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Full Length Research Paper

Effects of mulberry (*Morus alba* L) silage supplementation on the haematological traits and meat compositions of Hanwoo (*Bos taurus coreanae*) steer

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This study was conducted to evaluate the effect of dietary supplementation with mulberry silage on hematologic traits and meat compositions in Hanwoo (*Bos taurus coreanae*) steer fed total mixed rations and to determine application possibility of mulberry silage as a functional feed source in the beef industry. Twenty steer (622.5±12.7 kg) of the late fattening stage were assigned to either a group with mulberry silage supplementation (MSS) or without (control). No significant differences were found in serum total protein and albumin concentrations. There was no difference between control and MSS in serum cholesterol concentrations but a significant difference (P<0.05) was noted in serum cholesterol concentrations before and after the experiment for the MSS. Dry matter and ether extract content of *Longissimus dorsi* for MSS were significantly higher (P<0.05) than those for control. However, crude protein of *L. dorsi* was significantly lower (P<0.05) in MSS than in control. Although no significant change was apparent in content of saturated fatty acids in *L. dorsi* between control and MSS, some fatty acids including arachidate and behenate were significantly (P<0.05) changed with mulberry silage supplementation. Total free amino acids showed similar levels in the two groups. Levels of most free amino acids were significantly higher (P<0.05) in MSS than in control. In conclusion, MSS changed the serum biochemistry in Hanwoo steer of the late fattening stage and caused relative alterations of chemical composition in *L. dorsi.*

Key words: Hanwoo steer, mulberry silage, meat composition, fatty acid composition, free amino acid.

INTRODUCTION

Hanwoo (*Bos taurus coreanae*), Korean native cattle, are raised for beef cattle that has a good quality of meat in Korea. Feeding of Hanwoo is persistently increased due to customers' needs for high-quality beef. Recently, customers have focused on freshness, safety, and functionality of food (Han, 1999). The market, including the beef industry, is required to produce good quality and safe beef.

Mulberry trees (*Morus alba* L), native to China, have been cultivated for centuries in Asia, selected for their high nutritional value for feeding silkworms (Doran et al., 2007), and widely used in both agricultural and medicinal fields worldwide. Mulberry leaves, which have a high yield and are widely used as a silkworm diet and as alternative medicine in Korea, China, Thailand and Japan, have recently been reported to contain several biological activities. The leaves, barks, and branches have been used to treat fever, reduce blood pressure, and strengthen the joints (Miyahara et al., 2004; Zhishen et al., 1999). Mulberry leaves have antihyperglycemic, antihypertensive, and antioxidative effects because of the

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Table 1. Formulation* (%) of experimental diets.

Item	Control	MSS
Premix (Vitamins with Ca and P)	0.5	0.5
Commercial concentrates	7.0	5.0
Corn silage	45.0	35.0
Beet pulp	5.0	5.0
Ryegrass	5.0	5.0
Corn flake	5.0	5.0
Whole crop barley	5.0	5.0
Corn gluten feed	9.0	10.0
Ground corn	9.0	10.0
Molasses	3.5	3.5
Limestone	0.3	0.3
Corn fodder pellet	5.0	5.0
Urea	0.4	0.4
Bay salt	0.3	0.3
Mulberry silage	0.0	10.0

*As fed basis.

presence of many bioactive compounds including α-glucosidase inhibitor, 1-deoxynojirimycin (1-DNJ), γ-aminobutyric acid (GABA), phenolics, flavonoids and polyhydroxylated alkaloids (Du et al., 2003; Kang et al., 2006). In the past, in the active silk industry, the mulberry leaf was mainly used as a feed source for silkworms and as medicinal material for Oriental medicine in Korea. However, the silk industry has faded over the years and, therefore, cultivation areas of mulberry trees have declined consistently every year and many mulberry fields are lying fallow. Mulberry trees are present in many regions of the world and are a potential source of feed for ruminant livestock (Doran et al., 2007).

However, limited information is available on the mulberry as a functional feed source and the underlying mechanism of its activity. The aim of the present study was to evaluate the effect of dietary supplementation with mulberry silage on the hematologic traits and meat composition in Hanwoo (*Bos taurus coreanae*) steer fed a total mixed ration and to determine application possibility of mulberry silage as a functional feed source in the beef industry.

MATERIALS AND METHODS

Mulberry silage preparation and determination of active ingredient

Mulberries were harvested from an organic farm in Gyeongju, Korea during June 2008. Harvested mulberry leaves and stems were ensiled without additives after being chopped to a length of 2.3±1.7 cm. Mulberry silage was stored for 30 d, opened for feeding experiments to fattening steer, and sampled for analysis. Sampled mulberry silage was weighed and dried for 48 h at 65 4℃ in a convection dry oven. Dried mulberry silage samples were ground through a 1-mm sieve, and stored at -40℃ for determination of active ingredients and antioxidant activities.

The samples were analyzed for dry matter, crude protein, ether extract, crude fibre, and ash (AOAC, 1990). The concentration of 1-deoxynojirimicin (DNJ) was determined according to a previous procedure (Stead and Richards, 1996) with a slight modification. The DNJ standard was weighed accurately and dissolved in a mixture of acetonitrile and water (50:50; containing 6.5 mM ammonium acetate; pH 5.5), then prepared for a stock solution containing 5 mg/ml of DNJ, and stored at -4℃ until analysis. Mulberry DNJ was extracted from lyophilized samples (0.1 g) with 1 ml of a mixture of acetonitrile and water (50:50; containing 6.5 mM ammonium acetate; pH 5.5) in a microtube by sonication for 1 min. The resulting suspension was centrifuged at 15000 rpm for 5 min. The supernatant was filtered through a polytetrafluoroethylene filter (0.45 μm pore size; Advantec, Tokyo, Japan), and a 10 μl aliquot was applied to the high-performance liquid chromatography-evaporative light scattering detection/mass spectrometry (HPLCELSD/MS) system. DNJ concentration was calculated using the equation of the calibration curve. A method for analyzing GABA in mulberry silage by HPLC was developed. A gradient elution was used for the separation and quantification of GABA after pre-column derivatization with phenylisothiocyanate (PITC). The column was Pico × Tag for free amino acids. GABA was determined with a UV detector at 254 nm. Total phenolics were determined according to a previous procedure (Pastrana-Bonilla et al., 2003) with a slight modification. Briefly, samples of mulberry silage were extracted in 2% HCl in methanol for 24 h in the dark at room temperature. The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis. Then, 200 μl of sample extract was placed in a test tube, 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml sodium carbonate (7.5%) were added, and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a Shimadzu 300 UV-vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). The total phenolic content was expressed as garlic acid equivalents in microgram per milligram of sample.

Feeding trials of fattening steer and beef sample preparation

Feeding trials were conducted at the Hanwoo farm in Gyeongju, Korea from December, 14, 2008 to January, 19, 2009. All animal-based procedures were approved by the Institutional Animal Care and Use Committee at Konkuk University (KU10064). Twenty steer (622.5±12.7 kg) of the late fattening stage were assigned to either a group with (MSS) or without (control) mulberry silage supplementation. The control animals were only fed a total mixed ration for fattening steer and the MSS animals were fed a total mixed ration formulated with mulberry silage at the rate of 10%, fresh matter basis (Tables 1 and 2). All steer received experimental diets above 2.5% of initial body weight.

Experimental steer were slaughtered at the same time as the termination of the experiment and meat samples were collected for analysis. Meat was sampled at the loin part between the fifth and sixth back-bones. Beef samples collected from the two groups were freeze-dried and used to determine chemical composition, fatty acids and free amino acids.

Chemical analysis of meat samples

Chemical analysis for collected meat samples was performed by the method of the AOAC (1990) for determination of moisture, crude protein, crude fat, and crude ash. Fatty acid analysis for meat samples was conducted by the following procedure. Samples were first methylated using the method of Park and Goins (1994). About

Table 2. Chemical composition of experimental diet and mulberry silage.

Item[1]	Experimental diet	Mulberry silage
Dry matter (%)	62.13±4.23	28.41±3.12
Crude protein (%)	14.49±0.30	12.43±0.28
Ether extract (%)	3.20±0.28	2.47±0.18
Crude fiber (%)	37.74±0.66	20.29±0.75
Crude ash (%)	5.18±0.07	6.98±0.12
1-DNJ[2] (mg/g)		0.57±0.02
GABA[3] (pmol)		5936.22±12.32
Total phenol (μg/mg)		21.70±0.78

[1]Data are mean ± standard deviation. Values (*n*=3), [2]1-deoxynojirimycine, [3]γ-aminobutyric acid.

Table 3. Blood serum biochemical composition in Hanwoo steer supplemented with or without mulberry silage.

Items[1]		Total protein (g/dl)	Albumin (g/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	D. Bilirubin (mg/dl)
Beginning of	Control	36.40±2.50	11.70±0.79	410.00±24.25	31.00±4. 58	767.00±54.11	242.00±4.58	5.70±0.79
Experiment	MSS	38.30±2.11[a]	12.20±0.46[a]	432.00±7.94[a]	25.00±6.24[a]	813.00±64.97[a]	235.00±3.46[a]	7.10±1.05[a]
End of	Control	36.20±0.92	11.70±0.30	396.00±13.75	25.00±6.9 3	782.67±35.92	234.00±6.24	5.30±0.17
experiment	MSS	35.80±0.92[a]	11.50±0.17[a]	391.00±3.46[b]	25.00±4.58[a]	763.00±24.43[a]	208.00±9.64[b]	5.40±0.60[b]

[1]Means ± standard deviation [Values (n=10)], [ab]Means with the different superscripts in MSS before and after the experiment significantly differ (P<0.05).

3 ml of sample was trans-esterified to fatty acid methyl esters in benzene using 0.5 M NaOH/methanol for 10 min at 100°C. After cooling, the turbid preparation was neutralized with HCl/methanol and then reheated fatty acid and methyl esters were extracted with hexane and mastered by gas-liquid chromatography (HP 5890 II Series, Hewlett-Packard, Atlanta, GA, USA) using a capillary column (HP INNOWax, 30 m × 0.32 mm × 0.25 mm, Agilent Technologies Ltd., Santa Clara, CA, USA). The initial column temperature was programmed at 150℃ and increased to 20 0℃ at 5 /min. The components detected were identified by comparison with a standard mixture of fatty acid methyl esters. Composition of the free fatty acid fraction was expressed as a weight percentage of the total fatty acids.

Free amino acid contents of meat samples were assessed using reverse-phase HPLC. First, the hydrolysis of proteins of meat samples (1 g) was carried out in screw-capped tubes, using 0.01 N HCl acid (5 ml) at 110℃ for 24 h. Afterwards, 1 ml ali quots of a standard of free amino acids (Alltech Grom, Rottenburg–Hailfingen, Germany) or the hydrolyzed samples were derivatized with PITC as described by Bidlingmeyer et al. (1984). The chromatographic system was composed of a 2690-model separation module (Waters Corporation, Milford, MA, USA), equipped with a Waters 996 Photodiode Array detector and a C18 Symmetry (Waters) column (250 mm long 4.6 mm internal diameter and 5 mm pore size). The column temperature was maintained at 50℃ with a SP8 792 column heater (Spectra-Physics, San Jose, CA, USA). Samples were injected in a volume of 20 mg/ml. The solvent system consisted of two eluents: (A) 0.14 M pH 6.5 sodium acetate buffer and (B) 60% (v/v) acetonitrile in water. The solvent gradient was as follows: 0 min, 100%A; 20 min, 78%A–22%B; 40 min, 54%A–46%B; 42 min, 100%B; 44 min, 100%A. Elutions were followed at 254 nm, and spectra were taken between 205 and 400 nm.

Blood sample collection and analysis

Blood samples of about 10 ml were drawn from the caudal vein of all steer at the beginning and end of trial, respectively. Collected blood samples were centrifuged at 3000 rpm for 15 min to separate serum. The serum samples were analyzed using an auto blood biochemical analyzer Hitach 7170A (Hitachi, Tokyo, Japan) for blood biochemical substrates by application of photometry and ion selective electrode methods (Henry, 2001).

Statistical analysis

The main effects between groups were subjected to ANOVA using the general linear model procedure of SAS (1989, Version 6.0). The differences between means were assessed by the Student's *t*-test and statistical significance was defined at $P < 0.05$.

RESULTS

Table 3 shows the changes in serum constituents before and after the experiment. Serum total protein and albumin concentrations did not differ significantly (P>0.05). There was no difference between control and MSS in serum cholesterol concentrations, but a significant difference (P<0.05) was noted in serum cholesterol concentrations before and after the trial for the MSS group. Serum triglyceride and serum high density lipoprotein concentrations showed no significant

Table 4. Chemical composition of *longissimus dorsi* produced in Hanwoo steer.

Items[1]	Control	MSS
	---------- % in DM ----------	
Dry matter	37.1±1.9[b]	42.1±1.6[a]
Crude protein	53.2±2.9[a]	44.3±4.8[b]
Ether extract	41.5±4.3[b]	50.7±3.2[a]
Ash	2.5±0.3[a]	2.1±0.2[b]

[1]Means ± standard deviation [Values (*n*=10)], [ab] Means with the different superscripts in the same row significantly differ(*P*<0.05).

differences. Serum glucose and serum D-bilirubin concentrations were significantly decreased (P<0.05) in both groups before and after the trials, with little difference between groups.

Table 4 shows the changes in chemical composition of meat sample produced in Hanwoo steer with or without mulberry silage. Dry matter (42.1%) for MSS was significantly higher (P<0.05) than that (37.1%) for control. The content of ether extract was similar to that of dry matter (50.7 versus 41.5%). However, the content of crude protein was significantly lower (P<0.05) in MSS (44.3%) than in control (53.2%). The content of crude ash showed a similar tendency to that of crude protein (2.1 versus 2.5%).

Fatty acid composition of meat produced in Hanwoo steer with or without mulberry silage is shown in Table 5. Although no significant changes occurred in the content of saturated fatty acids between control and MSS groups, some fatty acids including arachidate and behenate had significant differences (P<0.05) between control and MSS. The total contents of ω 6 fatty acid were higher in MSS than in control. Oleate which is large proportion of unsaturated fatty acids and defines meat taste, was higher in MSS and in control but there was no significant difference. Although no significant difference was apparent in unsaturated fatty acid contents between control and MSS, it is thought to be useful to increase unsaturated fatty acid content in Hanwoo meat by feeding mulberry silage. The total contents of ω6 fatty acids and polyunsaturated acids were significantly higher (P<0.05) in MSS than in control but those of ω3 fatty acids did not differ significantly (P>0.05).

Free amino acids were measured in each of the meat samples produced in Hanwoo steer fed with or without mulberry silage. Table 6 is presented the results obtained in the two groups. Total free amino acids showed similar levels in control (587.55 mg/100 g) and MSS (583.21 mg/100 g). The arginine content, which is the highest proportion of free amino acids, was 352.15mg/100g in control and 323.30 mg/100 g in MSS, but no considerable change occurred. However, most free amino acids were significantly higher in MSS than in control. The alanine content was significantly higher in MSS (88.59 mg/100 g) compared to control (84.44 mg/100 g), with a significant difference (P<0.05). The glutamate content was significantly higher (P<0.05) in MSS (20.62 mg/100 g) compared to control (13.57 mg/100 g). Glutamine content was slightly higher in MSS than in control. Although the levels of tryptophan, valine, methionine, leucine, and phenylalanine, which are essential amino acids, were significantly higher (P<0.05) in MSS than in control, the level of histidine, also an essential amino acid, was significantly higher (P<0.05) in control compared to MSS

DISCUSSION

It is well known that blood constituents are affected by feed conditions. In this study, Hanwoo steer were fed a diet with the same level of nutrition and closely related similar serum protein and albumin concentrations. However, Kim et al. (2005) reported that serum cholesterol concentration in mice fed a high cholesterol diet was significantly decreased (P<0.05) with supplementation of 5 to 10% ground mulberry leaves. This result implies that mulberry feeding is closely associated with lipid metabolism in mice and thus we expected a similar lower level of serum cholesterol concentration in Hanwoo in this study.

There were several reports on chemical composition of Hanwoo meat depending on meat grade and feed condition. Cho et al. (2008) reported that the contents of moisture, protein, fat, and ash for the loin part of Hanwoo steer with 1^{++} grade were 58.2, 16.9, 24.7, and 0.6%, respectively, on the basis of fresh matter. In addition, Kim and Jung (2007) determined the effects of feeding dietary mugwort on beef quality in fattening Hanwoo and reported that meat had a moisture content of 63.6 to 66.9%, crude protein content of 18.7 to 20.4%, and ether extract content of 13.8 to 16.2% on the basis of fresh matter. These results converted with dry matter were similar to those of our results within the reference range and thus the effect of mulberry silage supplementation on the chemical composition of meat was not great with the exception of ether extract content.

Kim and Jung (2007) reported that the contents of unsaturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid of the loin area muscle in steer supplemented with mugwort was significantly higher (P<0.05) than contents in steer that did not have supplementation with mugwort: similar tendencies were seen for the contents of ω3 and ω6 fatty acids. Warren et al. (2008) also reported that the fatty acid composition of beef was different depending on dietary conditions.

Consequently, it is thought that the fatty acid composition of Hanwoo meat is changed by dietary conditions and fatty acid composition can affect meat quality through nutritional value and flavor index (Wood et al., 2003).

Koutsidis et al. (2008) reported that growth in cattle fed

Table 5. Fatty acid compositions of *longissimus dorsi* produced in Hanwoo steer.

Items[1]		Control	MSS
Fatty acid (%) Myristate	C14:0	3.15±1.07	2.90±0.65
Myristate	C14:0	3.15±1.07	2.90±0.65
Palmitate	C16:0	26.26±3.06	25.94±2.90
Stearate	C18:0	10.73±0.37	10.11±0.85
Arachidate	C20:0	0.09±0.02[a]	0.04±0.03[b]
Behenate	C22:0	0.00±0.00[b]	0.03±0.02[a]
Lignocerate	C24:0	0.05±0.04	0.05±0.02
Total SFA*		40.29±4.43	39.07±3.28
Myristoleate	C14:1n5	0.78±0.22	1.13±0.42
palmitoleate	C16:1n7	4.65±0.94	4.74±0.78
Oleate	C18:1n9	45.97±7.05	47.42±4.99
11-Eicosenoate	C20:1n9	0.31±0.12	0.42±0.09
Erudate	C22:1n9	0.01±0.02	0.02±0.02
Nervonate	C24:1n9	0.00±0.00	0.01±0.02
Total MUFA*		51.72±6.16	53.74±4.00
Linoleate	C18:2n6	1.55±0.19[b]	2.36±0.49[a]
11,14-Eicosadienoate	C20:2n6	0.22±0.10	0.18±0.09
Honogamma Linolenate	C20:3n6	0.12±0.07	0.14±0.04
Arachidonate	C20:4n6	0.02±0.05	0.02±0.04
Total ω 6		1.92±0.10[b]	2.71±0.49[a]
Linolenate	C18:3	0.03±0.03[b]	0.17±0.07[a]
11,14,17-Eicosatrienoate	C20:3	0.25±0.09	0.23±0.10
Docosahexaenoate	C22:6	0.00±0.00	0.00±0.00
Total ω 3		0.28±0.08	0.40±0.16
Total ω 6/ ω 3		7.28±2.03	7.44±2.04
Total PUFA*		2.20±0.14[b]	3.10±0.58[a]
Total PUFA/SFA		0.05±0.00[b]	0.08±0.02[a]
Unidentified		5.79±2.20	4.08±1.13

[1]Means ± standard deviation [Values (n=10)], [ab]Means with the different superscripts in the same row significantly differ (P<0.05), *MUFA, monounsaturated fatty acid; PUFA polyunsaturated fatty acid; SFA, saturated fatty acid.

Table 6. Free amino acid content (%) of *longissimus dorsi* produced in Hanwoo steer.

Item[1]	Control	MSS
Free amino acid (%) Asp	0.00±0.00	1.59±3.55
Glu	13.57±1.16 [b]	20.62±3.28[a]
Asn	3.26±0.37 [b]	4.38±0.61[a]
Ser	5.52±0.61 [b]	7.93±1.11[a]
Gln	52.27±23.63	55.84±8.87
Gly	9.87±2.37	11.62±1.52
His	12.94±10.76 [a]	6.39±3.14[b]
Arg	352.15±32.00	323.20±30.34
Thr	7.14±0.44 [b]	8.85±0.82[a]
Ala	84.44±15.33 [b]	88.59±14.51[a]
Pro	5.23±0.82 [b]	5.84±0.62[a]
Tyr	5.78±0.81 [b]	6.35±0.82[a]
Val	7.09±0.45 [b]	8.84±0.57[a]
Met	3.09±0.78 [b]	4.52±0.29[a]

Table 6. Contd.

Cys2	0.30±0.06 [b]	0.47±0.06[a]
Ile	4.44±0.70 [b]	5.69±0.45[a]
Leu	9.89±1.18 [b]	12.65±1.51[a]
Phe	3.79±0.84 [b]	5.05±0.44[a]
Trp	1.44±0.21	1.45±0.27
Lys	5.32±0.62	4.95±1.40
Total free amino acid	587.55±63.14	583.21±47.23

[1]Means ± standard deviation [Values (n=10)], [ab]Means with the different superscripts in the same row significantly differ(p<0.05).

on concentrate was faster than that in cattle fed on silage, but their muscle contained significantly lower concentrations of free amino acids. Cattle showed a faster rate of protein turnover due to a faster rate of growth. Therefore, during slaughter, a concentration of proteinase increases, which affects myofibrillar protein decomposition and collagen solubility (Miller et al., 1983). Prolong stress before slaughter creates a higher pH level in the carcass and a significant increase of m-calpain enzyme activity and mollification of *L. dorsi* on 7 days after slaughter (Beltran et al., 1981). The greater the weight of cattle, the slower is the freezing speed and lower pH of the carcass (Klont et al., 1999). According to Cho et al. (2008), among free amino acids in 1^{++} grade Hanwoo steer *L. dorsi*, glutamine had the highest content at 457.83 mg/100 g, with alanine second at 130.63 mg/100 g. The content of total free amino acids was 675.74 mg/100 g. In this study, the content of total free amino acids was 587.55 mg/100 g in control and 583.21 mg/100 g in MSS. In addition, arginine showed the highest proportion, followed by alanine, glutamine, glutamate, histidine, leucine, and valine. Consequently, free amino acids of Hanwoo *L. dorsi* changed due to conditions of feed, carcass characteristics, and storage methods. This study also showed that feed conditions actually can cause a change in free amino acid contents of Hanwoo *L. dorsi*.

Conclusion

Mulberry silage supplementation changed the serum biochemistry, especially cholesterol concentration, in Hanwoo steer of the late fattening stage and caused relative alterations of chemical composition in *L. dorsi*. Findings from the present study suggest that mulberry silage may be useful as a functional feed source for beef cattle by improving the haematological trait and composition of fatty acid and amino acid in Hanwoo meat.

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