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Effects of Non-ionic Surfactant Supplementation on Ruminal Fermentation, Nutrient Digestibility and Performance of Beef Steers Fed High-roughage Diets

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ABSTRACT : Three experiments were conducted to determine the effects of non-ionic surfactant (NIS) supplementation on ruminal fermentation, nutrient digestibility and performance of beef steers fed high-roughage diets. The objective of experiment 1 was to investigate the effects of NIS supplementation on *in vitro* ruminal fermentation of cultures administered with corn and barley as grain substrate and rice straw and timothy hay as roughage substrate. The *in vivo* ruminal fermentation, nitrogen balance and digestibility of nutrients were also examined with steers fed a high-roughage diet in experiment 2. The aim of experiment 3 was to determine the responses to NIS of growing steers fed a high-roughage diet. In experiment 1, ammonia nitrogen concentration for NIS supplementation was higher ($p<0.05$) than for the control with all substrates. However, concentrations of total volatile fatty acid (VFA), acetate, butyrate and valerate of the incubated roughage substrates, rice straw and timothy hay, were higher ($p<0.05$) for NIS supplementation than for the control whereas VFA concentrations in the cultures of corn and barley were unaffected. These results indicated that effects of NIS on ruminal fermentation are diet dependent, specifically on roughage sources. In experiment 2, ruminal pH of steers supplemented with NIS was lower ($p<0.05$) than the control. Ruminal concentrations of ammonia nitrogen, acetate, total VFA and urinary concentrations of purine derivatives were increased ($p<0.05$) by NIS supplementation. In experiment 3, supplementation of NIS increased ($p<0.05$) intakes of total feed and corn silage, average daily gain, and feed efficiency of growing steers although they varied depending on supplementation level. Due to the roughage-specific feature of NIS effects, NIS appears to enhance ruminal fermentation of fibrous parts of feeds and, consequently, performance of steers fed a high-roughage diet. (**Key Words :** Non-ionic Surfactant, Tween 80, Ruminal Fermentation, Nutrient Digestibility, Growing Steer)

INTRODUCTION

Roughage sources, such as silage, hay or straw, are fermented in the rumen at a slow rate thereby limiting the efficient use of roughage sources. Non-ionic surfactant (NIS) has been widely used to increase the ruminal fermentation of roughage because NIS enhances the enzymatic hydrolysis of cellulose in bioreactors by increasing stability of cellulase (Ooshima et al., 1986; Park et al., 1992; Helle et al., 1993). For instance, the beneficial effects of NIS have been observed on ruminal microbial growth rates (Lee et al., 2003; Goto et al., 2003b), ruminal enzyme activity (Lee and Ha, 2003; Lee et al., 2004), ruminal fermentation profiles (Wang et al., 2003b; Kim et al., 2004) and *in situ* disappearance of rice straw (Lee et al., 2007) by stimulating enzyme secretion and enhancing stability of enzymes secreted (Reese and Maguire, 1969; Park et al., 1992).

However, the responses to NIS supplementation have not always been positive. Hristov et al. (2000) demonstrated that adding NIS at 0.2% of dietary dry matter (DM) did not increase ruminal fermentation and nutrient digestion in steers fed a diet containing 70% barley. Non-ionic surfactant added to a medium at concentrations of 0.01 or 0.05% did not enhance *in vitro* fermentation of barley grain and barley silage in a ratio of 93:7 whereas the fermentation

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rate of grain and silage in a ratio of 58:42 was increased (Wang et al., 2004). In addition, Wang et al. (2003b) reported that effects of NIS on *in vitro* volatile fatty acid (VFA) production and gas production were dependent upon the silage type (alfalfa, corn and orchardgrass). On this basis, Wang et al. (2003b) suggested that the response of ruminal fermentation to NIS might be diet dependent. There is a need to know which characteristics of diets respond specifically to NIS. The responses of *in vivo* ruminal fermentation profiles to NIS supplementation have been examined mainly with steers fed diets with a high dietary concentrate : roughage ratio (Hristov et al., 2000; Lee et al., 2004; Kim et al., 2004) and it is unknown if similar effects would occur with high roughage-fed steers. The effects of NIS on performance of growing beef steers fed high roughage diets, have not been investigated.

Therefore, this study was conducted to determine the effects of NIS supplementation on ruminal fermentation, nutrient digestibility and performance of beef steers fed high-roughage diets. The first experiment was conducted to determine the effects of NIS supplementation on *in vitro* ruminal fermentation of cultures administered with various substrates. The *in vivo* ruminal fermentation, nitrogen (N) metabolism and nutrient digestibility were also examined in steers fed a high roughage-diet in the second experiment. The final aim of this study was to investigate the responses in performance of growing steers to NIS in the third experiment.

MATERIALS AND METHIDS

Experiment 1

Substrates, donor animal and inoculums : Corn, barley, rice straw and timothy hay, oven-dried (55°C, 48 h) and ground to pass through a 1-mm screen, were used as substrates. A ruminally-cannulated, Korean Hanwoo beef steer (568 kg) was used as a donor animal for ruminal fluid. The steer was fed 1.2 kg of rice straw and 4.8 kg of concentrate (as fed basis) per day. Concentrate ingredients and chemical composition of concentrate and rice straw are shown in Table 1. Diets were offered twice daily at 09:00 and 17:00 h. The steer had free access to water and free choice minerals. About 1,000 ml of ruminal fluid was collected from the bottom of the rumen before morning feeding, strained through four layers of cheesecloth into a pre-warmed thermos flask (39°C), which was previously flushed with O_2-free CO_2, and immediately transported to the laboratory.

Treatments and incubation method : Appropriate volumes of Tween 80 (polyoxyethylene (20) sorbitan monooleic acid, Uniqema, IL, USA) were added to the incubation solutions, prepared in four different flasks, to yield final concentrations of 0 (control), 0.05 (NIS5), 0.10 (NIS10) or 0.20% (v/v) Tween 80 (NIS20) in the incubation solution plus ruminal inoculum. The incubation solution, including 40 ml of macromineral solution, 40 ml of *in vitro* buffer, 20 μl of micromineral solution, 0.4 g of casein enzymatic hydrolysate and 80 ml of distilled water, was prepared according to Goering and Van Soest (1970). Pre-warmed (39°C) incubation solution was measured in 24 ml quantities into 50 ml serum bottles containing 300 mg of each substrate. Triplicate serum bottles were prepared for each substrate type (corn, barley, rice straw and timothy hay) and level of NIS (0, 0.05, 0.10 and 0.20%). After 6 ml of inoculum was added to each serum bottle, they were

Table 1. Ingredients of concentrate and compositon of concentrate and rice straw fed to steers[1] in experiments 1 and 2

Ingredients	% DM	Composition	% dry matter (DM)		
			Concentrate	Rice straw	Total[2]
Ground corn	47.80	DM, %	86.62	95.41	91.89
Wheat bran	41.00	Crude protein	13.28	6.12	8.98
Soybean meal	5.00	Ether extract	3.71	1.24	2.23
Rapeseed meal	5.00	Crude ash	4.92	9.71	7.79
Molasses	2.00	Neural detergent fiber	14.09	56.49	39.53
Limestone	2.00	Acid detergent fiber	2.95	37.68	23.79
Calcium phosphate	1.50				
Salt	0.40				
Vitamin-mineral additive[3]	0.20				
Rasalocid	0.10				

[1] Each steer had free access to water and a free choice mineral (Rincal 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, 40μg; NaCl, 380g) throughout the experiment.

[2] Calculated according to the concentrate:roughage ratio of 4:6.

[3] 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.

immediately flushed with O_2-free CO_2, sealed with butyl rubber stoppers and aluminium caps, and incubated anaerobically in a 38°C incubator without shaking for 0, 3, 6, 9, 12, 24 and 36 h for corn and barley and 0, 3, 6, 12, 24, 36 and 48 h for rice straw and timothy hay. Experiments were conducted in duplicate.

Measurement and sample analyses : At the desired interval, the serum bottle was removed from the incubator and the entire contents were transferred into large-neck tubes and pH was immediately measured (Pinnacle M530, Corning, NY, USA). Contents were then filtered through four layers of cheesecloth and 1% saturated $HgCl_2$ was added to stop microbial activity. The-filtrate was divided into two sub-samples for measurement of ammonia N and VFA.

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, 5 ml of sample was 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 (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 a helium carrier gas 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.

Feed samples fed to the donor animal and the four substrates were analyzed for moisture, crude protein (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).

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

$$Y_{ijk} = \mu + S_i + D_j + T_k + (D \times T)_{jk} + e_{ijk}$$

where S, D, T and D×T are square effects, diet effects, time effects and diet by time interactions, respectively. Duncan's multiple range test (Duncan, 1955) was used to determine significant differences ($p<0.05$) among treatments within each substrate and a trend was considered to exist where $0.05 \leq p \leq 0.10$.

Experiment 2

Animals, treatments and management : Three Hanwoo steers (mean 586.0±45.3 kg) fitted with permanent ruminal cannulae were used in a 3×3 Latin square experiment with three 14-day experimental periods. The three treatments were the basal diet (control) and the basal diet supplemented with 2 g/d (NIS2; 0.04% of dietary DM) or 4 g/d Tween 80 (NIS4; 0.08% of dietary DM). Because of its high viscosity, each Tween 80 supplement was mixed with 20 g of ground corn cobs prior to adding it to the daily concentrate fed. Steers were adapted to individual metabolic stalls for 10 days prior to commencement of the experimental period. During the experimental period, 2.4 kg/d of concentrate and 3.6 kg/d of rice straw (concentrate:roughage ratio of 40:60 as fed basis) were fed to steers. The same concentrate and rice straw used in experiment 1 was used in this experiment (see Table 1). Diets were offered twice daily at 09:00 and 17:00 h. 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 (6 kg/d). Representative samples of the experimental diets were collected daily, composited at the end of each period and ground through 1-mm screen for analysis.

On day 13 of each period, approximately 5 ml of 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.

On day 14 of each period, 100 ml of ruminal contents were collected just prior to morning feeding and also sampled at 0.5, 1, 1.5, 2, 4, 6 and 8 h post-feeding. After sampling, pH of ruminal contents was immediately measured and then contents were filtered through four layers of cheesecloth and 1% saturated $HgCl_2$ was added. To measure total tract digestibility, N balance and urinary concentrations of purine derivatives, 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 analyses.

Chemical analyses : Ruminal concentrations of

ammonia N and VFA and feed and fecal samples were analyzed according to the methods described in experiment 1. Additionally, starch concentrations in feed and fecal samples were determined according to procedure 920.40 of AOAC (1990). 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 in 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 blood urea nitrogen (BUN) and glucose using an automated blood analyzer (Express Plus, Ciba-Corning, CA, USA) according to the urease method of Roch-Ramel (1967) and the hexokinase method of Farrance (1987), respectively.

Statistical analyses : Data obtained from the analysis of blood, N balance and nutrient digestibility were 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. Duncan's multiple range test (Duncan, 1955) was used to determine significant differences ($p<0.05$) among treatments and a trend was considered to exist where $0.05 \leq p \leq 0.10$.

Data obtained from pH, ammonia N and profiles of VFA 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 diets by time interactions, respectively. Animal effect, animal by time interaction and error terms (e_{ijk} defined as between unit error and e_{ijkl} as within unit error) are multivariate normally distributed random effects with AR i) covariance structure. Differences among treatments were determined using LSMEANS with PDIFF and adjusted by the Tukey procedure (Kramer, 1956). Statistical significance was considered to exist where $p<0.05$, whereas a trend was considered to exist where $0.05 \leq p \leq 0.10$.

Experiment 3

Animals, treatments and management : Thirty-six, 11-month-old growing Hanwoo steers (260.22±24.30 kg) were used in a 108-day performance experiment. Steers were adapted to a basal diet for 4 weeks prior to the experiment. Steers were randomly assigned to pens (3 heads/pen) and adapted to the pens for 7 days. Steers were housed in sawdust-bedded pens (5×10 m) with overhead shade, concrete feed bunks (5 m long) and automatic drinkers. Four pens were randomly assigned to one of 3 dietary treatments in a randomized complete block design (3 animals per pen and 4 pens per treatment). Treatments comprised 0 (control), 15 (NIS15; 0.05% of assumed volume of ruminal liquid, 30,000 ml or 0.15% of dietary DM) and 30 g/d Tween 80 (NIS 30; 0.10% of 30,000 ml or 0.30% of dietary DM) added to diets which were group-fed. Each Tween 80 supplement was mixed with 150 g of ground corn cobs prior to adding it to the daily concentrate fed. Experimental diets were concentrate, rice straw and corn silage and corn silage was top-dressed on the concentrate for prolonged consumption of NIS. Concentrate feed was limit-fed (2.5 kg/d/head as fed basis) once daily in the morning at 09:00 h while corn silage and rice straw were fed in amounts adequate to allow *ad libitum* access to feed. According to the feed intake results obtained when experiment 3 was finished, the concentrate:roughage ratio of experimental diets was 30:70 on a DM basis. Feed bunks were monitored closely to ensure that steers consumed each concentrate completely and that fresh silage and rice straw were available at all times. Steers had free access to water throughout the experiment. Ingredients and chemical composition of the diets are shown in Table 2.

Measurement and sample analyses : Steers were weighed individually prior to morning feed delivery using a single livestock scale at the beginning and at the end of the experiment and at 28-day intervals. Daily concentrate feed was consumed completely because intake was restricted (2.5 kg/d as fed basis). The amounts of silage and rice straw fed and refused were weighed and recorded once daily. Representative samples of the experimental diets were collected weekly, composited at the end of the experiment, dried and ground through a 1-mm screen for analysis. Feed samples were analyzed according to the methods described in experiment 1.

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

$$Y_{ij} = \mu + P_i + T_j + e_{ij}$$

where P and T are pen and treatment effects, respectively. Duncan's multiple range test (Duncan, 1955)

Table 2. Ingredients of concentrate and compositon of concentrate, corn silage and rice straw fed to growing steers[1] in experiment 3

Ingredients	% DM	Composition	% dry matter (DM)			
			Concentrate	Silage	Rice straw	Total[2]
Ground corn	32.60	DM (%)	90.83	30.83	64.67	59.99
Ground wheat	14.00	Crude protein	16.30	8.56	3.61	8.21
Corn gem meal	11.50	Ether extract	3.93	2.53	1.58	2.44
Soybean meal	9.50	Crude ash	6.80	4.69	13.65	9.14
Rice bran	6.80	NDF[3]	9.96	32.31	50.83	35.18
Rapeseed meal	7.70	ADF[4]	2.24	19.09	30.97	20.34
Corn gluten	7.30					
Palm kernel meal	5.00					
Lupin	4.30					
Sodium bicarbonate	0.80					
Vitamin-mineral [mix5]	0.50					

[1] Each steer had free access to water.
[2] Calculated according to the ratio (2.4:3.2:4.4) of concentrate, silage and rice straw consumed.
[3] Neutral detergent fiber. [4] Acid detergent fiber.
[5] 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.

was used to determine significant differences ($p<0.05$) among treatments and a trend was considered to exist where $0.05 \leq p \leq 0.10$.

RESULTS

Experiment 1

The chemical composition of substrates used in experiment 1 is shown in Table 3. Composition of substrates was different among substrates; especially, NDF and ADF concentration of rice straw and timothy hay which were far higher than those in corn and barley. From the levels of NDF and ADF, rice straw and timothy hay represented a typical roughage source whereas corn and barley represented a grain source. Effect of levels of NIS on pH, ammonia and VFA during *in vitro* incubation of corn, barley, rice straw and timothy hay are shown in Table 4. Supplementation of NIS did not affect pH of ruminal mixed culture. However, the ammonia N concentration for NIS10 was higher ($p<0.05$) than for other treatments with all substrates. Total VFA concentrations of the incubation of rice straw were higher ($p<0.05$) for NIS10 and NIS20 than for the control, and that of timothy hay was higher ($p<0.05$) for NIS10 than for the control whereas total VFA concentrations in the cultures of corn and barley were unaffected. The increased concentration of total VFA by NIS was mainly due to the elevated concentration of acetate because acetate concentration showed the same significant differences as total VFA. However, supplementation of NIS increased ($p<0.05$) propionate concentration with all substrates except for timothy hay. In the cultures with rice straw and timothy hay, butyrate concentration was higher ($p<0.05$) for NIS treatments than the control. Valerate was also influenced by NIS supplementation in the cultures with roughage sources. Isobutyrate and isovalerate were also detected in small amounts with no differences observed among treatments.

Experiment 2

Effects of levels of NIS on ruminal metabolism and

Table 3. Chemical composition of substrates used in experiment 1

Items (% DM)	Substrates[1]			
	Corn	Barley	Rice straw	Timothy hay
Dry matter (%)	85.98	88.33	95.41	92.72
Crude protein	8.78	11.84	6.12	10.10
Ether extract	4.48	1.62	1.24	2.41
Crude ash	1.58	1.99	9.71	8.42
Neutral detergent fiber	11.87	23.90	56.49	41.73
Acid detergent fiber	0.74	2.41	37.68	26.40

[1] Substrates were oven-dried (55°C, 48 h) and ground to pass through a 1-mm screen.

Table 4. Effects of levels of non-ionic surfactant on pH, ammonia and volatile fatty acid (VFA) profiles of *in vitro* incubation of ruminal mixed culture administered with corn, barley, rice straw and timothy hay as a substrate

Items	Treatments[1]				SEM[2]	p value[3]
	Control	NIS5	NIS10	NIS20		
pH						
Corn	6.610	6.578	6.594	6.571	0.016	0.318
Barley grain	6.620	6.581	6.591	6.582	0.014	0.106
Rice straw	6.761	6.740	6.750	6.742	0.014	0.685
Timothy hay	6.725	6.695	6.699	6.694	0.012	0.252
Ammonia (mg/L)						
Corn	203.07^{b}	197.74^{b}	212.62^{a}	199.94^{b}	2.87	0.002
Barley grain	219.82^{b}	216.93^{b}	227.98^{a}	221.20^{ab}	3.07	0.050
Rice straw	265.27^{b}	258.81^{b}	276.67^{a}	260.36^{b}	2.89	0.001
Timothy hay	274.98^{b}	270.57^{b}	285.34^{a}	271.24^{b}	3.41	0.010
Total VFA (m*M*)						
Corn	159.80	169.29	156.59	166.82	8.89	0.723
Barley grain	165.32	155.90	174.30	174.39	10.96	0.592
Rice straw	171.34^{c}	192.49^{bc}	205.67^{ab}	222.74^{a}	8.69	0.002
Timothy hay	204.84^{b}	211.68^{b}	221.99^{a}	205.48^{b}	3.32	0.004
Acetate (m*M*)						
Corn	98.29	100.62	95.45	101.00	5.74	0.897
Barley grain	98.57	88.99	102.86	98.63	7.16	0.577
Rice straw	106.89^{c}	123.03^{bc}	131.04^{ab}	142.37^{a}	5.80	0.002
Timothy hay	131.42^{b}	133.82^{b}	140.84^{a}	132.45^{b}	2.11	0.007
Propionate (m*M*)						
Corn	33.31^{b}	41.82^{a}	32.67^{b}	39.84^{a}	1.82	0.002
Barley grain	38.64^{b}	42.41^{b}	43.05^{b}	50.60^{a}	2.20	0.006
Rice straw	32.69^{b}	35.99^{b}	36.95^{ab}	41.14^{a}	1.64	0.011
Timothy hay	40.65	42.38	42.85	41.27	0.83	0.132
Butyrate (m*M*)						
Corn	23.26	22.35	23.22	23.31	1.32	0.948
Barley grain	23.88	21.02	23.94	22.97	1.69	0.588
Rice straw	23.06^{c}	25.73^{bc}	27.31^{ab}	29.55^{a}	1.29	0.010
Timothy hay	24.53^{b}	26.64^{a}	28.18^{a}	27.61^{a}	0.53	0.001
Valerate (m*M*)						
Corn	2.26	2.60	2.82	2.72	0.13	0.670
Barley grain	2.26	2.16	2.36	2.45	0.09	0.164
Rice straw	3.61^{b}	3.88^{b}	5.95^{a}	5.64^{a}	0.34	0.001
Timothy hay	3.29^{c}	3.89^{bc}	5.56^{a}	4.13^{b}	0.24	0.001
Isobutyrate (m*M*)						
Corn	0.92	0.90	1.00	0.88	0.04	0.271
Barley grain	0.85	0.81	0.87	0.86	0.04	0.694
Rice straw	2.17	2.25	2.18	2.25	0.10	0.912
Timothy hay	2.27	2.34	2.34	2.23	0.08	0.704
Isovalerate, m*M*						
Corn	1.92	1.74	2.02	1.73	0.14	0.430
Barley grain	1.66	1.55	1.57	1.65	0.10	0.795
Rice straw	3.93	4.04	4.21	4.08	0.14	0.575
Timothy hay	3.58	3.43	3.93	3.67	0.15	0.195

[1] Treatments include incubation solutions added with 0 (control), 0.05 (NIS5), 0.10 (NIS10) and 0.20% Tween 80 (NIS20).
[2] Standard error of mean; n = 56. [3] Statistical significance of treatment effects by F-test.
[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 5. Effects of levels of non-ionic surfactant on ruminal metabolism and blood metabolites of steers fed a high-roughage diet

Items	Treatments[1]			SEM[2]	p value[3]
	Control	NIS2	NIS4		
Ruminal metabolism					
pH	6.95[a]	6.82[b]	6.81[b]	0.02	0.017
Ammonia (mg/L)	64.94[b]	73.46[a]	72.72[a]	3.05	0.047
Volatile fatty acids (m*M*)					
Acetate	38.64[b]	40.66[b]	53.75[a]	2.37	0.004
Propionate	12.09	11.17	12.42	0.93	0.651
Butyrate	7.89	7.57	8.11	0.79	0.889
Valerate	0.45	0.46	0.56	0.05	0.518
Isobutyrate	0.59	0.50	0.60	0.05	0.354
Isovalerate	0.70	0.66	0.76	0.06	0.546
Total	60.37[b]	61.03[b]	76.05[a]	2.83	0.007
Microbial nitrogen supply (g N/d)[4]	21.50[b]	28.40[ab]	31.87[a]	1.24	0.053
Blood metabolites					
Urea nitrogen (mg/dl)	7.17[B]	8.03[AB]	8.70[A]	0.29	0.097
Glucose (mg/dl)	62.17	62.83	63.43	0.98	0.228

[1] Treatments include the basal diet (control), supplemented with 2 g/d (NIS2) and 4 g/d Tween 80 (NIS4).
[2] Standard error of mean; n = 72 for pH, volatile fatty acids and ammonia, n = 27 for microbial nitrogen supply and n = 18 for total protein and urea nitrogen.
[3] Statistical significance of treatment effects by F-test. [4] Estimated from purine derivatives excreted in the urine.
[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).
[A, B] Means in the same row with different superscripts tend to differ ($p<0.10$).

blood metabolites of steers fed a high-roughage diet are shown in Table 5. Ruminal pH of steers fed NIS was lower ($p<0.05$) than the control. Ruminal ammonia N concentration was also affected by NIS supplementation with inclines ($p<0.05$) for NIS2 and NIS4. Ruminal acetate concentration was higher ($p<0.05$) for NIS4 than for other treatments whereas the rest of the VFA were unaffected by NIS. The increased acetate with NIS feeding contributed to the elevated concentration of total VFA. In addition, urinary concentrations of purine derivatives was increased by NIS supplementation ($p<0.05$). Although no differences in levels of blood glucose among treatments was observed, BUN level tended to be increased ($p<0.10$) by NIS. Effects of different supplementation levels of NIS on N metabolism of steers fed a high-roughage diet are shown in Table 6. Since steers consumed each meal completely due to restricted amounts fed, N intakes were similar among treatments. The amounts of fecal and urinary N excretions were unaffected by NIS. When expressed as output per unit N intake, fecal and urinary N excretions were also similar among

Table 6. Effects of levels of non-ionic surfactant on nitrogen (N) metabolism of steers fed a high-roughage diet

Items	Treatments[1]			SEM[2]	p value[3]
	Control	NIS2	NIS4		
N intake (g N/d)	80.90	80.90	80.90	-	-
N output (g N/d)					
Fecal N	35.51	35.58	34.93	4.66	0.830
Urinary N	34.54	33.69	34.63	0.35	0.316
N retention	10.85	11.85	11.34	4.10	0.776
N output (% of N intake)					
Fecal N	43.89	43.94	43.28	5.97	0.837
Urinary N	42.83	41.71	42.97	0.47	0.315
Retained N	13.28	14.35	13.75	4.34	0.780

[1] Treatments include the basal diet (control), supplemented with 2 g/d (NIS2) and 4 g/d Tween 80 (NIS4).
[2] Standard error of mean; n = 27. [3] Statistical significance of treatment effects by F-test.

Table 7. Effects of levels of non-ionic surfactant on the apparent nutrient digestibility of steers fed a high-roughage diet

Items (%)	Treatments[1]			SEM[2]	p value[3]
	Control	NIS2	NIS4		
Dry matter	50.37	55.30	50.55	3.38	0.294
Crude protein	56.11	56.06	56.72	2.39	0.417
Neutral detergent fiber	47.39	48.71	51.83	3.08	0.430
Acid detergent fiber	35.06	37.69	38.56	3.74	0.378
Starch	96.01	97.09	96.65	0.82	0.669

[1] Treatments include the basal diet (control), supplemented with 2 g/d (NIS2) and 4 g/d Tween 80 (NIS4).
[2] Standard error of mean; n = 27. [3] Statistical significance of treatment effects by F-test.

treatments. As a result, N retention, expressed as either daily excretion amount or output per unit N intake, was not influenced by NIS supplementation. Effects of levels of NIS on the apparent nutrient digestibility of steers fed a high-roughage diet are shown in Table 7. Apparent digestibilities of DM, CP, NDF, ADF and starch were not different among treatments.

Experiment 3

Effects of levels of NIS on feed intake, weight gain and feed efficiency of growing steers fed a high-roughage diet are shown in Table 8. Intake of corn silage was higher ($p<0.05$) for NIS30 than other treatments and intake of rice straw tended ($p<0.10$) to be higher for NIS30 than other treatments. As planned, concentrate intakes were the same among treatments because steers were fed restricted amounts. Attributable to increased intakes of corn silage and rice straw, total intake was higher ($p<0.05$) for NIS30 than for the other treatments. Average daily gain (ADG) was significantly higher ($p<0.05$) for NIS15 than other treatments. Hence, feed efficiency, expressed as a ratio of weight gain to total intake, was higher for NIS15 than other treatments. Similarly, the ratio of weight gain and silage intake and the ratio of weight gain and rice straw intake were the highest for NIS15 among treatments.

DISCUSSION

Results obtained in the *in vitro* study showed that the type of substrate and NIS concentration significantly affected some rumen fermentation characteristics. Although pH of cultures supplemented with NIS was numerically lower than the control, there was no significant difference between them, which is in contrast to the results of Goto et al. (2003b) and Lee and Ha (2003), who showed that adding NIS *in vitro* to ruminal culture with barley grain decreased pH. However, ammonia N concentration was affected by NIS. Ammonia N concentrations, which increased for

Table 8. Effects of levels of non-ionic surfactant on feed intake, weight gain and feed efficiency of growing steers fed a high-roughage diet

Items	Treatments[1]			SEM[2]	p value[3]
	Control	NIS15	NIS30		
Intake (kg DM/d)					
Concentrate	2.27	2.27	2.27	-	-
Corn silage	3.01^{b}	3.10^{b}	3.13^{a}	0.01	0.030
Rice straw	4.16^{B}	4.14^{B}	4.37^{A}	0.08	0.083
Total	9.53^{b}	9.51^{b}	9.78^{a}	0.09	0.049
Weight gain					
Initial weight (kg)	260.00	260.33	266.33	24.30	0.253
End weight (kg)	329.78	339.94	335.00	25.86	0.257
Average daily gain (kg/d)	0.646^{b}	0.737^{a}	0.636^{b}	0.019	0.050
Feed efficiency					
Gain:total intake	0.067^{b}	0.077^{a}	0.065^{b}	0.002	0.046
Gain:silage intake	0.208^{b}	0.236^{a}	0.203^{b}	0.006	0.047
Gain:rice straw intake	0.153^{b}	0.174^{a}	0.146^{b}	0.005	0.049

[1] Treatments include 0 (control), 15 (NIS15) and 30 g/d Tween 80 (NIS 30) added to experimental diets.
[2] Standard error of mean; n = 36 for gain and n = 12 for intake and feed efficiency. [3] Statistical significance of treatment effects by F-test.
[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).
[A, B] Means in the same row with different superscripts tend to differ ($p<0.10$).

NIS10 compared with the control, indicate that NIS either reduced ammonia N utilization or increased ammonia production. In fact, Lee et al. (2003) reported that the addition of NIS to the rumen of cows increased protease production in the rumen contents. Although ammonia N was affected with all substrates, concentrations of total VFA, acetate and butyrate were increased by NIS only in cultures with rice straw and timothy hay. This result indicates more extensive fermentation in the cultures grown with a roughage source, which is also substantiated by a higher ammonia N concentration in cultures. This is consistent with Goto et al. (2003b) who demonstrated that the extent of increase in total VFA resulting from 0.20% NIS supplementation was higher for culture grown with a roughage source (orchardgrass hay) than with a grain source (barley grain) (45.6 vs. 6.1 m*M*). Increasing roughage contents in the substrate also influenced the effect of NIS on ruminal fermentation in other studies. Wang et al. (2004) observed that the initial fermentation rate of ruminal culture with barley grain and barley silage in a ratio of 58:42 was increased by NIS added at concentrations of 0.01 or 0.05% but fermentation rate of culture with grain and silage in a ratio of 93:7 was not enhanced.

The reason that effects of NIS on ruminal fermentation are diet dependent may be attributed to an increase in activity of cellulolytic enzymes. *In vitro* ruminal studies (Lee et al., 2003; Lee and Ha, 2003) showed that when NIS was added at the level of 0.05 and 0.10%, the total and specific activities of extracellular cellulase and xylanase increased. In *in vivo* trials (Kim et al., 2004; Lee et al., 2004), the addition of NIS to the rumen of steers increased the activities of cellulase and xylanase in ruminal contents. These were attributed to stimulating effects of NIS on enzyme secretion (Reese and Maguire, 1969) and stability of enzymes secreted (Park et al., 1992). As a result of increased cellulolytic enzyme activity, hydrolysis of cellulose was increased (Castanon and Wilke, 1981; Helle et al., 1993) and digestion of cellulose by mixed ruminal bacteria was improved by NIS (Akin et al., 1980; Kamande et al., 2000). Supplementation of NIS also increased amylase (Lee et al., 2003; Lee and Ha, 2003; Lee et al., 2004) and barley glucanase activities (Lee et al., 2003) in ruminal contents. However, their effects on the degradation of starchy feeds may not be great because digestibility of nitrogen-free extract was unaffected by 5 or 10 g/d supplementation of NIS to steers whereas that of crude fiber was increased by NIS (Kim et al., 2004). It may be explained by the affinity of enzymes for starchy parts in substrates being already maximal and/or the degradability of starch that would be degraded at a maximal rate in the rumen without NIS supplementation. Thus, enhanced ruminal fermentation of roughage substrates may be attributed to increased activity of cellulolytic enzymes, resulting in increased degradation of fibrous parts of substrates in experiment 1.

In the present study, improved ruminal fermentation resulting from NIS supplementation was also observed *in vivo*. The ruminal pH of steers supplemented with NIS was lower than that of steers with no supplementation. However, the ruminal pH of all treatments remained high and all observations measured within 8 h after feeding were greater than 6.3 (time values not shown), which is the pH identified as critical for maintaining ruminal fiber digestion (Stewart, 1977; Hiltner and Dehority, 1983). This is probably due to the low dietary concentrate:roughage ratio (40:60) because consumption of straw results in high salivation rates, which helps maintained the ruminal pH at high levels (Nolan and Leng, 1972). Ruminal ammonia N concentration was higher for NIS treatments than the control. Ruminal ammonia N would accumulate in the rumen when release of energy was not coupled with the release of ammonia N in the early phase after feeding (Kennedy and Milligan, 1980; Nocek and Russell, 1988). Thus, increased ruminal ammonia N concentrations with NIS supplementation indicate that more plant protein degradation occurred in the rumen and that ammonia N released from dietary protein was probably uncoupled from energy levels that were similar among treatments. 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*. In the present study, regardless of treatment, ammonia N concentrations rarely dropped below 50 mg/L within 8 h after feeding (time values not shown). Although crude protein content in whole diets was approximately 9%, the main protein sources were ruminal-degraded proteins (Table 1). Thus, ammonia N concentrations were relatively high, which resulted in high urinary N excretion (Table 6). In the present study, ruminal microbial growth probably was not limited by a shortage of ruminal ammonia.

Similar to the *in vitro* result of experiment 1, concentrations of total VFA and acetate in the rumen of steers supplemented with NIS increased. The increased concentration of total VFA by NIS was mainly due to the elevated concentration of acetate. This indicates that increased activity of enzymes resulting from additions of NIS is specific for fibrous parts of the diets fed. The elevated VFA concentration is consistent with the results of previous studies in which dietary concentrate:roughage ratio was high. Lee et al. (2004) and Kim et al. (2004), feeding a diet containing 60% compound feed and 40% rice straw to steers, observed that total VFA and acetate concentrations increased with NIS supplementation. Enhanced ruminal

fermentation is also substantiated by increased microbial N supply resulting from feeding NIS. Estimated from purine derivatives excreted in the urine, microbial N supply was increased by NIS supplementation in the present study. *In vitro* ruminal fermentation trials have shown that NIS administration stimulates growth rate of ruminal microorganisms. Lee et al. (2003) reported that NIS added to the growth medium at concentrations of 0.05 or 0.10% increased the growth rates of ruminal noncellulolytic bacteria including *Bacteroides amylophilus*, *Megasphaera elsdenii*, *Prevotella ruminicola* and *Selenomonas ruminantium* and ruminal cellulolytic bacterium, *Fibrobacter succinogenes* which is considered to be the most effective in utilizing cellulose from plant tissues (Miller, 1959). Goto et al. (2003b) also showed that the growth of *Streptococcus bovis*, *Selenomonas ruminantium*, *Prevotella ruminicola*, *Megasphaera elsidenii*, *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* was higher at 0.01 or 0.20% concentration of NIS added to pure cultures. In the *in vivo* trial (Lee et al., 2004), the total viable bacteria population dramatically increased when NIS solution was administered to the rumen of steers. This may be attributed to differences in availability of ammonia N in the rumen, resulting from administration of NIS. Mean ammonia N concentrations were 64.94, 73.46 and 72.72 mg/L for control, NIS2 and NIS4, respectively. Thus, increased ammonia N concentration may contribute to elevated microbial N supply estimated by urinary purine derivatives.

In earlier studies, three different likely explanations of enhanced ruminal fermentation rate by NIS have been suggested: i) NIS may stimulate enzyme production from ruminal microorganisms (Kamande et al., 2000; Lee and Ha, 2003; Lee et al., 2003; Lee et al., 2004) through modification of cell permeability (Reese and Maguire, 1969); ii) NIS could increase stability of enzymes (Kim et al., 1982; Park et al., 1992; Kaar and Holzapple, 1998); iii) and/or NIS could affect structure of the substrate, and consequently improve accessibility of enzymes to substrates (Helle et al., 1993; Kaar and Holzapple, 1998). Lee et al. (2003) suggested that NIS considerably increased the release of microbial cell-bound enzymes, cellulase, xylanase, protease, amylase and glucanase into the ruminal fluid. Since cell-free cellulolytic enzymes are more important in ruminal forage digestion than cell-bound enzymes (Weimer et al., 1990), increased activity of extracellular cellulolytic enzymes may contribute to enhanced ruminal fermentation by increasing digestion of roughage in the rumen. The positive effect of NIS on production of enzymes may be attributed to cell permeability modified by NIS as NIS appears to promote the transport of compounds and release of membrane-bound enzymes through modification of cell permeability (Reese and Maguire, 1969). Another likely explanation is the beneficial effects of NIS on stability of enzymes secreted by ruminal microorganisms. Park et al. (1992) showed that hydrolysis of cellulose in newspaper increased through preventing inactivation of cellulase by NIS. Kim et al. (1982) also reported that surfactants could increase the stability of enzymes and reduce enzyme denaturation during hydrolysis of cellulose. Improved accessibility of enzymes to substrates by NIS may also contribute in part to the promotion of enzymatic hydrolysis. Non-ionic surfactant increases water and enzyme-holding capacities of forages by altering substrate structures (Goto et al., 2003a). Kim et al. (2006) also reported that NIS caused cellulose to swell and have more cracks and filaments. The hydration of substrate, enhanced by NIS, could increase the rate of microbial colonization and vulnerability to enzymatic attack (Helle et al., 1993; Kaar and Holtzapple, 1998; Kamande et al., 2000). Thus, NIS seems to increase the accessibility of enzymes to substrates in this manner.

Although concentrations of ruminal ammonia N and acetate and urinary concentrations of purine derivatives were increased by NIS, blood metabolites, N metabolism and nutrient digestibility were unaffected in experiment 2. This could indicate that enhanced ruminal metabolism by NIS did not positively affect parameters which could influence steer performance. It implies that improvements in performance of steers would not be expected from the NIS-supplemented diets used in the present *in vivo* experiment. The discrepancy between responses of ruminal and post-ruminal metabolism may arise from the low dosage (0.04 and 0.08% of dietary DM) of NIS supplementation. Shelford et al. (1996) reported that adding NIS to dietary DM at 0.2% concentration enhanced milk production in dairy cows but McAllister et al. (2000) showed that 0.02% of NIS supplemented to diets did not affect digestibility and performance of lambs. This suggests that the extent of responses of animal performance may be dose-related. Goto et al. (2003a) also suggested that the extent of enzymatic degradation of leaf blade fractions of orchardgrass was dependent on the dose of NIS. Another likely explanation is that NIS could be degraded by rumen bacteria (Goto et al., 2003b), possibly resulting in decrease in effects of NIS at low dosages. Thus, the supplementation levels determined to be optimal *in vitro* may not be enough to improve total tract digestive activity of steers.

Based on the inference discussed above, we decided to increase the NIS levels in the diet of growing steers in experiment 3 compared to the levels used in experiments 1 and 2. Supplementation levels of NIS in experiment 1 were from 0.05 to 0.20% of volume of incubation solution plus ruminal inoculums, and 0.10% was most effective. Thus, we added NIS at 0.05 and 0.10% of assumed volume of ruminal liquid of steers. As the volume of ruminal liquid of Korean native Hanwoo beef steers weighing 260 kg is

assumed to be 30,000 ml according to the Korean Feeding Standard for Korean Cattle Hanwoo (Ministry of Agriculture and Forestry of Korea, 2002). 15 and 30 g of NIS (approximately 0.15 and 0.30% of dietary DM, respectively) were added to the daily diet. Supplementation of NIS increased intakes of total feed, corn silage and rice straw, ADG and feed efficiency although they varied depending on supplementation level in experiment 3. Unlike these results, Hristov et al. (2000) observed that supplementing NIS at 0.20% level did not increase performance in steers fed a diet containing 70% barley. Plascencia et al. (2007) also observed no positive response in performance of feedlot cattle fed corn-based diets containing 14% forage supplemented with 0.22% NIS. However, Wang et al. (2003b) reported that 0.20% NIS supplementation increased ADG by 7% in back-grounding feedlot cattle fed diets with a concentrate:roughage ratio of 58:42. Performance of back-grounding feedlot cattle with a concentrate:roughage ratio of 52:48, DM intake, ADG and feed efficiency were also increased by addition of 0.60% NIS (Wang et al., 2003a). This discrepancy suggests that the performance response to NIS is possibly due to the substrate-related feature as evident from the *in vitro* results of experiment 1. It could be also be supported by the results of Wang et al. (2003a; b) with finishing feedlot cattle which showed that NIS did not increase performance of cattle fed diets with high concentrate:roughage ratios (94:06 and 90:10, respectively). As the concentrate:roughage ratio was 30:70 in the present experiment, presumably increased activity of cellulolytic enzymes in the rumen by NIS may have enhanced ruminal fermentation of fibrous feed and, consequently, performance of steers. More in-depth research is required to define optimum application rates of NIS under different feeding conditions and ensure that NIS supplementation in the diets of steers results in consistent positive responses in animal performance.

CONCLUSION

Results obtained in the present study showed that effects of NIS on ruminal fermentation are diet dependent, specifically affecting roughage based diets. Stimulating effects of NIS on activity of cellulolytic enzymes previously observed may result in the increase in degradation of fibrous parts of feeds and, consequently, the enhanced ruminal fermentation of roughage sources in the *in vitro* (rice straw and timothy) and *in vivo* trials (concentrate:roughage ratio of 4:6). Due to roughage-specific feature of NIS, performance of growing steers fed a high-roughage diet was improved in the growth trial (concentrate:roughage ratio of 3:7).

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