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Interactions Between Different Selenium Compounds and Essential Trace Elements Involved in the Antioxidant System of Laying Hens

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Abstract

The purpose of this study was to investigate the interactions between different selenium (Se) compounds including sodium selenite (SS), selenium-enriched yeast (SY), and nano-selenium (NS) and various essential trace elements involved in the antioxidant systems, and to evaluate the effects on laying performance and egg quality. A total of 288 21-week-old Hyline Sophie hens were allotted to four dietary treatments: (1) basal diet without Se supplementation; (2) basal diet supplemented with 0.3 mg/kg Se of SS; (3) basal diet supplemented with 0.3 mg/kg Se of SY; (4) basal diet supplemented with 0.3 mg/kg Se of NS. Each treatment had eight replicates with nine hens per replicate. The trial lasted for 35 days. Results demonstrated that NS supplementation decreased the egg production (EP) and increased the feed conversion rate (FCR) and eggshell thickness and that SY changed the egg shape index ($p < 0.05$). Supplementation with three Se compounds significantly increased serum Se concentration and glutathione peroxidase (GSH-Px) activity in all treatment groups, as well as total superoxide dismutase (T-SOD) activity in the SY and NS groups. Yolk iron (Fe) and copper (Cu) concentrations in the NS group were also increased with Se supplementation. While the serum zinc (Zn) concentration decreased in the NS and SY groups, as well as the yolk manganese (Mn) concentration in the SY group. And the total antioxidant capability (T-AOC) of yolk with 3 days of storage in the SY and NS groups, malondialdehyde (MDA) value in the NS group, and the T-SOD activity and MDA value of yolk with 10 days of storage in the SY group also decreased. Thus, the source of Se compounds may influence the balance between Se and other trace elements including Zn, Mn, Fe, and Cu, which is important for proper antioxidant defense in blood and egg yolk of laying hens.

Keywords Antioxidant system · Selenium · Trace elements · Laying hens

Introduction

Essential trace elements are important minerals for the normal function of all basic biochemical processes; deficiency of which may lead to poor health and production [1]. Selenium (Se) is an essential trace element, and it exerts antioxidant defense, immune responses, resists the free radicals, and improves reproduction in humans and animals [2–7]. It was reviewed that the broiler chicks were susceptible to dietary Se deficiency [8], which could lead to oxidative injury and alteration in the immune organs of broiler chickens [9]. In animal diets, there are two Se sources: organic and inorganic Se; organic sources of Se include selenium-enriched yeast (SY), normally explored as an alternative to an inorganic source, and sodium selenite (SS) supplementation, because of its higher rates of absorption, tissues deposition, and antioxidant activities [2, 4, 10–12]. Nano-selenium (NS) appears to be more effective than other forms of Se at scavenging free radicals; it also has additional benefits such as low toxicity and acceptable bioavailability [13–16], in that NS exhibits high specific surface area, small particle size, and good intestinal absorption [17, 18]. Se bioavailability increased egg deposition when organic sources were compared with inorganic sources, and organic sources led to a higher deposition of Se in egg contents [19]. Diet supplemented with 0.30 mg/kg of nano-Se could effectively increase the growth performance of chickens and the Se concentrations of the organ [18]. And about 0.3 mg Se/kg diet was suggested to be the suitable level [20].

In the past years, the role of trace elements in animal health has been well established, and their presence in feed in

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adequate quantities has been taken for granted [1]. However, a mineral intake with a high level has interaction effects on other minerals [21], and previous studies demonstrate that a high level of calcium supplementation may cause or accentuate poor utilization of zinc (Zn) from soy products [22, 23]. Specially, it was reported that Se deficiency would cause an overload of iron (Fe) and unbalance in vivo distributions of other elements, such as copper (Cu) and Zn, and the interactions in vivo might occur by a mechanism other than a simple mutual interaction [24]. Egg is an important international food commodity, and China accounts for approximately a quarter of the world's egg production at roughly 39.68 billion pounds; female birds may deposit minerals into the egg [25, 26].

The main objective of the present study was to investigate the effects of supplemental Se (0.3 mg/kg) from different Se compounds (NS, SY, and SS) in laying hens on antioxidant enzyme activity and the interactions between Se and other essential trace elements involved in the antioxidant system.

Materials and Methods

Ethics Statement

Experiments were carried out in accordance with the ethical guidelines of Hunan Agricultural University for the care and use of laboratory animals.

Animals and Management

A total of 288 21-week-old Hyline Sophie laying hens were selected from a commercial flock (Huangpu District, Guangzhou City, China) and randomly divided into four groups with 72 hens each group in eight replicates. And four treatments were as follows: (1) The CON group received basal diet without selenium supplementation; (2) the SS group received diet supplementation with 0.3 mg kg^{-1} Se of SS; (3) the SY group received diet supplementation with 0.3 mg kg^{-1} Se of SY; (4) and the NS group received diet supplementation with 0.3 mg kg^{-1} Se of NS. The trial lasted for 35 days. Three birds were housed in a (38 × 35 × 35 cm) wire cage with three ladders, and then three wire cages formed an experimental unit that was randomly distributed in the shed. The light regimen was 16 h light: 8 h darkness and the birds were fed twice (05:30 AM and 13:30 PM) ad libitum and had free access to water during the experiment period. Feed intake was recorded daily, and the feed conversion rate and daily average feed intake per bird were calculated for a 5-week period. The number of eggs per replicate was counted, and the eggs were weighed daily; the incidences of soft-shelled and cracked eggs were also recorded.

Diets

All hens were fed corn-soybean-based diets in mash form. The corn-soybean basal diet was formulated to contain adequate nutrient concentrations as suggested in the NRC (1994) for laying hens except for Se (Table 1). All four groups of hens received the basal layer diet during the 1-week adaptation period. After the adaptation period, three treatments of birds received basal diet supplemented with 0.3 mg kg^{-1} Se from SS, NS, and SY (provided by Tanke Bio-tech Co., Ltd., 510890, Guangzhou), respectively.

Table 1 Composition of the basal diet for the feeding trial

Item	Ingredients (g/kg)	
Corn	600.00	
Soybean meal (43%)	220.00	
Wheat bran	60.00	
Limestone	79.80	
Dicalcium phosphate	13.10	
NaCl	3.00	
Zeolite powder	20.9	
DL-methionine	0.6	
Premix[1]	2.6	
Total	1000.00	
Nutrient and energy content (g/kg)[2]		
ME (kcal/kg)	3059.03	
Crude protein	156.6	
Calcium (Ca)	36.0	
Total phosphorus	4.9	
Available phosphorus	3.5	
Methionine	3.0	
Methionine + cystine	5.7	
Lysine	7.6	
Treatment	Se added (mg kg^{-1})	Se determined (mg kg^{-1})
CON group (basal diet with no Se)	0	0.03 ± 0.01
SS group (with sodium selenite)	0.3	0.32 ± 0.02
SY group (with selenium-enriched yeast)	0.3	0.34 ± 0.03
NS group (with nano-selenium)	0.3	0.32 ± 0.02

[1] Supplied per kilogram of diet: 12,000 IU of retinyl acetate; 3000 IU of 1,25-hydroxy-cholecalciferol; 30 mg of dl-a-tocopheryl acetate; 6 mg of menadione; 3 mg of thiamine propyl disulfide; 9 mg of riboflavin tetrabutyrate; 6 mg of pyridoxine; 0.03 mg of cobalamin; 0.15 mg of D-biotin; 18 mg of D-pantothenic acid; 1.5 mg of folic acid; 6 mg of nicotinamide; 18.15 mg of ethoxyquin; 50 mg of choline chloride; 10 mg of phytase; 0.004 mg of ubiquitin calcium; 5.12 mg of copper (Cu), copper glycine form; 72 mg of iron (Fe), iron glycine form; 60 mg of zinc (Zn), zinc methionine form; 84.8 mg of manganese (Mn), manganese glycine form; 0.64 mg of iodine (I); 0.32 mg of cobalt (Co); 0.27 mg cystine; 0.76 mg lysine; 0.58 mg threonine; 0.18 mg tryptophan. (Iron glycine form, zinc methionine form, manganese glycine form, and copper glycine form were provided by Tanke Bio-tech Co., Ltd., 510890, Guangzhou.)

[2] Calculated values

Sample Collection and Preparation

On the 35th day of the trial, a total 64 birds, 16 birds per treatment (2 per replicate), were randomly selected, and blood samples were collected from the main wing vein. Blood was centrifuged for 15 min at 3000×*g* under room temperature; then, serum was obtained and stored at − 20 °C for subsequent analysis. In addition, a total of 128 normal eggs were randomly selected ($n = 32$ eggs/treatment) to evaluate the egg quality traits and receive yolk. Yolk and albumen were separated manually by a plastic egg separator, and trace element analysis and antioxidant enzyme activity of yolk were analyzed.

Egg Quality Traits

Egg quality traits including egg weight, eggshell strength, albumen height, Haugh unit, and shell thickness were evaluated. The egg weight, albumen height, and Haugh unit were determined by an egg quality analyzer (EA-01, Orka Food Technology Ltd., Ramat HaSharon, Israel). The eggshell strength was determined by an egg force reader (Orka Food Technology Ltd., Ramat HaSharon, Israel). The shell thickness was determined by a shell thickness gauge (Orka Food Technology Ltd., Ramat HaSharon, Israel) at three different locations (bottom, middle, and top of the egg) [27].

Trace Element Concentration in Feed, Yolk, and Serum

Total Se concentration of the feed, yolk, and serum samples was determined by an atomic fluorescence spectrophotometer (AFS-2000, Beijing, China). 0.5 g of feed or yolk samples and 1 ml of serum samples were mineralized in 5 ml of HNO_3 in closed vessels in a microwave oven (Topex, Shanghai, China). The vessels were heated at 140 °C for 10 min, and this temperature was maintained for 8 min. After cooling, the solution was diluted with 2 ml of $HCLO_4$ in a 100-ml Erlenmeyer flask, and heated at 140 °C until the appearance of white fumes. After cooling, the digested sample was transferred to a cuvette and made up to a final volume of 25 ml with 15 ml HCL and ultrapure water; the supernatant was measured directly on the machine [11].

For serum and yolk sample dilution and preparation of standards, ultrapure water and ultrapure acids (HNO_3:$HClO_4$ = 4:1) were used. Samples were analyzed with preliminary treatment electric oven digestion; then, Fe, Zn, Mn, and Cu content in ICP-OES (iCAP 6000 series, Shanghai, China) was determined [28].

Measurement of Antioxidant Enzyme Activity in Yolk

Total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, total antioxidant capability (T-AOC), and malondialdehyde (MDA) content in the serum and yolk were determined by assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions [29].

Statistical Analysis

This experiment was performed at a completely randomized design. All data statistical analyses were performed using SPSS 17.0 software for Windows (Chicago, IL, USA). A multivariate linear model was used to analyze the data where Se, Zn, Cu, manganese (Mn), and Fe were the concentration variables, while treatments and tissues (blood and yolk) were the fixed factors, and the model [30] was

$$Y = \text{treatment tissue} + (\text{tissue} \times \text{treatment})$$

where Y denotes Se, Zn, Cu, Mn, and Fe concentrations.

The statements of significance presented in this study were based on $p < 0.05$. In the table, the data are showed as the mean ± standard error (SE) of each of the main effects.

Other data in this experiment was shown as mean ± standard error of mean (SEM). Significant differences among treatment means were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (SPSS 17.0). The differences were considered significant when $p < 0.05$. The data of laying performance and laying ratio is the average value of 5 weeks.

Results

Effects of Supplemental Se on Laying Performance and Egg Quality Traits

The laying performances of hens are presented in Table 2. Se supplementation significantly decreased the average egg production and increased feed conversion ratios ($p < 0.05$) in the NS group when compared with the CON group. The results of egg quality traits are shown in Table 3. Se supplementation significantly increased the eggshell thickness ($p < 0.05$) in the NS group compared with the CON group. When compared with the SS group, Se supplementation decreased significantly the egg shape index in the SY group ($p < 0.05$).

Effects of Supplemental Se on Interaction Between Se and Other Trace Elements

Supplementation of diet with 0.3 mg/kg of Se from different Se compounds significantly ($p < 0.001$) increased Se concentration (data pooled for deposition in serum and yolk effects), compared with the Se concentration of laying hens fed the diet without Se supplementation (CON group) (Table 4). Se

Table 2 Effects of different Se compound supplementation (0.3 mg Se/kg) sources on laying performances

Laying performance	Dietary treatment				SEM	*p* value
	CON	SS	SY	NS		
Feed intake (g/bird/day)	91.61 ± 1.61	90.48 ± 1.79	90.53 ± 1.42	89.74 ± 1.98	1.70	0.895
Egg production (%)	87.63 ± 1.49^{a}	84.01 ± 2.71^{ab}	82.86 ± 1.30^{ab}	78.88 ± 3.15^{b}	2.16	0.012
Feed conversion ratio (g of feed/g of egg)	2.03 ± 0.03^{b}	2.09 ± 0.06^{ab}	2.13 ± 0.04^{ab}	2.26 ± 0.06^{a}	0.05	0.031
Egg mass (g of egg/bird/day)	52.00 ± 0.56	52.27 ± 0.32	51.78 ± 0.41	52.16 ± 0.45	0.44	0.872

Data are means of eight replicates of nine hens per dietary treatment. Values in the same row with different superscripts are significantly different ($p < 0.05$) by one-way ANOVA

CON control (no selenium supplementation); *SS* sodium selenite; *SY* selenium-enriched yeast; *NS* nano-Se

concentration was affected significantly ($p < 0.001$, data pooled for treatment to exacerbate deposition effects; Table 4). Se concentration in descending order was egg > blood, and this pattern was marked for all treatments as indicated by the significant treatment × tissue interactions ($p < 0.001$).

Zn concentration was not affected by different Se compound addition to laying hens' diets (Table 4). However, Zn concentration was 4.48 times greater in yolk than its concentration in serum ($p < 0.001$). Furthermore, the treatment × tissue interactions ($p = 0.040$) indicated that different Se compound supplementation had significant effects on Zn concentration. In detail, Zn concentration in serum of laying hens fed diets SY (5.78 ± 0.31 mg kg^{-1}) and NS (6.39 ± 0.46 mg kg^{-1}) was lower than that of laying hens fed the diet without Se supplementation (11.58 ± 0.86 mg kg^{-1}).

Different Se compound supplementation had not affected the concentration of Fe (Table 4). Fe concentration of the SS, SY, and NS groups was increased by 8.7, 11.1, and 15.1%, respectively, compared with the concentration of the CON group ($p = 0.125$). However, Fe concentration was significantly affected ($p < 0.001$), and Fe concentration in yolk was 4.1 times greater than that in serum ($p < 0.001$). In detail, Fe concentration in yolk of laying hens fed diet NS (61.94 ± 2.11 mg kg^{-1}) was higher than that of laying hens fed the diet without Se supplementation (52.99 ± 3.04 mg kg^{-1}).

Mn concentration was not affected by different Se compound addition (Table 4). Yolk contained higher ($p < 0.001$) concentration of Mn compared with serum. Furthermore, the treatment × tissue interactions ($p = 0.011$) indicated that different Se compound supplementation had significant effects on Mn concentration. The present study revealed significant interactions between treatment and tissue regarding Mn accumulation. In detail, Mn concentration in yolk of laying hens fed diet SY (0.82 ± 0.16 mg kg^{-1}) was lower than that of laying hens fed the diet without Se supplementation (1.16 ± 0.11 mg kg^{-1}).

Copper concentration was not affected by different Se compound addition (Table 4). Yolk contained higher ($p < 0.001$) concentration of Cu compared with serum. In detail, Cu concentration in yolk of laying hens fed diet NS (1.70 ± 0.05 mg kg^{-1}) was higher than that of laying hens fed the diet without Se supplementation (1.53 ± 0.06 mg kg^{-1}).

Table 3 Effects of different Se compound supplementation (0.3 mg Se/kg) sources on egg quality traits

Egg quality	Dietary treatment				SEM	*p* value
	CON	SS	SY	NS		
Egg weight (g)	53.18 ± 1.09	54.00 ± 0.77	53.06 ± 0.66	54.11 ± 0.80	0.83	0.743
Egg shape index	1.30 ± 0.01^{ab}	1.31 ± 0.01^{a}	1.28 ± 0.00^{b}	1.29 ± 0.01^{ab}	0.01	0.023
Albumen height (mm)	6.15 ± 0.51	6.04 ± 0.23	6.86 ± 0.27	6.74 ± 0.13	0.29	0.194
Yolk color	5.41 ± 0.57	5.54 ± 0.23	6.16 ± 0.34	5.04 ± 0.41	0.39	0.283
Haugh unit (HU)	79.41 ± 3.67	79.11 ± 1.61	83.90 ± 0.94	84.61 ± 1.48	1.93	0.178
Eggshell strength (kg)	3.77 ± 0.19	3.73 ± 0.12	3.79 ± 0.09	3.85 ± 0.18	0.14	0.955
Eggshell thickness (mm)	0.34 ± 0.00^{b}	0.35 ± 0.01^{ab}	0.34 ± 0.01^{b}	0.37 ± 0.01^{a}	0.01	0.022

Data are means of eight replicates of nine hens per dietary treatment. Values in the same row with different superscripts are significantly different ($p < 0.05$) by one-way ANOVA

CON control (no selenium supplementation); *SS* sodium selenite; *SY* selenium-enriched yeast; *NS* nano-Se

Table 4 Effects of different Se compound supplementation (0.3 mg Se/kg) sources on serum and yolk mineral concentration

Factor studied	Trace element (mg/L)				
	Se	Zn	Fe	Mn	Cu
Treatment					
CON group	0.10 ± 0.01^{b}	29.72 ± 1.28	31.78 ± 1.44^{b}	0.72 ± 0.06^{ab}	1.04 ± 0.03
SS group	0.34 ± 0.01^{a}	28.76 ± 1.45	34.56 ± 1.64^{ab}	0.75 ± 0.07^{ab}	1.05 ± 0.04
SY group	0.35 ± 0.01^{a}	28.27 ± 1.28	35.30 ± 1.44^{ab}	0.60 ± 0.06^{b}	1.05 ± 0.03
NS group	0.35 ± 0.01^{a}	26.79 ± 1.28	36.59 ± 1.44^{a}	0.79 ± 0.06^{a}	1.12 ± 0.03
Tissue					
Serum	0.13 ± 0.01^{b}	8.76 ± 0.97^{b}	11.33 ± 1.09^{b}	0.31 ± 0.05^{b}	0.54 ± 0.03^{b}
Yolk	0.44 ± 0.01^{a}	48.01 ± 0.90^{a}	57.78 ± 1.01^{a}	1.12 ± 0.04^{a}	1.59 ± 0.02^{a}
Source of variation	p value				
Treatment	< 0.001	NS ($p = 0.442$)	NS ($p = 0.125$)	NS ($p = 0.167$)	NS ($p = 0.361$)
Tissue	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment × tissue	< 0.001	0.040	NS ($p = 0.253$)	0.011	NS ($p = 0.60$)

Data are means of eight replicates of nine hens per dietary treatment. Values in the same row with different superscripts are significantly different ($p < 0.05$) by one-way ANOVA

Effects of Supplemental Se on Antioxidant Capacity in Serum and Yolk

The effects of dietary supplementation with different Se compounds on antioxidant enzyme activity in serum were shown in Table 5. The serum T-SOD activity in the NS and SY groups was significantly higher than that in the CON group ($p < 0.05$). Meanwhile, SS, SY, and NS supplementation all improved the GSH-Px activity in serum of the hens compared with the CON group ($p < 0.05$).

The effects of dietary supplementation with different Se compounds on antioxidant enzyme activity in yolk were shown in Table 6. After 3 days in the storage at room temperature, the yolk T-AOC values decreased significantly in the SY and NS group in comparison with CON group, as well as the yolk MDA value in NS group significantly ($p < 0.05$). After 10 days in the storage at room temperature, the yolk T-SOD activity and MDA values decreased significantly in the SY group in comparison with the CON group ($p < 0.05$).

Discussion

Some previous studies show that dietary supplementation with different levels and sources of Se (from 0.18 to 0.51 mg/kg of SY, Se-enriched kale sprouts, or other organic Se source) has no effect on egg production or feed intake [2, 31, 32–36], but in our present study, NS supplementation decreased the EP and increased FCR; a potential reason for such result may be due to higher bioavailability but low metabolism requirements for Se in poultry. Meanwhile, NS supplementation increased the eggshell thickness, because during the process of eggshell formation, sources of mineral nutrients play an important role in maintaining eggshell quality [37]; it was speculated that NS supplementation may help improve eggshell quality via interactions with other mineral nutrients.

An interaction exists among different ions, and changes in the ion concentration may play some roles in the regulation of physiological processes [38]. Optimal nutrition guarantees proper functions of the organism, among which the

Table 5 Effects of different Se compound supplementation (0.3 mg Se/kg) sources on serum antioxidant enzyme activity

Item (U/ml)	Dietary treatment				SEM	p value
	Con	SS	SY	NS		
GSH-Px	188.72 ± 7.18^{c}	3773.79 ± 264.64^{a}	3809.07 ± 254.86^{a}	2873.60 ± 97.30^{b}	205.60	0.000
T-AOC	8.89 ± 0.41	6.64 ± 0.71	6.77 ± 0.67	8.57 ± 0.96	0.69	0.066
T-SOD	223.50 ± 3.03^{b}	218.23 ± 3.71^{b}	244.24 ± 3.48^{a}	241.94 ± 4.55^{a}	3.69	0.000
MDA	5.00 ± 0.69	4.14 ± 0.42	4.66 ± 0.87	4.62 ± 0.57	0.63	0.832

Data are means of eight replicates of nine hens per dietary treatment. Values in the same row with different superscripts are significantly different ($p < 0.05$) by one-way ANOVA

CON control (no selenium supplementation); *SS* sodium selenite; *SY* selenium-enriched yeast; *NS* nano-Se

Table 6 Effects of different Se compound supplementation (0.3 mg Se/kg) sources on antioxidant enzyme activity in yolk

Item (U/ml)	Dietary treatment				SEM	*p* value
	CON	SS	SY	NS		
Yolk-3d						
T-AOC	92.55 ± 13.41^{a}	109.42 ± 14.66^{a}	40.92 ± 4.15^{b}	56.90 ± 5.80^{b}	9.50	0.000
T-SOD	135.23 ± 7.14^{ab}	111.41 ± 10.82^{b}	159.48 ± 7.61^{a}	140.96 ± 11.85^{a}	9.36	0.013
MDA	32.62 ± 3.53^{a}	25.96 ± 2.68^{ab}	30.41 ± 3.14^{ab}	24.09 ± 1.91^{b}	2.81	0.045
Yolk-10d						
T-AOC	4.27 ± 0.74	3.60 ± 0.71	3.86 ± 0.88	4.94 ± 0.95	0.82	0.689
T-SOD	86.05 ± 3.07^{a}	85.36 ± 1.98^{a}	65.94 ± 7.79^{b}	82.43 ± 6.03^{ab}	4.72	0.037
MDA	71.96 ± 3.40^{a}	77.63 ± 3.33^{a}	53.43 ± 6.12^{b}	70.58 ± 6.55^{a}	4.60	0.007

Data are means of eight replicates of nine hens per dietary treatment. Values in the same row with different superscripts are significantly different ($p < 0.05$) by one-way ANOVA

CON control (no selenium supplementation); *SS* sodium selenite; *SY* selenium-enriched yeast; *NS* nano-Se

most important are structural, physiological, catalytic, and regulatory [39]. In chicken muscles, Mn and Cu are significantly influenced by Se deficiency, and an imbalance in the level of Cu and Mn influences the activity of enzymes [40]. It is reported that Se in the body is first to supply the tissues and organs that need it and is distributed in various tissues and organs through blood circulation [41]. This study revealed that Se compound supplementation in laying hens' diet increased the Se concentration of serum and egg yolk. Eggs have been very useful in studying the absorption of various Se compounds, and egg Se concentration is significantly affected by the dosage level and source of Se [10, 11, 34, 35]. In the present study, Se supplementation with SY or NS or SS increased Se concentrations in egg yolk which is consistent with the result in the published literature [10, 19, 33–35]. Furthermore, different Se compounds affected other trace elements' retention in egg yolk, whereas in the present work, supplemental SY decreased Mn concentration and NS increased Fe and Cu concentration, which was speculated that trace elements might interact with each other at the levels of absorption, distribution, and retention [30]. It is reported that there may be an antagonistic effect between Se (sodium selenite) and Mn, Cu, and other elements in chicken muscles [40], and Se (sodium selenite) supplementation increases some essential microelements, such as Zn in the chicken liver [39]. Se (sodium selenite) also reduces cadmium (Cd) accumulation and antagonizes Cd-triggered apoptosis in the chicken pancreas, and Se has interactions with heavy metal, which exerts its toxicity via changing the ion homeostasis in animal organisms [42]. It was suggested that the complex interactions might occur between Se and other elements in laying hens' blood and egg, not only synergistic and antagonistic effects but also some complicated modulating effects among these ions. And different Se compounds may be related to these changing ion profiles in laying hens.

In addition, Mn, as an essential trace element, is a crucial component of the metalloenzyme, Mn superoxide dismutase (MnSOD), which is an antioxidant enzyme for detoxification of reactive oxygen species (ROS) [43]. Cu and Zn are also involved in the metabolism of oxygen and the biochemistry of redox reactions included in enzyme CuZn superoxide dismutase (SOD), which catalyzes the dismutation of superoxide, and Se was positively correlated with Zn, Cu, and Fe [24]. And Fe is an essential micronutrient for various physiological functions including energy metabolism, electron transfer and oxygen transport, and redox reactions, but excessive storage and intake of Fe cause various diseases [44–46]. Subsequently, the trace elements' retention in egg yolk in the present study may affect the egg yolk antioxidant status, which may be related to the egg quality.

Se has antioxidant effects because it forms selenocysteine, a part of the active center of glutathione peroxidase (GSH-Px), which has an antioxidative action by catalyzing the reduction of hydrogen peroxide and lipid peroxides [9, 47, 48]. It has been reported that inorganic, organic, or nano-Se affects the GSH-Px activity previously. Broilers supplemented with 0.3 mg/kg of NS increased the GSH-Px activity compared with the control group without supplementation [15]. And Jing et al. reported that the organic sources (SY and Se-Met) exhibited a greater ability to increase the GSH-Px activity than the SS that was added at 0.3 mg Se kg^{-1} [49]. Previous work also suggests that there was significantly higher serum GSH-Px activity in NS-supplemented groups of broilers [47], similarly in laying hens [50]. Consistent with this, the present study showed that serum GSH-Px activities were all increased in the Se compound supplementation treatments. However, in contrast to the previous result that total superoxide dismutase activity in serum of broilers supplemented with 0.3 mg/kg was not affected by nano-Se levels [15], in the present study, dismutase activity in the SY or NS group was increased. A potential reason for such discrepancies may be due to differences

in the length and process control of the experiments. Many ions are involved in important biological processes, and normal biochemical function in animals requires the proper amount of minerals, and Se could alleviate ion metabolism to some extent [5]. In this study, the alteration of serum Zn balance both in the SY and NS groups may concomitantly influence the antioxidant defense systems due to the positive Se-Zn correlation attributed to metallothionein (MT) [30]. Therefore, SY or NS supplementation may improve the T-SOD activity of serum in laying hens to ensure laying performance.

In the present study, the SY or NS supplementation increased the total antioxidant capacity in yolk of stored eggs at room temperature for 3 days, and it was speculated that SY and NS might improve egg quality during storage. Egg yolk is a natural supramolecular assembly of proteins and lipids with different organization levels [51]. The increase of Se content in broiler feed increases linolenic acid in thigh muscle tissue of broilers [52]. And Se sources (SS or SY) decrease the MDA content of chicken breast meat [53]. The NS supplementation affected the reduction of MDA content in yolk of stored eggs at room temperature for 3 days, and SY supplementation affected MDA content in yolk of stored eggs at room temperature for 10 days. MDA is the product of lipid peroxidation, and an increasing MDA level is an index of enhanced lipid peroxidation [5, 54]. It was speculated that supplementation SY or NS might be beneficial for egg yolk lipid retention, and a higher concentration of organic selenium in eggs may be a factor that positively affected the indicators of egg freshness [55]. However, further studies should be carried out to investigate the underlying mechanism on how other nutrients such as Se, Cu, Zn, and Mn metabolize and how they are related to the layer's oxidative status.

Conclusions

In conclusion, the supplementation of NS with 0.3 mg kg^{-1} decreased the EP and increased FCR and eggshell thickness. And serum Zn concentration was decreased in both the SY and NS groups; the egg yolk Mn retention was also decreased in the SY group, whereas Fe and Cu concentrations were increased in the yolk of the NS group. The supplementation of SY and NS trended to increase the serum GSH-Px and T-SOD activities. It demonstrated that egg production, egg quality, serum trace mineral concentration, antioxidative capacity, egg yolk trace mineral concentration, and antioxidant status were affected by different Se compounds. And there may be existing complex interactions between different Se compounds and other elements in laying hens, including synergistic, antagonistic, and modulating effects among these ions; thus, the trace elements' retention in eggs affected the egg antioxidant status. Based on these findings, it was speculated that the source of Se compounds may play important and complex roles in the alteration of the balance and correlation to other trace elements, such as Zn, Mn, Fe, and Cu, and may then affect the antioxidant defense system in blood and egg of laying hens, which provides a reference for choosing a suitable Se source.

Authors' Contributions Yurong Zhao and Jiahua He designed the experiment; Xue Lin analyzed the data; and Xue Lin, Ting Yang, and Yinli Ji performed the experiments. Xue Lin wrote the manuscript, and Yurong Zhao, Jianhua He, and Hua Li revised it. Xue Lin, Ting Yang, and Yinli Ji contributed to data collection and analysis. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Experiments were carried out in accordance with the ethical guidelines of Hunan Agricultural University for the care and use of laboratory animals.

Competing Interests The authors declare that they have no conflict of interest.

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