Effects of nano-selenium on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers

S. J. Cai,* C. X. Wu,† L. M. Gong,* T. Song,† H. Wu,* and L. Y. Zhang*[1]

*State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, P. R. China, 100193; and †Hebei Tianyin Biotech Co. Ltd., Langfang, Hebei, P. R. China, 065600

ABSTRACT This study was conducted to investigate the effect of nano-selenium (nano-Se) on performance, meat quality, immune function, oxidation resistance, and tissue selenium content in broilers. A total of five hundred forty 1-d-old male Arbor Acres broilers were randomly allotted to 1 of 5 treatments with each treatment being applied to 6 replicates of 18 chicks. The 5 treatments consisted of corn-soybean meal-based diets supplemented with 0.0, 0.3, 0.5, 1.0, or 2.0 mg/kg of nano-Se. The selenium content of the unsupplemented control diet was 0.09 mg/kg for the starter phase (0 to 21 d) and 0.08 mg/kg for the grower phase (22 to 42 d). There were no significant differences ($P > 0.05$) in performance, meat color, or immune organ index (thymus, bursa, and spleen) due to supplementation with nano-Se. On d 42, a significant quadratic effect of nano-Se was observed on glutathione peroxidase activity, free radical inhibition, contents of IgM, glutathione, and malondialdehyde in serum, on glutathione peroxidase activity, free radical inhibition in liver, and on glutathione peroxidase activity in muscle, with birds fed 0.30 mg/kg of nano-Se exhibiting the best effect and birds fed 2.0 mg/kg of nano-Se showing the worst effect on these parameters. Liver and muscle selenium content increased linearly and quadratically as the dietary nano-Se level increased ($P < 0.01$), and reached the highest value when 2.0 mg/kg of nano-Se was fed. Based on a consideration of all experiment indexes, 0.3 to 0.5 mg/kg is suggested to be the optimum level of supplementation of nano-Se, and the maximum supplementation of nano-Se could not be more than 1.0 mg/kg in broilers.

Key words: nano-selenium, performance, meat quality, oxidation resistance

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INTRODUCTION

Selenium is a trace element essential in animal nutrition and exerts multiple actions related to animal production, fertility, and disease prevention (Mervyn, 1985). Selenium is an integral part of the enzyme glutathione peroxidase, which serves as an antioxidant enzyme that helps to control levels of hydrogen peroxide and lipid peroxides that are produced during normal metabolic activity (Rotruck et al., 1973). In addition, dietary selenium is essential for the activity of virtually all arms of the immune system (Surai and Dvorska, 2002).

The NRC (1994) recommendations established a minimum level of 0.15 mg/kg for selenium supplementation of broilers. There is widespread concern in the animal industries that the NRC minimum recommendation is not sufficient to prevent production losses due to selenium deficiency syndromes; therefore there is continued research into alternative selenium sources and alternative selenium supplementation levels. The bioavailability of selenium is associated with its physical form. Currently, sodium selenite is the most common selenium source used in animal feeds, whereas organic forms such as selenium-enriched yeast and seleno-methionine are also used in many countries (Federal Register, 2002; European Union, 2006; Ministry of Agriculture, 2008). Selenium, like all biologically essential trace elements, can be toxic when provided at levels in excess of the biological requirement. Trace elements typically demonstrate what has been referred to in toxicology as a U-shaped response curve (Hayes, 2008), which describes the negative effects of selenium deficiency and the negative effects of selenium at excessive dietary inclusion levels. Selenium has long been known to be toxic and there are concerns about its effect on animals and animal products. To ensure feed safety, maximum levels for selenium in complete feeds have been set at 0.5 mg/kg in the European Union (2004) and China (Ministry of Agriculture, 2010) and 2.0 mg/kg for the United States (AAFCO, 2011).

With the recent development of nanotechnology, nano-selenium (**nano-Se**) has attracted widespread at-

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[1]Corresponding author: zhangliying01@sina.com

Table 1. Composition and nutrient content of basal diets

Item	Starter phase (d 1 to 21)	Grower phase (d 22 to 42)
Ingredient (%)		
Corn	59.63	61.55
Soybean meal	32.50	31.70
Fish meal	2.00	0.00
Soybean oil	2.00	3.00
Dicalcium phosphate	1.50	1.70
Limestone	1.34	1.13
DL-Methionine (98%)	0.23	0.12
Sodium chloride	0.30	0.30
Vitamin-mineral premix[1]	0.50	0.50
Nutrient content[2] (%)		
ME (kcal/kg)	2,960	3,020
CP	21.54	20.02
Calcium	1.02	0.91
Total phosphorus	0.68	0.66
Methionine	0.57	0.43
Lysine	1.21	1.09
Selenium (mg/kg)	0.09	0.08

[1]Provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 3,000 IU; vitamin E, 24 mg; vitamin K_3, 1.8 mg; vitamin B_1, 2.0 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.1 mg; riboflavin, 5.0 mg; nicotinic acid, 40 mg; pantothenic acid, 15 mg; pyridoxine, 1.0 mg; biotin, 0.05 mg; choline chloride, 500 mg; iron, 80 mg; copper, 20 mg; zinc, 90 mg; manganese, 80 mg; iodine 0.35 mg.

[2]All nutrient levels except ME were analyzed, and values are the means of 2 determinations.

tention because nanometer particulates exhibit novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability, and low toxicity (Wang et al., 2007; Zhang et al., 2008). It has been reported that nano-Se possesses comparable efficiency to selenite and Se-methylselenocysteine in upregulating selenoenzymes but with dramatically decreased toxicity (Zhang et al., 2008).

Zhou and Wang (2011) supplemented 0, 0.1, 0.3, and 0.5 mg/kg of nano-Se in diets fed to Guangxi Yellow broilers and concluded that 0.30 mg/kg of nano-Se was effective in improving feed conversion, the selenium content of tissues, and the quality of the meat. However, no research has been reported to study the effect of supplementing higher nano-Se levels in broilers. Therefore, the purpose of this study was to evaluate the effect of supplementing nano-Se at recommended and maximum limit levels on performance, meat quality, immune function, oxidation resistance, and selenium retention in broilers.

MATERIALS AND METHODS

The animal welfare committee of China Agricultural University (Beijing, China) approved the animal care protocol used for this experiment.

Diets and Experimental Design

A total of five hundred forty 1-d-old male Arbor Acres broilers (Arbor Acres Poultry Breeding Company, Beijing, China), weighing an average of 42.5 g, were individually wing-banded, weighed, and housed in wire-floored pens (90 × 60 × 40 cm). There were 5 dietary treatments with each treatment being applied to 6 replicates of 18 chickens. The 5 treatments consisted of corn-soybean meal-based diets supplemented with 0.0, 0.3, 0.5, 1.0, or 2.0 mg/kg of nano-Se. The selenium in diets was analyzed according to method 996.16 (AOAC International, 2000). Other nutrients were determined according to the methods described by Wu et al. (2011). The actual analyzed selenium content of the 5 experimental diets was 0.09, 0.35, 0.54, 1.10, and 2.06 mg/kg for the starter phase (0 to 21 d) and 0.08, 0.33, 0.56, 1.05, and 2.09 mg/kg for the grower phase (22 to 42 d).

The diets were formulated to meet or exceed NRC (1994) recommendations for all other nutrients (Table 1). All diets were fed in mash form, and the feed and water were provided ad libitum throughout the experiment.

The nano-Se was provided by the Hebei Tianyin Biotech Co. Ltd. (Hebei, China). The product was a pink powder with a measured selenium content of 2,550 mg/kg. The size of the nano-Se was 10 to 150 nm, and the median size was 80 nm. Limestone was used as a carrier to dilute the concentration of nano-Se to a suitable level for blending the ration to achieve the desired levels for diet formulation.

Sampling and Analytical Methods

Body weight and feed consumption per pen were recorded on d 21 and 42, and these values were used to calculate weight gain, feed intake, and feed conversion of the broilers for the periods between d 0 and 21, d 21 and 42, as well as for the overall experiment. Mortalities were recorded daily and averaged less than 1% across treatments.

At the end of each feeding phase, 3 birds from each replicate were randomly selected for blood sampling. The serum was collected and stored at −20°C for later analysis (Wu et al., 2011). The birds slaughtered on d

42 were eviscerated and the liver, thymus, bursa and spleen were removed and weighed. The immune organ index was calculated expressed as the weight of the organ as a percentage of total BW. The center parts of the left pectoralis major of the slaughtered chicks were also taken and weighed and then cut into a 30 × 15 × 5 mm cube. The muscle was hung in a transparent polyethylene bag full of air to keep the meat sample clear of the bag and stored in a chilling room at 4°C. At 24 h, the meat was taken out and weighed after it was dried by filter paper. The drip loss was calculated as the percentage of breast meat yield (g). Meat color (L* = lightness, a* = redness, b* = yellowness) was measured by a Minolta Chroma Meter CR-410 (Osaka, Japan).

Antibody levels (IgA, IgM, and IgG) in serum were determined by turbidimetry with commercial kits (Sanwei Biotech Firm, Weifang, China). This test gave the total level of nonspecific IgA, IgM, and IgG. Serum samples were analyzed for glutathione peroxidase activity, total superoxide dismutase activity, total antioxidant capacity, free radical inhibition, and the concentration of glutathione and malondialdehyde; liver samples were analyzed for glutathione peroxidase activity, total superoxide dismutase activity, free radical inhibition and malondialdehyde concentration; and muscle samples were analyzed for glutathione peroxidase activity, total superoxide dismutase activity, and malondialdehyde concentration. All enzymatic assays were conducted within 24 h of extraction. The kits used for analysis of oxidation resistance, and other measures of antioxidant status were purchased from the Jiancheng Institute of Biological Engineering (Nanjing, China). The UV-visible spectrometer used was the TU-1901 produced by Puxi General Instrument Corporation (Beijing, China).

For selenium analysis in tissues, 0.1 g of liver or muscle was weighed into a digestion tube and 8 mL of HNO_3 was added. The solution was mixed and subsequently digested in a microwave digestion system. After removing some of the acid using an adjustable electric heating plate at 160°C to leave 1 mL of solution, deionized water was added to the solution to produce a volume of 10 mL. The selenium content in the solution was determined following the procedure of Wahlen et al. (2005). The inductively coupled plasma–mass spectrometer used was the Agilent 7500 series (Agilent Technologies, Santa Clara, CA).

An ANOVA was conducted using replicate means. Differences between treatments were examined using one-way ANOVA (SAS Institute, 1996). Means were separated post-hoc by Student-Newman-Keul's multiple comparison test. The linear and quadratic effects of nano-Se concentration were assessed using orthogonal polynomials. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Performance and Meat Quality

During the periods from 0 to 21 d, 22 to 42 d, and 0 to 42 d, weight gain, feed intake, and feed conversion did not differ between treatments ($P > 0.05$; Table 2). On d 42, nano-Se supplementation linearly and quadratically ($P < 0.01$) reduced the drip loss percentage (Table 3). There were no differences in muscle meat color due to treatment.

Immune Function

On d 21, dietary treatments had no effect (ANOVA, $P > 0.05$) on serum IgG, IgM, and IgA (Table 4). However, IgM levels were linearly ($P < 0.01$) and quadratically ($P < 0.05$) increased as the level of nano-Se increased. On d 42, a significant effect of nano-Se was observed for IgG and IgM (ANOVA, $P < 0.01$) with birds fed 0.30 mg/kg of nano-Se having the highest IgG and IgM levels. There was a significant quadratic ($P < 0.01$) relationship between IgM and dietary Nano-Se levels. Bursa, thymus, and spleen indexes were unaffected by dietary treatment.

Oxidation Resistance

On d 21, serum glutathione peroxidase activity and total antioxidant capacity showed linear and quadratic

Table 2. Performance of Arbor Acres broilers fed diets containing different levels of nano-Se[1,2]

	Level of nano-Se (mg/kg)						*P*-value		
Item	0.00	0.30	0.50	1.00	2.00	SEM	ANOVA	Linear	Quadratic
Starter (d 0 to 21)									
Weight gain (g/d)	26.1	26.2	27.3	26.0	27.2	0.47	0.15	0.22	0.47
Feed intake (g/d)	32.3	31.5	32.2	35.0	32.8	1.12	0.22	0.22	0.47
Feed conversion	1.24	1.21	1.18	1.35	1.21	0.04	0.05	0.56	0.84
Grower (d 22 to 42)									
Weight gain (g/d)	68.5	70.0	69.1	69.7	70.5	1.02	0.66	0.24	0.51
Feed intake (g/d)	122.3	122.9	123.6	120.8	123.7	1.07	0.32	0.82	0.93
Feed conversion	1.79	1.76	1.79	1.73	1.75	0.02	0.31	0.19	0.42
Overall (d 0 to 42)									
Weight gain (g/d)	47.3	48.1	48.2	47.9	48.9	0.64	0.57	0.16	0.38
Feed intake (g/d)	77.3	77.2	77.9	77.9	78.2	0.69	0.82	0.24	0.51
Feed conversion	1.64	1.61	1.62	1.63	1.60	0.02	0.81	0.47	0.78

[1]Values are the means of 6 replicates of 18 chickens.

[2]There were no differences between treatments ($P > 0.05$).

Table 3. Meat quality of Arbor Acres broilers fed diets containing different levels of nano-Se (d 42)[1]

	Level of nano-Se (mg/kg)						*P*-value		
Item	0.00	0.30	0.50	1.00	2.00	SEM	ANOVA	Linear	Quadratic
Drip loss percentage (%)	3.42[a]	3.22[b]	3.10[b]	3.08[b]	3.06[b]	0.07	<0.01	<0.01	<0.01
Meat color									
L* (lightness)	38.60	38.73	38.81	38.72	38.91	0.37	0.99	0.62	0.89
a* (redness)	9.30	9.55	9.25	9.26	9.35	0.23	0.90	0.79	0.97
b* (yellowness)	13.33	13.45	13.42	13.03	13.10	0.34	0.89	0.42	0.68

[a,b]Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]Values are the means of 6 replicates of 3 chickens.

($P < 0.01$) responses to nano-Se (Table 5). In contrast, total superoxide dismutase activity, free radical inhibition, and the content of glutathione and malondialdehyde were not affected by dietary nano-Se levels ($P > 0.05$). On d 42, nano-Se supplementation had significant quadratic effects on glutathione peroxidase activity, glutathione content, free radical inhibition, and malondialdehyde concentration in serum. Birds supplemented with 0.3, 0.5, 1.0, and 2.0 mg/kg of nano-Se showed increased glutathione peroxidase activity compared with the control group, but there was no difference among the 4 treatments fed supplemental nano-Se. Higher glutathione content and free radical inhibition and lower malondialdehyde content were observed in the birds receiving 0.3 mg/kg of nano-Se compared with the control group or birds receiving 1.0 and 2.0 mg/kg of nano-Se, but no difference was found between birds supplemented with 0.3 and 0.5 mg/kg of dietary nano-Se. Total superoxide dismutase activity and total antioxidant capacity in serum were not affected by nano-Se levels.

Regarding the liver oxidation resistance on d 42, a quadratic response was observed in glutathione peroxidase activity ($P = 0.02$) and free radical inhibition ($P < 0.01$; Table 6). Birds supplemented with 0.3 mg/kg of nano-Se showed increased glutathione peroxidase activity compared with the control group and birds receiving 2.0 mg/kg of nano-Se, but there was no difference among birds supplemented with 0.3, 0.5, and 1.0 mg/kg of nano-Se. The treatment supplemented with 0.3 mg/kg of nano-Se showed the highest free radical inhibition and lowest malondialdehyde content compared with other treatments. No significant differences ($P > 0.05$) were found in total superoxide dismutase activity in liver across all the treatments on d 42.

Supplementing different levels of dietary nano-Se had a quadratic ($P < 0.01$) effect on glutathione peroxidase activity in muscle (Table 7). Although there was no difference among birds supplemented with 0.3, 0.5, 1.0, and 2.0 mg/kg of nano-Se, these 4 groups also showed significantly higher glutathione peroxidase activity compared with control group. However, nano-Se did not affect the total superoxide dismutase activity and malondialdehyde content across all treatments in muscle ($P > 0.05$).

Tissue Selenium Content

Table 6 and Table 7 shows the results of the determination of selenium content in liver and breast muscle of broilers supplemented with different levels of nano-Se on d 42. The nano-Se supplementation had significant linear ($P < 0.01$) and quadratic ($P < 0.01$) effects on the selenium content in liver and muscle. As the supplemental nano-Se increased, liver and muscle selenium content increased. Liver selenium content increased significantly ($P < 0.01$) when the level of nano-Se supplementation reached 2.0 mg/kg. However, there were no

Table 4. Immune status of Arbor Acres broilers fed diets containing different levels of nano-Se[1]

	Level of nano-Se (mg/kg)						*P*-value		
Item[2]	0.00	0.30	0.50	1.00	2.00	SEM	ANOVA	Linear	Quadratic
21 d									
IgG (g/L)	3.64	3.93	3.72	3.76	4.17	0.18	0.23	0.12	0.23
IgM (g/L)	1.79	1.93	2.06	2.15	2.25	0.14	0.15	<0.01	0.03
IgA (g/L)	1.16	1.09	1.24	1.02	1.30	0.08	0.09	0.40	0.37
42 d									
IgG (g/L)	3.58[a]	4.74[b]	3.44[a]	3.53[a]	3.64[a]	0.25	<0.01	0.19	0.32
IgM (g/L)	1.39[a]	2.00[c]	1.74[b]	1.67[b]	1.30[a]	0.12	<0.01	0.20	<0.01
IgA (g/L)	1.19	1.25	1.18	1.21	1.23	0.07	0.95	0.89	0.98
Bursa index (g/kg)	1.24	1.33	1.36	1.30	1.31	0.04	0.39	0.47	0.25
Thymus index (g/kg)	2.06	2.10	2.35	2.21	2.56	0.22	0.52	0.11	0.28
Spleen index (g/kg)	0.95	1.02	1.10	0.98	1.02	0.10	0.76	0.42	0.69

[a–c]Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]Values are the means of 6 replicates of 3 chickens.

[2]IgG, IgM, and IgA levels are for serum. The organ index is expressed as the weight of the organ as a percentage of total BW.

Table 5. Oxidation resistance in serum of Arbor Acres broilers fed diets containing different levels of nano-Se[1]

	Level of nano-Se (mg/kg)						*P*-value		
Item	0.00	0.30	0.50	1.00	2.00	SEM	ANOVA	Linear	Quadratic
21 d									
Glutathione peroxidase activity (U/mL)	1,170[a]	1,465[b]	1,603[b]	1,643[b]	1,538[b]	87.69	<0.01	<0.01	<0.01
Glutathione (mg/L)	5.9	6.0	6.0	6.2	6.4	0.24	0.62	0.10	0.26
Total superoxide dismutase activity (U/mL)	147	160	148	162	153	5.99	0.28	0.43	0.56
Total antioxidant capacity (U/mL)	10.7[a]	12.2[b]	13.8[c]	12.1[b]	12.6[b]	0.54	<0.01	0.03	<0.01
Free radical inhibition (U/mL)	511	521	52	523	516	31.94	0.99	0.91	0.97
Malondialdehyde (nmol/mL)	12.4	12.0	11.1	12.3	12.3	0.37	0.05	0.88	0.10
42 d									
Glutathione peroxidase activity (U/mL)	1,175[a]	1,409[b]	1,393[b]	1,368[b]	1,358[b]	53.45	<0.01	0.05	<0.01
Glutathione (mg/L)	5.0[a]	5.7[c]	5.5[bc]	5.2[ab]	5.1[a]	0.17	<0.01	0.42	0.01
Total superoxide dismutase activity (U/mL)	146	171	156	159	158	7.15	0.20	0.64	0.42
Total antioxidant capacity (U/mL)	9.0	11.1	11.3	10.1	10.6	0.86	0.35	0.44	0.29
Free radical inhibition (U/mL)	656[a]	842[c]	802[bc]	768[b]	685[a]	35.22	<0.01	0.88	<0.01
Malondialdehyde (nmol/mL)	3.9[a]	3.2[c]	3.3[bc]	3.4[b]	3.7[a]	0.13	<0.01	0.78	<0.01

[a–c]Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]Values are the means of 6 replicates of 3 chickens.

significant differences in liver selenium content when the selenium addition ranged from 0.3 to 1.0 mg/kg.

Regarding muscle selenium content, supplementing 0.3 to 2.0 mg/kg selenium increased muscle selenium content of broilers compared with the control group ($P < 0.01$). Significantly higher selenium content ($P < 0.01$) was found in muscle of birds supplemented with 1.0 and 2.0 mg/kg of nano-Se compared with birds receiving 0.3 and 0.5 mg/kg of nano-Se.

DISCUSSION

Growth Performance

The effects of Se on growth performance in animals are somewhat variable. In the present study, supplementing 0.3 to 2.0 mg/kg of nano-Se had no effect on the growth performance in broilers. Similar results have also been found in other studies, suggesting that increasing the level of selenium supplementation does not improve weight gain or feed intake of broilers fed various concentrations (0 to 0.5 mg/kg) of Se (Miller et al., 1972). Even when the Se supplemental level reached 8 mg/kg, BW and feed efficiency were unaffected by the dietary Se from 3 to 6 wk of age; and no adverse effect of Se on the growth of the chickens was observed during the experimental period of 42 d (Ryu et al., 2005). However, the present results do not agree with the results reported by Ševčíková et al. (2006) and Dlouhá et al. (2008), who found an increase in live weight due to dietary Se in broilers. The differences among studies might be associated with the fact that their chicks were Se- and vitamin E-depleted at hatch, whereas our broilers were not deficient in nutrients at the time of hatch.

Most studies of selenium supplementation in broilers do not discover any effect of selenium on growth performance and feed conversion. However, it is frequently reported from the field that under practical conditions of commercial production, broilers have an apparent need for selenium that exceeds the recommendations of the NRC. Higher levels of selenium are often provided to reduce the mortality associated with marginal selenium deficiency syndromes. Under the conditions of this study, there was no association of selenium level with mortality. In commercial production where birds may encounter microbial challenges, mycotoxicoses, and oxidative stress, increased immune function and improved antioxidant status can contribute to reduced morbidity and mortality.

Meat Quality

In previous studies, it is reported that chicken muscle drip loss could be decreased by adding organic selenium (Perić et al., 2009) and nano-Se (Zhou and Wang, 2011). That is in agreement with the current study. The

Table 6. Oxidation resistance and selenium content in liver of Arbor Acres broilers fed diets containing different levels of nano-Se[1]

	Level of nano-Se (mg/kg)						*P*-value		
Item	0.00	0.30	0.50	1.00	2.00	SEM	ANOVA	Linear	Quadratic
Glutathione peroxidase activity (U/mg)	22[a]	27[b]	26[ab]	25[ab]	23[a]	1.25	0.04	0.80	0.02
Total superoxide dismutase activity (U/mg)	289	298	293	293	282	12.48	0.95	0.65	0.72
Free radical inhibition (U/mg)	231[a]	286[d]	268[c]	259[bc]	250[b]	9.29	<0.01	0.77	<0.01
Malondialdehyde (nmol/mg)	8.9[a]	4.7[d]	7.8[bc]	8.7[ab]	6.9[c]	0.71	<0.01	0.99	0.61
Selenium content (mg/kg)	0.31[a]	0.49[b]	0.52[b]	0.56[b]	0.68[c]	0.06	<0.01	<0.01	<0.01

[a–d]Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]Values are the means of 6 replicates of 3 chickens.

Table 7. Oxidation resistance and selenium content in breast muscle of Arbor Acres broilers fed diets containing different levels of nano-Se[1]

	Level of nano-Se (mg/kg)						*P*-value		
Item	0.00	0.30	0.50	1.00	2.00	SEM	ANOVA	Linear	Quadratic
Glutathione peroxidase activity (U/mg)	18[a]	21[b]	21[b]	21[b]	20[b]	0.66	0.02	0.12	<0.01
Total superoxide dismutase activity (U/mg)	272	300	278	291	284	10.38	0.37	0.64	0.60
Malondialdehyde (nmol/mg)	4.7	3.6	4.2	4.2	3.9	0.32	0.23	0.35	0.46
Selenium content (mg/kg)	0.07[a]	0.19[b]	0.26[b]	0.43[c]	0.46[c]	0.07	<0.01	<0.01	<0.01

[a–c]Means within a row with different superscripts are significantly different ($P < 0.05$).

[1]Values are the means of 6 replicates of 3 chickens.

ability of muscle proteins to attract water and hold it within the cells is of paramount importance to meat quality. It is well understood that selenium is vital for the intra- and extra-cellular antioxidant systems of the body (Mahan and Parrett, 1996; Surai and Dvorska, 2002). In addition, the correlation between meat quality and oxidation resistance of muscle has been well documented. Huff-Lonergan and Lonergan (2005) reported that differences in the antioxidant defense system between animals, muscles, or both, would affect calpain activity, proteolysis, and thus quality characteristics influenced by proteolysis such as tenderness and water-holding capacity. In the present study, birds supplemented with nano-Se showed higher glutathione peroxidase activity in serum and tissue compared with the control group, and as a result, decreased drip loss was observed in the birds fed nano-Se. The improved antioxidant status may promote the maintenance of cell membrane integrity (Cheah et al., 1995), which could ultimately result in reduced drip loss.

Immune Function

No differences were found in IgG, IgM, and IgA levels across all treatments on d 21, but increased IgM level was found in groups supplemented with 0.3 to 1.0 mg/kg of nano-Se and birds fed 0.30 mg/kg of nano-Se exhibited the highest IgG and IgM levels (ANOVA, $P < 0.01$). This indicated that the number of days chickens were supplemented with Se might be an important factor in evaluating the humoral immunity. It is in agreement with Reffett et al. (1988), who reported that supplemental selenium may enhance serum IgM in calves. Droke and Loerch (1989) reported that steers given a selenium and vitamin E injection had higher serum IgG titers. Furthermore, this study showed that a high level (0.5 to 2.0 mg/kg) of nano-Se supplementation decreased the IgG and IgM levels compared with the 0.3 mg/kg group. Similar relationship between high selenium level and immune function was found by Vega et al. (2007), who reported that excess selenomethionine intake (2 mg/kg) resulted in B cell toxicity and a significant decrease in the number of B cells in mice. It is also reported by Wang et al. (2007) that despite being an essential trace element, selenium in fact is toxic at a level not much higher than the requirement. This suggests that feeding a diet containing 0.30 mg/kg of nano-Se produced the greatest improvement in chickens for the humoral immunity, and the nano-Se supplementation should not be more than 1.0 mg/kg according to the present results.

Selenium deficiency could result in histopathological changes in a variety of tissues including immune organs such as bursa, thymus, and spleen (Marsh et al., 1986), and then results in the decrease of their relative weight. However, according to this study, dietary treatment had no effect on broiler immune organ index on d 42. Similar results were reported by Swain and Johri (2000), who revealed that the relative weights of spleen, thymus, and liver were not affected by supplemental vitamin E (0 to 300 IU/kg) and selenium (0 to 1 mg/kg) in broilers. In the study of Peng et al. (2009), no significant differences were noted in relative weight of bursa among birds receiving 0, 1, 5, 10, and 15 mg/kg Se at d 7, 14, and 42. Therefore, the results of this study suggest that no symptoms of selenium deficiency exist in the immune organ indexes, and nano-Se can improve the immune function by enhancing the humoral system rather than increasing the relative weight of immune organs.

Oxidation Resistance

Selenium is an essential trace element that upregulates a major component of the antioxidant defense mechanism by controlling the body's glutathione pool and its major Se-containing antioxidant enzyme (Jiang et al., 2009). Glutathione peroxidase and superoxide dismutase are the primary enzymic defense against toxic oxygen reduction metabolites, with each enzyme playing an integral role in free radical modulation (Maestro, 1991). Glutathione is one of the most important intracellular nonenzymatic antioxidants, and considered to be the largest component of an endogenous cellular redox buffer (Hasspieler et al., 1994; Storey and Braz, 1996). Total antioxidant capacity is also relevant to the capacity of the antioxidant system and high total antioxidant capacity indicates the increase of oxidation resistance. Malondialdehyde is one of the final products of polyunsaturated fatty acid peroxidation in the cells and is a marker of oxidative stress (Gawel et al., 2004). Jiang et al. (2009) reported that dietary Se-methionine

supplementation raised activities of antioxidant enzymes and concentrations of antioxidants and reduced the production of protein and lipid peroxidation products in plasma and breast muscle.

In this study, increased glutathione peroxidase activity was found in groups fed nano-Se compared with the control group in serum and tissue. The results were consistent with those of Yoon et al. (2007), Wang and Xu (2008), Leeson et al. (2008), Wang et al. (2009), and Zhou and Wang (2011). The current results also indicate that feeding nano-Se could improve the glutathione peroxidase activities, and the elevation of the glutathione peroxidase activities in serum, liver, and muscle may be optimized with the supplementation of 0.3 mg/kg of nano-Se.

Regarding the total antioxidant capacity, the highest value was found in birds supplemented with 0.5 mg/kg of nano-Se and the lowest value was observed in the control group on d 21. The results reveal that supplementing 0.5 mg/kg of nano-Se has the same effect as 0.3 mg/kg, and sometimes better than 0.3 mg/kg on oxidation resistance.

However, according to the quadratic response, when the supplementation reached 2.0 mg/kg, lowest glutathione content, lowest inhibiting ability of hydroxyl free radical, and highest malondialdehyde level in serum was found compared with the 0.3, 0.5, and 1.0 groups on d 42. In liver, as the nano-Se supplementation increased, the glutathione peroxidase activity and free radical inhibition decreased and had the smallest value in birds receiving 2.0 mg/kg of nano-Se. This appears to be clear evidence of a negative toxic effect of nano-Se at the higher levels. Perhaps when the nano-Se supplementation is more than 1.0 mg/kg, serum and liver could not endure the toxicity and as a result the antioxidant ability becomes poor.

Therefore, these findings suggested that dietary nano-Se enhanced the antioxidant ability and oxidative stability. Supplementing 0.3 to 0.5 mg/kg of nano-Se seemed to be effective and advantageous in improving the oxidation resistance, and the maximum supplementation of nano-Se should not be more than 1.0 mg/kg.

Tissue Selenium Content

Supplementing nano-Se had a marked effect on selenium content in broiler liver and muscle (Table 6 and Table 7). Similar results were reported by Petrovič et al. (2006), who investigated the effects of feed supplemented with selenium yeast on parameters of selenium status in laying hens. The linear and quadratic relationship between nano-Se supplementation and tissue selenium content observed in this study is consistent with the study of Yoon et al. (2007), who found that the whole blood Se concentration increased (linear, $P = 0.01$; quadratic, $P = 0.01$; cubic, $P = 0.02$) as the concentration of supplemental Se increased (0 to 0.3 mg/kg) in diets of broilers. Zhou and Wang (2011) pointed out that the accumulation of selenium in liver of Guangxi Yellow broilers was dose dependent when the nano-Se supplementation ranged from 0.1 to 0.5 mg/kg. Bou et al. (2005) also reported that broiler meat seems to accumulate Se in response to feeding level. An explanation for this phenomenon may be that modest nano-Se supplementation can easily saturate selenoenzymes, and thereby markedly increasing the tissue content of selenium. This study suggests that the liver and muscle selenium content reflected the supplemental nano-Se level in the diet and that a certain relationship exists between nano-Se metabolism and liver function and meat quality.

The current study demonstrated that the supplementation of nano-Se in broiler diets could improve meat quality, immune function, oxidation resistance, and the selenium content of liver and muscle. Nano-Se supplementation exhibited significant quadratic effects with the optimum responses at a nano-Se level of 0.3 mg/kg. Negative effects appeared at nano-Se levels above 1.0 mg/kg. Liver and muscle selenium content increased with dietary nano-Se level. A recommendation for nano-Se supplementation of broiler diets of 0.3 to 0.5 mg/kg is supported by these findings.

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