ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Lemongrass Supplementation on Antioxidant Enzyme Activity within the *Longissimus* Muscle of Hanwoo (*Bos taurus coreanae*) heifer.

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ABSTRACT

This study was conducted to evaluate the effect of dietary supplementation with lemongrass on the antioxidant activities in the beef produced by Hanwoo (*Bos taurus coreanae*) heifer. Twenty heifers in the last stage of the fattening (26 months of age) with a mean initial body weight of 563.0 ± 49.8 kg were fed total mixed ration (TMR) with or without lemongrass. The heifers were randomly assigned to four groups with (T1, T2 and T3) or without (control) lemongrass. T1, T2 and T3 groups were supplemented with 25g, 50g and 100g/day/head of lemongrass for 130 days, respectively. In the present study, the oxidative stress biomarkers such as superoxide dismutase (SOD) and catalase (CAT) activities were significantly improved in the lemongrass supplementation groups comparing to control group ($P < 0.05$). Total glutathione (GSH) and glutathione peroxidase (GPx) were showed the highest activities in T2 group, although there were no significant differences. Taken together, lemongrass supplementation, especially, 50g/head/day, may be useful as a functional feed by improving the antioxidant enzyme activities for beef cattle.

Keyword: Hanwoo, heifer, lemongrass, antioxidant enzyme activity

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ISSN: 0975-8585

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 customer's needs for high-quality beef [12]. Consumers are increasingly concerned about health and safety as well as quality of meat products and the demand for these products is increasing [11].

Oxidative processes in meat lead to quality deterioration and a loss of nutritional value [14]. Meat has endogenous antioxidants and pro-oxidants and living cells have several mechanisms of protection against oxidative processes, including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Especially, CAT and GPx are considered as the major peroxide-removing enzymes located in the cytosol [5] [10]. In addition, Owele [17] reported that natural antioxidants can be incorporated into the muscle through dietary delivery in beef to improve meat quality. One of such plant with a potential to be used as an antioxidant is lemongrass.

Lemongrass (*Cymbopogon citratus* Stapf.) is tropical perennial herb and is native to India and Sri Lanka. It is also thought to have its origin in Malaysia and can be found growing in most parts of South East Asia [3]. Lemongrass is also used throughout many regions of the world as a medicinal plant. Effects of lemongrass on antioxidant [6] and anti-inflammatory [18] activities have been studied. Especially, lemongrass has been reported to include antioxidant activity due to higher amount of polyphenols [7]. However, very limited information on the antioxidant activity of lemongrass as a functional feed additive and the underlying mechanism is available. Therefore, the present study was conducted to evaluate the effect of dietary supplementation with lemongrass on antioxidant enzyme activity of beef produced by Hanwoo heifer and to optimise the use of lemongrass in the beef industry.

MATERIALS AND METHODS

Management of experimental animal

Twenty Hanwoo (Bos taurus coreanae) heifers under the last stage of fattening (26 months of age) with a mean initial body weight of 563.0 ± 49.8 kg were used in an experiment to investigate the effects of different levels lemongrass of TMR based diets. Field trial was conducted at commercial farm of Icheon, Korea from 15th March, 2013 to 23th July, 2013. The fattening heifers were randomly assigned to four groups with or without lemongrass, each group housed in the 9 × 8 m pen and were allowed a free access to water. The Control group was only fed on standard total mixed ration (TMR), while lemongrass treatment groups were fed on TMR formulated with lemongrass at the rate of 25g (T1 group), 50g (T2 group), and 100g (T3 group)/head/day for 130 days. The composition of TMR was analyzed and presented in Table 1. In the present study, the chemical composition of lemongrass, in which dry matter 94.63±0.15 %, crude protein 5.97±0.23 %, crude fat 3.19±0.18 %, crude fiber 27.92±0.19 %, crude ash 7.63±0.18 %, total phenol 9.17±0.89 μg/mg were contained. All heifers received TMR based diets above 2.5% of average body weight during the experimental periods.

Meat sample preparation

Experimental heifers were slaughtered at the termination of the experiment. Twenty-four hours after slaughter, 300 g of meat was taken from the *longissmus* muscle at 13th rib. Beef samples were freeze-dried and used to determine chemical composition and antioxidant enzyme activity.

Chemical composition of sample

Chemical composition of the samples was determined using the procedures described by the Association of Official Analytical Chemists [2] for determination of moisture, crude protein, crude fat, crude fiber and ash contents. Total phenol was determined according to a previous procedure [16] with a slight modification. Briefly, samples were extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis. 200 μL of sample extract was introduced in a test tube, 1.0 mL of Folin-Ciocalteu reagent and 0.8 mL of 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.

Determination of antioxidant enzyme activity of meat sample

Each meat samples were homogenized individually into ice-cold homogenizing buffer containing 50% KCl, 1 M TRIS-HCl, and 0.5 M EDTA (pH 7.0) at 1:10 w/v concentration. Homogenate was centrifuged (3000 rpm at 4°C) for 10 min. The supernatant was used for antioxidant enzyme assays. Superoxide dismutase (SOD) activity was measured using a spectrophotometer by Marklund and Marklund [15]. The activity of SOD was expressed inhibition rate %. Catalase (CAT) activity was determined by the rate of hydrogen peroxide (H_2O_2) degradation [1], and was expressed as µmol H_2O_2/min/mg protein. Total glutathione (GSH) was determined enzymatically according to the method of Floreani et al. [9] with slight modification. Briefly, the supernatant (0.05 mL) was mixed with 100 mM phosphate buffer (pH 7.4, 0.39 mL) containing 5 mM EDTA, 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (0.025 mL), and 5 mM NADPH (0.08 mL). After 3 min of equilibration at 25°C, the reaction was started by adding 2 units of glutathione reductase (GR) (type III from baker's yeast). The formation of 5,5'-dithio-2-nitrobenzoic acid was continuously recorded at 412 nm with an ultraviolet/visible spectrophotometer. The total amount of GSH in the samples was determined from a standard curve obtained by plotting known amounts of GSH versus the rate of change of absorbance at 412 nm. Glutathione peroxidase (GPx) was determined following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) with t-butyl hydroperoxide as a substrate [19] and expressed in nmol NADPH/min/mg protein.

Statistical analyses

All data were presented as the means ± standard deviation, and statistical analyses were performed using Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, USA). The differences between means were assessed by the Duncan's multiple range tests, and statistical significance was defined at $P < 0.05$.

RESULTS

Table 1: Chemical composition of total mixed rations (TMR)

	Total mixed rations (TMR)
Premix	0.3
Concentrates	14
Corn grain	34.4
Beet pulp	1
Brewer's grain wet	14
Whole cottonseed	3.7
Alfalfa	0
Tall fescue	7
Water	16
Molasses	2.5
Salt	0
Limestone	0
Corn fodder pellet	2.5
Tallow	0.6
Soybean curd residue	0
Probiotics	4
Total	100
Dry matter (%)	84.30±2.34
Crude protein (%)	13.15±0.20
Crude fat (%)	3.71±0.17
Crude fiber (%)	9.10±0.55
Crude ash (%)	5.40±0.70

Data are mean ± S.D. values (n=3)

ISSN: 0975-8585

The chemical composition of TMR diet is shown in Table 1. The content of dry matter, crude protein, crude lipid, crude fiber and ash of TMR were 84.30 ± 2.34 %, 13.15 ± 0.20 %, 3.71 ± 0.17 %, 9.10 ± 0.55 % and 5.40 ± 0.70 %, respectively. Total phenolic content of lemongrass accounted for 9.17 µg/mg.

The chemical compositions of *longissimus* muscle of heifer were not significantly affected by lemongrass supplementation (Table 2).

Table 2: Chemical composition of *longissimus* muscle of Hanwoo heifer supplemented with or without lemongrass

	Control	T1*	T2	T3
Dry matter (%)	36.97±1.21 ^{NS}	37.64±0.97	37.03±1.52	36.95±0.85
Crude protein (% DM basis)	43.93±2.57 ^{NS}	43.91±3.04	44.74±3.39	44.93±3.05
Crude fat (% DM basis)	49.91±3.00 ^{NS}	50.93±1.97	48.94±3.00	50.03±2.90
Ash (% DM basis)	3.62±0.86 ^{NS}	3.72±0.64	3.61±0.63	3.52±0.32

Data are mean ± SD values (n = 5 per group)
^{NS} Not significantly different among the groups.
*T1: 25 g/day/head lemongrass supplementation, T2: 50 g/day/head lemongrass supplementation, T3: 100 g/day/head lemongrass supplementation

In the present study, the effects of lemongrass supplementation on the antioxidant enzyme activities in meat sample are shown in Fig. 1. The SOD activity was significantly higher in the T3 group compared to those of the control and T1 groups (P < 0.05). Also, the CAT activity was markedly increased in the lemongrass treatment groups compared to that of the control group (P < 0.05). Especially, T2 group was observed the highest CAT activity and declined as follows T1, T3 and control (0.164, 0.153, 0.137, 0.107 µmol H_2O_2/min/mg protein, respectively) (P < 0.05). Although there was no difference between groups, in GSH and GPx activity, it did tend to increase in lemongrass supplemented groups than in control group. These results supported that the lemongrass supplementation may have contributed to a reduced oxidative stress in beef cattle via improving the antioxidant enzyme activities.

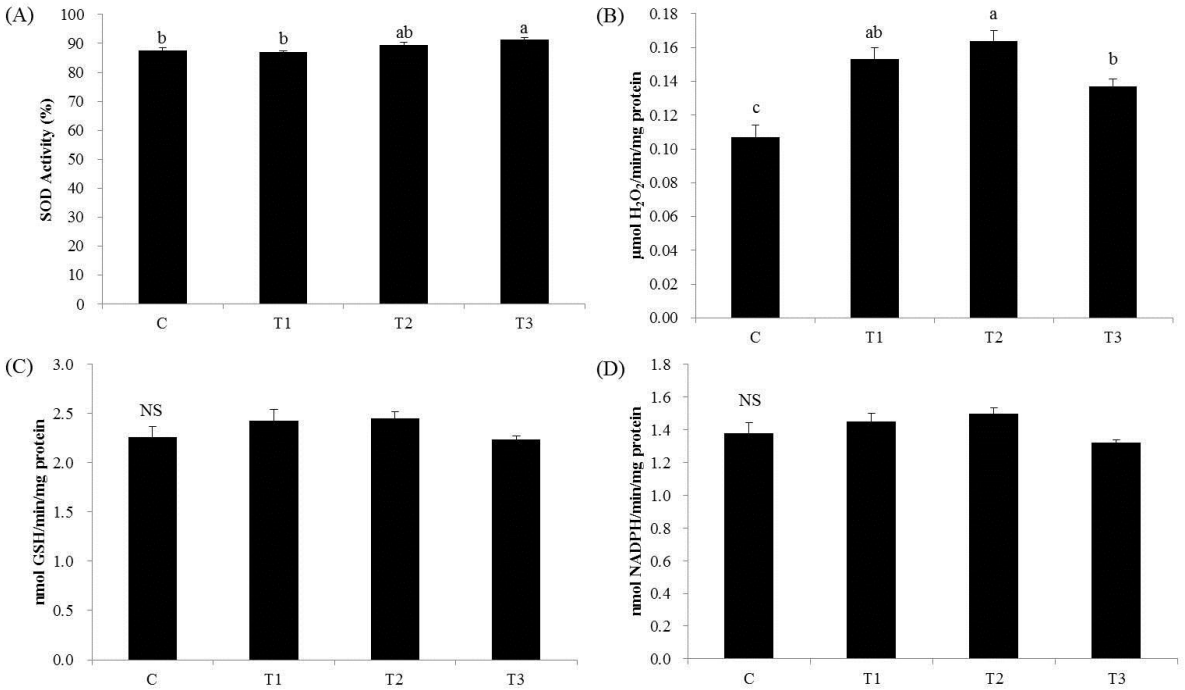

Figure 1: Antioxidant and antioxidant enzyme activities in *longissimus* muscle of Hanwoo heifer by fed lemongrass. (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) total glutathione and (D) glutathione peroxidase (GPx) activities of *longissimus* muscle of Hanwoo heifer supplemented with lemongrass. Data are presented as means ± SEM bar (n = 5 per group). ^{NS} Not significantly different among the groups. $^{a, b, c}$ Means with different superscript among the groups are different (P < 0.05). T1: 25 g/day/head lemongrass supplementation, T2: 50 g/day/head lemongrass supplementation, T3: 100 g/day/head lemongrass supplementation.

ISSN: 0975-8585

DISCUSSION

In the present study, the total polyphenol content of natural lemongrass was 9.17 μg/mg. Tsai *et al*. [20] reported that the major contributors to the phenolic content of lemongrass are chlorogenic acid, caffesic acid and myricetin. More recently, Chae *et al.* [4] reported that the content of total polyphenol was 72.03 μg/mg, and total flavonoids content was 67.58 μg/mg in methanol extract from lemongrass, and those bioactive compounds is related to antioxidant and antibacterial activities. Especially, total flavonoids content of lemongrass was higher than other herbs such as gu-jeol-cho, lavender, rosemary, mok-hyang and calendular [4]. Therefore, it is considered that lemongrass is a source for antioxidants and thus may assist in the prevention of cattle diseases and improve beef products, potentially making it an attractive component in beef cattle diets.

It has been known that SOD is a metalloprotein that is involved in the antioxidant defense mechanism, which plays an important role in the protection of cells against reactive oxygen system (ROS) by lowering the steady state of superoxide anions [17]. Also, SOD converted superoxide radical to hydrogen peroxide and molecular oxygen which in turn can be counteracted by catalase or glutathione peroxidase reaction thereby reducing the level of cellular damage [8] [17]. In our present study, the oxidative stress biomarkers such as SOD of *longissimus* muscle were improved in the lemongrass supplement groups. Also, GSH and GPx activity tend to increase in lemongrass supplemented groups than in control group. Similarly with our results, Qwele *et al*. [17] reported that the consumption of lemongrass by the animals increased the activity of SOD which indicated its ability to protect the animal body and cells from cellular damage by quenching free radicals so as to maintain the meat quality. On the other hand, CAT is one of the enzymatic antioxidants widely distributed in all animal tissues, which prevents the generation of hydroxyl radical and protects cellular constituents from oxidative damage in peroxisomes [13]. In the present study, CAT activities in the lemongrass supplement groups were markedly increased compared to that of the control group. Especially, CAT activity in the T2 group was significantly higher than those of other groups. Taken together, lemongrass supplementation resulted in beneficial effects on the antioxidant enzyme activities of meat products in Hanwoo heifer.

CONCLUSIONS

In the present study, lemongrass supplementation especially 50g/head/day resulted in beneficial effects on the antioxidant enzyme activities in heifer meat. These results suggested that the lemongrass supplementation as the cattle feed may have contributed to reduced oxidative stress in beef cattle via an improvement in antioxidant capacity that was evidenced by the increased concentrations of antioxidant enzyme activities.

ACKNOWLEDGEMENT

This work was carried out with the support of the “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ907038)” Rural Development Administration, Republic of Korea.

REFERENCES

[1] Aebi HE. 1974, Methods for enzymatic analysis Hans-Ulrich Bergmeyer ed. (Weinheim: Verlag Chemie)
[2] Association of Official Analytical Chemists. 1995, Official methods of analysis. 16th ed. (AOAC International: Arlington, USA)
[3] Carlini EA, Contar JDP, Silva-Filho AR, Silveira-Filho NG, Frosshtengarten ML and Bueno OF. J Ethnopharmacol 1986; 17: 37-64.
[4] Chae IG, Kim HJ, Yu MH, Kim HI and Lee IS. J Korean Soc Food Sci Nutr 2010;39:1411-1417.
[5] Chan KM and Decker EA. Crit Rev Food Sci Nutr 1994;34:403-426.
[6] Cheel J, Theoduloz C, Rodrìguez J, and Schmeda-Hirschmann G. J Agr Food Chem 2005;53:2511–2517.
[7] Choi HS, Song HS, keda HU and Sawamura M. Food Chem 2000;48:4156-4161.
[8] Curtis JJ and Mortiz M. Gastroenterol 1972;62:84-92.
[9] Floreani M, Petrone M, Debetto P, Palatini P. Free Rad Res 1997;26:449–455.
[10] Hernandez P, Zomeno L, Arino B and Blasco A. Meat Sci 2004;66:525–529
[11] Hoffman L and Wiklund E. Meat Sci 2006;74:197–208.
[12] Jeon BT, Kim KH, Kim SJ, Kim DH, Kim ET, Cho WM, Hwang IH, Choi NJ and Moon SH. African J Agr Res

2012;7:662-668.
[13] Kumar NA and Pari L. J Med Food 2003;6:255-259.
[14] Ladikos D and Lougovois V. Food Chem 1990;35:295-314.
[15] Marklund S and Marklund G. European J Biochem 1974;47:469–474.
[16] Pastrana-Bonila E, Akoh CC, Sellappan S, Krewer G. J Agr Food Chem 2003;51:5497-5503.
[17] Qwele K, Hugo A, Oyedemi SO, Moyo B, Masika PJ and Muchenje V. Meat Sci 2013;93, 455-462.
[18] Runnie I, Salleh MN, Mohamed S, Head RJ and Abeywaedna MY. J Ethnopharmacol 2004;92:311-316.
[19] Tamura M, Oschino N and Chance B. J Biochem 1982;92:1019–1031.
[20] Tsai TH, Tsai TH, Chien YC, Lee CW and Tsai PJ. Food Chem 2008; 110:859-864.