Dietary supplementation with selenomethionine enhances antioxidant capacity and selenoprotein gene expression in layer breeder roosters

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ABSTRACT This study's objective was to investigate the effects of dietary Se (in the form of selenomethionine) on the antioxidant activity and selenoprotein gene expressions in layer breeder roosters. One hundred and eighty, 36-wk-old Jingfen layer breeder roosters were randomly allocated to one of 5 dietary treatments (0, 0.25, 0.5, 1, or 2 mg/kg Se) for 6 wk on a corn-soybean meal-based diet. Antioxidant parameters and selenoprotein gene expressions were assessed at the end of the experiment. The results showed that Se supplementation significantly increased the activity of T-SOD, CAT, GSH-Px, and superoxide anion scavenging ability in plasma ($P \leq 0.05$), and activities of T-SOD, CAT, GSH-Px, superoxide anion scavenging ability, and hydroxyl radical scavenging ability in the liver, kidney, and testis ($P < 0.05$). Moreover, MDA levels were significantly reduced in plasma, liver, kidney, and testis ($P < 0.01$), compared to the control group. Furthermore, the dietary administration of Se significantly increased TrxR2 and GPx4 mRNA levels in kidney and testis, and ID1 mRNA levels in liver and kidney. Most of the antioxidant parameters and selenoprotein-related gene expressions significantly increased, and MDA significantly decreased at dietary supplementation with 0.5 mg/kg Se. Whereas a higher dose of Se level (1 or 2 mg/kg) inhibited the activities of some of the antioxidant enzymes and selenoprotein-related gene expressions in selected tissues. In conclusion, dietary Se supplementation with 0.5 mg/kg significantly improved roosters' antioxidant status and selenoprotein-related gene expression in liver, kidney, and testis, while higher doses led to inhibit these; dietary Se might increase reproductive performance by enhancing their antioxidant status in roosters.

Key words: breeder rooster, antioxidant status, Se, selenoprotein

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INTRODUCTION

As one of the essential elements for the health of animals, selenium (**Se**) has key functions in balancing the redox system (Huang et al., 2003). Se has been recognized as an essential trace element involved in resistance to oxidative stress (Brenneisen et al., 2005). Its deficiency in mammals and poultry has been associated with various disorders, such as impaired fertility or hatchability, abortion, lower growth performance, and increased embryonic mortality (Cantor and Scott, 1974; Mateo et al., 2007). Accumulating evidence indicates that Se is required for the maintenance of the critical balance between germ cell death and proliferation during apoptosis in spermatogenesis (Ranawat and Bansal, 2009; Shi et al., 2014).

Oxidative stress related to the generation of reactive oxygen species (**ROS**) has been associated with cell proliferation, differentiation, and apoptosis in various systems (Abrahan et al., 2009; Guo et al., 2010). Spermatogenesis is a complex process by which undifferentiated germ cells residing on the basement membrane of seminiferous tubules in testis divide and mature into millions of spermatozoa (Shi et al., 2014), which is easy to have side effects under oxidative stress. Furthermore, spermatozoa are richly endowed with substrates, such as polyunsaturated fatty acids (**PUFA**), for free radical attack and deficient in protective enzymes (Aitken et al., 2006; Bongalhardo et al., 2009). Therefore, rooster spermatozoa are extremely susceptible to ROS. Se is the catalytic center of several antioxidant enzymes and proteins such as thioredoxin reductase (**TrxR**), glutathione

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peroxidase (**GPx**), and deiodinase iodothyronine (**ID**), which are involved in the regulation of the oxidative microenvironment (Hawkes and Alkan, 2010; Li et al., 2012).

Our previous result shows that dietary selenomethionine (**SeMet**) supplementation significantly improved semen quality, and blood reproductive hormones in, and the fertilization and hatching rate by breeder cocks (Daheng) (Yu et al., 2014). In the current study, we hypothesize that dietary Se increased reproductive performance by enhancing their antioxidant status in roosters. Furthermore, Se requirements for the full expression of selenoproteins in roosters might be higher than the current Se requirement (0.3 mg/kg) of China's Nutrition Parameter and Feed Standard for animals, for optimum growth performance. Therefore, this study aimed to determine the effect of different Se levels on antioxidant parameters as well as selenoprotein mRNA expression in roosters and determine the optimal dietary Se level of roosters fed a practical corn-soybean meal diet.

MATERIAL AND METHODS

Birds and Diets

All experimental protocols were approved by the Animal Care and Use Committee of Beijing University of Agriculture. A total of 180 healthy 36-wk-old Jingfen layer breeder roosters (1.96 ± 0.12 kg BW) were randomly allocated to one of 5 dietary treatments. Each treatment had 6 replicates of 6 birds with 1 bird per cage. All birds had free access to diets and water and were assigned to a corn-soybean meal-based diet containing 0 (control), 0.25, 0.5, 1, or 2 mg/kg Se for 6 wk. Se was supplied by organic selenium (SeMet, Diamond V Co., IA). The composition and nutrient levels of the corn-soybean meal-based diet (without additional Se) are shown in Table 1.

Table 1. Composition and nutrient levels of basal diet (air-dry basis).

Ingredients	Content (%)	Nutrition level	
Corn	74.18	Metabolizable energy [MJ/kg]	11.92
Soybean meal	19.10	Crude protein [%]	14.20
DL-Methionine	0.13	DL-Methionine [%]	0.36
L-Lysine HCl	0.14	L-Lysine [%]	0.79
Limestone	3.50	Calcium [%]	1.61
Dicalcium phosphate	1.45	Total phosphorus [%]	0.55
Salt	0.30	Available phosphorus [%]	0.36
50% Choline chloride	0.20		
Premix*	1.00		
Total	100.00		

*Premix provides the following per kg of diet:Cu 10.04 mg; Fe 60 mg; Mn 95.4 mg; Zn 103.5 mg; Vitamin (V) A 28,000IU; VD_3 6,000IU; VE 20 IU; VK 6 mg; VB_1 4 mg; VB_2 16 mg; VB_6 6 mg; VB_{12} 0.026 mg; folic acid 1.6 mg; pantothenic acid 24 mg; nicotinic acid 80 mg; biotin 0.2 mg.

Sample Collection

At the end of the trial, one bird from each replicate was chosen randomly, and sacrificed by cervical dislocation. Plasma samples were obtained from the blood after centrifugation at 3,000 rpm for 15 min at 4°C and then stored at −20°C for further analysis. Tissues of the liver, kidney, and testis were sampled carefully, blotted free of blood, and immediately frozen in liquid nitrogen, then stored at −80°C for further analysis. The tissues were chopped into small pieces on ice. A 10 % (w/v) homogenate was prepared in 10 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatants were collected and stored at −80°C to measure antioxidant parameters.

Antioxidant Indices Assays

The antioxidant capacities of plasma and tissue homogenates were analyzed by the same method in duplicate. Total superoxide dismutase (**SOD**) activity was determined using the xanthine/xanthine oxidase system for superoxide anion generation. This anion reduces nitroblue tetrazolium to formazan, which was monitored at 550 nm. Malondialdehyde (**MDA**) was assayed using the thiobarbituric acid method by measuring spectrophotometrically reactive products at 532 nm. Glutathione peroxidase (**GSH-Px**) activity was determined based on the enzyme-catalyzed oxidation of GSH by cumene hydroperoxide coupled to the reduction of oxidized GSH by NADPH. Units of GSH-Px activity were expressed as micromole oxidized NADPH per minute. Catalase (**CAT**), hydroxyl radical scavenging ability (**HRSA**) and superoxide anion scavenging ability (**SASA**) in seminal plasma were measured using commercial Colorimetric kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Quantitative PCR Analysis

Total RNA was extracted from tissues of the liver, kidney, and testis using the TRIzol reagent (Ambion, Austin, TX) according to the manufacturer's instructions. The A260/A280 ratio was determined to estimate the RNA purity, which was then visually confirmed by examining the 18S and 28S bands in a 1% agarose gel stained with ethidium bromide. The gene expression levels were determined using the Bio-Rad iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The primer sequences used for quantitative PCR (**qPCR**) are listed in Table 2. qPCR reactions were performed using a programmable thermal cycler (denaturation at 94°C for 10 min followed by 40 cycles of denaturation at 94°C for 10 s, annealing at 60°C for 30 s). Each sample was measured in triplicate. The relative expression level of mRNA was normalized to the endogenous control gene GAPDH using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Table 2. Primer sequences used for qPCR.

Gene	Primer sequence (5′ - 3′)	Fragment size
GAPDH	*F:* 5′- CATTCCTCCACCTTTGATGCG -3′ *R:* 5′- GTGCCTGGCTCACTCCTTG-3′	147
TrxR2	*F:* 5′- TGAAAGAGATGCTGCTCAGTGT-TAT-3′ *R:* 5′-AGCATTTGGTCCAATGAAAT-GAAGG-3′	151
ID1	*F:* 5′-AGAGCAGAATGAACCAGAACC -3′ *R:*5′-GTCTTCCAGCCTCCTCCAC -3′	113
GPx 4	*F:* 5′-TCAACCGTGAGGGCCAAGT-3′ *R:* 5′- CTCGGCACGCAGCTCTAC -3′	100

Statistical Analysis

All data were analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL). Data related to the effect of dietary selenomethionine levels on antioxidant capacity, and selenoprotein gene expression were analyzed by one-way analysis of variance with orthogonal linear and quadratic contrasts. Duncan's multiple comparison test was used to examine statistical differences among treatments. Differences were considered as significant at $P \leq 0.05$.

RESULTS

Antioxidant Status in Plasma and Tissues

In this study, in SeMet supplemented groups the antioxidant capacity of plasma and tissues of roosters was increased. As is shown in Table 3, dietary SeMet supplementation of 1 or 2 mg/kg Se significantly increased plasma T-SOD activity compared with the control group ($P = 0.02$). With dietary SeMet addition, the CAT activity and SASA in plasma increased quadratically ($P < 0.01$) and reached the maximum value with 0.5 mg/kg Se addition. Dietary Se supplementation linearly improved ($P < 0.01$) the activity of GSH-Px in plasma of roosters. There was no significant difference in HRSA between the control and the Se-added groups. Compared with the control group, dietary Se supplementation remarkably reduced ($P < 0.05$) the MDA concentrations except for the 2 mg/kg Se group.

In Table 4, with an increase in dietary Se, the activities of T-SOD, CAT, GSH-Px, and HRSA in liver increased quadratically ($P < 0.01$). MDA concentrations decreased quadratically ($P < 0.01$). The 0.25 and 0.5 mg/kg doses of dietary Se significantly increased ($P < 0.05$) T-SOD activity compared to the control group. CAT activity was higher ($P = 0.03$) in the 0.5 mg/kg Se supplemented group than in other groups. SASA in the 0.5 mg/kg Se supplemented group was significantly increased ($P < 0.05$) in comparison to the control group. MDA concentration in this group was lower ($P < 0.05$) than any other groups.

Table 5 shows that dietary Se quadratically increased ($P < 0.01$) the antioxidant capacity in kidneys of roosters. T-SOD, CAT, and GSH-Px activities in the 0.5 mg/kg Se treated group were significantly higher ($P < 0.05$) than that in other groups. Dietary addition of 0.25, 0.5, and 1 mg/kg of Se significantly increased ($P < 0.05$) SASA compared to the control group. HRSA was higher ($P < 0.05$) in the 0.5 and 1 mg/kg Se treated groups compared to other groups.

As is shown in Table 6, dietary 0.5 and 1 mg/kg of Se treatment significantly increased ($P < 0.05$) T-SOD and CAT activities in testis compared to the control group. The activity of GSH-Px was significantly improved by adding Se, with GSH-Px activity in the 0.5 mg/kg Se group being higher than that in other groups ($P < 0.05$). Compared with the control group, testicular SASA significantly increased ($P < 0.05$) from the 0.25 to the 1 mg/kg Se groups. Dietary Se quadratically increased the testicular HRSA ($P < 0.01$), and no difference was observed between the Se groups. MDA concentrations were reduced quadratically ($P < 0.01$) by dietary Se supplementation.

Selenoprotein-Related Gene Expression in Tissues of Roosters

No differences were observed in TrxR2 mRNA levels among the groups in liver tissue. However, supplementation of dietary Se with 0.5 and 2 mg/kg significantly increased ($P < 0.05$) TrxR2 mRNA levels in kidneys compared to the control group. Only the 1 mg/kg of Se treated group showed significantly enhanced ($P < 0.05$) testicular TrxR2 gene expression (Figure 1).

Table 3. Effects of dietary Se on antioxidant activity in plasma of roosters.

	Dietary Se supplemental level [mg/kg]						*P* value		
Item	0	0.25	0.5	1	2	SEM	ANOVA	Linear	Quadratic
T-SOD[1] [U/mL]	322.89^{b}	353.62^{ab}	354.37^{ab}	364.55^{a}	358.90^{a}	5.14	0.08	0.02	0.12
CAT[2] [U/mL]	2.42^{b}	2.94^{a}	3.03^{a}	2.79^{ab}	2.53^{b}	0.07	0.01	0.87	< 0.01
GSH-Px[3] [U/mL]	1,242.31^{b}	1,321.15^{b}	1,694.23^{a}	1,911.54^{a}	1,801.92^{a}	57.95	< 0.01	< 0.01	0.06
SASA[4] [U/mL]	312.22^{b}	325.56^{ab}	351.11^{a}	344.45^{a}	304.44^{b}	5.16	< 0.01	0.91	< 0.01
HRSA[5] [U/ L]	447.77	454.24	459.03	449.22	448.22	1.9	0.28	0.76	0.07
MDA[6] [nmol/mL]	11.86^{a}	7.94^{b}	6.96^{b}	6.67^{b}	10.30^{a}	0.47	< 0.01	0.05	< 0.01

$^{a-d}$Means within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Total superoxide dismutase.
[2]Catalase.
[3]Glutathione peroxidase.
[4]Superoxide anion scavenging ability.
[5]Hydroxyl radical scavenging ability.
[6]Malonaldehyde.

Table 4. Effects of dietary Se on antioxidant activity in liver of roosters.

Item	Dietary Se supplemental level [mg/kg]					SEM	*P* value		
	0	0.25	0.5	1	2		ANOVA	Linear	Quadratic
T-SOD[1] [U/mg prot]	198.22^{b}	245.51^{a}	231.15^{a}	213.18^{b}	208.44^{b}	3.8	< 0.01	0.47	< 0.01
CAT[2] [U/mg prot]	19.26^{c}	28.28^{b}	32.35^{a}	25.24^{b}	25.71^{b}	0.91	< 0.01	0.03	< 0.01
GSH-Px[3] [U/mg prot]	11.74^{c}	15.46^{a}	15.14^{a}	14.19^{ab}	13.77^{b}	0.3	< 0.01	0.05	< 0.01
SASA[4] [U/mg prot]	171.96^{b}	178.28^{ab}	190.36^{a}	176.31^{ab}	177.60^{ab}	2.58	0.26	0.57	0.11
HRSA[5] [U/mg prot]	27.09^{c}	31.20^{b}	34.13^{a}	32.28^{ab}	31.92^{ab}	0.57	< 0.01	< 0.01	< 0.01
MDA[6] [nmol/mg prot]	11.80^{a}	8.75^{b}	6.83^{c}	8.24^{b}	9.18^{b}	0.33	< 0.01	< 0.01	< 0.01

$^{a-d}$Means within a row with no common superscripts differ significantly ($P < 0.05$).
$^{1\text{-}6}$The same as legend of Table 3.

Table 5. Effects of dietary Se on antioxidant activity in kidney of roosters.

Item	Dietary Se supplemental level [mg/kg]					SEM	*P* value		
	0	0.25	0.5	1	2		ANOVA	Linear	Quadratic
T-SOD[1] [U/mg prot]	117.45^{c}	142.20^{b}	163.00^{a}	141.72^{b}	121.53^{c}	3.3	< 0.01	0.44	< 0.01
CAT[2] [U/mg prot]	8.19^{c}	10.59^{b}	13.05^{a}	7.16^{cd}	5.68^{d}	0.67	< 0.01	< 0.01	< 0.01
GSH-Px[3] [U/mg prot]	15.88^{c}	18.42^{b}	22.40^{a}	18.96^{b}	16.90^{c}	0.46	< 0.01	0.11	< 0.01
SASA[4] [U/mg prot]	158.31^{b}	191.32^{a}	198.74^{a}	191.28^{a}	165.00^{b}	3.37	< 0.01	0.27	< 0.01
HRSA[5] [U/mg prot]	29.26^{b}	32.78^{b}	38.74^{a}	37.86^{a}	31.62^{b}	0.9	< 0.01	0.04	< 0.01
MDA[6] [nmol/mg prot]	16.26^{a}	12.59^{b}	10.68^{c}	11.27^{bc}	11.17^{c}	0.42	< 0.01	< 0.01	< 0.01

$^{a-d}$Means within a row with no common superscripts differ significantly ($P < 0.05$).
$^{1\text{-}6}$The same as the legend of Table 3.

Table 6. Effects of dietary Se on antioxidant activity in testis of roosters.

Item	Dietary Se supplemental level [mg/kg]					SEM	*P* value		
	0	0.25	0.5	1	2		ANOVA	Linear	Quadratic
T-SOD[1] [U/mg prot]	51.08^{c}	55.97^{bc}	65.38^{a}	60.04^{ab}	57.91^{bc}	1.28	0.15	0.03	< 0.01
CAT[2] [U/mg prot]	3.79^{b}	3.95^{b}	6.95^{a}	6.24^{a}	4.51^{b}	0.29	< 0.01	0.01	< 0.01
GSH-Px[3] [U/mg prot]	11.76^{d}	15.09^{b}	17.05^{a}	14.63^{b}	13.34^{c}	0.35	< 0.01	0.01	< 0.01
SASA[4] [U/mg prot]	289.69^{c}	323.64^{ab}	339.77^{a}	315.65^{b}	309.97^{bc}	4.25	< 0.01	0.16	< 0.01
HRSA[5] [U/mg prot]	50.27^{b}	62.14^{a}	61.25^{a}	59.45^{a}	56.59^{a}	1.08	< 0.01	0.09	< 0.01
MDA[6] [nmol/mg prot]	17.00^{a}	14.70^{b}	12.26^{c}	13.47^{bc}	14.33^{b}	0.39	0.01	< 0.01	< 0.01

$^{a-d}$Means within a row with no common superscripts differ significantly ($P < 0.05$).
$^{1\text{-}6}$The same as the legend of Table 3.

No differences were observed in GPx4 mRNA levels among the groups in liver tissue. The dietary supplementation with 0.5 mg/kg of Se, however, significantly increased ($P < 0.05$) GPx4 mRNA levels in kidney and testis compared to the control group (Figure 2).

Dietary supplementation with 0.5 mg/kg of Se significantly increased ($P < 0.05$) ID1 mRNA level in liver,

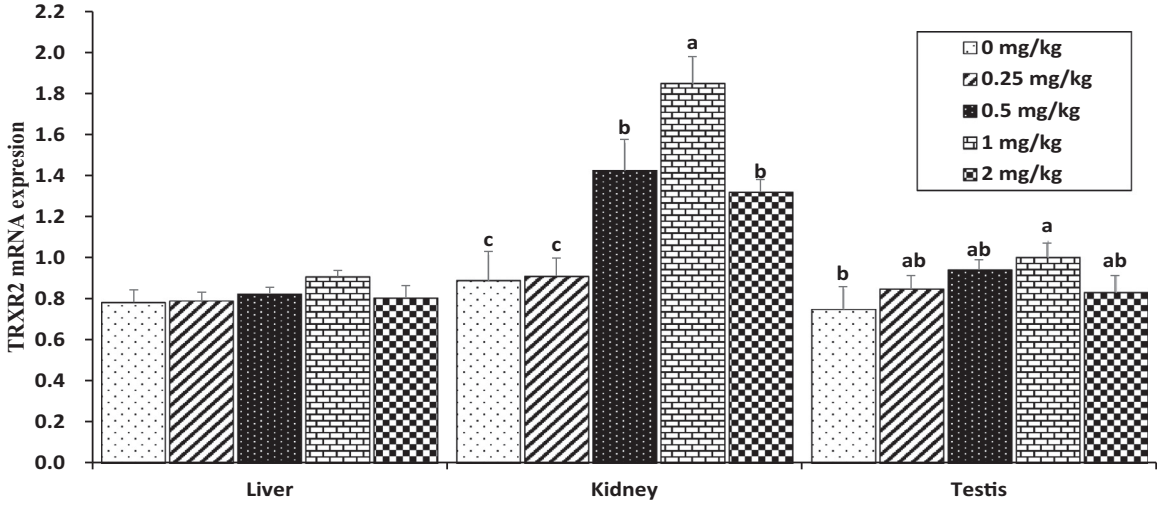

Figure 1. The effects of dietary Se supplementation on the TrxR2 mRNA level in liver, kidney, and testis. The values are expressed as means ± SEM of 6 birds per treatment. Means without a common letter differ significantly ($P < 0.05$). Abbreviations: Se, selenium; TrxR2, thioredoxin reductase.

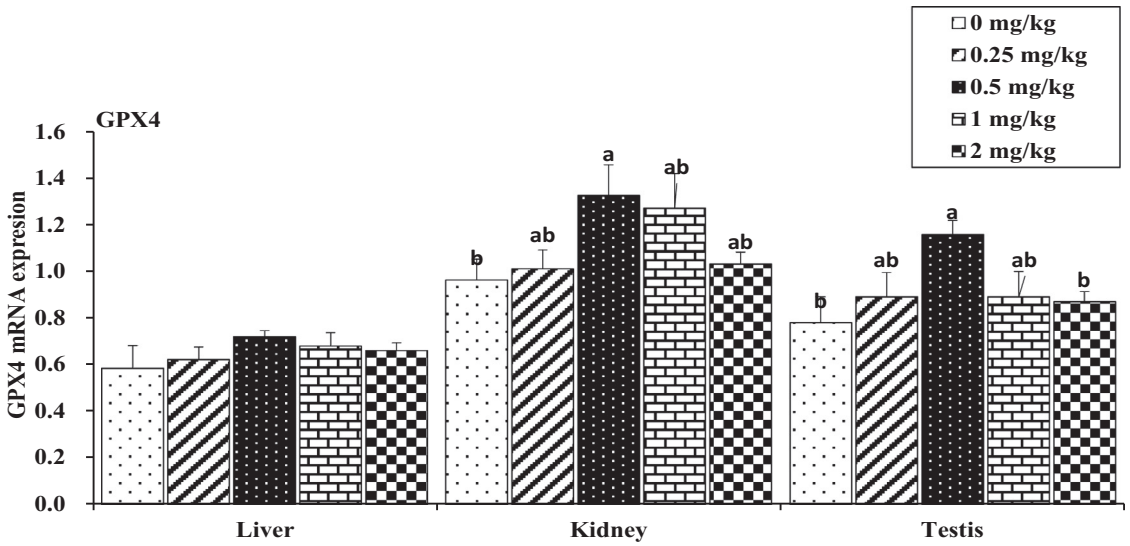

Figure 2. The effects of dietary Se supplementation on the GPx4 mRNA level in liver, kidney, and testis. The values are expressed as means ± SEM of 6 birds per treatment. Means without a common letter differ significantly ($P < 0.05$). Abbreviation: Se, selenium.

whereas the 1 mg/kg of Se group showed significantly enhanced ($P < 0.05$) testicular ID1 gene expression (Figure 3).

DISCUSSION

Selenium is an essential trace element with vital functions in poultry. Our results showed that dietary SeMet significantly increased the antioxidant activity and selenoprotein gene expression of roosters, but depending on the dose provided. Redox balance plays an important role in body metabolism and function. Once the balance is destroyed, a series of metabolic disorders and free radical chain reactions will be triggered (Babior et al., 2003; Djordjevic, 2004). Rooster spermatozoa are extremely susceptible to ROS, as they are richly endowed with substrates, such as PUFA, for free radical attack, while at the same time deficient in protective enzymes (Aitken et al., 2006; Bongalhardo et al., 2009). Antioxidant enzymes, such as SOD, CAT, and GSH-Px, play critical roles in antioxidative protection (Sadi et al., 2018; Tuncsoy et al., 2019). SOD as the first line of defense against oxidation can convert superoxide anions into hydrogen peroxide ($\mathbf{H_2O_2}$) and oxygen ($\mathbf{O_2}$) (Miao et al., 2017). Then, H_2O_2 is degraded by CAT to H_2O and O_2 (Han et al., 2004). GSH-Px not only breaks down H_2O_2 into H_2O but also decomposes lipid peroxides into non-toxic substances to prevent cell damage (Reiter et al., 1995). In the current study, we observed that the activities of T-SOD, CAT, and GSH-Px in the plasma, liver, kidney, and testis significantly increased after feeding dietary SeMet. This was in line with previous studies. It has been reported that dietary SeMet supplementation at 0.225 mg/kg level significantly enhanced the activities of T-SOD, CAT, and GSH-Px in plasma of broilers (Jiang et al., 2009). Moreover, supplementation with SeMet (0.3 mg/kg) increased GSH-Px and SOD activities in breast muscles of broilers (Wang et al., 2011; Chen

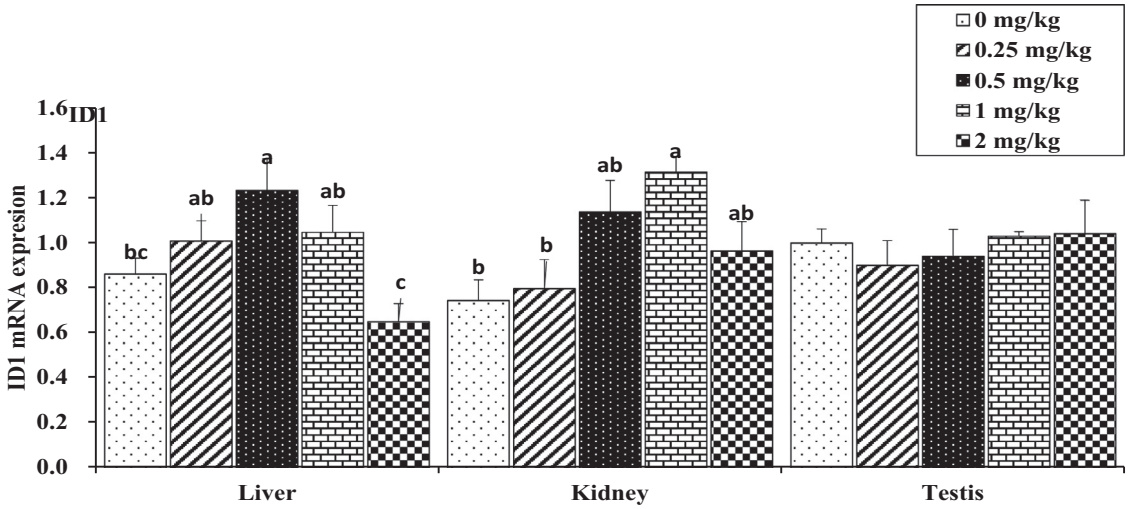

Figure 3. The effects of dietary Se supplementation on the ID1 mRNA level in liver, kidney, and testis. The values are expressed as means ± SEM of 6 birds per treatment. Means without a common letter differ significantly ($P < 0.05$). Abbreviation: Se, selenium.

et al., 2014). In the current research, the antioxidant enzyme activities of tissues (liver, kidney, and testis) in the 2 mg/kg Se supplemented group were significantly lower than that in the 0.5 mg/kg group, which indicated that dietary supplementation of a (too) high dose of Se could inhibit the activity of antioxidant enzymes, like GSH-Px. A similar result was observed in a study by Shi et al. (Shi et al., 2014), wherein the highest GSH-Px level was observed when the animals were fed 0.5 mg/kg dietary Se, and the GSH-Px activity decreased with the increasing Se level in the diet, suggesting that the GSH-Px activity could be inhibited by the high Se level (2 mg/kg). This is in agreement with the results reported by previous studies in Hy-Line Variety white roosters (Davis et al., 2006; Shi et al., 2014).

Free radicals (e.g., superoxide anion radical and hydroxyl radical) are defined as molecules having an unpaired electron in the outer orbit (Gilbert, 2000), which combine with membrane PUFA, inducing free radical chain reactions that result in damage to the membrane structure and function, leading to various diseases (Cheeseman and Slater, 1993). Excessive levels of free radicals result in the disturbance of the balance between the oxidant and antioxidant defense systems, causing lipid peroxidation and oxidative damage to proteins, DNA, and other biological molecules (Yang et al., 2010). In the present study, dietary Se supplementation significantly increased SASA and HRSA in the liver, kidney, and testis of Jingfen roosters. This was in line with previous studies, which showed that Se had good free radical scavenging effects on different free radicals, such as DPPH, free radicals generated by APPH, superoxide anion, singlet oxygen, and NO (Huang et al., 2003; Yu et al., 2016). Lipid bilayers of the cell membrane are the common biological target of free radicals. MDA is the principal product of PUFA peroxidation, and its levels can be used to monitor the extent of lipid peroxidation (**LPO**). In the current study, dietary Se supplementation significantly decreased the concentration of MDA in the tissues (liver, kidney, and testis) and plasma compared to the control group. These were consistent with the findings of Li (Li et al., 2018) and Cong (Cong et al., 2017), who both show that dietary Se supplementation significantly decreased the concentration of MDA in broilers. That might be because Se supplementation increased the antioxidant capacity in roosters by elevating the activity of antioxidant enzymes and reducing the generation of oxidation products (Li et al., 2018). However, the MDA content in the treatment of 2.0 mg/kg was significantly higher than that in the 0.5 mg/kg groups in the plasma, liver, and testis. It seems that LPO can be caused by high Se content (2 mg/kg), but that Se deficiency could more easily lead to LPO. As discussed before, this might be because the GSH-Px activity could be inhibited by the high Se level (2 mg/kg).

Moderate Se in diets can thus enhance the body's antioxidant status by up-regulation of selenoprotein-related gene expression and protect the body from peroxide and free radical damage, so as to keep the body healthy. Selenoprotein constitutes an integral part of enzymes, including TrxR, GPx, and ID. Selenium is an essential trace element with vital functions in animals, which is required for the expression of the selenoenzymes as a cofactor. In the current study, supplementation with dietary Se significantly increased TrxR2 mRNA levels in kidneys (0.5, 1, and 2mg/kg) and testis (1 mg/kg) compared to the control group in roosters. This was in line with previous studies, which show that TrxR2 mRNA expression significantly improved with the increase of dietary Se supplementation in mice testis (Zheng et al., 2013; Liu et al., 2014). Meanwhile, dietary Se supplementation also increased GPx4 mRNA level in kidney (0.5 mg/kg) and testis (0.5 mg/kg) compared to the control group of roosters. Furthermore, supplementation with dietary Se significantly increased ID1 mRNA levels in liver (0.5 mg/kg) and kidney (1 mg/kg). This was in line with a previous study, which showed that the optimal dietary Se level is about 0.5 mg/kg based on the kidney Se concentration and GPx activity (Liao et al., 2021). TrxR, GPx4, and ID play an important role in the suppression of free radicals and the inhibition of NADPH-dependent lipid peroxidation as well as in the prevention of lipid peroxidation via inhibiting glutathione depletion (Fang et al., 2002). Therefore, dietary Se supplementation could improve roosters' antioxidant status (as discussed above) through upgrading selenoprotein-related gene expression.

CONCLUSIONS

In conclusion, the results from the current study indicate that dietary Se supplementation with 0.5 mg/kg significantly improved roosters' antioxidant status and selenoprotein-related gene expression in liver, kidney, and testis, while higher doses led to inhibition of these. Therefore, Se supplementation with 0.5 mg/kg, which is almost twice as high as the current China Nutrition Parameter and Feed Standard recommendation for animals, is recommended to improve the reproductive performance of Jingfen roosters in practice.

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DISCLOSURES

The authors declare they have no conflict of interest.

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