Effects of Dietary Addition of Surfactant Tween 80 on Ruminal Fermentation and Nutrient Digestibility of Hanwoo Steers*

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ABSTRACT : A non-ionic surfactant, Tween 80 has been known to exert a number of positive effects on degradative enzymes in *in vitro* aerobic and anaerobic microbial cultures. An experiment was conducted to examine effects of supplementation of Tween 80 on ruminal fermentation and nutrient digestibility of Hanwoo steers. The experiment was designed as a 3×3 Latin square with duplication and six Hanwoo steers with rumen cannulae, average weight 497 (SE 61.1) kg. For the experiment the animals were given a basal diet consisting of rice straw and compound feed mixed at 4:6 ratio. The three experimental treatments were (1) the basal diet, supplemented with (2) 5 g/d Tween 80 and (3) 10 g/d Tween 80. Ruminal pH was significantly ($p<0.05$) affected by Tween 80 supplementation at 6 h after feeding. Increasing supplementation levels of Tween 80 linearly increased the total VFA concentration. CMCase activity by the 10 g/d supplementation of Tween 80 were significantly increased ($p<0.05$) by 24.4% compared with that of control. Digestibility of crude fiber was significantly increased ($p<0.05$) in Hanwoo steers fed the diet supplemented with 10 g/d Tween 80 compared with those of control, whilst digestibility of ether extract (EE) was linearly increased by increasing Tween 80 supplementation level ($p<0.05$). In other nutrient components, their digestibilities of Hanwoo fed diets supplemented with Tween 80 tended to increase. It is concluded that Tween 80 has a potential for industrial application as a feed additive to improve ruminant production. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 337-342)***

Key Words : Tween 80, Ruminal Fermentation, Digestibility, Hanwoo Steers

INTRODUCTION

Non-ionic surfactants (NIS) have been shown *in vitro* to exert a number of positive effects on degradative enzymes, such as preventing inactivation of cellulase (Park et al., 1992), increasing enzymatic hydrolysis of cellulose (Helle et al., 1993) and improving digestion of cellulose by mixed ruminal bacteria (Akin, 1980). Further more NIS increases production of enzymes by rumen microorganisms (Lee et al., 2003). The surfactant Tween 80, an oleate ester of sorbitol and its anhydrides copolymerized with ethylene oxide, is well known as an effective surfactant that stimulates the release of enzymes from a range of aerobic fungi (Reese and Maguire, 1969; Schewale and Sadana, 1978; Deshpande et al., 1987; Hung et al., 1988; Yazdi et al., 1990; Long and Knapp, 1991). Several researchers reported that effects on aerobic microorganisms are due to action on the cell membrane causing increased permeability (Reese and Macguire, 1969), promotion of the release of bound enzymes (Reese and Maguire, 1969) and decrease in growth rate by reduced oxygen supply (Hulme and Stranks, 1970). Lee et al. (2003) and Lee and Ha (2003) suggested potential uses of the surfactant Tween 80 as a feed additive for ruminant animals. Lee et al. (2003) reported that when Tween 80 was included at a concentration of 0.05% (v/v) in the growth medium, this material increased the growth rate of rumen bacteria and fungi and the rate of cereal grain digestion, succinate and lactate dehydrogenase activities, and the activities of polysaccharide-degrading enzymes. Further research (Lee and Ha, 2003) indicated that supplementation of 0.05% Tween 80 to *in vitro* cultures of mixed rumen microorganisms growing on barley grain and orchard grass hay significantly increased cellulase, xylanase and amylase activities. Besides, adding Tween 80 (0.2% wt/wt) to diets for lactating cows increased milk production by 2.5 to 3.5 kg/d (Shelford et al., 1996). In spite of some previous reports, animal response with Tween 80 has rarely examined. Thus, the experiment was conducted to examine effects of supplementation of Tween 80 on ruminal fermentation and nutrient digestibility of Hanwoo steers fed compound feed and rice straw.

MATERIALS AND METHODS

Experimental animals and their management

Six Hanwoo steers with rumen cannulae, average

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Table 1. Formula and chemical composition of compound feed and rice straw for Hanwoo steers in the experiment

Formula of compound feed		Chemical composition (%, DM basis)		
Ingredients	%, DM basis		Compound feed	Rice straw
Yellow corn, ground	35.12	Dry matter	87.78	87.07
Wheat bran	28.00	Crude protein	15.81	4.03
Soybean meal	12.60	Ether extract	2.77	0.86
Fish meal	1.00	Crude fiber	6.67	29.10
Corn gluten feed	10.00	Crude ash	4.87	11.77
Soybean hull	8.00	NDF[a]	29.32	64.02
Molasses	3.00	ADF[b]	10.37	38.45
Limestone	1.76	TDN[c]	81.63	42.63
Salt	0.50	Calcium	1.02	0.34
Lasalocid sodium	0.02	Phosphorus	0.34	0.11

[a] Neutral detergent fiber. [b] Acid detergent fiber. [c] Total digestible nutrients.

weight 497 (±61.1) kg were used. They were housed in individual metabolic stalls. Feed was provided in two equal meals each day at 09:00 and 17:00 h, and water and mineral block were freely available at all times.

Experimental diet and treatments

For the experiment the animals were given a basal diet consisting of rice straw and compound feed mixed at 4:6 ratio at fresh weight. The steers were given a diet at 1.75% of body weight at fresh. The formula of compound feed and the chemical composition of the rice straw and compound feed are shown in Table 1.

The three experimental treatments were (1) the basal diet, supplemented with (2) 5 g/d Tween 80 and (3) 10 g/d Tween 80. Each Tween 80 supplement was mixed with 5 g wheat bran for premix prior to adding it into a daily ration of compound feed because Tween 80 is highly viscous and so it is difficult to directly mix the supplement with compound feed.

Experimental plan and procedures

The experiment was designed as a 3×3 Latin square with duplication and with 3 week periods. The complete output of feces was collected on days 18, 19, 20 and 21 of each period just before the morning ration. Samples of rumen contents were taken through the rumen cannulae by suction, on the last day of each period at 10:00 (before feeding), 11:00, 13:00, 17:00 and 19:00 h.

The pH of the ruminal samples was measured immediately after they were withdrawn. The samples were quickly strained through 4 layers of muslin cloth and centrifuged to remove solids; the supernatant fluid was stored at -20°C until analyzed.

Determination of cellulolytic enzyme activity

Extracellular enzyme activity against carboxymethyl cellulose (CMC) was determined by incubating 0.5 ml of supernatants from rumen fluids in each treatment with 0.5 ml of 2% (w/v) CMCellulose in 0.1 M sodium acetate buffer (pH 5.0). After 2 h incubation, the reaction was stopped by the addition of 0.25 ml of 8% Na_2CO_3. Aliquots were centrifuged at 12,000×g for 5 min and reducing sugars in the supernatants were assayed colorimetrically by using the DNS (dinitrosalicylic acid) method (Miller, 1959). One international unit (IU) of enzyme activity was defined as the amount of enzyme that liberated 1 μmol of glucose equivalent per min under the condition described above.

Chemical analysis

Samples of feed were analyzed, as appropriate, for dry matter (DM) by drying at 100°C. Crude ash was determined by ashing at 550°C. Total N was determined by a Kjeldahl procedure. Analyses of neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) were done by the methods of Van Soest and Wine (1967) and Van Soest (1963), respectively. Ruminal ammonia N was analyzed by colorimetric method (Chaney and Marbach, 1962). VFA concentration of rumen fluid was analyzed with gas chromatography (Varian 3800, USA) by the method proposed by Erwin et al. (1961) after samples were strained through 0.45 μm of disposable micro filter.

Statistical analysis

The data were analyzed as a 3×3 Latin square design with a replication using GLM (general linear model) procedures of SAS (1996) and statistical significance among treatment means was determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

Ruminal fermentation characteristics of Hanwoo steers fed a basal diet containing compound feed and rice straw supplemented with different levels of Tween 80 are presented in Figure 1 and Table 2. In the present experiment, ruminal fermentation was improved by Tween 80 supplementation and especially the general effect was maximized by the 10 g/d supplementation level of Tween 80. In a recent report (Lee et al., 2003), when 30 ml NIS

Table 2. Daily mean values for ruminal fermentation variables of Hanwoo steers fed diets supplemented with different levels of Tween 80

	Supplementation levels (g/d) of Tween 80			SEM[1]	P value[2]
	0	5	10		
pH	6.72	6.65	6.65	0.027	0.289
NH_3-N (mg/dl)	8.05	8.60	7.31	0.712	0.587
Total VFA (mmol)	71.85^{b}	80.26^{ab}	85.21^{a}	2.427	0.106
Acetic acid (mmol)	50.37^{b}	57.09^{a}	60.69^{a}	1.795	0.023
Propionic acid (mmol)	10.90	11.45	11.97	0.405	0.781
Butyric acid (mmol)	7.51	8.44	8.97	1.353	0.601
Valeric acid (mmol)	0.95	1.14	1.16	0.048	0.313
Isobutyric acid (mmol)	0.78	0.79	0.89	0.024	0.166
Isovaleric acid (mmol)	1.34	1.35	1.52	0.049	0.495

[1] SEM, standard error of means. [2] Statistical significance of treatment effects by F-test.
[a, b] Means in the same row with unlike superscripts differ significantly ($p<0.05$).

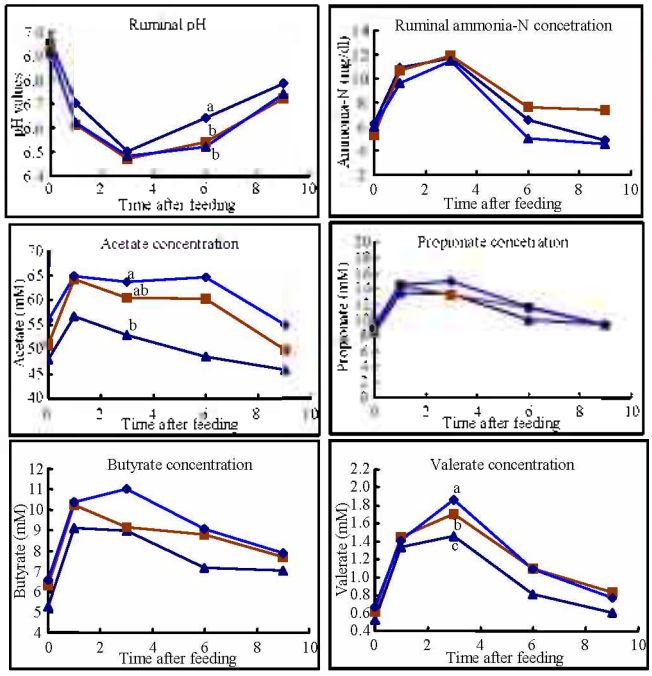

Figure 1. Daily pattern of variation in ruminal pH, ammonia-N and individual VFA of Hanwoo steers fed diets with different levels of Tween 80. Tween 80 was supplemented with 0 (▲--▲), 5 (■--■) and 10 g/d (♦--♦) in the basal diet. The letters indicate statistical significance: means with different letters are significantly different ($p<0.05$).

was administrated through the rumen cannulae at two times a day, ruminal pH, ammonia-N and total VFA were affected by the administration of NIS.

Tween 80 supplemented Hanwoo groups tended to have lower ruminal pH than control group. Ruminal pH was significantly ($p<0.05$) affected by Tween 80 supplementation at 6 h after feeding. pH values (6.65 to 6.72) in the experiment were considered to be within the optimum range for both proteolysis and celluloysis as reviewed by Tamminga (1979). The present experiment used a diet containing high amount of readily fermentable carbohydrate in the form of starch. Rapid fermentation results in an inhibition of cellulolysis, which has been variously attributed to low pH (Terry et al., 1969) or feedback inhibition of key fiber-digesting enzymes (Murphy, 1989). Therefore, it was expected to reduce ruminal pH rapidly after feeding. However, this was not the case in the present experiment. The ruminal pH remained at surprisingly high levels between the treatments. This is probably due to the consumption of straw led to high salivation rates, which maintained the ruminal pH at high levels. According to Nolan and Leng (1972), the amount of saliva produced can be greatly influenced by the physical structure of the diet, i.e. it increases with increasing proportion of roughage in diet. Feeding a high concentrate diet would be expected to decrease ruminal pH to lower than 6.0 after feeding, but this effect seems to be prevented by feeding straw.

Ammonia-N concentration in the rumen of Hanwoo was not influenced by the supplementation of Tween 80. Samples at 9 h after feeding from the control and 10 g/d Tween 80 supplemented groups showed the ammonia-N concentrations of 4.88 and 4.52 mg/dl in the rumen below the levels of 5 mg/d required to maximize microbial protein synthesis (Satter and Slyter, 1974) and not to depress the rate of microbial fermentation (Wilson and Kennedy, 1996). However, throughout the day, the ruminal concentrations of ammonia-N concentrations in the rumen in all treatments met the minimal requirement, ranging from 7.31 to 8.60 mg/dl.

The total VFA concentration was linearly increased by increasing supplementation levels of Tween 80 and especially, the 10 g/d Tween 80 supplementation significantly increased ($p<0.05$) total VFA concentration up to 13.36 mmol compared with the control treatment. In terms of individual VFA, both supplementation levels of Tween 80 increased ($p<0.05$) acetic acid concentration in the rumen. Although it was not significant, concentrations of propionic, butyric, isobutyric and isovaleric acids tended to be linearly increased by increasing supplementation levels. Concentrations of acetic and valeric acids were

Table 3. The daily pattern of variation in extracellular CMCase activity (μmol/ml/min) in the rumen of Hanwoo steers fed diets supplemented with different levels of Tween 80

Time after feeding (h)	Supplementation levels (g/d) of Tween 80			SEM[1]	P value[2]
	0	5	10		
0	13.57	11.77	24.48	3.676	0.325
1	141.89	139.83	151.19	5.391	0.454
3	61.64	78.51	77.85	6.452	0.415
6	16.73	13.66	36.93	5.770	0.163
9	27.56	29.55	34.68	6.765	0.747
Daily mean	52.28[b]	54.66[b]	65.03[a]	3.310	0.108

[1] SEM, standard error of means. [2] Statistical significance of treatment effects by F-test.
[a,b] Means in the same row with unlike superscripts differ significantly ($p<0.05$).

Table 4. Effects of different supplementation levels of Tween 80 on dry matter intake (kg/d) and the nutrient digestibility (%, DM basis) of Hanwoo steers fed compound feed and rice straw

Items	Supplementation levels (g/d) of Tween 80			SEM[1]	P value[2]
	0	5	10		
Dry matter intake	7.65	7.63	7.68	0.166	0.5000
Digestibilities					
Dry matter	59.52	60.24	61.45	0.559	0.3671
Crude protein	59.78	59.83	62.15	0.907	0.4013
Ether extract	76.73[c]	78.31[b]	80.38[a]	1.116	0.0130
Crude fiber	44.96[a]	45.75[ab]	47.01[a]	0.542	0.0884
Neutral-detergent fiber	48.89	50.10	50.89	0.590	0.5102
Acid-detergent fiber	69.86	70.89	70.74	0.636	0.6270
Crude ash	9.68	11.41	11.30	1.341	0.7179
Nitrogen-free extract	68.07	68.92	69.93	0.483	0.3010

[1] SEM, standard error of means. [2] Statistical significance of treatment effects by F-test.
[a,b,c] Means in the same row with unlike superscripts differ significantly ($p<0.05$).

highest ($p<0.05$) in the highest supplementation group at 3 h after feeding. The elevated concentration of total VFA in the 10 g/d supplementation of Tween 80 was primarily due to the increased concentrations of acetic and propionic acids. Lee et al. (2003) reported that the acetic and propionic acids were higher in the non-ionic surfactant (NIS) treatment, increasing total VFA concentration. Wang, et al. (2003) reported that in *in vitro* cultures of mixed rumen microorganisms, total VFA and gas production were increased by 0.2% supplementation of Tween 80 during fermentation of all silage substrates. However, in contrast to our results of the higher concentrations of acetic acid in the rumen of Hanwoo fed diets supplemented 5 and 10 g/d Tween 80, they reported that the 0.2% supplementation into the cultures of mixed rumen microorganisms increased molar percentages of propionic acid resulting in lower A:P ratios in all silage substrates. They suggested that the effects of Tween 80 on ruminal fermentation are diet dependent, expressed either through altering the species composition of the rumen microbial population (as opposed to affecting the capacity of microbial populations to produce enzymes) or through altering the interaction between the enzymes and the target substrates. Thus, the response of ruminal fermentation to Tween 80 may be both dose-related and diet dependent.

The time courses of CMCase activity in the different treatments are shown in Table 3. CMCase activity rapidly increased just after feeding and the 10 g/d supplementation of Tween 80 showed relatively increased CMCase activity at 0, 1, 6 and 9 h after feeding but these are not statistically significant ($p>0.05$). Lee and Ha (2003) reported that the addition of 0.1% Tween 80 showed the highest CMCase activity at 6 h after incubation in *in vitro* cultures of mixed rumen microorganisms grown with barley grain ($p<0.05$). They also reported that in the cultures grown with Orchardgrass hay the addition of 0.05% Tween 80 resulted in the higher activity than control at 12, 24, 72 and 96 h and the addition of 0.1% Tween 80 resulted in the higher CMCase activity at 24, 48, 72 and 96 h compared with control. In the present experiment, daily mean value of CMCase activity from the 10 g/d supplementation of Tween 80 was significantly increased by 24.4% than that of control. An increased activity from Tween 80 supplementation in the permeability of the anaerobic microbial cell membrane, thus permitting more enzymes to be released, as postulated for aerobic fungal species (Demain and Birnbaum, 1968; Reese and Maguire, 1969; Schewale and Sadana, 1978; Yazdi et al., 1991). As indicated from the study carried out by Lee et al. (2003), Tween 80 supplementation in Hanwoo steers can elicit an increase in the amounts of certain cellulolytic enzymes.

Nutrient digestibility of Hanwoo steers fed diets

supplemented with different levels of Tween 80 is shown in Table 4. Dry matter intakes were similar between all treatments ranging from 7.63 to 7.68 kg/d because animals were restricted in feeding. Digestibility of crude fiber (CF) was significantly increased ($p<0.05$) in Hanwoo steers fed the diet supplemented with 10 g/d Tween 80 compared with that of control, whilst digestibility of ether extract (EE) was linearly increased by increasing the NIS supplementation level ($p<0.05$). Although digestibilities of DM, crude protein, NDF, ADF, crude ash and NFE were not significantly improved in the all supplementation treatments compared with those of control, by the increasing Tween 80 supplementation level into the basal diet the digestibilities tended to linearly increase without statistical differences.

In contrast to the results of the work of McAllister et al. (2000) in which Tween 80 applied at 0.02% to both forage and concentrate diets for lambs affected neither digestibility nor animal performance, the present experiment demonstrated that the positive responses of Tween 80 addition on digestion and release of enzymes related to digestibility in previous *in vitro* research works can be applicable to ruminant animals. Lee et al. (2003) indicated that Tween 80 stimulated release of cell-free and cell-bound enzymes from mixed anaerobic rumen microorganisms into the rumen fluids. Yazdi et al. (1990) demonstrated that the secretion of several cellulolytic enzymes of *Neurospora crassa* is intimately linked to membrane lipid composition, and the increased release of these enzymes can be explained through an alteration of membrane fluidity due to an increase in the proportion of unsaturated lipids. Again, Wang et al. (2003) summarized that a number of positive effects on degradative enzymes of NIS are due to preventing inactivation of cellulase (Park et al., 1992), increasing enzymatic hydrolysis of cellulose (Helle et al., 1993) and improving digestion of cellulose by mixed ruminal bacteria. The higher EE and fiber digestibilities in Hanwoo steers fed a diet supplemented with 10 g/d Tween 80 may be due to increase cellulolytic enzyme activities and changes of membrane lipid composition in rumen microbes. Again, it is likely that the slightly improved digestibilities of nutrients by the supplementation of Tween 80 in the present experiment was caused by improved rumen fermentation (Figure 1 and Table 2) due to increased release of cellulase, xylanase, protease, amylase and glucanase in the cell free fraction (Lee et al., 2003).

Due to the complete import liberalization of livestock animals since year 2001, Korean livestock producers have mainly been focusing on high quality production and reduction of production cost, which could possibly compete against the lower priced imported animal products in domestic markets. Therefore, the work like the present one to improve the feeding value using non-ionic surfactant such as Tween 80 could be important of beef and milk production in Korea, not only improving quality but also to lowering feed cost. Further research is required to define the optimum application rates of Tween 80 under different feeding regimes and in addition, to examine animal performance with Tween 80 supplementation.

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