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Effect of Supplemental Selenomethionine on Growth Performance and Serum Antioxidant Status in Taihang Black Goats*

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ABSTRACT: An experiment was conducted to evaluate the effect of different levels of supplemental selenomethionine (Se-Met) on growth performance and serum antioxidant status in Taihang Black goats. Fifty 16-week-old goats with an average body weight of 12.5±0.5 kg were randomly assigned to five treatments fed a basal diet (0.049 mg Se/kg DM) supplemented with 0 (control), 0.10, 0.30, 0.50 and 1.00 mg of Se/kg DM (form Se-Met) for 80 days. Average daily gain and feed efficiency were higher (p<0.05) in the groups supplemented with 0.30 to 0.50 mg Se/kg DM compared with the control group. However, Se-Met supplementation had no influence on average daily feed intake (p>0.05). Se-Met supplementation significantly increased (p<0.01) the activity of glutathione peroxidase enzymes (GSH-Px) and superoxide dismutase (SOD) in serum. The group supplemented with 0.50 mg Se/kg DM had the highest activity of GSH-Px compared with other groups (p<0.05). Serum SOD activity was higher (p<0.05) in goats supplemented with both 0.30 and 0.50 mg Se/kg DM than in control goats and goats supplemented with 1.00 mg Se/kg DM. Serum glutathione-S-transferase (GST) activity and malondialdehyde (MDA) concentration were significantly decreased (p<0.05) in goats supplemented with 0.30, 0.50 and 1.00 mg Se/kg DM compared with control values. These results indicated that Se-Met supplementation markedly improved the antioxidant status in goats. Blood Se concentration increased linearly (p<0.001) and quadratically (p<0.001) as the level of supplemental Se-Met increased. The concentration of Se in the control diet (0.049 mg Se/kg DM) did not satisfy the Se requirement in goats as indicated by reduced growth rate, feed efficiency, activities of GSH-Px and SOD in serum, and blood Se concentrations. In conclusion, it is recommended that 0.30 to 0.50 mg of Se/kg DM from Se-Met (total diet Se of 0.349 to 0.549 mg/kg DM) be supplied in the diet of Taihang Black goats to enhance growth performance and improve antioxidant status. (Key Words : Taihang Black Goats, Selenomethionine, Growth Performance, Blood Se, Antioxidant Status)

INTRODUCTION

Selenium (Se) is an essential trace element for maintaining normal physiological processes in animals and humans. Se exerts multiple actions on the antioxidant (Arthur, 2000; Tapiero et al., 2003), reproductive (Maiorino et al., 1999), endocrine (Beckett and Arthur, 2005), and immune systems (McKenzie et al., 1998; Beck et al., 2005). It exists in nature in organic and inorganic forms. The main Se supplement that has been used in animal diets is the inorganic form (sodium selenite or selenate). However, absorption of inorganic selenium is much lower in

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ruminants than in non-ruminants. Wright and Bell (1966) reported that absorption of orally administrated ⁷⁵Se was only 34% in sheep compared with 85% in swine, which is due to reduction of selenate and selenite to insoluble selenide and elemental Se in the rumen environment (Hidiroglou et al., 1968). Some studies indicated that organic selenium from selenomethionine (Se-Met) or Seenriched yeast is an ideal additive because animals absorb and retain it more than inorganic selenium (Ortman and Pehrson, 1997). Organic Se supplementation did not affect growth performance but increased serum and tissue Se concentration in growing-finishing pigs (Tian et al, 2006a, b) and in broilers (Choct and Nylor, 2004; Payne and Southern, 2005; Yoon et al., 2007). Ehlig et al. (1967) found higher tissue selenium retention by lambs fed selenomethionine than fed selenite, which results from incorporation of part of selenomethionine into microbial protein by rumen microorganisms (Paulson et al., 1968; Hidiroglou et al., 1973). Recent studies by Juniper et al.

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 Table 1. The ingredients and chemical composition of the basal diet (% DM basis)

Ingredients	
Chinese wildrye	20.84
Alfalfa	20.96
Corn stalk	37.11
Cracked corn	11.27
Wheat bran	2.91
Soybean meal	3.39
Sunflower meal	2.52
Salt	0.50
Limestone	0.30
Trace mineral mix ¹	0.20
Chemical composition ²	
Metabolizable energy (MJ/kg DM)	9.10
Crude protein	10.02
Acid detergent fiber	32.22
Neutral detergent fiber	51.16
Calcium	0.60
Phosphorus	0.30
Selenium (mg/kg)	0.049

¹ Provided per kilogram of the diet: 40 mg of Zn as ZnSO₄·7H₂O; 25 mg of Mn as MnSO₄·H₂O; 1.0 mg of I as KI; 45 mg of Fe as FeSO₄·7H₂O; 15 mg of Cu as CuSO₄·5H₂O; 1,500 IU of Vitamin A; 250 IU of Vitamin D and 20 IU of Vitamin E.

² Analyzed values except metabolizable energy.

(2006; 2008) and Steen et al. (2008) have indicated improved bioavailability of Se when using organic selenium. The tolerance of ruminant animals (dairy cattle, beef cattle, calves and lambs) to a high dose of a selenium-enriched yeast was at least 20 times the maximum permitted within the United States (0.30 mg/kg of DM) (Juniper et al., 2008).

Se is a component of glutathione peroxidase enzymes (GSH-Px) (Rotruck et al., 1973), which are antioxidant enzymes that catalyze the reduction of hydrogen peroxide and lipid hydroperoxides to destroy free radicals produced during normal metabolic activity. GSH-Px activity increased in Se-supplemented broilers (Yoon et al., 2007), lambs (Qin et al., 2007), dairy cows (Zhao et al., 2008) and beef calves (Beck et al., 2005). However, Payne and Southern (2005) reported that Se source and concentration did not affect the plasma glutathione peroxidase activity.

Se deficiency in soils of northern China has resulted in low Se concentrations of the plants that have affected productivity of sheep (Masters et al., 1993). The addition of a dietary Se supplement may be required in areas of northern China. Unfortunately, there is little information available concerning the optimal level of Se-Met supplementation in goat diets, the effect of Se-Met supplementation on growth performance and serum antioxidant status in goats. Therefore, the objectives of the present study were to evaluate the effect of different levels of supplemental Se-Met on growth performance and serum antioxidant status of Taihang Black goats.

MATERIALS AND METHODS

Animals, diets and feeding

Before the trial, goats were grazed extensively on a mountain pasture (containing 0.03-0.06 mg Se/kg DM), at the Breeding Institute of Taihang Black goat, in Lichen county, Shanxi province, North China. Fifty 16-week-old goats with an average body weight of 12.5±0.5 kg were randomly assigned in equal number to five groups: the control group was fed with the basal diet only (containing 0.049 Se mg/kg DM), while the basal diet of the other four groups were fed with either 0.10, 0.30, 0.50 or 1.00 mg Se/kg DM from Se-Met (Se concentration ≥1,500 ppm, Zhejiang Jiande Weifeng Corporation). The basal diet was formulated to meet all nutrient requirements for goats with the exception of Se (NRC, 1981) (Table 1). All of the goats were housed in individual wooden pens $(1.0 \text{ m} \times 1.2 \text{ m})$ with concrete floors in an open-sided barn. Animals were fed the basal diet for 2 weeks, and then gradually switched to the experimental diets. The experiment lasted for 80 days. Feed was offered daily at 07:00 and 17:00 in equal allotments. Feed intake was adjusted every 20 days during the 80-day feeding trail. Coarsely chopped (2 cm) corn stalk, Chinese wildrye hay and alfalfa hay were fed first and concentrate was fed 30 minutes later. Selenium was added as Se-Met to the premix using finely ground maize flour as a carrier and was mixed with the concentrate. Water was freely available at all times.

Collection of data and samples

Body weights were obtained before the goats were fed in the morning on two consecutive days at the start and end of the experiment. Daily feed offerings and refusals were measured to obtain net feed intake for each animal. Average daily gain (ADG), dry matter intake (DMI) and gain efficiency were calculated for each goat. Blood samples (20 ml) were obtained by jugular venipuncture prior to the morning meal on the last day of the experiment. One aliquot of blood was transferred to a tube containing ethylenediaminete tracetic acid (EDTA 1.5 mg/ml blood) anticoagulant and stored at -30°C for blood selenium (Se) analysis, and another aliquot was centrifuged at 3,000×g for 15 min to obtain serum. Serum was separated and stored at -30°C prior to analysis for GSH-Px, superoxide dismutase (SOD), glutathione-S-transferase (GST) and malondialdehyde (MDA).

Analytical methods

Feed samples were analyzed for dry matter (DM, i.d.: 934.01, AOAC, 1990), crude protein (CP, i.d.: 984.13, AOAC, 1990), neutral detergent fiber (Van Soest et al., 1991) and acid detergent fiber (Robertson and Van Soest, 1981). The Se concentration of feed and blood samples was

Table 2. Effect of dietary Se-Met supplementation on performance of goats

Items ¹	Se-Met supplemental levels (mg/kg)				sem ²	p-values		
	0	0.10	0.30	0.50	1.00	SEM	Linear	Quadratic
Initial BW (kg)	12.66	12.34	12.62	12.84	12.88	0.240	0.595	0.853
Final BW (kg)	18.76	18.80	20.66	20.40	19.76	0.311	0.529	0.073
ADG (g)	76.30 ^c	80.83 ^{bc}	101.83 ^a	94.50^{ab}	86.00^{bc}	2.30	0.717	0.001
DMI (g)	504.3	444.5	451.9	461.8	474.4	10.51	0.887	0.480
G/I (g/g)	0.151 ^c	0.190^{b}	0.238^{a}	0.205^{ab}	0.178^{b}	0.006	0.592	< 0.001

Means within the same row with different letters (a-c) are significantly different (p<0.05).

¹ BW = Body weight; ADG = Average daily gain; DMI = Dry matter intake; G/I = Average daily gain/dry matter intake.

 2 SEM = Standard error of mean, where n = 10 per treatment.

determined using inductively coupled plasma-mass spectrometry (Agilent 7500c, Aglient Technologies, Co, Ltd. USA) as described by Taylor (2005).

Serum GSH-Px, SOD, GST and MDA determination

GSH-Px activity in serum was measured according to the method of Paglia and Valentine (1967) and using an improved coupled test procedure with hydrogen peroxide as substrate (Günzler et al., 1974). The SOD activity in serum was determined using the system of xanthine-xanthine oxidase and nitroblue tetrazolium (NBT) (Sun et al., 1988). GST activity was assayed using 1-chloro-2,4-dinitrobenzene reagent (CDNB) (Habig et al., 1974). The activity of GSH-Px, SOD and GST was expressed as units per milliliter of serum. The concentration of MDA was determined using the thiobarbituric acid technique (Wong et al., 1987).

Statistical analysis

Data were analyzed using the GLM procedure of SAS (2001). The following model was employed:

 $Y_{ij} = \mu + T_i + \varepsilon_{ij},$

Where Y_{ij} = dependent variable; μ = overall mean; T_i = the effect of Se supplementation (I = 1, 5); ε_{ij} = the random error.

Results are presented as treatment means and SEM. Duncan's multiple range tests were used to detect the statistical significance between different treatment groups. Differences were considered significant at p<0.05. Linear or quadratic relationships were used to determine effects of increasing Se concentration on performance and serum antioxidant status in goats.

RESULTS AND DISCUSSION

Performance

Effects of Se-Met supplementation on BW, DMI, ADG and gain efficiency of goats are shown in Table 2. The main finding was that ADG increased in a quadratic fashion (p = 0.001) as supplemental Se increased. The highest responses for ADG were at the 0.3 and 0.5 mg Se concentrations.

Similar responses were observed in sheep (McDonald, 1975) and beef cattle (Perry et al., 1976; Johnson et al., 1979), but not in pigs (Mahan et al., 1999; Tian et al., 2006 a,b) and chickens (Payne and Southern, 2005). Inconsistency of response between experiments may have been due to varying levels of Se in the basal diets. In addition, the response in ADG was reflected by feed efficiency (ADG/DMI) where DMI was not influenced by supplementation. Hence, the change in animal growth was considered to result from a change in feed efficiency. Yoon et al. (2007) also observed improved feed efficiency in broiler chickens supplemented with Se but Ryu et al. (2005) did not. Nevertheless, reasons why feed efficiency in the goat is enhanced are not understood, but may be explained in a future study that examines the effects of Se on nutrient digestibility.

No adverse effect of Se (Se-Met) supplementation on growth of the goats was observed at the highest level of Se supplementation (1.0 mg Se/kg DM), a result in agreement with Juniper et al. (2008). The highest level of 1.0 mg in the current study is almost twice that permitted by the European Union (currently 0.568 mg Se/kg DM, Council Directive 2001/79/EC) but approximately six times less than the amount tested by Juniper et al. (2008) in dairy cows, beef cattle, calves and lambs. They indicated that there were no adverse outcomes on health and performance for these ruminant animals.

Although final BW tended to increase at the higher levels of Se supplementation quadratically, as reflected by ADG, the relationship was not significant (p = 0.073). Cantor et al. (1982) observed a quadratic relationship in their experiment with young turkeys fed Se-Met, while Ryu et al. (2005) reported no response in BW to Se supplementation in broiler chickens.

DMI was not influenced by Se-Met supplementation in the present study, a result consistent with the findings of Payne and Southern (2005) for broilers, while Tian et al. (2006 a) showed that pigs fed organic Se had a greater DMI compared with unsupplemented animals fed during the growing phase.

Based on the results of the current study, it is recommended that 0.30 to 0.50 mg of supplemental Se/kg

Items		Se-Met supplemental levels (mg/kg)				SEM ²	p-values	
	0	0.10	0.30	0.50	1.00	SLIVI	Linear	Quadratic
GPX-Px (U/ml)	21.64 ^c	28.46 ^b	29.66 ^b	34.80 ^a	27.71 ^b	1.03	0.013	< 0.001
SOD (U/ml)	115.49 ^c	235.43 ^{ab}	289.19 ^a	266.26 ^a	204.65 ^b	12.90	0.152	0.001
GST (U/ml)	15.32 ^a	13.73 ^{ab}	9.79 ^b	8.54^{b}	10.97 ^b	0.71	0.027	0.092
MDA (nmol/ml)	2.50^{a}	2.10 ^{ab}	1.28^{b}	1.48^{b}	1.62 ^b	0.12	0.021	0.018
Blood Se (ng/ml)	52.87 ^e	91.23 ^d	106.18 ^c	143.90 ^b	178.01^{a}	9.01	< 0.001	< 0.001

Table 3. Effect of dietary Se-Met supplementation on serum antioxidant status¹ in goats

Means within the same row with different letters (a-c) are significantly different (p<0.05).

¹ GSH-Px = Glutathione peroxidase; SOD = Superoxide dismutase; GST = Glutathione-S-transferase; MDA = Malondialdehyde.

 2 SEM = Standard error of mean, where n = 10 per treatment.

DM from Se-Met (total diet Se of 0.349 to 0.549 mg/kg DM) be fed to the goats to achieve optimal growth performance.

may affect SOD activity in animals.

Antioxidant status

Effects of Se-Met supplementation on antioxidant status in goats are shown in Table 3. The activity of GSH-Px increased (linearly p = 0.013; quadratic p<0.001) as the levels of supplemental Se-Met increased. The supplemented groups had higher activity of GSH-Px than the control groups (p<0.05). The group supplemented with 0.50 mg Se/kg DM had the higher activity of GSH-Px compared with other groups (p < 0.05). The results of the present study agreed with those in previous reports in which Se supplementation increased plasma GSH-Px activity in broilers (Canter et al., 1982; Hassan et al., 1988; Yoon et al., 2007), pigs (Adkin and Ewan, 1984; Mahan et al., 1999), beef cattle (Beck et al., 2005) and sheep (Qin et al., 2007). Lack of a response in plasma GSH-Px activity to Se supplementation in chicks (Cantor et al., 1975) and broilers (Payne and Southern, 2005) may have been due to either the levels of Se supplemented or to the concentration of Se in the basal diets.

Serum SOD activity was higher (p<0.05) in goats supplemented with both 0.30 and 0.50 mg Se/kg DM than in control goats and goats supplemented with 1.00 mg Se/kg DM (Table 3). The present study showed that serum SOD activity increased quadratically as the levels of supplemental Se-Met increased, a result in agreement with that of Gao et al. (2006); in that study plasma SOD activity in pigs supplemented with Se probiotics was significantly higher than in control animals. The increase in SOD activity may be attributed to an increase in liver MnSOD expression (Shilo et al., 2008). Zhang et al. (2005) reported that selenite administration at a dose of 6 mg/kg BW caused a significant decrease in liver SOD activity of mice compared with the control and Nano-Se treatment (Nano-Se are the particles of elemental Se (Se⁰), which possess low toxicity, and nanometer particulates possess a quantum size effect, increased surface area and high surface activity), suggesting the development of selenite toxicosis. Their results and those of the present study indicate that Se supplementation Serum GST activity was significantly decreased (p<0.05) in goats supplemented with 0.30, 0.50 and 1.00 mg Se/kg DM compared with the control (Table 3). Arthur et al. (1987) reported that Se deficiency in rats produced significant increases in the activity of hepatic GST, whereas Zhang et al. (2005) demonstrated that Nano-Se and selenite at a dose of 6 mg/kg BW elevated activity of hepatic GST in mice. This discrepancy in results is due to total Se concentration in the diet, one is deficient and the other supranutritional. In the current study, higher GST activity in the control group may indicate low Se status or deficiency. There is little information available about the effect of Se supplementation on serum or plasma GST activity in livestock.

Serum MDA concentration was measured to determine lipid peroxidation. Serum MDA concentration decreased linearly (p = 0.021) and quadratically (p = 0.018) as Se-Met supplementation increased (Table 3). MDA concentration in pigs also decreased with Se probiotic supplementation (Gao et al., 2006). Reduction in MDA concentration in the current study could result from the elevation of serum GSH-Px activity and SOD activity, suggesting that Se-Met supplementation improved the antioxidant status in goats. Alternatively, lower serum GSH-Px and SOD activity and higher serum GST activity and MDA concentration in the control goats than animals supplemented with Se-Met (p<0.05), indicates that 0.049 mg Se/kg DM in the control diet is inadequate. Based on the results of antioxidant status, 0.30 to 1.00 mg supplemental Se/kg DM from Se-Met (total diet Se of 0.349 to 1.049 mg/kg DM) can improve antioxidant status in goats.

Blood Se concentration

The effect of Se-Met supplementation on blood Se concentration in goats is shown in Table 3. Blood Se concentration increased linearly (p<0.001) and quadratically (p<0.001) as the level of supplemental Se-Met increased. This finding agrees with results for broilers (Canter et al., 1982; Hassan et al., 1988; Ryu et al., 2005; Yoon et al., 2007), pigs (Adkin and Ewan, 1984; Mahan et al., 1999; Tian et al., 2006a,b), beef cattle (Perry et al.,

1976; Beck et al., 2005) and sheep (Qin et al., 2007).

IMPLICATIONS

The present study revealed that supplementation of 0.30 to 0.50 mg supplemental Se/kg DM from Se-Met (total diet Se of 0.349 to 0.549 mg/kg DM) enhanced performance and feed efficiency, elevated activities of GSH-Px and SOD (antioxidant enzymes) in serum, reduced GST activity and MDA concentration in serum, and increased blood Se concentration. Goats fed the control diet with a Se concentration of 0.049 mg/kg DM grew more slowly, converted feed less efficiently and displayed lower activities of serum GSH-Px and SOD. It is contended that nutrient content of the control diet, specifically Se, was inadequate for achieving optimal growth rate in goats. Therefore, it is recommended that the level of Se-Met supplementation for Taihang Black goats be 0.30 to 0.50 mg Se/kg DM.

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