 h^{-1} for rice straw and other feeds, respectively.

On day 12 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 analyzed.

During the last 2 days of each period, 100 ml of RD and OD and 100 g of duodenal digesta were collected. On day 13, samples were taken from 09:00 to 19:00 h, and on day 14, samples were taken from 10:00 to 20:00 h at 2 h intervals for each day. Thus, a total of 12 samples were taken. Ruminal digesta was sampled from the rumen via the ruminal cannula and OD was collected using a tube (14 mm i.d.) that was passed through the ruminal cannula and

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

Items	Brans[2]		Experimental diets[3]		
	SH	WB	Control	SH diet	WB diet
Ingredients (% dry matter (DM))					
Ground corn			53.8	26.4	38.5
Corn gluten feed			4.0	2.0	2.9
Beetpulp pellet			20.9	10.3	14.9
Salt			0.3	0.3	0.3
Calcium phosphate			0.8	0.8	0.8
Vitamin-mineral additive[4]			0.2	0.2	0.2
Soybean hull			-	40.0	-
Wheat bran			-	-	22.4
Rice straw			20.0	20.0	20.0
Total			100.0	100.0	100.0
Composition (% DM)					
DM (%)	90.3	88.4	87.0	87.6	86.7
Ruminal DM degradability (%)[5]	57.2	65.5	50.6	50.8	52.5
Crude protein (CP)	13.0	16.1	9.2	11.2	11.3
Rumen-degraded protein (RDP)[5]	5.2	9.7	4.1	5.4	6.6
RDP (% CP)	52.3	79.8	44.1	48.0	58.6
Ether extract	2.0	3.9	3.4	3.3	3.4
Ash	3.9	5.1	6.1	6.6	7.0
Neutral detergent fiber	66.8	40.1	30.5	51.2	34.3
Acid detergent fiber	31.4	9.6	12.9	30.7	13.2
Starch	ND[6]	ND	52.4	31.9	48.4
Calorie (gross energy, Mcal/kg DM)	ND	ND	3,787.4	3,793.0	3,720.2

[1] Each steer had free access to water and a free choice mineral (Rineal block, Daehan New Pham, Seoul, Korea; provided 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] SH = Soyhulls, WB = Wheat bran.
[3] Control; basal diet without SH and WB (CP 9.2%), SH diet; soyhulls (RDP 52.3%)-based diet (CP 11.2%), WB diet; wheat bran (RDP 79.8%)-based diet (CP 11.3%).
[4] Provided 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.
[5] Calculated as the effective degradability of DM and CP from *in situ* incubations (Ørskov and McDonald, 1979); using a rumen outflow rate of 0.02 h^{-1} and 0.04 h^{-1} for rice straw and other feeds, respectively.
[6] ND = Not determined.

positioned in the omasal canal according to the procedure outlined by Huhtanen et al. (1997), with modifications described by Ahvenjärvi et al. (2000) as follows: i) larger sampling tube (14 vs. 9.5 mm i.d.), ii) solenoid valves instead of a three way ball valve to control vacuum and pressure phases in the pump and iii) a 0.5 kg weight inserted into the abomasum for securing the sampling device in the omasum. Duodenal digesta was sampled from the duodenum via the duodenal cannula by using a plug covering the passage of the duodenal cannula toward the jejunum for securing collection of whole duodenal digesta. After sampling, pH of RD was immediately measured (Pinnacle M530, Corning, NY, USA) and RD and OD were filtered through four layers of cheesecloth and 1% saturated $HgCl_2$ was added to prevent microbial activity. Composite duodenal samples, formed by combining 100 g from each duodenal sample, were lyophilized and ground through a 1-mm screen for analysis. The duodenal flow of digesta was measured using chromic oxide as an external marker (Merchen, 1988). The marker was mixed with concentrate and fed to steers at each feeding (14 g/d as 2 g/kg of diet). To measure total tract digestibility, N balance and microbial N supply, total feces and urine were collected for the last 3 days of each period. Collection boxes were emptied once daily at 09:00 h and samples were stored frozen at -20°C. After thawing, fecal samples were dried at 60°C for 96 h

and ground through a 1-mm screen for analysis.

Chemical analyses

Feed samples were analyzed for moisture, CP, ether extract, and ash according to AOAC procedures 934.01, 976.05, 920.39, and 927.02, respectively (AOAC, 1990). Concentration of neutral detergent fiber exclusive of residual ash (aNDFom) was determined with the use of a heat stable amylase and sodium sulphite according to the methods of Van Soest et al. (1991), and level of acid detergent fiber exclusive of residual ash (ADFom) was determined according to procedure 973.18 of AOAC (1990). Starch concentration was determined according to procedure 920.40 of AOAC (1990). Gross energy concentration was measured using a bomb calorimeter (CA-3, Shimadzu corporation, Kyoto, Japan). Fecal and duodenal samples were analyzed for moisture, CP and ash. Chromic oxide in duodenal samples was analyzed by the method of Fenton and Fenton (1979). Urinary samples were analyzed for total N and allantoin according to procedure 976.05 of AOAC (1990) and the method of Borchers (1977), respectively. Before analyzing allantoin, urinary samples were mixed with xanthine oxidase and stood for 2 h at room temperature to convert hypoxanthine and xanthine to uric acid and, subsequently, mixed with uricase and stood for 2 h to convert uric acid to allantoin. Microbial N supply was computed from total absorption of microbial purines, calculated as total purine derivatives, hypoxanthine, xanthine, uric acid and allantoin in the urine, according to the equations of Chen and Gomes (1992).

Plasma was analyzed for total protein and blood urea N (BUN) using an automated blood analyzer (Express Plus, Ciba-Corning, CA, USA) according to the biuret method of Flack and Woollen (1984) and the urease method of Roch-Ramel (1967), respectively. To determine ruminal ammonia (NH_3) 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 volatile fatty acid (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) as an internal standard (980 g/kg 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 Oh et al. (2008) as follows. Ruminal digesta and OD were centrifuged at 1,000×g for 10 min at 4°C to eliminate small particles and protozoa, followed by high-speed centrifugation (10,000×g for 60 min at 4°C) to eliminate 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 NH_3 N while the TCA-precipitated pellet was comprised of protein and some NH_3. Each fraction of SNAN in the sample was estimated as follows: i) free amino acids as N from supernatant without acid-hydrolysis, ii) peptides as N from the difference between hydrolyzed supernatant (6 M HCl at 110°C for 24 h) and free amino acid N, and iii) 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 treated 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, N balance and nutrient digestibility was subjected to statistical analysis using the GLM procedure of SAS (2002; version 9.1) according to the following statistical model:

$$Y_{ijk} = \mu+A_i+P_j+D_k+e_{ijk}$$

where A, P and D are animal, period, diet effects, respectively.

Data obtained from pH, NH_3 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:

$$Y_{ijkl} = \mu+A_i+P_j+D_k+e_{ijk}+T_l+(A\times T)_{il} +(P\times T)_{jl}+(D\times T)_{kl}+ e_{ijkl}$$

where T is time effect, and A×T, P×T and D×T are animal by time, period by time and diet by time interactions, respectively. Animal effect, animal by time interaction and error terms (e_{ijk} defined as between unit error and e_{ijkl} as

Table 2. Effects of protein supply from soyhulls and wheat bran on ruminal metabolism and blood metabolites

Items	Diets[1]			SEM[2]	Statistical significance of (p<)[3]	
	Control	SH	WB		C_1	C_2
Ruminal parameters						
pH	6.58	6.64	6.58	0.05	0.485	0.302
Volatile fatty acids (m*M*)						
Acetate	61.75	64.55	57.46	10.38	0.605	0.256
Propionate	18.17	17.3	18.96	2.52	0.497	0.392
Isobutyrate	0.93	0.96	0.83	0.14	0.827	0.512
Butyrate	12.11	9.94	11.09	2.40	0.402	0.587
Isovalerate	1.65	1.92	1.32	0.30	0.946	0.222
Valerate	0.90	1.01	0.91	0.12	0.608	0.470
Total	95.51	96.11	90.57	15.49	0.571	0.340
Ammonia (mg/L)	31.26	38.74	72.71	2.71	0.002	0.001
Microbial nitrogen supply (g N/d)[4]	21.05	29.30	39.87	0.91	0.007	0.015
Blood metabolites						
Total protein (g/dl)	7.28	7.38	7.27	0.05	0.607	0.270
Urea nitrogen (mg/dl)	5.44	7.75	9.65	0.29	0.011	0.042

[1] Control; basal diet without SH and WB (crude protein 9.2%), SH; soyhulls (rumen-degraded protein 523 g/kg)-based diet (crude protein 11.2%), WB; wheat bran (rumen-degraded protein 79.8%)-based diet (crude protein 11.3%).
[2] Standard error of mean; n = 108 for pH, volatile fatty acids and ammonia, n = 27 for microbial protein supply and n = 18 for total protein and urea nitrogen.
[3] C_1, control vs. bran diets; C_2, SH vs. WB.
[4] Estimated from purine derivatives excreted in the urine.

within unit error) are multivariate normally distributed random effects with AR (1) covariance structure. Orthogonal contrasts used in post-ANOVA comparisons were as follows; i) effect of increasing protein level with brans (control vs. bran diets), ii) comparison between low and high RDP contents in bran sources (SH- vs. WB-based diet).

RESULTS AND DISCUSSIONS

Nutrient composition and *in situ* degradation of feeds

The ingredients and chemical composition of diets are shown in Table 1. Diets were computed to be isoenergetic, with 3,800 Mcal/kg of dietary DM. The CP concentrations of the experimental diets were close to expected values, at approximately 9 and 11% DM for control and bran diets, respectively. The SH diet had more NDF and ADF but less starch content compared to other treatments. This was due to the high fiber content and low starch content of SH. However, fiber in SH is highly digestible as shown by *in vivo* digestibility and *in situ* disappearance (Nguyen et al., 2008). In fact, ruminal DM degradabilites of diets, calculated as the effective DM degradability obtained from the present *in situ* determinations, were similar among treatments. Greater ruminal CP degradabilities of WB than SH resulted in higher content of RDP for the WB diet than the SH diet.

Ruminal fermentation parameters and blood metabolites

Effects of protein supply from SH and WB on ruminal metabolism and blood metabolites are shown in Table 2. Ruminal pH and concentrations of ruminal VFA were unaffected by diet ($p>0.05$). Klopfenstein and Owen (1987) reported that feeding SH prevented drastic reductions in ruminal pH. However, pH for the SH diet was not significantly different from the other treatments. This discrepancy could be attributed to relatively high ruminal pH in the present study. Within 12 h after feeding, the ruminal pH levels of all treatments were ≥6.3 (time data not shown), which is the pH identified as critical for maintaining ruminal fiber digestion (Stewart, 1977; Hiltner and Dehority, 1983).

Bran diets increased ($p<0.01$) ruminal NH_3 N concentration compared with the control. In addition, ruminal NH_3 N was higher ($p<0.001$) for the WB than for the SH diet. These observations are consistent with other studies in which increasing CP content (Oh et al., 2007) and RDP fraction (Davidson et al., 2003) in the diet increased ruminal NH_3 N concentration. Ruminal NH_3 N accumulates in the rumen when release of energy is not coupled with the release of NH_3 N in the early phase after feeding (Kennedy and Milligan, 1980; Nocek and Russell, 1988). Thus, increased ruminal NH_3 N concentrations with increasing CP level in bran diets were expected because the increased

dietary RDP (4.1 vs. 6.0% DM for control and bran diets (average of SH and WB diets), respectively; Table 1) was probably uncoupled from energy levels that were similar among treatments. Similarly, the WB diet contained more RDP than the SH diet, which explains the nearly two-fold increase in NH_3 N in the present study.

Microbial N supply was higher for bran diets ($p<0.01$) than for the control and for WB ($p<0.05$) than for the SH diet. Increasing dietary CP concentration and RDP fraction has been reported to increase microbial N supply. Olmos Colmenero and Broderick (2006) found that omasal flow of total bacterial non-NH_3 N showed a linear increase from 425 to 480 g/d when dietary CP increased from 13.5 to 19.4%. Similarly, omasal flows of microbial non-NH_3 N increased from 384 to 470 g/d when RDP level increased from 10.6 to 13.2% (Reynal and Broderick; 2005). The effect of diet on microbial N supply is attributed to differences in availability of NH_3 N, amino acids and peptides in the rumen (Russell et al., 1983). Ruminal NH_3 N concentrations for the control and SH diet were lower than 50.0 mg/L which is widely accepted as the minimum concentration at which maximum microbial growth occurs (Satter and Slyter, 1974). Thus, microbial growth for the control and SH diets was probably limited by low concentrations of ruminal NH_3 N which were lower than 50.0 mg/L. In the present study, increased CP in the bran diets compared with the control and increasing RDP with WB compared with the SH diet resulted in increases in concentrations of free amino acids and peptides in the rumen (64.97, 87.62 and 108.36 mg N/L for control, SH and WB diets, respectively; see Table 6), which would in part have stimulated microbial N supply (Russell et al., 1983).

Although no differences in levels of total protein in blood among treatments were observed, BUN level was higher ($p<0.05$) for bran diets than for the control, and for WB than for the SH diet. The bran diets would be expected to increase BUN because ruminal NH_3 N concentrations for bran diets were higher. Similarly, the effect of increased RDP on levels of BUN was expected. In the present study, ruminal NH_3 N concentration for the WB diet was higher than for the SH diet and, as a result, BUN for the WB diet were greater compared with the SH diet.

Nitrogen output and retention

Effects of protein supply from SH and WB on N output and retention are shown in Table 3. Fecal N excretion tended ($p<0.051$) to decrease in response to bran diets compared with the control. When output was expressed as a proportion of N intake, fecal N excretion significantly ($p<0.05$) declined in bran diets. However, ruminal protein degradability did not affect fecal N excretion. Urinary N excretion increased ($p<0.05$) in response to the bran diets compared with the control and on WB compared with the SH diet. The bran diets and WB diet also increased ($p<0.01$) urinary N excretion when output was expressed as a proportion of N intake.

The mean difference in N intake between the control and bran diets was almost 15 g/d. As a result, significant differences in N excretion were observed. The most distinctive effect of bran diets on N excretion was on urinary N excretion. Increasing protein supply may have resulted in the excess N being excreted as urea in the urine. It has been widely accepted that as dietary protein increases, urinary N increases linearly and becomes the primary route for N excretion from cattle (Susmel et al., 1995; Tamminga, 1992). Increase in urinary N excretion with the WB diet may be related to ruminal NH_3 N concentration. Since energy supply was equal among treatments in the present study, increased urinary N excretion might be indicative of inefficient capture of ruminal NH_3 N for microbial protein

Table 3. Effects of protein supply from soyhulls and wheat bran on nitrogen (N) output and retention

Items	Diets[1]			SEM[2]	Statistical significance of (p<)[3]	
	Control	SH	WB		C_1	C_2
N intake (g/d)	89.70	105.59	103.65	2.21	-	-
N output (g/d)						
Fecal N	48.36	37.34	31.66	2.65	0.051	0.269
Urinary N	18.21	26.88	46.69	1.78	0.014	0.016
N retention	23.13	41.38	21.65	3.06	0.112	0.065
N output (% N intake)						
Fecal N	53.90	35.35	30.60	2.99	0.029	0.379
Urinary N	20.44	25.47	44.99	1.16	0.009	0.007
Retained N	25.67	39.18	24.41	3.20	0.258	0.082

[1] Control; basal diet without SH and WB (crude protein 9.2%), SH; soyhulls (rumen-degraded protein 52.3%)-based diet (crude protein 11.2%), WB; wheat bran (rumen-degraded protein 79.8%)-based diet (crude protein 11.3%).
[2] Standard error of mean; n = 27.
[3] C_1, control vs. bran diets; C_2, SH vs. WB.

Table 4. Effects of protein supply from soyhulls and wheat bran on apparent crude protein disappearance in the gastrointestinal tract of steers

Items	Diets[1]			SEM[2]	Statistical significance of (p<)[3]	
	Control	SH	WB		C_1	C_2
Intake (g/d)	560.65	659.96	674.83	13.81	-	-
Duodenal flow (g/d)	918.31	1167.84	1054.73	14.20	0.041	0.092
Fecal flow (g/d)	302.24	233.35	197.90	16.55	0.051	0.269
Disappearance (g/d)						
Rumen	-357.65	-507.88	-406.91	36.42	0.155	0.189
Intestine	616.07	934.49	856.83	38.68	0.080	0.302
Total tract	258.41	426.61	449.93	23.45	0.040	0.727
Disappearance (%)[4]						
Rumen	-63.79	-76.96	-62.81	5.41	0.460	0.205
Intestine	67.09	80.02	81.24	2.21	0.077	0.605
Total tract	46.09	64.64	69.45	2.85	0.056	0.856

[1] Control; basal diet without SH and WB (crude protein 9.2%), SH; soyhulls (rumen-degraded protein 52.3%)-based diet (crude protein 11.2%), WB; wheat bran (rumen-degraded protein 79.8%)-based diet (crude protein 11.3%).
[2] Standard error of mean; n = 27. [3] C_1, control vs. bran diets; C_2, SH vs. WB.
[4] Disappearance as per cent of flow to the segment.

synthesis (Wright et al., 1998). When expressed as a proportion of N intake, urinary N excretion became the primary route of excretion for approximately 45% of total consumed N in the WB diet.

Although N retention was not affected by bran diets, steers fed SH tended (p<0.065) to retain more N than steers fed the WB diet. Similarly, Castillo et al. (2001) reported that N retention for cows fed highly degradable protein (RDP, 63.9% CP) was lower than for cows fed low degradable protein (RDP, 38.9% CP) (5.2 vs. 40.0 g/d). Wright et al. (1998) also reported that dietary protein degradability altered N retention with a decline for a highly degradable protein-fed treatment. Changes in protein degradability resulted in a significant repartitioning of excreted N between the urine and feces, contributing to the trend toward difference in retained N between SH and WB diets. Higher retained N for the SH diet was mainly attributed to lower urinary N excretion than for the WB diet.

Apparent crude protein and starch digestion in the gastrointestinal tract

Effects of protein supply from SH and WB on apparent digestion of CP in the gastro-intestinal tract are shown in Table 4. Flow of CP at the duodenum was higher (p<0.05) for bran diets than for the control and tended (p<0.092) to be higher for SH compared with the WB diet. Fecal CP flow tended (p<0.051) to decrease with the bran diets but protein degradability did not affect it. A lower fecal CP flow with bran diets would suggest that the treatments applied to increase dietary CP level with SH and WB may have increased total tract CP digestibility. Ruminal CP digestion was not affected by treatment. Negative digestibility values for ruminal CP digestion would have arisen from a great quantity of N being recycled to the rumen as urea and NH_3 and by the contribution of sloughed cells from the rumen wall (Richards et al., 2002). Although CP digestion was not affected by protein degradability in the rumen, intestine and total tract, bran diets tended (p<0.080) to increase quantity of CP apparently disappearing in the intestine, resulting in an increased quantity for the total tract (p<0.05). In addition, when expressed as per cent of flow, intestinal (p<0.077) and total tract (p<0.056) CP digestion for bran diets tended to be higher compared with the control. Our results showed that CP flowing to duodenum was more digestible in the intestine for bran diets. Increased total tract digestibility for bran diets, mainly due to enhanced intestinal digestibility, is consistent with previous studies (Cressman et al., 1980; Grieve et al., 1980) that have shown increased apparent N digestibility as dietary protein increased.

Effects of protein supply from SH and WB on apparent digestion of starch in the gastro-intestinal tract are shown in Table 5. Due to lower starch content in the SH diet than the other diets, differences in starch intake, duodenal flow, fecal flow and quantity apparently disappearing in the total tract among treatments were observed. However, ruminally digested starch was not affected by either bran diets or protein degradability when expressed as per cent of flow. Although protein degradability did not affect intestinal and total tract digestion of starch, intestinal digestibility tended (p<0.085) to be increased and total tract digestibility was increased (p<0.05) by the bran diets compared with the control. Increasing dietary CP supply has been reported to

Table 5. Effects of protein supply from soyhulls and wheat bran on apparent starch disappearance in the gastrointestinal tract of steers

Items	Diets[1]			SEM[2]	Statistical significance of (p<)[3]	
	Control	SH	WB		C_1	C_2
Intake (g/d)	3,161.21	1,943.89	2,909.23	61.37	-	-
Duodenal flow (g/d)	1,127.57	835.48	779.72	16.77	0.048	0.830
Fecal flow (g/d)	333.14	128.18	194.38	22.12	0.024	0.169
Disappearance (g/d)						
Rumen	2,033.65	1,108.40	2,129.51	131.75	0.124	0.032
Intestine	794.43	707.30	585.33	49.56	0.504	0.622
Total tract	2,828.07	1,815.71	2,714.84	39.28	0.007	0.004
Disappearance (%)[4]						
Rumen	64.43	56.77	73.26	6.01	0.944	0.192
Intestine	69.41	84.06	74.89	3.61	0.085	0.214
Total tract	89.51	93.40	93.33	0.70	0.047	0.955

[1] Control; basal diet without SH and WB (crude protein 9.2%), SH; soyhulls (rumen-degraded protein 52.3%)-based diet (crude protein 11.2%), WB; wheat bran (rumen-degraded protein 79.8%)-based diet (crude protein 11.3%).
[2] Standard error of mean; n = 27. [3] C_1, control vs. bran diets; C_2, SH vs. WB.
[4] Disappearance as per cent of flow to the segment.

increase total tract starch digestibility. Veira et al. (1980) reported that total tract starch disappearance increased linearly with increasing CP in the diet of Holstein calves. Increased total tract starch digestion of the bran diets may be attributed to a trend toward enhanced starch digestion in the intestine. This result is consistent with that of Taniguchi et al. (1995) in which small-intestinal starch disappearance increased in sheep infused with casein into the abomasum. Enhanced intestinal starch digestion of the bran diets might be associated with increased duodenal CP flow (918 vs. 1,111 g/d for control and bran diets (average of SH and WB diets), respectively) because increasing abomasal protein flow of steers has been reported to increase quantity of starch disappearing in the small intestine (Richards et al., 2002). Nocek and Tamminga (1991) reported an increase in synthesis and secretion of pancreatic amylase when protein flow to the duodenum of steers increased. Richards et al. (2003) also reported that concentration and total secretion of pancreatic α-amylase increased in steers infused with increasing quantities of protein into the abomasum. Thus, increased duodenal CP flow by increasing CP supply from SH and WB would have been responsible for enhanced intestinal digestion of starch by increasing secretion of pancreatic α-amylase.

Soluble non-ammonia nitrogen in the rumen and omasum

Effects of protein supply from SH and WB on concentrations of free amino acids, peptides, soluble protein and total SNAN in the RD and OD are shown in Table 6. Concentrations of peptides in the RD ($p<0.01$) and OD ($p<0.05$) increased with the bran diets compared with the control but those of amino acids were unaffected. However, concentrations of the sum of free amino acids and peptides in RD and OD were significantly higher ($p<0.01$) for the bran diets than for the control. Similarly, the WB diet had increased ($p<0.05$) concentrations of the sum of free amino acids and peptides in RD and OD compared with the SH diet, although both free amino acids and peptides were unaffected by protein degradability. Discrepancy of statistical significance between individual and sum of free amino acids and peptides may be related to the analytical method for SNAN fractions; accurate distinction between amino acids and peptides is hard to estimate. Ninhydrin reacts with free amino acids and with the terminal amino groups of peptides in a mixed solution containing free amino acids and peptides (Rosen, 1957). Thus, concentrations of free amino acids and peptides determined by the method used in the present study may be somewhat different from real values. However, estimations of the sum of peptide and free amino acid concentrations can represent the real values because all peptides are hydrolyzed to ninhydrin-reactive amino acids.

Soluble protein concentrations were higher for the bran diets ($p<0.001$) than for the control, and for the WB diet ($p<0.05$) than for the SH diet only in OD. Total SNAN concentrations in both RD and OD showed similar responses to the sum of free amino acids and peptides. With a cut off at around 10 amino acids, TCA is a common precipitant applied to feed (Licitra et al., 1996) with a 5% concentration causing maximum precipitation (Greenberg and Shipe, 1979). However, due to the fluffy nature of some of the pellets formed after centrifugation, some soluble protein could remain in the supernatant, possibly resulting

Table 6. Effects of protein supply from soyhulls and wheat bran 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]			SEM[2]	Statistical significance of (p<)[3]	
	Control	SH	WB		C_1	C_2
FAA[4]						
Ruminal	19.95	17.16	23.10	1.80	0.939	0.075
Omasal	24.30	23.56	26.94	2.73	0.767	0.404
Peptide						
Ruminal	45.02	70.46	85.26	5.79	0.008	0.139
Omasal	58.46	78.28	95.16	8.47	0.040	0.215
FAA+peptide						
Ruminal	64.97	87.62	108.36	5.95	0.010	0.047
Omasal	82.76	101.84	122.10	5.53	0.004	0.036
Soluble protein						
Ruminal	16.59	20.35	22.33	2.21	0.149	0.496
Omasal	20.32	24.15	26.97	1.84	0.001	0.032
SNAN[5]						
Ruminal	79.40	105.81	127.66	7.52	0.042	0.041
Omasal	103.08	125.99	149.07	5.86	0.002	0.025

[1] Control; basal diet without SH and WB (crude protein 9.2%), SH; soyhulls (rumen-degraded protein 52.3%)-based diet (crude protein 11.2%), WB; wheat bran (rumen-degraded protein 79.8%)-based diet (crude protein 11.3%).
[2] Standard error of mean; n = 108. [3] C_1, control vs. bran diets; C_2, SH vs. WB. [4] FAA; free amino acid.
[5] Calculated as the sum of free amino acid, peptide and soluble protein.

in underestimation of soluble protein (Reynal et al., 2007). In the present study, to minimize fluffy pellets in the supernatant, high-speed centrifugation (10,000×g for 60 min) was employed.

Increasing dietary protein level with protein supplements has been previously reported to enhance concentrations of SNAN fractions. Robinson et al. (1998) reported that urea supplementation increased concentrations of free amino acids and peptides (83.6 vs. 105.3 mg N/L for control and urea, respectively) and soluble protein (11.5 vs. 17.0 mg N/L for control and urea, respectively) in RD, compared with the control. Chen et al. (1987) reported that concentrations in the rumen and estimated flow to the omasum of peptides (including free amino acids) 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). In OD, concentrations of peptides (56.0, 81.0, 78.4 and 72.8 mg N/L for control, fish meal, SBM and CGM, respectively) and total SNAN (69.8, 98.8, 95.5 and 92.0 mg N/L for control, fish meal, SBM and CGM, respectively) were increased with protein supplementation (Choi et al., 2002c). Choi et al. (2002b) also observed that protein supplementation (skimmed milk powder, wet distiller's solubles, rapeseed meal) increased concentrations of total SNAN and each SNAN fraction in both RD and OD compared with the control, except for peptides. In the present study, increasing dietary protein level with SH and WB showed similar results to using protein supplements. Bran diets supplied more RDP than the control (Table 1) and thus would have resulted in increased proteolysis of protein released from SH and WB. The increased proteolysis may contribute to increased supply of components of SNAN in the rumen and, consequently, the omasum.

Previous experiments have reported that concentrations of SNAN were not different among diets supplemented with different types of protein supplements in RD (Chen et al., 1987; Robinson and McQueen, 1994; Robinson et al., 1998; Choi et al., 2002b) and OD (Choi et al., 2002b; 2002c). However, degradability of protein supplements, fish meal and SBM used by Choi et al. (2002c) and blood meal and SBM used by Robinson et al. (1998), was not measured. In addition, the differences in CP degradability (RDP, % CP) among protein supplements, untreated (83.0) and heat-treated rapeseed meals (81.7) used by Choi et al. (2002b), blood meal+CGM (75.7) and SBM (85.2) used by Robinson and McQueen (1994) and SBM (69.8), extruded SBM (66.2) and fish meal (65.4) used by Chen et al. (1987), were small. Thus, these studies did not show the effect of degradability of dietary protein on concentrations of SNAN. In a recent study (Oh et al., 2007), investigating the effect

of protein degradability of CGM and SBM (RDP, 23.4 vs. 62.1% CP for CGM and SBM, respectively), concentrations of SNAN fractions increased in RD and OD of steers fed a SBM diet compared with a CGM diet, indicating that higher protein degradability increases SNAN concentrations.

Similarly, RDP fraction in the WB diet was higher than for the SH diet in the present study (Table 1). It is expected that high RDP would be accompanied by relatively high ruminal protein degradation and, in many cases, by high solubilization of protein, which could contribute to increased SNAN fractions by release of free amino acids and peptides in the rumen after hydrolysis. In addition, increasing RDP supply in the rumen probably results in enhanced microbial protein synthesis because microbes can benefit from amino-N for growth. In the study of Choi et al. (2002b) in which flow of microbial non-NH_3 N into the omasal canal increased from 159 to 241 g N/d, concentrations of total SNAN in OD and soluble N originating from microbial protein also increased from 97 to 173.3 mg N/L and from 66.8 to 105.5 mg N/L, respectively. Thus, increasing RDP supply from WB would have resulted in increased components of SNAN coming from the 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 is the end product of ruminal metabolism. Therefore, the current findings indicate that increasing level and degradability of CP derived from brans increases concentrations of SNAN as a result of ruminal metabolism and, subsequently flows to the small intestine.

CONCLUSIONS

Results obtained in the present study show that increasing protein level with SH and WB enhanced concentrations of ruminal NH_3 N and BUN, microbial N supply, digestion of CP and starch in the intestine and total tract, and concentrations of SNAN in RD and OD of steers. In addition, ruminal CP degradability of SH and WB affected ruminal and N metabolism and SNAN concentrations. These results indicate that protein supply from SH and WB having a low level of protein could affect ruminal metabolism and nutrient digestion, if inclusion level is relatively high (>20%).

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