Effects of Dietary Supplemented Inorganic and Organic Selenium on Antioxidant Defense Systems in the Intestine, Serum, Liver and Muscle of Korean Native Goats

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ABSTRACT : The present study was designed to assess whether dietary inorganic and organic selenium (Se) could affect antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST), and the level of malondialdehyde (MDA), a marker of lipid peroxidation, in the intestine, serum, liver, and *gastrocnemius* muscle of Korean native goats. A total of eighteen Korean native goats was allotted into three dietary groups, consisting of basal diet (CON), or basal diet with either 0.25 ppm inorganic (IOSEL) or 0.25 ppm organic Se (ORSEL), and fed the corresponding diets for 5 wks. Growth performance, including body weight and total gain, and blood biochemical profiles, including GSH-Px, were not significantly different between the three dietary groups. Also, the specific activities of SOD, GSH-Px, and GST, and the level of MDA in the intestinal mucosa and liver from goats were not substantially affected by either inorganic Se or organic Se. However, goats fed the diet containing organic Se showed a significant increase in GSH-Px and GST activities in the *gastrocnemius* muscle compared with those fed the basal diet. In conclusion, increased muscle GSH-Px and GST activities suggest that dietary organic Se may affect, at least in part, the antioxidant defense system in muscle of Korean native goats under the conditions of our feeding regimen. (**Key Words :** Inorganic Se, Organic Se, Korean Native Goats, Antioxidant Enzymes, SOD, GSH-Px, GST, MDA)

INTRODUCTION

Dietary antioxidants, by virtue of their beneficial effects such as enhancing meat quality and consumer health as well as preventing certain diseases, have been widely used as feed additives in animal production. Moreover, considerable emphasis has been placed on the deposition of intramuscular fat and modification of fatty acid composition via intensive feeding strategies and dietary incorporation of certain fatty acids, respectively, for improvement of meat quality (Lauridsen et al., 1999; Song et al., 2000). However, modification of increased intramuscular fat content and fatty acid composition accelerated lipid peroxidation in meat products. It is important, thus, to use dietary antioxidants to produce high-quality livestock products, since they are able to protect lipid peroxidation, prolong the color stability of meat products and provide benefits to both animal and human health (Surai, 2002).

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Among potential antioxidants, selenium (Se) plays a central role in enzymatic defense pathways against oxidative damage in tissues. The effects of Se are mediated in antioxidant metabolism by GSH-Px in which Se is incorporated in the core of selenocysteine (Yeh et al., 1997). Numerous studies have demonstrated the beneficial effect of dietary Se on the prevention of biological membrane peroxidation as well as several diseases of animals (O'Grady et al., 2001; Pallares et al., 2002). The FDA initially (till 1974) allowed use of up to 0.1 ppm of Se as a feed additive for all major food-producing animals, which was subsequently increased to 0.3 ppm Se in 1987 (FDA, 1987). Apparently, selenium is found naturally in two forms, namely inorganic or organic Se. The dietary supplementation of inorganic forms, such as selenite and selenide, could result in toxicity, interaction with other minerals, relatively poor absorption by the intestinal tract and subsequent less retention of Se in the body (Pond et al., 1995). In contrast, organic Se, which exists in yeast products, primarily in the form of Se-methionine, resulted in a higher level of Se in animal products compared with

inorganic Se (Enjalbert et al., 1999; Ortman and Pehrson, 1999).

It has been reported that Se from the diet accumulates very well when incorporated into muscle of lambs (Molnar et al., 1996), beef (Ekholm et al., 1991) and pigs (Tian et al., 2006). These observations provide evidence that dietary Se, at least partially, plays a role in not only preventing lipid peroxidation of various tissues for animals but also in supplying organic Se for humans, depending on the bioavailability of Se in tissues from particular animal species. Much research literature exists on the functions and effects attributed to dietary Se, however little study has been done on the application of dietary Se to Korean native goats. Korean native goats are widely produced in Korea for medical purposes as well as a meat protein source. If a beneficial effect of the Se-mediated antioxidant defense system is revealed, it could be applicable to the production of valuable meat.

Recognising the above facts, the present study was conducted to investigate the effects of inorganic and organic Se on the activity of antioxidant enzymes and lipid peroxidation in serum, the intestine, the liver and the *gastrocnemius* muscle of Korean native goats.

MATERIALS AND METHODS

Experimental design

A total of 18 male Korean native goats obtained from local market was offered a basal concentrate diet at 2% of BW and rice straw *ad libitum* for a 4 wk-adjustment period. Immediately thereafter, 18 goats (17.77±1.12 kg) were housed in steel cages with 3 animals per cage and randomly allotted to three dietary groups consisting of the basal diet (CON), the basal diet plus 0.25 ppm sodium selenite (IOSEL), and the basal diet plus 0.25 ppm organic Se (ORSEL). The sources of Se were sodium selenite (Acros Organics) and Se-yeast (Taehan Pharm). The basal diet was a ground corn-based commercial diet which contained 13.86% crude protein, 3.18% crude fat, 6.2% crude fiber, 0.83% Ca, 0.55% P, and 0.15 ppm Se. Rice straw contained 3.48% crude protein, 29.88% crude fiber, 0.2% Ca, and 0.1% P. All goats were fed the corresponding concentrate diets twice a day at 2% of BW and rice straw *ad libitum* during a 5 wk experimental period. Body weight was measured on the initial and final days of the experiment.

At the end of the feeding trial, the 18 goats (6 goats per group) were sacrificed by cutting the jugular vein. Serum was isolated from blood drawn from the jugular vein. The small intestine, liver and *gastrocnemius* muscle (muscle of hindlimb) were carefully removed from the goats and stored at -70°C.

Serum biochemical analysis

Serum biochemical components including glucose, total protein, cholesterol, triglyceride, AST, ALT, and LDH were assayed by Automatic Biochemical Analyzer (Hitachi 747, Japan) using the corresponding kits.

Tissue harvest and preparation

The harvested small intestine was perfused with ice-cold saline, and gently squeezed to remove remaining digesta. After measuring intestinal weight, sixty percent of the upper region was designated as the proximal intestine, and the rest of the region as the distal part. The length of each segment was rinsed in three successive baths containing mannitol buffer (5 mM $MgCl_2$, 150 mM mannitol, 10 mM Tris succinate, 5 mM K_2HPO_4, and 1 mM $MnCl_2$; pH 7.4). The mucosal surface of the proximal and distal regions was removed by gentle scraping with a glass slide, and scrapings were mixed in an aluminum pan seated on a bed of ice. Residual fat and digesta were removed from harvested mucosal scraping by resuspending twice in equal volumes of mannitol buffer followed by centrifugation at 4,500×g at 4°C for 10 min. Immediately after withdrawal of the whole liver, it was rinsed with ice-cold saline and blotted to remove moisture. To isolate the fractions enriched in peroxisomes, microsomes, and cytosol from tissues, the whole intestinal mucosa, liver and muscle tissues were homogenized with 1:6 volume of 0.25 M sucrose buffer in a tissue homogenizer. The crude homogenized tissues were centrifuged at 600×g for 10 min, and the resulting supernatant was centrifuged at 10,000×g for 20 min to isolate the fractions enriched in peroxisomes and lysosomes. The remaining supernatant from the second centrifugation was recentrifuged at 105,000×g for 90 min in a Beckman ultracentrifuge. The pellets obtained from centrifugation were suspended in phosphate buffer containing 150 mM KCl (pH; 7.4) to adjust protein concentration. The cytosol and suspended pellets thus obtained were frozen in liquid nitrogen, and stored at -70°C until assay.

Analytical assays of antioxidant enzymes and lipid peroxidation

Protein was assayed as per the BCA method (Pierce Assay) using an ELISA (Molecular Devices). The activity of superoxide dismutase (SOD) was determined in cytosolic fractions using a xanthine and xanthine oxidase system for the production of superoxide radical and subsequent measurement of cytochrome c as a scavenger of the radicals (McCord and Fridovich, 1969). The SOD activity was expressed as units/mg of protein, where one unit of activity is the amount of enzyme required to inhibit the rate of reduction of cytochrome c by 50%. Glutathione peroxidase (GSH-Px) was measured at 37°C with cumen hydroperoxide as a substrate (Tappel, 1978). The GSH-Px

Table 1. Effects of dietary supplementation of inorganic (IOSEL) and organic Se (ORSEL) on body weight in Korean native goats

Item	Treatment		
	CON	IOSEL	ORSEL
Initial body weight (kg)	17.7±1.16	18.2±0.74	17.5±1.17
Final body weight (kg)	22.2±0.66	22.4±0.49	22.3±0.75
Gain for 35 days (kg)	4.5±0.22	4.2±0.13	4.8±0.23

Values are means±SE (n = 6).

coupled reduction of cumen hydroperoxide from the oxidation of NADPH by glutathione reductase and concomitant oxidation was monitored in a spectrophotometer with the decrease in absorbance at 340 nm. One unit of GSH-Px is expressed as the amount of GSH-Px needed to oxidize 1 μmol of NADPH per min. Glutathione S transferase (GST) was determined with 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, and monitored by absorbance at 340 nm (Habig et al., 1974). One unit of activity was expressed as the amount of enzyme catalyzing the conjugated CDNB per min. One unit of activity was expressed as the amount of enzyme catalyzing the decomposition of one μmole of H_2O_2 per min at 25°C and pH 7.0. The specific activity of all enzymes assayed was expressed as activity per mg protein. The estimation of lipid peroxidation in the microsomes was quantified by measuring the 2-thiobarbituric acid (TBA) reactive substances with a spectrophotometer at 532 nm (Bidlack and Tappel, 1973). TBA-material is described as nmoles of malondialdehyde (MDA) per milligram of protein.

Statistical analysis

Data on diet-associated difference were analyzed by PROC-GLM (SAS package, 1996) procedures appropriate for completely randomized designs. Mean differences between dietary treatments were evaluated by the Duncan multiple test at p<0.05.

RESULTS AND DISCUSSION

Growth performance and serum biochemical components

Growth performance and serum biochemical profiles in goats fed the basal diet containing either inorganic or organic Se are given in Table 1 and 2. Although the body weight gain of animals in the ORSEL group was 6.6% higher, the differences were non-significant. In this study, the gain, feed intake, and feed conversion were not affected by dietary inclusion of Se source or Se level. Similar results have been reported in beef steers (Lawler et al., 2004), pig (Mahan et al., 1999), and poultry (Payne and Southern, 2005). Growth performance in goats was not significantly influenced by dietary supplementation of either an inorganic or organic form of Se at various levels in this study.

As shown in Table 2, dietary supplementation with 0.25 ppm of inorganic and organic Se had no significant effect on glucose, total protein, cholesterol, triglyceride, AST, ALT, and LDH levels in serum. Although nonsignificant, serum cholesterol level was lower by 20.3% in goats fed organic Se diet compared with those fed the basal diet. The effect of dietary antioxidants on blood biochemical components seemed to vary according to the level of dietary Se, dietary level of fat, feeding period, and age. Since a preventive effect of Se against atherosclerosis has been shown (Scott et al., 1991), the relationship between antioxidants and cholesterol level was investigated. However, so far the effect of dietary Se on blood cholesterol and triglyceride level is much less clear. Several studies reported that dietary inclusion of antioxidants including Se and vitamin E under a normal dietary feeding regimen did not affect blood cholesterol in humans and rats (Meydani et al., 1994; Choi et al., 1995). However, it was reported that rats fed a high-cholesterol diet with Se had reduced levels of triglyceride, cholesterol and free fatty acid in serum (Kang et al., 1998; Iizuka et al., 2001). Therefore, it is postulated that supplementation of Se during atherosclerosis may result in a significant improvement in blood parameters associated with lipid metabolism. The AST, ALT, and LDH are intracellular enzymes involved in amino acid or carbohydrate metabolism. These are present in high concentrations in the liver, muscle, and brain. Elevated concentrations of these enzymes in blood indicate liver

Table 2. Effects of dietary supplementation of inorganic (IOSEL) and organic Se (ORSEL) on serum biochemical profiles and GSH-Px activity in Korean native goats

Item	Treatment		
	CON	IOSEL	ORSEL
Glucose (mg/dl)	80.66±6.42	86.66±5.30	77.00±6.12
Total protein (mg/dl)	8.03±0.74	6.90±0.50	6.40±0.69
Cholesterol (mg/dl)	63.33±4.36	62.33±3.66	52.66±5.67
Triglyceride (mg/dl)	7.50±0.88	6.00±1.71	6.66±1.83
AST (U/L)	110.83±8.34	100.66±8.10	102.66±9.52
ALT (U/L)	27.66±3.59	20.66±1.60	25.66±7.48
LDH (U/L)	1,503.33±196.57	1,249.00±108.01	1,253.00±147.58
GSH-Px (U/mg/protein)	0.81±0.18	0.86±0.21	0.63±0.13

Values are means±SE (n = 6).

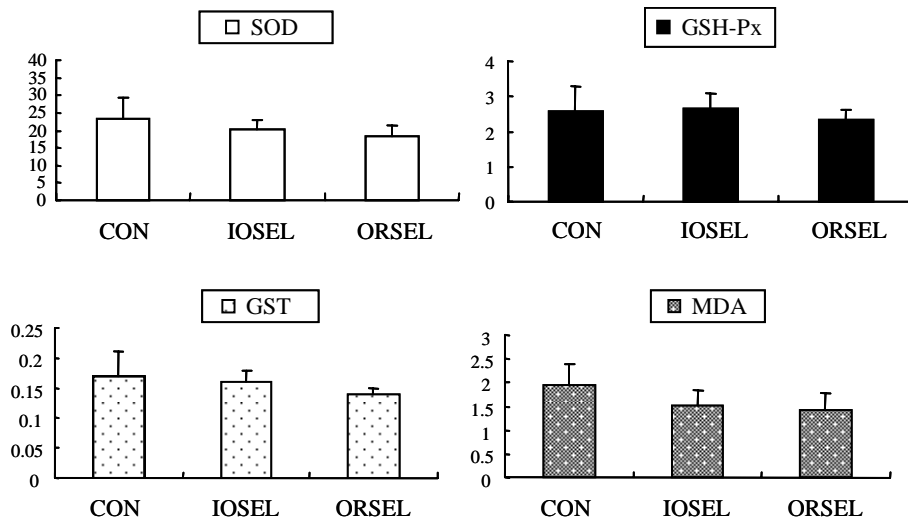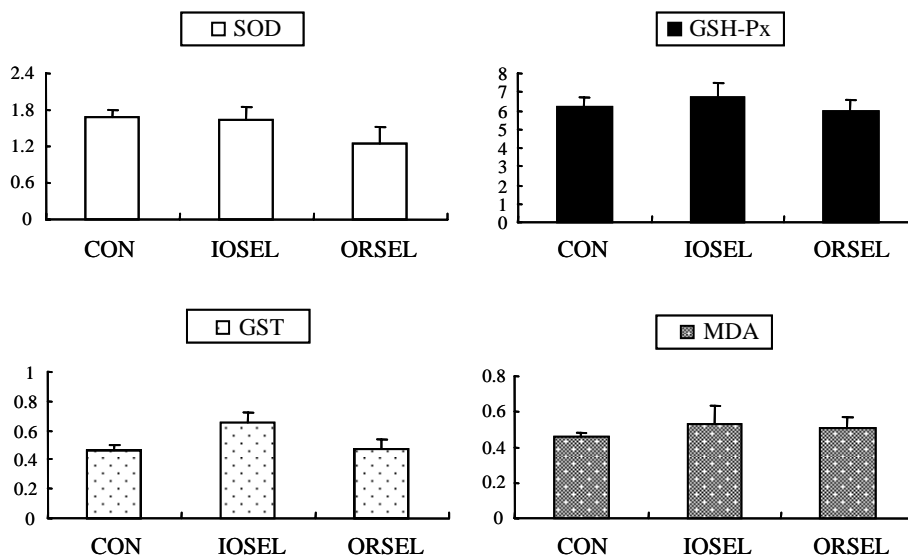*Chung et al., (2007) Asian-Aust. J. Anim. Sci. 20(1):52-59*

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Figure 1. Effects of dietary inorganic Se (IOSEL) and organic Se (ORSEL) on the specific activities of SOD (U/min/mg protein), GSH-Px (U/min/mg protein), and GST (U/min/mg protein), and lipid peroxidation (MDA, nM/mg protein) in the intestinal mucosa of Korean native goats. Bar represents mean±SE (n = 6).

Figure 2. Effects of dietary inorganic Se (IOSEL) and organic Se (ORSEL) on the specific activities of SOD (U/min/mg protein), GSH-Px (U/min/mg protein), and GST (U/min/mg protein), and lipid peroxidation (MDA, nM/mg protein) in the liver of Korean native goats. Bar represents mean±SE (n = 6).

necrosis or disease (Murray et al., 1990). Kim and Mahan (2001) reported that 20 ppm dietary inorganic Se significantly increased serum AST of pigs, suggesting that a high dose of inorganic Se may cause hepatic tissue damage in pigs. Elevated serum AST and LDH activity has been reported in nutritional muscular dystrophy which is caused by Se- deficiency in lambs and calves, and partly results from enhanced tissue damage due to lipid peroxidation (Pond et al., 1995). But, we did not observe any differences in serum AST, ALT and LDH in goats fed the basal diet alone or supplemented with either inorganic or organic Se. The potential benefit of dietary supplementation of Se is to

protect liver damage under some circumstance (Choi et al., 1995). Gehringer et al. (2003) reported that dietary Se protected against toxins, leading to decreased serum ALT and lipid peroxidation levels. Thus, the level of supplemented Se used in our study could be applied to goats without the occurrence of adverse effects.

Specific activities of antioxidant enzymes in serum and tissues

Table 2 and Figure 1, 2 and 3 present changes in the specific activities of antioxidant enzymes or lipid peroxidation in various tissues from Korean native goats
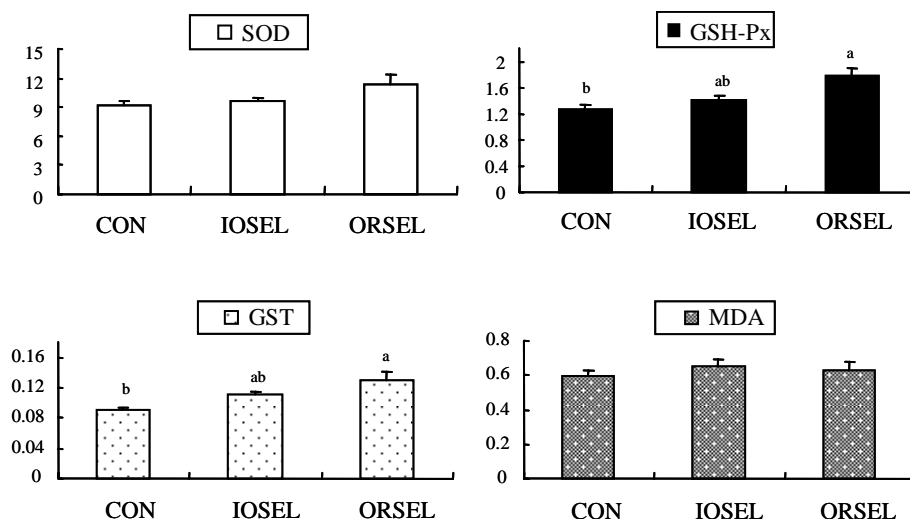
Figure 3. Effects of dietary inorganic Se (IOSEL) and organic Se (ORSEL) on the specific activities of SOD (U/min/mg protein), GSH-Px (U/min/mg protein), and GST (U/min/mg protein), and lipid peroxidation (MDA, nM/mg protein) in the *gastrocnemius* muscle of Korean native goats. Bar represents mean±SE (n = 6). [a, b] Values with different superscripts differ significantly (p<0.05) between treatments.

fed diets containing either inorganic or organic Se. In serum, dietary Se did not noticeably affect the specific activities of GSH-Px, which is a Se-containing antioxidant enzyme (Table 2). Also, we did not observe a significant difference in the specific activities of SOD, GSH-Px, and GST, and the MDA value in the intestinal mucosa (Figure 1). In hepatic tissues, there were no differences in the specific activities of SOD, GSH-Px, and GST in response to dietary inorganic and organic Se (Figure 2). Dietary supplementation of both inorganic and organic Se did not influence MDA level in liver microsomes either (Figure 2). In muscle, significant (p<0.05) increases in the specific activities of GSH-Px and GST were observed in goats fed 0.25 ppm of organic Se (ORSEL) compared with those fed the basal diet (CON) (Figure 3). However, we did not see any difference in the activities of antioxidant enzymes (SOD, GSH-Px and GST) and lipid peroxidation (MDA) in the *gastrocnemius* muscle tissues between IOSEL and ORSEL groups (Figure 3).

Excess oxidants causing cellular damage in tissues are captured by SOD and GSH-Px. First, SOD converts superoxide anion to hydrogen peroxide in a cellular antioxidant reaction (Liu and Mori, 1993). Thereafter, GSH-Px, a Se-containing enzyme, independently detoxifies the hydrogen peroxide produced into H_2O (Tappel, 1978). GST is a family of multifunctional enzymes that catalyze the conjugation of glutathione with a large number of compounds with an electrophilic center and render the products more water soluble (Egaas et al., 1995). In the antioxidant defense mechanism, therefore, dietary supplementation of Se is an important method to increase Se content and Se containing- GSH-Px in tissues, which are closely correlated with dietary level of Se (Schrauzer, 2000). It is known that bioavailability of Se may be affected by the

gastrointestinal physiology of species, the forms of Se, and the level of dietary Se (Pond et al., 1995). Due to the complicated factors influencing Se bioavailability, lack of information on the effect of dietary Se on the activities of GSH-Px in various tissues of goats has been released.

After ingestion, dietary Se is transported into plasma via intestinal mucosal cells and assimilated into all tissues where it is deposited as a component of selenoproteins such as GSH-Px (Yeh et al., 1997). Although Se is present in all tissues, an especially higher concentration is found in the liver, kidney and muscle, which largely depends on the level of dietary supplementation (Pond et al., 1995). GSH-Px also exists in all tissues with highest levels in the liver and red blood cells (Pond et al., 1995). Therefore, most studies have focused on the effect of dietary supplementation of Se on GSH-Px activity in blood and the liver. We examined the effect of dietary inorganic and organic Se on the antioxidant defense mechanism not only in serum and the liver of goats but also in the intestine and muscle. The main site of Se absorption is the small intestine, which acts as the primary membrane barrier between the external and internal environment and subsequently has many antioxidant defense mechanisms against ingested chemicals (Nijhoff and Peters, 1992). The liver, containing the highest level of Se and GSH-Px, plays the central role in coordinating metabolism of antioxidants. However, changes were not observed in intestinal and hepatic antioxidant enzymes, including GSH-Px, SOD, GST, and lipid peroxidation, in response to either dietary inorganic or organic Se. Studies with sheep and cattle indicated that serum GSH-Px activity was not correlated with dietary level of Se (Podoll et al., 1992). Podoll et al. (1992) reported that sheep fed a diet supplemented with 0.3 ppm

inorganic Se for 56 days did not show a significant increase in serum GSH-Px, although serum Se contents significantly increased. Plasma GSH-Px activity did not reflect Se supply, because the majority of GSH-Px (98%) in blood was attributed to red blood cells (Gerloff, 1992). Mahan et al. (1999) reported that serum GSH-Px activity reached a plateau when pigs were fed diets containing 0.05-0.1 ppm of Se. In ruminants, organic Se, such as selenomethionine, is predominantly absorbed well compared with inorganic Se, partly due to incorporation of inorganic Se into amino acids by ruminal microbes (Pond et al., 1995). Thus, organic Se was much more effectively absorbed into blood compared with inorganic Se, leading to increased GSH-Px activity in dairy cows (Ortman and Pehrson, 1999). By contrast, study with monogastric animals indicated that both inorganic and organic Se may be effective in maintaining GSH-Px activity of similar magnitude (Mahan and Parrett, 1996).

Compared with other tissues, results showing a significant increase in muscle GSH-Px and GST activities in response to organic Se are interesting. Increased muscle GSH-Px activity in goats fed organic Se suggested that dietary addition of organic Se could be eventually incorporated into muscle which is known to have a relatively lower GSH-Px activity among the various organs. Several studies reported that muscle Se was correlated with dietary Se level in lambs (Molnar et al., 1996) and beef cattle (Ekholm et al., 1991). Furthermore, muscle GSH-Px activity was closely associated with dietary level of Se in several meat-producing animals (Moksnes and Norheim, 1983; Yeh et al., 1997). Ekholm et al. (1991) reported that bovine muscle Se content showed a linear increase when dietary Se was added to the feed ranging from 0.03 ppm to 0.4 ppm Se. Mahan et al. (1999) also found that organic Se was more effective in the retention of Se in muscle of pigs, although neither organic or inorganic Se gave a similar effect on the retention of Se in the liver of pigs. Similarly, broilers fed a diet supplemented with organic Se had significantly increased muscle and plasma Se levels compared with those fed the control diet or the diet with selenite, although plasma GSH-Px activity was not changed by Se source or level (Payne and Southern, 2005). Less bioavailability of selenite in ruminants in comparison with monogastric animals is due to conversion to less-soluble forms such as elemental Se or selenide in the rumen (Cousins and Cairney, 1961). One feasible reason for increased activity of muscle GSH-Px is that organic Se can be readily deposited in muscle, leading to the maximal synthesis of muscle GSH-Px in goats. However, we did not measure Se content in tissues from goats.

Consideration has to be given to the dietary regimen when interpretating results. The Se content of the basal diet is a crucial factor for the evaluation of dietary added Se.

The basal diet used for this study already contained 0.15 ppm of inorganic Se, which is considered to be the optimal requirement for maintaining physiological function in goats. The recommended dietary requirement of Se for goats and sheep is 0.1 ppm (Morand-Fehr, 1981) and 0.2 ppm per head daily, respectively (NRC, 1984). Under the conditions of this study, no differences in GSH-Px activity of the intestine, serum and liver from goats in response to dietary addition of Se might be attributed to reaching a plateau level of Se with regard to synthesis of GSH-Px even on the basal diet (0.15 ppm of Se).

The elevated GST in muscle in response to organic Se may reflect an important role in biotransformation for deposited Se (de Waziers et al., 1988). Therefore, dietary organic Se may have a role in reducing peroxidation and the subsequent self-protective mechanism from repairing oxidized fatty acid of the lipid membrane of muscle. It is generally accepted that enhancing one or more of the antioxidant enzymes may reduce lipid peroxidation (Xia et al., 1995).

In conclusion dietary organic Se may exert a favorable effect on antioxidant ability through enhancing GSH-PX and GST activities, which may be associated with increased Se contents in muscle tissues of Korean native goats. However, more detailed investigation on the assessment of deposited Se in various tissues of Korean native goats is needed to elucidate the relationship between GSH-Px activity and dietary level of Se.

ACKNOWLEDGEMENT

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