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Effects of Dietary Addition of Bentonite and Probiotics on Meat Characteristics and Health of Hanwoo (*Bos taurus coreanae*) Steers fed Rice Straw As a Sole Roughage Source (a Field Study)

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ABSTRACT

A study was conducted to determine the dietary effects of Na-bentonite (NaB) and probiotics on meat characteristics and health of Hanwoo steers fed rice straw as a sole roughage source. A total of 24 growing Hanwoo steers (avg BW 232 kg) were assigned to two treatments which included a control diet (concentrate mix and rice straw) and a treatment diet (control diet + 0.5-1.0% NaB + 0.5-1.0% probiotics. The diets were fed for 22 months up to the time the animals were slaughtered. Dietary treatment increased ($p<0.05$) concentrations of trace minerals such as Zn, Cu, and Fe in the *longissimus* muscle compared to the control. The treatment diet did not affect cold carcass weight, yield traits such as backfat thickness, *longissimus* muscle area, yield index, yield grade and quality traits such as marbling score, meat color, fat color, texture, maturity and quality grade. Blood profiles of growing steers were within the normal ranges for healthy cattle. In conclusion, feeding a combination of clay mineral and probiotics to Hanwoo steers fed rice straw as a sole roughage source could have a desirable effect on improving trace mineral retention in *longissimus* muscle without any deleterious effects on carcass traits of steers.
(**Key words** : Bentonite, Probiotics, Trace mineral, Meat quality, Hanwoo)

Ⅰ. INTRODUCTION

Na-bentonite (NaB) is an expanded lattice clay of the montmorillonite group of minerals (Bates and Jackson, 1980) with a high ion exchange capacity that binds a wide range of cations (Fenn and Leng, 1989). Incorporated into diets, it has improved wool growth of sheep (Fenn and Leng, 1989; Cobon et al., 1992), decreased ruminal ammonia concentrations, and improved feed and bacterial protein flow to the small intestine of ruminants (Ivan et al., 1992). Because acid buffering capacity has been correlated with total cations and total ash (Jasaitis et al., 1987), mineral buffers such as NaB might be effective in alleviating acid stress in ruminants under intensive feeding and management programs. The swelling capacity of NaB might provide a

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desirable habitat niche for probiotic microbes in the ruminant digestive tract.

It is well known that probiotic microbes improve gut microbial balance, feed intake, weight gain and feed efficiency in ruminants (Yoon and Stern, 1995; Krehbiel et al., 2003). Kim et al. (2007) reported that feeding probiotic cultures improved growth, meat quantity and meat quality of Hanwoo steers. However, there is no research conducted on feeding effects of NaB and probiotics together on cattle. It is, therefore, postulated that the addition of probiotics with NaB might show a desirable effect on meat quality and quantity.

Based on previous reports of the beneficial effects of bentonite and probiotic cultures incorporated into diets of ruminants, a study was conducted to determine the effects of these dietary additives on meat quality and quantity by Hanwoo steers.

Ⅱ. MATERIALS AND METHODS

1. Animals and treatments

All animal care protocols were approved by the Konkuk University Institutional Animal Care and Use Committee. Twenty four-Hanwoo (*Bos taurus coreanae*) steers at 9 mon of age (average BW 232 kg) were allotted in groups of 4 steers to each of 6 pens. Four of the pens were located at Farm 1, and 2 at Farm 2 in Boeun County, Chungbuk Province, Korea. Steers were fed one of two rations. Each ration was fed to steers in two pens located at Farm 1 and one pen at Farm 2. The steers were fed a control diet (concentrate mix and rice straw), and a treatment diet (the control diet + 0.5-1.0% NaB + 0.5-1.0% probiotics). Periods for growing, fattening and finishing were 6, 8, and 8 mon, respectively. Diets were fed for 22 mon until the animals were slaughtered.

Feeds and feed additives were supplied daily to the steers in a manner shown in Table 1. The concentrate mix (Table 2) was fed in a restricted manner to achieve 0.8 kg levels of average daily gain (ADG) during the growing period, and over 0.9 kg ADG during the fattening period. Animals had free access to rice straw at all times. The NaB and probiotics were top-dressed at each feeding time. Feed was supplied twice a day at 07:00 h and 18:00 h. During the finishing period, the concentrate mix was fed *ad libitum* and rice straw was restricted at a level of approximately 10% of concentrate mix.

Animals were observed for health status, and body weight was measured on a monthly basis throughout the study. Samples of concentrate mix and rice straw were collected every 2 wk for proximate analysis.

The chemical composition of the commercial concentrate mix and rice straw fed to the steers is presented in Table 2. The bentonite product (Bionit, Korea Sud Chemical, Korea) used in the study was an extra-purified powder for animal use, and was composed of 75~85% Montmorillonite, 58% SiO_2, 20% Al_2O_3, 6% Fe_2O_3, 3.5% MgO, 2.5% CaO, 2% Na_2O, and 1% K_2O. Based on individual minerals, it contained 1.41% Ca, 0.04% P, 0.10% Mg, 1.78% K, 0.66% Na, 1,631 ppm Fe, 7.9 ppm Zn, 5.0 ppm Cu, and 212 ppm Mn. The pH was about 10. The swelling volume was 9 mL/g, and the cation exchange capacity was 80 meq/100 g. The specific surface area was 400-600 m^2/g. The probiotics used in the study was a mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae* at a viable

Table 1. Diets for growing, fattening and finishing periods for Hanwoo steers[1)]

Item	Control	Treatment
Growing period		
Concentrate mix, % live wt	1.44	1.44
Rice straw	FA[3)]	FA
Na-Bentonite[2)], % conc. mix	0	1.0
Probiotics[2)], % conc. mix	0	1.0
Fattening period		
Concentrate mix, % live wt	1.50	1.50
Rice straw	FA	FA
Na-Bentonite[2)], % conc. mix	0	0.5
Probiotics[2)], % conc. mix	0	0.5
Finishing period		
Concentrate mix	FA	FA
Rice straw, % conc. mix	10	10
Na-Bentonite[2)], % conc. mix	0	0.5

1) Dry matter basis.
2) Each of Na-bentonite and probiotics was top-dressed on concentrate (conc.) mix at each feeding time.
3) FA means ‘free access’ to rice straw or concentrate mix all the time.

Table 2. Chemical composition of concentrate mix and rice straw[1),2),3)]

Item	Concentrate mix			Rice straw
	Growing	Fattening	Finishing	
	……………………………… % ………………………………			
Dry matter	88.0	88.0	85.8	86.8
Organic matter	92.3	91.5	93.2	89.8
Ether extract	3.6	3.1	3.0	0.7
Crude protein	15.7	14.1	14.0	3.7
Neutral detergent fiber	29.8	27.2	25.8	75.7
Acid detergent fiber	13.9	19.6	15.3	46.9
Crude ash	7.7	8.5	6.8	10.2
Minimum target TDN	80.7	80.7	81.8	–

1) Dry matter basis.
2) Means of 3 observations.
3) Ingredient composition (as-fed basis) of concentrate mix was as follows: corn grain 22.5%, wheat 18.0%, molasses 5.5%, wheat bran 21.0%, corn gluten meal 4.5%, rapeseed meal 7.0%, coconut meal 7.0%, palm meal 11.0%, NaCl 0.6%, limestone 2.0%, vitamin premix 0.1%, mineral premix 0.1% and other additives 0.7% for the growing period; corn grain 22.0%, wheat 18.0%, molasses 5.5%, tapioca 8.0%, wheat flour 3.0%, wheat bran 12.7%, rapeseed meal 4.4%, coconut meal 7.0%, palm meal 11.0%, mixed hays 5.0%, NaCl 0.6%, limestone 2.0%, vitamin premix 0.1%, mineral premix 0.1% and other additives 0.6% for the fattening period; corn grain 29.7%, wheat 18.0%, molasses 6.0%, tapioca 8.0%, wheat bran 4.0%, corn gluten meal 10.3%, distiller’s rice 1.0%, coconut meal 5.05%, palm meal 11.0%, mixed hays 4.0%, NaCl 0.6%, limestone 1.5%, vitamin premix 0.1%, mineral premix 0.1% and other additives 0.6% for the finishing period.

cell concentration in excess of 10^6 cfu/g for each culture. The cultures were grown on rice bran and the culture mixture had 85% DM, 16.0% CP, 14.0% ether extract (EE), 6.3% crude fiber (CF) and 10.3% crude ash. Based on the individual minerals, the probiotics contained 0.19% Ca, 1.41% P, 0.25% Mg, 1.79% K, 0.04% Na, 231 ppm Fe, 40 ppm Zn, 6.9 ppm Cu, and 184 ppm Mn.

The control diet (concentrate mix + rice straw) was lack in trace mineral contents as shown in Table 3. Dietary Zn levels in growing, fattening and finishing periods, respectively were lower than NRC (2000) requirement of beef cattle. Dietary Cu levels according to the periods were much lower than the requirement (NRC, 2000). When the control diet was added with NaB and probiotics, the levels of Zn and Cu (48 and 12 ppm, respectively) were higher than the NRC requirement (NRC, 2000).

2. Sampling and chemical analysis

Feed samples taken from troughs prior to feeding were dried and ground to pass through a 1 mm screen using a Sample Mill (Cemotec, Tecator, Sweden). The content of dry matter was determined by drying samples at 105℃ for 24 h to constant weight. The contents of crude protein, EE, acid detergent fiber (ADF), and ash were determined by the AOAC methods (2000). The content of ash free neutral detergent fiber (NDF) was determined according to the method of Van Soest et al. (1991). Organic matter (%) was determined as 100 minus ash %.

Steers were withdrawn from the experimental diets 24 h before slaughter. Following a 24-h carcass chill, yield and quality grades were assigned to each carcass using Korean carcass grading standards specified in the attached list No. 4 of Korean Livestock Enforcement Regulation (KLER, 2007). The 12th to 13th rib *longissimus* muscle was removed, retained from each steer and frozen until later analysis.

For mineral analysis of the rib muscle, samples were analyzed for Ca, P, Ma, K, Na, Mn, Fe, Zn and Cu by inductively coupled argon plasma emission spectroscopy (ICP-OES 5300DV, Perkin Elmer, USA) as described by Braselton et al. (1997).

For animal health diagnosis, blood samples were taken from jugular vein of steers during the growing period and an equal portion was divided into bottles with or without anti-coagulant EDTA. Serum profiles were analyzed using an Automatic Biochemical Analyzer (Hitachi 7170A, Hitachi Ltd., Tokyo, Japan) based on photometer and ion selective electrode methods, and whole blood profiles were analyzed with an Automatic Blood

Table 3. Zn and Cu levels in the control diet according to feeding periods[1)]

	Period			Requirement of beef cattle[2)]
	Growing	Fattening	Finishing	
	ppm			
Zn	28	19	21	30
Cu	6	4	5	10

[1)] Dry matter basis.
[2)] Cited from NRC (2000).

Analyzer (Coulter STKS, Beckman Coulter Co., Miami, FL, USA) based on impedance and VCS (volume, conductivity, light scattering) methods.

3. Statistical analysis

Data were analyzed using farms as a block in a randomized complete block design by the General Linear Model (SAS Institute, Inc., 1990). Comparison of means between control and NaB treatment was made using studentized-t test (SAS Institute, Inc., 1990). Significant differences were detected at $p < 0.05$.

Ⅲ. RESULTS AND DISCUSSION

1. Meat mineral profile

The effect of the dietary treatment on the meat mineral profiles of steers is presented in Table 4. Feeding a diet (treatment) added with NaB and probiotics affected ($p < 0.05$) the mineral concentrations in the *longissimus* muscle. Compared with the control, the treatment increased concentrations of Zn, Cu, Fe, P, Mg and Na, but concentrations of Mn, Ca and K were not affected. The *longissimus* muscle of steers assigned to the treatment had higher levels of Zn (49.0%), Cu (100%), Fe (34.5%), P (49.3%), Mg (46.1%), and Na (43.9%) than the control group. Individual herd data showed little variation associated with the increasing rates of the specific minerals (data not presented). The mineral concentrations ranged between the values reported by Westing et al. (1985) and Williams et al. (1983). However, Cu levels in the present study were rather low compared with those reported by Salles et al. (2008). This difference was attributed to the different analytical method. In a short term study with lambs Walz et al. (1998) reported a diet with 0.75% bentonite did not affect concentrations of Zn, Cu, Mn, Fe, Ca, Mg, and K in bone, liver and kidney.

The control diet in this study contained more Fe, Mn, K and Na and less Zn, Cu, and Mg (data not presented) than the dietary requirement specified in KFSEC (2007) and NRC (2000). Dietary supplementation of these deficient minerals

Table 4. Mineral profiles (ppm) in the *longissimus* muscle of Hanwoo steers fed different diets[1)]

Item	Control	Treatment	SE
Trace minerals			
Zn	39.4	58.7	4.1[a]
Cu	2.2	4.4	0.9[a]
Fe	26.7	35.9	2.5[a]
Mn	0.12	0.11	0.03
Major minerals			
Ca	146.2	145.7	23.1
P	2,225	3,323	238[a]
Mg	232	339	25[a]
K	4,250	6,061	438
Na	618	889	63[a]

[a] Control diet differs from treatment ($p<0.05$).
[1)] Means of 12 observations.

especially for the treatment resulted in a higher Zn, Cu, and Mg retention in the *longissimus* muscle. Zinc and Cu are essential trace minerals and perform important biochemical functions. These minerals are deficient in typical human diet; however, Cu deficiency is more common and important in the elderly diet (Subar et al., 1998; Ma and Betts, 2000). Cao et al. (2000) reported that Zn supplementation of ruminant diets that were deficient in Zn resulted in increased Zn content of the ruminant muscle.

The biological reasons for the improved effects of dietary NaB on mineral metabolism are not apparent. The improved bioavailability of mineral might be due to the high swelling capacity of NaB resulting in a slowing in the rate of digesta passage through the gastro-intestinal tract or might be related to the high cation exchange capacity of NaB.

The use of microorganisms in animal diets has been shown to improve mineral balance. Yoon and Stern (1995) reported that yeast culture supplementation improved retention of minerals (Cu, Fe and K) in growing ruminants and Cole et al. (1992) reported that lambs fed yeast culture tended to have a better balance of Zn and Fe. The present study showed there might be a desirable trace mineral retention when a diet was supplemented with both NaB and probiotics. This phenomenon may be possibly due to a better microbial habitat attributed to the swelled NaB in the digestive tract and also due to an improved mineral uptake by the microbes.

2. Meat characteristics

The effects of the dietary treatments on meat characteristics are presented in Table 5. The cold carcass weights were 20 kg higher for the treatment than for the control, but the difference was not significant. Also, the dietary treatment did not affect carcass yield traits including backfat thickness, *longissimus* muscle area, yield index and yield grade and quality traits including marbling score, meat color, fat color, texture, maturity and quality grade. For the meat quality grades the number of carcasses graded as 1^{++}, 1^{+}, 1, 2, and 3 grades were 2, 3, 4, 2, and 1 for the control group and 1, 2, 8, 1, and 0 for the treatment group.

Overall the dietary treatment had a beneficial effect on the carcass quality grades. In another study (Walz et al., 1998), feeding 0.75% NaB did not affect carcass weight, *longissimus* muscle area, backfat thickness, quality grade nor yield grade of lambs. The differences in response to NaB could be due to different feeding periods and contents. When beef steers were supplemented with Zn, carcass weights were increased (Spears and Kegley, 2002). A previous study by Kim et al. (2007) reported no effect on meat characteristics when 1% of probiotic culture was fed to steers.

3. Blood profiles

The blood profiles of the Hanwoo steers are presented in Table 6. For blood nutrients, the dietary treatment did not show any differences in blood concentrations of triglyceride, cholesterol, high density lipoprotein, low density lipoprotein, glucose and protein. These results indicate that fat, energy and protein metabolisms within the animal body were not affected by the treatment.

The blood electrolytes Ca, P and K were not affected by the treatment, but blood Na and Cl levels were lower ($p<0.05$) compared to steers

Table 5. Meat characteristics of Hanwoo steers fed different diets[1)]

Item	Control	Treatment	SE
Cold carcass weight, kg	403	423	20
Yield traits			
Backfat thickness, mm	16.8	14.4	1.6
Longissimus muscle area, cm^2	82.3	84.9	3.1
Yield index	61.9	63.3	1.2
Yield grade[2)]	2.34	2.38	0.16
Quality traits			
Marbling score[3)]	4.88	4.50	0.65
Meat color[4)]	4.50	4.31	0.16
Fat color[5)]	2.84	2.94	0.07
Texture[6)]	1.31	1.19	0.16
Maturity[7)]	2.19	2.06	0.14
Quality grade[8)]	2.75	2.88	0.37

[1)] Means of 12 observations.
[2)] Scored : grade A = 1(lean), B = 2, C = 3(fat).
[3)] Scored : grade 1 = poor, grade 9 = excellent.
[4)] Scored : grade 1 = scarlet, grade 7 = dark red.
[5)] Scored : grade 1 = white, grade 7 = yellow.
[6)] Scored : grade 1 = good, grade 3 = bad.
[7)] Scored : grade 1 = fully mature, grade 9 = least mature.
[8)] Scored : grade 1^{++} = 1(best), 1^{+} = 2, 1 = 3, 2 = 4, 3 = 5 (poorest).

on the control diet. In a similar study (Ha et al., 1985), feeding 2% NaB to lambs did not affect concentrations of blood Ca, P, and K, but Na content decreased. Also, in another study of Pulsipher et al. (1994), feeding NaB decreased blood Na and Cl levels. The exact reason for decreased blood Na and Cl leves was not explained. But they also could be associated with non-dietary factors such as hormone levels of the animals on the different diets (Church and Pond, 1982).

Blood enzyme analyses showed little effect of the dietary treatments on liver and kidney function because steers on the treatment had similar concentrations of blood aspartate aminotransferase and lactate dehydrogenase. Blood cell counts were not affected by the treatment. Generally, values for all blood constituents were within the normal range for healthy cattle (Church and Pond, 1982; Wallach, 1974). In a similar study, Cho et al. (2001) reported that feeding clay minerals to growing Hanwoo steers did not affect their red and white blood cell counts. Pulsipher et al. (1994) reported that feeding 42 g/d NaB to lambs did not affect their blood nutrients, electrolytes, and enzymes, but there was a decrease in serum Na and Cl concentrations. None of the steers showed

Table 6. Blood profiles of Hanwoo steers fed different diets[1)]

Item	Control	Treatment	SE
Triglyceride, mg/dL	29.8	24.6	2.9
Cholesterol, mg/dL	154.7	143.6	11.4
High density lipoprotein, mg/dL	121.3	113.8	8.5
Low density lipoprotein, mg/dL	29.9	27.6	4.1
Glucose, mg/dL	69.8	71.0	3.0
Total protein, g/dL	6.58	6.63	0.17
Electrolytes			
Calcium (Ca^+), mg/dL	9.43	9.58	0.22
Inorganic phosphorus (P^-), mg/dL	8.16	8.03	0.35
Potassium (K^+), mM/L	5.40	5.09	0.14
Sodium (Na^+), mM/L	145.8	144.1	0.8a
Chlorine (Cl^-), mM/L	103.7	101.8	0.8a
Albumin, g/dL	3.01	2.93	0.07
Globulin, g/dL	3.58	3.70	0.16
Albumin/globulin	0.87	0.80	0.05
Uric acid, mg/dL	1.22	1.13	0.06
Total bilirubin, mg/dL	0.10	0.11	0.01
Alkaline phosphatase, IU/L	539	486	64
Alanine aminotransferase, IU/L	25.3	23.9	1.8
Aspartate aminotransferase, IU/L	85.1	91.5	4.9
r-glutamyltransferase, IU/L	21.9	21.7	2.2
Lactate dehydrogenase, IU/L	1285	1349	49
Amylase, IU/L	27.6	27.8	2.2
Urea-N, mg/dL	10.7	10.5	0.9
Creatinine, mg/dL	1.26	1.27	0.06
White blood cell counts, $10^3/\mu L$	25.0	32.4	6.5
Red blood cell counts, $10^6/\mu L$	7.27	6.91	0.31
Platelet counts, $10^3/\mu L$	241	299	55

[a] Control diet differs from treatment ($p < 0.05$).
[1)] Means of 12 observations.

abnormal health problems throughout the experimental periods.

In summary, the addition of NaB and probiotics to the diet of Hanwoo steers increased concentrations of trace minerals (Zn, Cu, and Fe) in the *longissimus* muscle of steers. In conclusion, the combined use of clay mineral and probiotics in the animal diet improved mineral retention in muscle without any deleterious effects on carcass traits of Hanwoo steers.

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