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Effects of dietary addition of *Bacillus and Saccharomyces* culture on blood profiles, growth, and meat characteristics of Hanwoo (*Bos taurus coreanae*) steers

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Abstract

A study was conducted to determine the dietary effects of a mixed microbial (*Bacillus* and *Saccharomyces*) culture on growth, production, and meat characteristics of Hanwoo steers. Two treatment diets fed to steers included a control diet (concentrate mix and rice straw) and a treated diet (control + 1.0 and 0.5% direct-fed microbe (DFM) culture during the growing and fattening periods, respectively). Feeding a DFM (*Bacillus and Saccharomyces*) culture did not affect (P>0.05) growth during the growing, fattening and finishing periods, carcass yield and quality traits after slaughtering. The *longissimus* muscle of the DFM-fed steers had higher (P<0.05) concentrations of P, Mg, K, Na, Fe, and Zn than the control steers. The study demonstrated that the dietary addition of a mixed micriobial (*Bacillus* and *Saccharomyces*) culture could be effective in improving mineral bioavailability to beef steers.

Keywords: Microbe, Blood, Meat, Mineral, Beef cattle

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Introduction

It is well known that direct-fed microbes (DFM) have been demonstrated to be beneficial in animal agriculture production (Kwak and Kang, 2006; Yang et al., 2006; Zhou et al., 2009). Benefits derived from the use of DFM include improved ruminal acidosis prevention, gut microbial balance, feed intake, improved weight gain and feed efficiency in ruminants (Yoon and Stern, 1995; Krehbiel, et al., 2003). Feeding yeast culture increased feed intake by lactating Holstein cows (Williams et al., 1991: Robinson and Garrett, 1999) and Hereford steers (Adams et al., 1981), resulting in better animal performance. Addition of live yeast did not show positive effects on feed utilization (Mir and Mir, 1994). Kim et al. (2007) reported that feeding DFM cultures improved growth and meat quantity of Hanwoo steers.

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 (K, Cu and Fe) in growing ruminants and Cole et al. (1992) reported that lambs fed yeast culture tended to have a better balance of Zn and Fe. However, data are absent for Hanwoo steers fed a mixed microbial (*Bacillus and Saccharomyces*) culture.

Based on previous reports of the beneficial effects of microbial cultures incorporated into diets of ruminants, a study was conducted to determine the effects of feeding a DFM (*Bacillus and Saccharomyces*) culture on blood profiles and muscle mineral retention as well as growth performance and meat quality of Hanwoo steers fed rice straw as a main roughage source.

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Materials and Methods

Animals and treatments

All animal care protocols were approved by the Konkuk University Institutional Animal Care and Us Committee. Twenty four Hanwoo (*Bos taurus coreanae*) steers at 9 months of age (avg BW 232kg) 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. As presented in Table 1, the steers were fed a control diet (concentrate mix and rice straw), and a treated diet (DFM-added diet). The treated diet was a control diet + 1.0% DFM culture fed during the growing and 0.5% DFM culture fed during the fattening periods. The treated diet was top-dressed with 1% DFM culture based on the total diet. As the feed intake rapidly increased with the growth of the steers, the amount of the DFM culture fed became too much and extremely dusty and was deemed to be impractical to continue. To reduce the dusty condition of the diet the quantity of dry DFM culture was reduced by half from 1% each to 0.5% each at the beginning of the fattening period. The dusty condition of the diet could have been avoided by mixing the dry DFM culture into the total diet but a feed mixer was not available at the farm where the feeding trial was conducted. Mixing the culture into the total ration and pelleting the ration would have been the preferred method of feeding the DFM-added ration. The DFM culture was not fed during the finishing period when its efficiency can be doubtful. Periods for growing, fattening and finishing were 6, 8, and 8 months, respectively. This feeding trial was conducted for 22 months until the animals were slaughtered.

Table 1: Experimental feeding design for the growing, fattening and finishing periods for Hanwoo steers[1]

Item	Control	DFM treatment[2]
Growing period		
Concentrate mix, % live wt	1.44	1.44
Rice straw	FA[3]	FA
DFM culture[2], % conc.mix	0	1.0
Fattening period		
Concentrate mix, % live wt	1.50	1.50
Rice straw	FA	FA
DFM culture[2], % conc.mix	0	0.5
Finishing period		
Concentrate mix	FA	FA
Rice straw, % conc. Mix	10	10

[1]Dry matter basis; [2]A direct-fed microbe (DFM) culture 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.

The concentrate mix was fed in a restricted manner to achieve 0.8% levels of ADG during the growing period, and over 0.9kg ADG during the fattening period. Animals had free access to rice straw at all times. The DFM culture was top-dressed at each feeding time. Feed was supplied twice a day at 0700 h and 1800 h. During the finishing period, the concentrate mix was fed *ad libitum* and rice straw was restricted at a level of 1kg daily. The corn-based concentrate mix was formulated to be a minimum of 80.7% of TDN and 14.0% of CP on a dry basis. Rice straw was fed in the form of large bales (400-500kg).

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 fed to the steers was DM 88%, EE 3.6%, CP 15.7%, NDF 29.8%, ADF 13.9% and ash 7.7% for the growing period. The composition during the fattening period was DM 88%, EE 3.1%, CP 14.1%, NDF 27.2%, ADF 19.6% and ash 8.5%. During the finishing period the concentrate mix composition was DM 86%, EE 3.0%, CP 14.0%, NDF 25.8%, ADF 15.3% and ash 6.8%. The chemical composition of the rice straw fed during the whole period was DM 86.8%, EE 0.7%, CP 3.7%, NDF 75.7%, ADF 46.9%, ash 10.2% on a dry basis. The DFM used in the study was a mixture of *Bacillus subtilis* and *Saccharomyces cerevisiae* at a viable cell concentration in excess of 10^6cfu/g for each culture. The anaerobically fermented 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 DFM culture 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.

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). Dry matter was determined by drying samples at 105°C for 24 h to constant weight. Crude protein, EE, NDF, ADF, and ash were determined by the AOAC methods (2000).

Health diagnosis was made during the growing period of steers. Blood samples were taken from jugular veins 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 Analyzer (Coulter STKS, Beckman Coulter Co., Miami, FL, USA) based on impedence and VCS (volume, conductivity, light scattering) methods.

Steers were withdrawn from the experimental diets 24h before slaughter. Following a 48-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 (KMAF, 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, Mg, 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).

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 DFM treatment was made using studentized-*t* test (SAS Institute, Inc., 1990). Significant differences were detected at $P < 0.05$.

Results and Discussion

Blood profiles of the growing steers

The blood profiles of the Hanwoo steers were analyzed during the growing period and presented in Table 2. For blood nutrients, the dietary DFM addition did not show any differences ($P > 0.05$) in blood concentrations of triglyceride, cholesterol, high density lipoprotein, low density lipoprotein and glucose of steers. These results indicate that fat and energy metabolisms were not affected by the dietary addition of DFM. When the DFM was fed to Hanwoo growers, there was an increase in blood protein levels. The increased total protein content in blood was caused by the increased globulin content. This phenomenon can be explained by the theory that microbial protein in the fermented culture induced more amino acid absorption through the blood and consequent more protein synthesis.

The blood electrolytes Ca, P and K were not affected by dietary addition of DFM. Blood enzyme analyses showed little effect of the dietary treatment on liver and kidney function although steers on the DFM treatment had higher concentrations of blood aspartate aminotransferase and lactate dehydrogenase. These enzymes are used as markers of tissue damage. But, this phenomenon can not be explained by the results of this study. Blood cell counts were not affected by the treatments. Generally, values for all blood constituents were within the normal range for healthy cattle (Wallach, 1974; Church and Pond, 1982).

None of the steers showed abnormal health problems throughout the experimental periods.

Feed intake and body weight change of steers during the whole feeding periods

During the growing and fattening periods, steers were fed restricted amounts of concentrate mix, but had free access to rice straw. Daily voluntary rice straw intake measured during these periods decreased gradually from 1.0 to 0.83% of live weight during the growing period and from 0.80 to 0.35% of live weight during the fattening period. Based on body weights measured each month, the concentrate mix was controlled to achieve a planned ADG during the growing (0.8kg levels) and fattening (>0.9kg) periods. Under these conditions, feeding 1.44% BW of concentrate mix resulted in an ADG of 0.82 to 0.85kg during the growing period. When concentrate mix was fed at 1.50% of BW the ADG was 0.91 to 0.93kg

Table 2: Blood profiles of Hanwoo steers fed different diets[1]

Item	Control	DFM treatment[2]	SE	P
Triglyceride (mg/dl)	27.6	24.6	2.9	0.538
Cholesterol (mg/dl)	146.9	143.6	7.1	0.535
High density lipoprotein (mg/dl)	118.0	113.8	8.5	0.707
Low density lipoprotein (mg/dl)	26.3	27.6	4.1	0.432
Glucose (mg/dl)	67.5	71.0	2.8	0.452
Total protein (g/dl)	6.24	6.63	0.17	0.083
Electrolytes				
Calcium (mg/dl)	9.38	9.58	0.18	0.857
Inorganic phosphorus (mg/dl)	8.66	8.03	0.35	0.096
Potassium (mmol/l)	5.24	5.09	0.14	0.341
Sodium (mmol/l)	144.4	144.1	0.8	0.097
Chlorine (mmol/l)	102.5	101.8	0.6	0.191
Albumin (g/dl)	2.9	3.0	0.05	0.050
Globulin (g/dl)	3.34[a]	3.70[b]	0.14	0.194
Albumin/globulin	0.86	0.80	0.04	0.949
Uric acid (mg/dl)	1.15	1.13	0.06	0.220
Total bilirubin (mg/dl)	0.10	0.11	0.01	0.998
Alkaline phosphatase (IU/l)	379[a]	539[b]	62	0.018
Alanine aminotransferase (IU/l)	22.6	23.9	1.5	0.165
Aspartate aminotransferase (IU/l)	76.4	85.0	4.4	0.066
r-glutamyltransferase (IU/l)	20.6	21.7	1.7	0.588
Lactate dehydrogenase (IU/l)	1,191[a]	1,285[b]	34	0.012
Amylase (IU/l)	26.5	27.8	2.1	0.651
Urea-N (mg/dl)	11.2	10.5	0.8	0.594
Creatinine (mg/dl)	1.33	1.27	0.05	0.261
White blood cell counts (10^3/µl)	23.6	32.4	6.5	0.808
Red blood cell counts (10^6/ µl)	7.10	6.91	0.31	0.587
Platelet counts (10^3/µl)	271	299	55	0.884

[a]Control differs from a DFM treatment ($P<0.05$); [1]Means of 12 observations; [2]A direct-fed microbe (DFM) culture was top-dressed on concentrate mix at each feeding time.

during the fattening period irrespective of the dietary treatment.

Body weight data for the Hanwoo steers were recorded for the growing, fattening and finishing periods, and these are shown in Table 3. The dietary DFM addition did not affect (P>0.05) body weight gain of the steers during the growing and fattening periods. The effects of DFM on body weight gain are not shown either during the growing or fattening periods. The little differences attributed to the treatment could have been predicted by the restricted intake of concentrate mix throughout the feeding periods.

Table 3: Production characteristics of Hanwoo steers fed different diets during the growing, fattening, and finishing periods[1]

Item	Control	DFM treatment[2]	SE	P
		Kg		
Growing period				
Initial weight	232	233	10	0.895
Final weight	384	381	15	0.798
Gain	152	148	10	0.539
Average daily gain	0.85	0.82	0.05	0.541
Fattening period				
Initial weight	432	426	13	0.769
Final weight	601	591	17	0.611
Gain	170	165	8	0.122
Average daily gain	0.93	0.91	0.04	0.122
Finishing period				
Initial weight	603	593	20	0.566
Final weight	727	732	21	0.807
Gain	124	139	8	0.082
Average daily gain	0.60	0.67	0.04	0.085

[a]Control differs from a DFM treatment (P<0.05); [1]Means of 12 observations; [2]A direct-fed microbe (DFM) culture was top-dressed on concentrate mix at each feeding time.

Table 4: Meat characteristics of Hanwoo steers fed different diets[1]

Item	Control	DFM culture[2]	SE	P
Cold carcass weight, kg	420	423	14	0.835
Yield traits				
Backfat thickness, mm	14.8	14.4	1.6	0.836
Longissimus muscle area, cm^2	81.8	84.9	3.0	0.300
Yield index	62.7	63.3	1.2	0.660
Yield grade[3]	2.13	2.38	0.16	0.172
Quality traits				
Marbling score[4]	5.00	4.50	0.65	0.446
Meat color[5]	4.50	4.31	0.16	0.256
Fat color[6]	3.00	2.94	0.07	0.408
Texture[7]	1.38	1.19	0.16	0.256
Maturity[8]	2.19	2.06	0.14	0.351
Quality grade[9]	2.69	2.88	0.37	0.564

[a]Control differs from a DFM treatment (*P*<0.05); [1]Means of 12 observations; [2]A direct-fed microbe (DFM) culture was top-dressed on concentrate mix at each feeding time; [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).

During the finishing period the effect of earlier feeding of DFM on weight gain of steers was not significant (P>0.05). However, the ADG was 12.1% higher for the DFM-fed steers than the ADG for the control steers. The higher ADG of these groups of steers was attributed to the compensation phenomenon for the earlier retarded growth, which was accelerated by the higher daily intake of concentrate mix for the treatment group (9.30kg) than the control group (8.58kg). The treatment steers consumed about 8.4% more than the control steers. It was assumed that the DFM had a beneficial effect on the gastro-intestinal tract which contributed to active voluntary intake during the finishing period when normally the feed intake of cattle is gradually reduced. Adams et al. (1981) reported that feeding yeast culture did not affect ADG and feed efficiency, but feed DM intake was greater for steers fed yeast culture than the control steers.

Meat characteristics after slaughtering of steers

Yield and quality profiles

The effects of the DFM treatment on meat characteristics are presented in Table 4. Cold carcass weight and carcass yield traits including *longissimus* muscle area, yield index and yield grade and quality traits including marbling score, meat color, texture, maturity and quality grade were not affected ($P > 0.05$) by the dietary addition of DFM. In a previous study, Kim et al. (2007) reported no effect on meat characteristics when 1% of DFM culture was fed to steers. In another study (Mir and Mir, 1994) using Hereford steers, feeding live yeast did not affect carcass characteristics such as carcass weight, backfat thickness, loin eye area, yield index and dressing percentage.

Meat mineral profile

The effect of the dietary DFM treatment on the meat mineral profiles of steers is presented in Table 5. The DFM treatment affected (**P**<0.05) the mineral concentrations in the *longissimus* muscle of steers. The *longissimus* muscle of steers assigned to the DFM treatment had higher (P<0.05) P, Na, Fe and Zn contents. In detail, the muscle of the treatment group had 22.5% higher P, 16.1% higher Mg, 17.7% higher K, 16.3% higher Na, 22.3% higher Fe, 19.7% higher Zn, 15.8% higher Cu levels than the control group. Individual herd data showed little variation associated with the increasing rates of the specific minerals; however, the *longissimus* muscle of animals fed the DFM culture had consistently higher concentrations of most of the minerals than that of the control animals. The mineral concentrations ranged between the values reported by Williams et al. (1983) and Westing et al. (1985). However, Cu levels in this study were rather

low compared with those reported by Salles et al. (2008). This difference was attributed to the different analytical method.

Table 5: Mineral profiles (ppm) in the *longissimus* muscle of Hanwoo steers fed different diets[1]

Item	Control	DFM treatmemt[2]	SE	P
Ca	125	128	20	0.119
P	2,770[a]	3,393[b]	197	0.0047
Mg	292[a]	339[b]	15	0.004
K	5,151[a]	6,061[b]	355	0.018
Na	754[a]	877[b]	45	0.019
Mn	0.10	0.13	0.03	0.266
Fe	29.1[a]	35.6[b]	2.5	0.014
Zn	49.3[a]	59.0[b]	2.6	0.009
Cu	3.8	4.4	0.9	0.365

[a] Control differs from a DFM treatment (P<0.05); [1]Means of 12 observations; [2]A direct-fed microbe (DFM) culture was top-dressed on concentrate (conc.) mix at each feeding time

The control diet in this study contained more K, Na, Fe and Mn and less Mg, Zn and Cu (data not presented) than the dietary requirement specified in KFSEC (2007) and NRC (2000). Dietary supplementation of these deficient minerals for the treatment resulted in a higher Mg, Zn and Cu retention in the *longissimus* muscle. 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 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 (K, Cu and Fe) in growing ruminants and Cole et al. (1992) reported that lambs fed yeast culture tended to have a better balance of Zn and Fe. In the present study the dietary addition of DFM improved bioavailability of minerals by Hanwoo steers possibly due to an improved mineral uptake by the microbes and subsequent bodily utilization.

Conclusions

Feeding a DFM (*Bacillus* and *Saccharomyces*) culture to Hanwoo steers during the growing and fattening periods appeared to alleviate feed intake reduction rate during the finishing period, indicating that feeding DFM could be conducive to the gastro-intestinal health of the animal. The use of DFM in the diet showed higher mineral bioavailability by Hanwoo steers. These results might suggest that the concentrate mix–rice straw feeding system formulated for this study might have been limiting in certain essential dietary minerals for Hanwoo steers, whereas the commercial concentrate mix was supplemented with a mineral premix which had an adequate supply of all or most of the required dietary minerals. In conclusion, feeding a DFM culture could be helpful in raising healthier Hanwoo steers.

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