Comparative study of DL-selenomethionine vs sodium selenite and seleno-yeast on antioxidant activity and selenium status in laying hens

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ABSTRACT The aim of this study was to compare the effect of DL-selenomethionine (SM) with 2 routinely used Se sources, sodium selenite (SS) and seleno-yeast (SY), on relative bioavailability based on antioxidant activity and tissue Se content. Six hundred thirty 131-day-old brown laying hens were randomly assigned to 7 treatments for 168 d (24 wks) with 6 replicates of 15 hens per replicate. The SS and SY animals were supplemented a cornmeal and soybean diet that supplied a total Se 0.3 mg/kg whereas SM was added at 4 different levels to the total Se at 0.1, 0.3, 0.5 and 0.7 mg/kg. All hens fed the Se-supplemented diet showed higher glutathione peroxidase (GSH–Px) activity ($P < 0.01$), higher superoxide dismutase (SOD) activity ($P < 0.05$), lower malondialdehyde (MDA) content ($P < 0.05$) in plasma, and greater Se contents in egg yolks, albumen, leg muscle, breast muscle, liver, and plasma compared with those fed the control diet ($P < 0.01$). The organic sources (SY and SM) exhibited a greater ability to increase the GSH–Px activity ($P < 0.01$) and Se content in albumen ($P < 0.01$), leg, and breast muscles ($P = 0.0099$ and $P = 0.0014$, respectively) than the SS that was added at 0.3 mg Se/kg. The higher SM added levels increased the GSH–Px activity until the dose of 0.5mg Se/kg ($P < 0.01$).The greater Se concentrations in albumen, muscle and liver appeared in the higher SM-added level, as well as above the dose of 0.1 mg Se/kg ($P < 0.01$). In addition, hens fed the diet with SM accumulated more Se in albumen, leg, and breast muscle than those fed diets with SY ($P < 0.05$). These results confirmed the higher ability of organic Se sources to increase the antioxidant activity and Se deposition in egg albumen, leg, and breast muscles compared with SS, and demonstrated a significantly better efficiency of SM compared with SY for albumen and muscle Se enrichment.

Key words: selenium, laying hen, antioxidant, selenium deposition

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INTRODUCTION

Selenium (i.e., Se) is regarded as an essential trace element that exerts many functions in several biological processes in animals (Holben and Smith, 1999). It has been well -demonstrated to play vitally important roles in antioxidant and redox reactions (Bleys et al., 2009), reproduction, immune function (McKenzie et al., 1998), and muscle function (Ruan et al., 2012; Zhang et al., 2012). Adding Se to diets is a common practice in poultry as well as in other animal species industries (Pappas et al., 2005; Pavlović et al., 2009). Sodium selenite (**SS**) has been the most practical source of Se added to animals' diets to ensure an optimal supply. However, over the last few years, some researchers have been interested in finding organic sources of Se that have a high bioavailability and low toxicity to replace the inorganic Se (Surai, 2002; Yoon et al., 2007; Reis et al., 2009).

Many studies have established that seleno-yeast (**SY**) is a commonly used organic selenium to provide an improved antioxidant ability and bioavailability when compared with SS in poultry nutrition (Pan et al., 2007, Yoon et al., 2007, Wang and Xu, 2008, Arpasova et al., 2009). However, the main Se compound present in SY is L-selenomethioninebased on previous research (Mendez et al., 2000; Huang et al., 2005). Additionally, L-selenomethionine represents the exclusive form in natural selenomethionine compounds (Cukierski et al., 1989), and the other stereoisomer is the D-selenomethionine form. DL-selenomethionine (**SM**) is a synthetic product that has an equimolar mixture of D-selenomethionine and L-selenomethionine.

Considering the bioavailability of SM, Deagen et al. (1987) indicated that SM was more effective than SS in improving the Se deposition in the liver, muscle, and brain tissue of weaning rats fed 0.2 to 0.4 mg SM/kg for 9 wks. Wang et al. (2011) found that the addition of D-selenomethionine increased the Se concentrations in different organs and improved the antioxidant capacity of broilers compared with SS. Wu et al. (2011) reported that SM had similar effects on hen performance and was superior to L-selenomethionine to enrich the Se content

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in eggs and tissues. EFSA (2014) reported that chickens used for fattening can tolerate up to 1.5 mg Se supplemented/kg feed and SM is an effective source of Se for fattening in chickens. However, there has not been sufficient research conducted to compare SM with SS and SY, or to compare the effects of different SM-added levels in improving the antioxidant activity and selenium status of laying hens. Furthermore, previous research that has been conducted in laying hens only lasted several weeks, which may overlook changes in laying hens over a long-term period.

Therefore, the present study was designed to compare the antioxidant activity and Se levels in laying hens that were fed diets supplemented with different doses or sources of Se over a 168-d period. Furthermore, a specific goal was to assess the long-term efficacy of SM in increasing the antioxidant activity and Se levels in the eggs and muscles of laying hens.

MATERIALS AND METHODS

Experimental Design and Diets

All procedures were approved by the Beijing Administration Office of Laboratory Animals. Six hundred and thirty 131-day-old commercial Hy-Line Brown hens were used in this study and randomly allotted to 7 dietary treatments varying in selenium sources and levels. Six replicates (experimental units) of 15 hens were assigned to each of the 7 treatments. Each replicate consisted of 5 adjacent cages (40 × 38 × 38 cm; length × width × height) with 3 hens per cage. Hens were kept with ad libitum access to food and water and the temperature was maintained at 22 ± 3°C. The house for the hens was provided with programmed lighting with a maintained 16-h photoperiod. All the hens were kept healthy during the study period.

The basal diet was corn/soybean meal-based and was formulated to meet the NRC requirements (NRC, 1994) for all nutrients except Se. The composition of the basal diet is shown in Table 1. The dietary Se addition was based on calculated levels for each source. The dietary treatments were composed with the same basal diet but differed only in Se sources and levels (Table 2): the control group was fed the basal diet without any Se supplementation; the SS group was fed the diet added 0.3 mg Se/kg from SS (1.0% Se content, Tiandichun Chemical Reagent Co. Ltd., China), the SY group was fed the diet added 0.3 mg Se/kg from Sel-Plex (a kind of seleno-yeast product, 2,000 mg Se/kg content, America Alltech (China) Co., Ltd.); the 4 SM groups were fed the diet added 0.1 mg Se/kg (SM-0.1), 0.3 mg Se/kg (SM-0.3), 0.5 mg Se/kg (SM-0.5), and 0.7 mg Se/kg (SM-0.7) from SM (2,000 mg Se/kg content, Novus International Inc., MO, USA). All Se additives were added to a premix then used for the manufacturing of the complete feedstuff.

The basal diet contained 0.029 mg Se/kg (dry matter basis), and the final Se levels of the 7 treatments are listed in Table 2. The representative feed samples were mixed from each diet every 4 wk and stored at −20°C until analysis.

Table 1. Composition and nutrient content of basal diet for the laying hens (dry matter basis).

Ingredients	Composition%	Nutrient[2]	Levels
Corn	64.00	ME (MJ/kg)	11.68
Soybean meal	24.50	CP (%)	17.00
Soybean oil	0.50	Calcium (%)	3.71
Limestone power	8.50	Available phosphorus (%)	0.39
Dicalcium phosphate	1.20	Lysine (%)	0.79
Salt	0.30	Methionine + cysteine (%)	0.63
Premix[1]	1.00	Methionine (%)	0.34
Total	100		

[1]Premix provided following (per kg of diet): vitamin A (trans-retinyl acetate) 8,000 IU; vitamin D_3 2,500 IU; vitamin E (all-rac-α-tocopherol acetate) 4,900 IU; vitamin K_3 1.5 mg; vitamin B_{12} 0.02 mg; thiamine (thiamine mononitrate) 4.6 mg; riboflavin 8.2mg; calcium pantothenate 14.0 mg; nicotinic acid 40.6 mg; folic acid 2.3 mg; pyridoxine 8.6 mg; biotin 0.2 mg; Mn 83.0 mg; I 0.5 mg; Fe 60.0 mg; Zn 71.4 mg; Cu 8.0 mg.

[2]Obtained by calculation.

Table 2. Selenium sources and levels supplemented in diets (mg/kg).[1]

		Se level	
Treatment[2]	Diet	Supplemental	final total
Control	Basal diet	0.0	0.029
SS-0.3	Basal diet+SS	0.3	0.28
SY-0.3	Basal diet+SY	0.3	0.36
SM-0.1	Basal diet+SM	0.1	0.13
SM-0.3	Basal diet+ SM	0.3	0.34
SM-0.5	Basal diet+SM	0.5	0.53
SM-0.7	Basal diet+SM	0.7	0.81

[1]Presented Se levels for the supplemental Se and the basal diet are calculated values, whereas the final total Se levels are the chemically analyzed values.

[2]SS, sodium selenite; SS-0.3, SS at 0.3mg Se/kg feed; SY, seleno-yeast; SY-0.3, SY at 0.3mg Se/kg feed; SM, DL-selenomethionine; SM-0.1, SM at 0.1mg Se/kg feed; SM-0.3, SM at 0.3mg Se/kg feed; SM-0.5, SM at 0.5mg Se/kg feed; SM-0.7, SM at 0.7mg Se/kg feed.

Data Collection

Growth Performance. Before the formal study began, production of laying hens was recorded to assure the similar values. Egg production was recorded daily on an individual treatment during the experimental period. Feed intake was determined on a replication basis by weighing back all feed every week.

Sample Collection and Preparation. Two eggs per replicate of each treatment were collected on Day 5, 10, 15, 20, 30, 60, 90, and 168 at random and stored at 4°C until analysis. Each liquid egg was separated into 2 parts, the yolk and albumen, and was homogenized with an electric blender under chilled conditions; the homogenate was collected in chilled 10-mL plastic tubes and stored at −20°C for the determination of Se.

Blood samples from 2 hens per replicate of each treatment were collected on Days 120, 150 and 168. Five milliliters of blood was drawn from the main wing vein prior to feeding the laying hens and stored at −20°C for analysis. At the end of the experiment, 2 hens per replicate of each treatment were slaughtered at random by cervical dislocation to in order to harvest liver, breast, and leg muscles. In total, 10 g each muscle tissue was collected and stored at −20°C for total Se analysis. Egg and tissue samples were previously lyophilized and mixed prior to analysis.

Sample Analysis. Total Se concentrations in diet, egg, and tissue samples were determined by inductively coupled plasma–mass spectrometry (ICP–MS, Agilent 7500cx; Agilent, Tokyo, Japan). This method is validated over a range of 0 to 100 ng/mL selenium in digested material. The procedure for the egg and tissue samples was as follows: briefly, we added the homogenized sample to the microwave Xpress vessel and then added 5 mL nitric acid to allow the sample to predigest for at least 15 min. We added 2 mL hydrogen peroxide, and capped and vortexed the sample. We then starting on the inside row of the turntable, evenly distributed the vessels, removed the vessels from the turntable, and allowed them to cool in the fume hood until reaching room temperature. We slowly opened the vessels, quantitatively transferred the contents to a 25-mL volumetric flask, and triple-rinsed the content using Milli-Q water. We added 0.25 mL internal standard mix, 0.25 mL methanol, and Milli-Q water to reach the final volume. Next, we capped, mixed, and transferred the mixture to a 15-mL autosampler tube. Samples were prepared in singlet with duplicate injections per sample.

The thiobarbituric acid reactive sbustances content, expressed as malondialdehyde (**MDA**) equivalents, and the activities of glutathione peroxidase (**GSH–Px**) and superoxide dismutase (**SOD**) in plasma were determined using the commercial assay kits purchased from Nanjing Jiancheng Institute of Bioengineering (Jiangsu, China) following the standard procedures described by the manufacturer. The water used in the chemical analysis was ultrapurified.

Statistical Analyses

All data were analyzed using SAS software (SAS Institute Inc., Cary, NC). The effects of treatment, sampling days, and their interactions were analyzed in relation to Se concentrations in yolks and albumen using repeated-measures 2-way ANOVA (the software's MIX procedure). The sampling days was added to the model as a repeated factor. For Se levels, was analyzed using the software's GLM procedure. Comparisons of means for each significant effect were analyzed by Tukey's test using the least -squares mean statement. Final data are presented as the mean ± SEM. A P-value of 0.05 or less was considered significant.

Table 3. Effects of dietary Se sources on performance of laying hens (%).[1]

	Treatment					
Study period (d)	Control	SS-0.3	SY-0.3	SM-0.3	SEM	P-value
Day 1 to 15	30.7	30.1	25.04	26.1	1.30	0.332
Day 16 to 30	87.4	87.0	85.11	87.9	0.92	0.750
Day 31 to 45	91.8	93.5	92.5	93.9	1.02	0.798
Day 46 to 60	92.6	95.3	94.7	93.8	0.86	0.737
Day 61 to 75	91.5	95.3	96.1	94.2	0.84	0.198
Day 76 to 90	90.5	93.8	94.5	93.1	0.82	0.307
Day 91 to 105	82.9	83.6	87.8	84.6	0.74	0.081
Day 106 to 120	86.5	88.2	90.4	89.5	0.82	0.377
Day 121 to 135	85.8	87.9	88.4	84.2	0.88	0.784
Day 136 to 150	87.6	86.1	83.8	86.4	0.93	0.673
Day 151 to 168	86.3	87.1	85.6	85.3	1.14	0.858
Day 1 to 168	83.4	84.4	84.3	84.0	0.58	0.933

[1]Data are reported as least-squares means; n = 6.

RESULTS

Production Performance

Performance of laying hens fed diet added with different Se sources during the whole trial period is summarized in Table 3. There are no differences in the performance of laying hens fed with organic and inorganic sources of Se at the same additional level. The addition of different levels of Se to the feed for 168 consecutive days did not significantly affect egg production of laying hens (Table 4). However, during the whole study period, average egg production was decreased 2.7% in the group added with 0.7 mg Se/kg (80.7%) compared with the control group (83.4%), and the highest and lowest egg production (averaged 84.4 and 80.7%, respectively) was observed in the birds receiving 0.3 mg Se/kg from SS, and 0.7mg Se/kg from SM. Feed consumption was not influenced by addition of different source of Se during all the study period (Table 5). However, from Day 120 to 168, feed consumption linearly ($P < 0.05$) increased with increasing SM level in the diet (Table 6).

Determined Se Levels in the Diets of Each Treatment

The Se content of each treatment is shown in Table 2. The Se levels in the diet were slightly above the supplemental levels. Only the SS-0.3 diet had a lower concentration than the supplemented value. All the other higher Se contents can be explained based on the natural occurrence of Se in the diet ingredients. The final total did not indicate major discrepancies among the treatments of different sources and doses.

Antioxidant Activities in Plasma

The MDA content and the activities of GSH–Px and SOD in the plasma of different sources are shown in Figure 1. The MDA concentrations ($P < 0.05$) and the activities of GSH–Px ($P < 0.01$) and SOD ($P < 0.05$) in plasma were affected by different sources of Se at the

Table 4. Effects of dietary SM supplementation on performance of laying hens for 168 d (%).[1]

	Treatment						*P*-value	
Study period (d)	Control	SM-0.1	SM-0.3	SM-0.5	SM-0.7	SEM	Linear	Quadratic
Day 1 to 15	30.7	27.0	26.1	22.5	28.4	1.15	0.523	0.078
Day 16 to 30	87.4	86.7	87.9	86.2	84.9	0.90	0.355	0.665
Day 31 to 45	91.8	92.1	93.9	93.3	90.4	0.78	0.250	0.712
Day 46 to 60	92.6	93.5	93.8	93.2	91.1	0.78	0.300	0.937
Day 61 to 75	91.5	92.1	94.2	93.5	90.6	0.78	0.345	0.533
Day 76 to 90	90.5	90.8	93.1	91.8	88.8	0.78	0.234	0.724
Day 91 to 105	82.9	83.0	84.6	83.2	82.0	0.72	0.458	0.880
Day 106 to 120	86.5	86.4	89.5	87.7	84.0	0.78	0.081	0.873
Day 121 to 135	81.7	81.4	82.9	81.4	80.0	0.82	0.012	0.789
Day 136 to 150	85.8	84.2	83.8	87.3	87.1	0.76	0.359	0.224
Day 151 to 168	87.3	81.6	86.1	85.6	82.9	0.85	0.258	0.905
Day 1 to 168	83.4	83.0	84.0	82.5	80.7	0.59	0.100	0.529

[1]Data are reported as least-squares means; n = 6.

Table 5. Effects of dietary Se sources on feed intake of laying hens (g/bird per day).[1]

	Treatment					
Study period (d)	Control	SS-0.3	SY-0.3	SM-0.3	SEM	*P*-value
Day 1 to 30	110.4	110.5	109.7	110.3	0.100	0.501
Day 31 to 60	119.1	119.5	119.4	119.5	0.139	0.684
Day 61 to 90	119.0	119.4	119.4	119.6	0.107	0.341
Day 91 to 120	119.4	119.8	119.7	119.8	0.079	0.164
Day 120 to 150	118.5	119.5	119.0	119.1	0.176	0.256
Day 150 to 168	115.3	117.1	115.9	115.7	0.423	0.842

[1]Data are reported as least-squares means; n = 6.

dose of 0.3 mg Se/kg on different sampling times. When comparing the group of hens fed diets with SS at 0.3 mg Se/kg, the activities of GSH–Px were significantly increased ($P < 0.01$) in the groups with the equivalent dose of organic sources of Se, but no differences were observed between SY and SM. No effects were observed in the MDA content or SOD activity due to the sources of Se.

The MDA content and the activities of GSH–Px and SOD in the plasma of different doses are shown in Table 7. Hens fed diet with different SM added levels have a linear response on Day 120 with respect to the MDA content and the difference was no significant on Day 150 and 168. Results of the activities of GSH–Px herein obtained showed a linear and quadratic response in response to the increase of Se levels, and the difference was not significant between the groups that were fed at the dose of 0.5 mg Se/kg and 0.7 mg Se/kg. No differences due to the dose of SM added to the diet were observed on Day 120, but the addition of 0.1 mgSe/kg and 0.3 mgSe/kg SM levels increased ($P < 0.05$) the SOD activity on Day 150 and 168.

Selenium Concentrations in Yolks and Albumen

As shown in Table 8, hens fed Se-added diets exhibited a higher yolk Se concentration than those fed the control diet from Day 10 ($P < 0.01$). No significant effects were observed between the 2 organic sources in egg yolks for all the study days. The selenium content was greater ($P < 0.05$) in egg albumen from hens supplemented with SM than those from hens provided the equivalent amount of SY and SS for all days studied except on Day 168. On Day 168, the organic groups exhibited higher ($P < 0.01$) Se content in yolks and albumen than the inorganic group and no response was observed between the organic groups. The influence of Se sources, sampling days, and their interactions on the Se content in albumen and yolks was remarkable ($P < 0.01$; Table 9).

The average Se concentrations in yolk and albumen at the different sampling days relative to the levels of SM in the diet are shown in Table 10. The various supplemented SM levels increased the Se concentrations to a different extent, and the Se concentrations in the

Table 6. Effects of dietary SM supplementation on feed intake of laying hens (g/bird per day).[1]

	Treatment						*P*-value	
Study period (d)	Control	SM-0.1	SM-0.3	SM-0.5	SM-0.7	SEM	Linear	Quadratic
Day 1 to 30	110.4	110.3	110.3	110.4	110.2	0.066	0.201	0.831
Day 31 to 60	119.1	119.6	119.5	119.3	119.8	0.097	0.094	0.640
Day 61 to 90	119.0	119.5	119.6	119.5	119.8	0.087	0.116	0.437
Day 91 to 120	119.4	119.9	119.8	119.8	120.0	0.067	0.071	0.525
Day 120 to 150	118.5	119.4	119.1	119.3	119.7	0.133	0.034	0.287
Day 150 to 168	115.3	116.5	115.7	117.0	117.9	0.323	0.019	0.133

[1]Data are reported as least-squares means; n = 6.

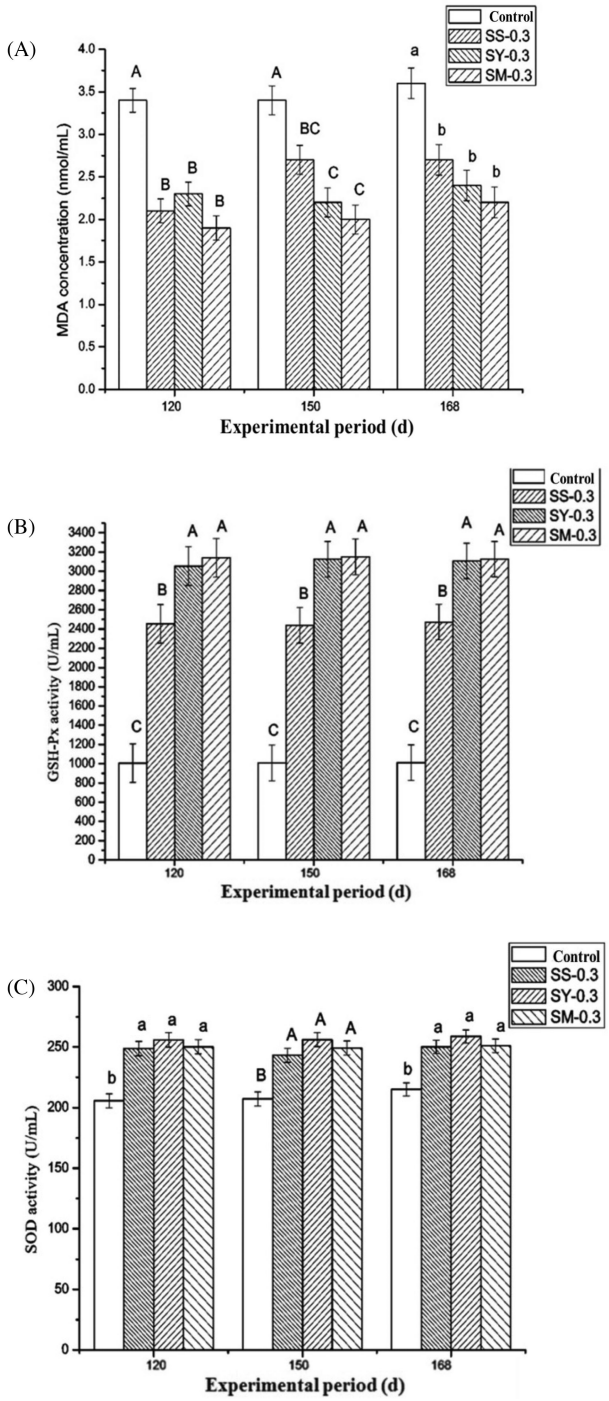

Figure 1. Effects of different Se sources on MDA concentrations (A) and activities of GSH–Px (B) and SOD (C) in plasma. Error bar represents the SEM. [a,b]Mean values with different letters were significantly different ($P < 0.05$) among the groups on the same day. [A,B,C]Mean values with different letters were significantly different ($P < 0.01$) among the groups on the same day.

egg yolk and albumen of the groups fed with SM at the dose of 0.5 mg Se/kg and 0.7 mg Se/kg were higher than the other two treatments from Day 15 ($P < 0.01$); Se concentrations in yolks and albumen increased linearly ($P < 0.01$) and quadratically ($P < 0.01$) with increasing SM level in the diet during every sampling day, but the difference was no significant between the groups that were fed the dose of 0.5 mg and 0.7 mg Se/kg. Moreover, Se concentrations in yolks and albumen were affected ($P < 0.01$) by Se levels, sampling days, and their interaction (Table 11).

Plasma and Tissue Se Concentrations

Table 12 shows the effect of different Se sources on Se concentrations in leg muscle, breast muscle, liver, and plasma. The Se concentrations in liver ($P = 0.0087$) and plasma ($P = 0.0014$) were higher in all groups of hens that were fed different sources of Se at the 0.3 mg Se/kg level. No effect was observed on the liver and plasma Se content among the 3 treatments at the dose of 0.3 mg Se/kg. Indeed, the Se content in muscles in the 2 treatments supplemented with the organic Se sources at the dose of 0.3 mg Se/kg of diet was significantly increased compared with the inorganic group and the control group ($P < 0.01$). No response in Se content was found in the leg and breast muscle of the hens fed with SS compared with the control diet. The results also showed that SM at the dose of 0.3 mg Se/kg has the ability to increase the Se content of leg and breast muscle more effectively compared with the equivalent amount of SY ($P < 0.01$).

Figure 2 presents the Se concentrations in leg muscle, breast muscle, liver and plasma, from the different supplementation levels of SM. Changes in the Se concentrations in leg muscle, breast muscle. and liver tissue showed a dose-dependent pattern in the groups of hens fed diets supplemented with SM, and the higher Se supplement level resulted in greater Se concentrations. Hens fed 0.5 and 0.7 mg Se/kg had greater ($P < 0.01$) plasma Se concentrations than hens fed 0.1 and 0.3 mg Se/kg, but no differences were observed between each other.

DISCUSSION

In the present study, production performance of hens fed a diet added with different sources or levels of Se for 168 d (consecutive) was not significantly different from the control diet that was fed without Se addition during the same age period. The results are consistent with the results obtained in previous reports. Payne et al. (2005) did not observe differences on percentage hen-day production of hens fed by sources or level of Se. Chinrasri et al. (2009) reported that different Se sources additional on the level of 0.3 mg Se/kg had no effects on egg production of hens. Furthermore, Chantiratikul et al. (2008) reported that supplementations with 0.3 to 3.47 mg/kg Se from sodium selenite or zinc-L-selenomethionine in the diets did not affect the performance of laying hens. However, Pavlović et al. (2009) found no effects when selenite or se-enriched yeast added to poultry diet on the first 8 wks, whereas from the ninth wk, diets supplemented with SY exhibits higher egg production than SS. Egg production for the entire flock reached a peak averaged 96.1% on Days 61

Table 7. Effect of different levels of Se on MDA content and activities of GSH–Px and SOD in plasma[1] (n = 6).

	Treatment						*P*-value	
Days	Control	SM-0.1	SM-0.3	SM-0.5	SM-0.7	SEM	Linear	Quadratic
MDA (nmol/mL)								
Day 120	3.4^{A}	$2.5^{B,C}$	1.9^{C}	$2.8^{A,B}$	2.1^{C}	0.14	0.034	0.356
Day 150	3.4^{a}	2.3^{b}	2.0^{b}	$2.6^{a,b}$	2.0^{b}	0.17	0.199	0.917
Day 168	3.6^{a}	$2.6^{a,b}$	2.2^{b}	2.3^{b}	2.1^{b}	0.18	0.152	0.210
GSH–Px (U/mL)								
Day 120	$1{,}007^{D}$	$2{,}393^{C}$	$3{,}140^{B}$	$3{,}685^{A}$	$3{,}812^{A}$	201	<0.001	<0.001
Day 150	$1{,}009^{D}$	$2{,}401^{C}$	$3{,}150^{B}$	$3{,}336^{A,B}$	$3{,}696^{A}$	186	<0.001	<0.001
Day 168	$1{,}012^{D}$	$2{,}389^{C}$	$3{,}126^{B}$	$3{,}353^{A,B}$	$3{,}582^{A}$	184	<0.001	<0.001
SOD (U/mL)								
Day 120	206^{B}	249^{A}	250^{A}	242^{A}	242^{A}	5.84	0.670	0.608
Day 150	207^{b}	249^{a}	250^{a}	$241^{a,b}$	244^{a}	5.77	0.567	0.662
Day 168	215^{b}	250^{a}	251^{a}	$243^{a,b}$	$242^{a,b}$	5.44	0.888	0.785

^{a,b}Means within a row with no common superscripts differ significantly ($P < 0.05$).

$^{A–D}$Means within a row with no common superscripts differ highly significantly ($P < 0.01$).

Table 8. Effects of the different Se sources on the selenium concentration in egg yolk and albumen (μg/kg, wet weight basis, n = 6).

	Specific day of Se treatment						
Treatment	5	10	15	20	30	60	168
Selenium concentration in yolk							
Control	837^{A}	$712^{B,Y}$	$503^{D,C,X}$	$561^{C,Y}$	$407^{D,Y}$	$251^{E,Y}$	$408^{D,X}$
SS-0.3	$814^{A,B}$	$886^{A,Y,Z}$	$864^{A,Y}$	$864^{A,Z}$	$780^{A,B,Z}$	$688^{B,Z}$	$742^{A,B,Y}$
SY-0.3	$863^{A,B}$	$911^{A,Z}$	$876^{A,B,Y,Z}$	$884^{A,B,Z}$	$825^{B,Z}$	$746^{C,Z}$	$873^{A,B,Z}$
SM-0.3	854^{C}	$1033^{A,Z}$	$984^{A,B,Z}$	$899^{B,C,Z}$	$825^{C,Z}$	$788^{C,Z}$	$888^{B,C,Z}$
SEM	28.62	28.25	40.42	36.78	38.28	46.84	41.64
P-value	0.939	0.001	<.001	0.001	<.001	<.001	<.001
Selenium concentration in albumen							
Control	$74^{B,Y}$	$75^{B,W}$	$107^{A,B,Z}$	$146^{A,Y}$	$121^{A,B,Z}$	$136^{A,Z}$	$133^{A,Z}$
SS-0.3	$89^{E,Y}$	$106^{D,E,X}$	$158^{A,B,Z}$	$128^{C,D,Y}$	$149^{B,C,Z}$	$126^{C,D,Z}$	$178^{A,Y}$
SY-0.3	$112^{D,Y}$	$155^{C,Y}$	$231^{A,Y}$	$174^{B,C,Y}$	$197^{A,B,Y}$	$190^{B,C,Y}$	$245^{A,X}$
SM-0.3	$206^{C,Z}$	$257^{A,Z}$	$354^{A,B,X}$	$310^{B,C,Z}$	$282^{C,X}$	$261^{C,X}$	$246^{B,C,X}$
SEM	0.26	14.54	21.74	8.93	13.82	11.84	10.45
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001

^{A,B,C}Means within a row with no common superscripts differ highly significantly ($P < 0.01$).

$^{V–Z}$Means within a column with no common superscripts differ highly significantly ($P < 0.01$).

Table 9. Model and ANOVA results, for Se concentrations in yolks and albumen, affected by Se sources and sampling days.

		Se in albumen		Se in yolk	
Variable	df[1]	*F*	*P*	*F*	*P*
Se source[2]	3	48.67	<0.001	90.55	<0.001
Day[3]	6	631.13	<0.001	27.08	<0.001
Source×day	18	6.90	<0.001	4.41	<0.001
Error	140				
Total	167				

[1]Degree of freedom was the same for both variables.

[2]Se sources (SS, SY, and SM).

[3]Days effect (sampling on Day 5, 10, 15, 20, 30, 60 and 168).

to 75 in the SY-0.3 group. Among all the test phases, the highest mean egg production for hens supplemented with SM was appeared from the hens fed diet supplementing 0.3 mg Se/kg. The present results indicated that supplementation of 0.3 mg Se/kg diet from SM can be safely applied for laying hens without decreasing the performance. Feed intake for the entire study was not influenced by Se sources. Chinrasri et al. (2009) also observed that different sources of Se had no effect on feed intake. However, feed intake was increased in hens fed diets supplemented with SM compared with the basal diet during Days 120 to 150 and 151 to 168 in this study. It was in summer during the 2 periods, and the increased feed intake may be attributable to the addition of Se to diet reduced negative effects of heat stress. The same phenomenon was observed by Sahin and Kucuk (2001) in quail.

The selenoenzyme GSH–Px works well to reveal the antioxidant status of cell bodies and functions to remove hydrogen and organic peroxides (Kyriakopoulos and Behne, 2002). The results of this study revealed that the activity of GSH–Px in plasma was significantly increased by Se dietary supplementation. These results

Table 10. Effects of different SM added levels on selenium concentration in egg yolks and albumen (μg/kg, wet weight basis).

	Treatment						*P*-value	
Specific days	Control	SM-0.1	SM-0.3	SM-0.5	SM-0.7	SEM	Linear	Quadratic
Selenium concentration in yolk								
5	837	$717^{X,Y}$	854^{X}	887^{X}	$1{,}041^{X}$	41.3	0.004	0.010
10	712^{E}	$851^{C,Z}$	$1033^{B,Z}$	$1{,}036^{B,Y}$	$1{,}341^{A,Z}$	31.1	<0.001	<0.001
15	503^{E}	$708^{D,X,Y}$	$984^{C,Y,Z}$	$1{,}191^{B,Z}$	$1{,}345^{A,Z}$	50.4	<0.001	<0.001
20	561^{D}	$686^{C,X,Y}$	$899^{B,X,Y}$	$1{,}099^{A,Y,Z}$	$1{,}255^{A,Y,Z}$	38.2	<0.001	<0.001
30	407^{D}	$738^{C,Y}$	$825^{C,X}$	$1{,}069^{B,Y,Z}$	$1{,}267^{A,Y,Z}$	46.3	<0.001	<0.001
60	251^{D}	$563^{C,W}$	$788^{B,X}$	$1{,}005^{A,X,Y}$	$1{,}053^{A,X}$	57.5	<0.001	<0.001
168	408^{D}	$600^{C,X,W}$	$888^{B,X,Y}$	$1{,}068^{A,Y,Z}$	$1{,}135^{A,X,Y}$	47.4	<0.001	<0.001
Selenium concentration in albumen								
5	74^{C}	$124^{C,V}$	$206^{B,C,W}$	$310^{A,B,Y}$	$365^{A,W}$	32.5	<0.001	<0.001
10	75^{E}	$130^{D,V,W}$	$257^{C,X,Y}$	$346^{B,Y}$	$499^{A,X,Y}$	28.5	<0.001	<0.001
15	107	$164^{D,X,Y}$	$354^{C,Z}$	$440^{B,Z}$	$561^{A,Y,Z}$	32.6	<0.001	<0.001
20	146	$191^{D,Z}$	$310^{C,Y,Z}$	$435^{B,Z}$	$590^{A,Z}$	31.1	<0.001	<0.001
30	121	$166^{D,X,Y}$	$282^{C,X,Y}$	$428^{B,Z}$	$587^{A,Z}$	32.4	<0.001	<0.001
60	139	$148^{D,X,W}$	$261^{C,X,Y}$	$397^{B,Z}$	$481^{A,X}$	25.7	<0.001	<0.001
168	133	$179^{E,Y,Z}$	$243^{C,X,W}$	$334^{B,Y}$	$363^{A,W}$	16.6	<0.001	<0.001

$^{A–E}$Means within a row with no common superscripts differ highly significantly ($P < 0.01$).

$^{V–Z}$Means within a column with no common superscripts differ highly significantly ($P < 0.01$).

Table 11. Model and ANOVA results, for Se concentrations in yolks and albumen, affected by Se levels and sampling days.

		Se in albumen		Se in yolk	
Variable	df[1]	*F*	*P*	*F*	*P*
Se level[2]	4	579.32	<0.001	216.74	<0.001
Day[3]	6	31.80	<0.001	19.83	<0.001
Level×day	24	6.31	<0.001	4.25	<0.001
Error	140				
Total	167				

[1]Degrees of freedom were the same for both variables.

[2]Se levels (SM, DL-selenomethionine; SM at 0.1mg Se/kg feed; SM at 0.3mg Se/kg feed; SM at 0.5mg Se/kg feed; SM at 0.7mg Se/kg feed).

[3]Days effect (sampling on Day 5, 10, 15, 20, 30, 60, and 168).

on the relationship between dietary Se supplement and the activity of GSH–Px are similar to previous reports (Hassan et al., 1988; Toyoda et al., 1990; Illek and Pechová, 2001; Spears et al., 2003; Surai and Fisinin, 2014). Recently, other reports in humans found that the plasma GSH–Px concentrations were higher in the adults who ingest Brazil nuts (the highest known food source of selenium) than those from the placebo group (Thomson et al., 2008). Likewise, studies have also shown that Se supplementation enhanced the GSH–Px mRNA level and the activity of GSH–Px in hepatocytes (Wu et al., 2010). As in this study, studies in animals have described unequal efficiencies of the different Se sources with regard to increasing the activity of GSH–Px. It has been reported that different Se sources affect the GSH–Px activity (Cantor et al., 1982) and the GSH–Px mRNA levels in the tissue of pigs that were fed organic sources were higher than those fed SS (Gan et al., 2013). Further, the improved effects of SM when compared with SS may be due to the better bioavailability of SM for functional selenprotein activity to express GSH–Px mRNA and the formation of superoxide anions. Moreover, methionine which is an analog to the selenomethionine compounds plays important roles to support cysteine for GSH synthesis. This may be attributed to the supplementation of SM

Table 12. Effects of the different Se sources on Se concentrations in leg muscle, breast muscle, liver, and plasma of laying hens at Day 168 of the feeding period (μg/kg, n = 6).

	Treatment					
Item	Control	SS-0.3	SY-0.3	SM-0.3	SEM	*P*-value
Leg muscle[1]	754^{C}	826^{C}	$1{,}315^{B}$	$1{,}544^{A}$	51.7	0.010
Breast muscle[1]	641^{C}	692^{C}	$1{,}010^{B}$	$1{,}423^{A}$	56.6	0.001
Liver[1]	1728^{B}	2403^{A}	$2{,}395^{A}$	$2{,}777^{A}$	84.1	0.009
Plasma[2]	103^{b}	191^{a}	190^{a}	195^{a}	11.0	0.002

^{a,b}Means within a row with no common superscripts differ significantly ($P < 0.05$).

^{A,B,C}Means within a row with no common superscripts differ highly significantly ($P < 0.01$).

[1]Sample was analyzed in dry basis.

[2]Sample was analyzed in wet basis.

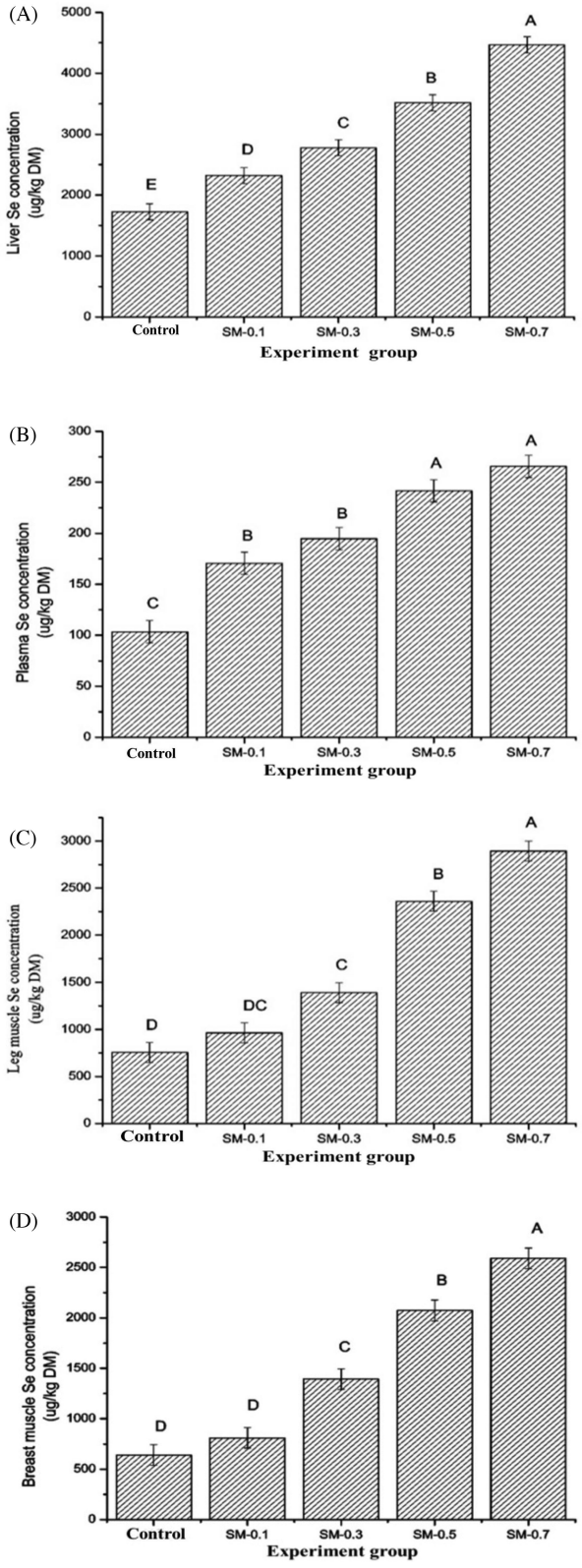

Figure 2. Effects of different SM added levels on Se concentrations in liver (A), plasma (B), leg muscle (C), and breast muscle (D) of laying hens at Day 168 of the feeding period. Error bar represents the SEM. [a,b]Mean values with different letters were significantly different ($P < 0.05$) among the groups on the same day. [A–E]Mean values with different letters were significantly different ($P < 0.01$) among the groups on the same day.

which increased the activity of GSH–Px in the plasma. The dose-dependent responses for activity of GSH–Px have both linear and quadratic terms, indicating that GSH–Px activity increased more quickly with the sampling days go along.

Superoxide dismutases function as an essential antioxidant enzyme defense system against radicals by protecting critical targets of superoxides (Zelko et al., 2002). The increased SOD activity indicated an increase in antioxidant activity (Wu et al., 2003). A previous study showed that a Se-deficient diet caused a significant decrease in mRNA expression for SOD, but Se supplementation increased the expression of SOD in tissues (Kurz et al., 2002). Our observation on the relationship between dietary Se supplementation and the activity of SOD in plasma is also similar to previous reports (Reddi and Bollineni, 2001; Kurz et al., 2002;). We observed that there was an increased SOD activity of hens fed diets supplemented with Se at a lower level, but the activity decreased above the 0.5 mg Se/kg level. This result appears to be associated with the expression of SOD enzymes in plasma, as above the 0.5 mg Se/kg level indicated a passive expression of plasma SOD enzymes.

The plasma concentration of MDA is commonly used biomarker that provides an indication of the lipid peroxidation level (Nielsen et al., 1997). Lipid oxidation results a number of secondary products that are highly toxic (Del Rio et al., 2005). As stated in the results, an inverse correlation between the selenium supplemented in animal diets and the MDA concentration in plasma was observed. No differences in the plasma MDA content were related to the sources of supplemented Se. This result may suggest that the hens fed diets with added Se could maintain the plasma antioxidant capacity. Otherwise, the lack of differences among the sources and levels of Se (not including the basal treatment) may have been due to the presence of more than adequate levels of vitamin E in the diet. Furthermore, the observed relationship between MDA and GSH–Px in our study is consistent with a previous study that reported the increased MDA concentration was accompanied by lower GSH–Px activity (Balogh et al., 2004).

Previous studies reported that eggs from hens fed diets added with Se showed higher Se concentration in yolks and albumen and the hens fed with organic Se exhibited a higher Se concentration compared with those supplemented with the inorganic form (Kralik et al., 2009; Bennett and Cheng, 2010; Invernizzi et al., 2013). In this study, a sources × sampling days interaction revealed that the Se addition of different sources to diet was effective in increasing Se concentration as prolonging the sampling period. Supplementary organic source, regardless of the sampling days, was associated with an increase in albumen Se concentration from Day 10. The 2 organic sources exhibited the same effect in increasing the Se concentration on Day 168. Our results confirmed the greater ability of organic Se sources (SM and SY) to increase albumen Se content than SS

at the same supplement level. However, there were no significantly differences observed in increasing Se content in yolks. This result is most likely due to the different forms of metabolites produced by the different pathways. Davis and Fear (1996) reported that different concentrations and forms of Se could affect the Se deposition and distribution between yolk and albumen. Moreover, Briens et al. (2013) reported that the different absorption mode between organic and inorganic form led to the different digestibility rates, with the inorganic form having a lower digestibility than the organic form.

Furthermore, as shown in the results, average Se concentration in albumen was lower than in yolk on fresh basis for all the examined days. Previous studies reported that most trace minerals are deposited in the yolk, while the organic sources result in greater Se concentrations in albumen and the yolk contains 40 to 60% Se (Li et al., 2007, Bargellini et al., 2008). Paton et al. (2002) also reported that the Se in the dietary was proportionally more accumulated in the yolk with different Se sources and there may be due to the deposition of a consequence of mineral-binding lipoproteins during the yolk accretion. However, the present study demonstrated that long-term supplementation of hens with organic Se elevate more concentration of the element in the albumen than in the yolk and the Se deposition in the eggs from the SS group was mainly in egg yolks.

It has been well-demonstrated that the Se concentration in eggs depends on the level of Se that is supplemented. As expected, our study showed a significant increase in Se content in yolk as the addition of Se increased in the diet. These results appear to be highly consistent with previous studies (Payne et al., 2005; Payne and Southern, 2005b; Pappas et al., 2006; Skřivan et al., 2008), with the main conclusion that the Se levels of the whole eggs of hens with Se at different levels increased as dietary Se levels increased. Furthermore, in our study, it was observed that the Se content in yolks increased from Day 10 onward whereas it increased from Day 5 in albumen. This phenomenon is due to the yolk take about 10 to 12 d to develop, while the albumen is secreted daily and could use absorbed Se right away.

In the present study, the linear and quadratic responses were found to be significant for Se-added level effect on Se concentration in yolks and albumen, which increased with the increase of Se levels and the highest Se content appeared at the 0.7 mg Se/kg supplement level. The presence of a dose-dependent Se increase in yolks and albumen may suggest that the increase of Se concentrations was generally proportional to the level of the dietary Se supplementation. Results also agree with those reported by (Gjorgovska et al., 2012, Pan et al., 2011, Wu et al., 2011). The significant interaction observed between Se levels and sampling days indicated that yolks and albumen from eggs sampled on Day 168 had significantly differences of Se concentrations among different treatments than that from eggs sampled on Day 5.

Regarding the muscle and liver Se concentration, measurements showed a significant increase based on the different Se sources. Moreover, a greater Se content was observed in the organic Se sources group compared with the SS source or control diet, in leg and breast muscles. The results were consistent with the findings reported by other authors (Dlouha et al., 2008; Markovic et al., 2008; Wang and Xu, 2008). Indeed, when comparing the 2 organic Se sources, the muscles from hens fed SM showed greater Se content than those fed SY at the same Se level. Thus, using leg and breast muscles Se content as indicators, the Se bioavailability of SM is higher than that of SY in hens. Hence, the amount of Se uptake and its absorption in muscle and egg of laying hens can be strongly determined by the chemical form of Se in different organic sources. The results from Cantor et al. (1975) also suggested that the main factor that affects the biological availability of dietary Se depend on its chemical nature. Indeed, further studies are needed to elucidate the complete metabolic pathway of SM.

The selenium concentration in plasma reflects short-term changes in the selenium concentration in tissues (Thomson et al., 2008). Zachara et al. (2004) previously demonstrated that the plasma of human whose diets were supplemented with Se at a greater concentration was likely to exhibit incorporation of selenomethionine in the albumen fraction. Payne et al. (2005a) reported that the low plasma Se concentration was independently associated with poor antioxidant levels due to the protection against oxidizing radiation of Se. The authors concluded that the plasma Se concentration was greater in the Se-supplemented groups compared with the control and the changes of SOD activity depicted related results. Thus, the effects of plasma selenium on the activity of SOD were determined and we could predict that high SOD activity may be a predictor of the high plasma Se concentration in laying hens.

Our study demonstrated that the organic sources of Se resulted higher bioavailability than the inorganic source using GSH–Px activity and Se content in albumen, breast, and leg muscle as indicators. Compared to organic Se, using the increase of muscle and albumen as indicator, the bioavailability of SM is higher than that of SY; using the activity of GSH–Px and SOD, MDA concentration, and liver, yolk, and plasma Se content as measurement criteria, the Se bioavailability of SM was equal to SY. Moreover, the study clearly demonstrates that the source and levels of Se, and sampling period has a large influence on the amount of Se deposited to the eggs.

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