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Effect of Selenium Source and Level in Hen's Diet on Tissue Selenium Deposition and Egg Selenium Concentrations

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The present study was conducted in a 2 × 4 factorial arrangement in a randomized complete block (RCB) design to compare the effects of a commercial inorganic Se source (sodium selenite, SS) with a commercial organic Se source (Se-enriched yeast, SY) on tissue Se distribution and blood and whole-egg Se concentrations in laying hens. Both Se sources were added into the basal diet at 0, 0.2, 0.5, and 1.0 mg/kg of Se. Seven hundred 68 week old Rohman laying hens were fed with a basal diet containing 0.15 mg/kg DM (dry matter) of Se for 2 weeks, and then, they were allocated randomly into seven groups and were investigated for 28 days. Each group was replicated five times with five cages of four hens per cage in each replicate. During the experiment, two eggs per replicate from each treatment were collected every 7 days and blood was sampled on days 0, 14, and 28 for whole-egg and whole-blood Se analyses. At the end of the experiment, two hens per replicate from each treatment were slaughtered, and muscle (cardiac and breast muscles), liver, spleen, and kidney were sampled for the determination of Se concentrations. The results showed that the addition of Se from either source caused a significant increase in whole-egg and whole-blood Se concentrations ($p < 0.01$) and Se concentrations in liver, kidney, spleen, and cardiac and breast muscles ($p < 0.05$) of hens in comparison to the control. Both Se sources and Se levels significantly influenced ($p < 0.01$) Se concentrations in egg, blood, and the above-mentioned tissues. There was a more significant increase in the Se concentrations in egg ($p < 0.01$), spleen ($p < 0.05$), and breast muscle ($p < 0.01$) and a decrease ($p < 0.01$) in whole-blood and kidney from hens fed SY than those from hens fed SS. The order of Se distribution was liver > kidney > spleen > cardiac muscle > egg > blood > breast muscle, irrespective of the addition level or source. It was concluded that meat and eggs from hens fed commercial SY are a potential source of Se for humans.

KEYWORDS: Selenium-enriched yeast; sodium selenite; selenium deposition; hens; eggs

INTRODUCTION

Selenium (Se) is an essential trace element for animal and human health. The importance of Se to human health has become a focus in recent years (*1*). Selenium deficiency in food can lead to Ke-shan disease, Kaschin-beck disease (*2*, *3*), hypothyroidism, and a weakened immune system (*4*). In the past decade, it has become more evident that Se has many potential health benefits beyond meeting basic nutritional requirements.

Although rare in most countries, selenium deficiency is common in China, due to low Se levels in the soil (*5*). Selenium enters the food chain through plants, which take it up from the soil (*1*). An alternative or complementary approach is to enhance the Se concentration of carcass meat and eggs by appropriate Se supplementation of animals. Thus, there are dual benefits from the Se supplementation of animals, namely, to improve the health and performance of animals and to influence the quality of a product (meat, milk, eggs, etc.), in a controlled way, to improve the Se status of humans.

In recent years, considerable attention has been given to comparing supplementation of organic and inorganic Se. Organic Se from plants and Se-enriched yeast (SY) possess antioxidant properties and have higher bioavailability and rates of tissue accumulation as well as lower toxicities as compared to inorganic Se from sodium selenite (SS) (*6*). Publications on the effects of inorganic Se (SS) vs organic Se (SY) on egg selenium concentration in laying hens have consistent results. Egg Se concentration is increased by SS and SY supplementation as dietary levels increase, but the SY supplementation is more potent to increase egg Se than SS (*7*−*10*).

The soil of most areas in China has severely low Se levels. It is common practice in the poultry industry in China to supplement Se in laying hen diets. Traditionally, the Se source is SS, despite the above-mentioned fact that organic Se has some advantages over inorganic Se (*6*). Currently, organic Se supplementation is advocated and approved by some nutritionists and/or researchers (*11*). Despite the extensive research that has

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Table 1. Ingredients and Nutrient Levels of Basal Diet

ingredients	%	nutrient levels	
corn	65.0	ME (MJ/kg)	11.59
soybean meal	9.0	crude protein (%)	15.40
bran	2.0	crude fiber (%)	3.60
clover	5.6	lysine (%)	0.75
fishmeal (55.5% CP)	8.0	methionine (%)	0.31
bone powder	1.0	cystine (%)	0.24
salt	0.35	calcium (%)	3.54
vitamins[a]	0.05	phosphorus (%)	0.74
trace minerals[b]	1.0	Se (mg/kg)	0.15
limestone	8.0		

[a] The vitamins provided per kg diet: vitamin A, 12000 IU; vitamin D_3, 3000 IU; vitamin E, 20 IU; menadione, 2 mg; vitamin B_{12}, 0.015 mg; thiamin, 1.5 mg; riboflavin, 4.5 mg; biotin, 0.1 mg; folacin, 0.5 mg; niacin, 20 mg; pantothenic acid, 10.0 mg; and pyridoxine, 3.0 mg. [b] The trace minerals provided per kg diet: manganese, 60.0 mg; zinc, 50.0 mg; iron, 25.00 mg; copper, 5.0 mg; and iodine, 0.5 mg.

been conducted comparing Se from SS with Se from SY, there have been few papers published about the effect of SY supplementation on accumulation and distribution of Se in multitissues or organs of laying hens. On the other hand, in autumn and winter, Chinese people usually have a habit of stewing laying hen meat and viscera as tonic foods. Obviously, it is important to know the accumulation and distribution of Se in tissues of laying hens fed with commercial SY supplementation. Therefore, the main purpose of this trial was to compare Se contents in whole egg, whole blood, muscle (cardiac and breast muscles), liver, spleen, and kidney when hens were fed diets supplemented with various levels of SS or SY over a 28 day period.

MATERIALS AND METHODS

Experiment Animals and Treatment. Seven hundred 68 week old Rohman laying hens, with an egg rate of 85%, were randomly distributed to seven treatments of 100 hens each and housed in wire cages. Chipboards were inserted into the feeders between the different treatments' cages to prevent hens in one treatment from eating another treatment's diet. Each cage was equipped with a nest, perch, and two fresh water nipple drinkers. Each treatment was replicated five times with five cages of four hens per cage in each replicate. A corn−soybean meal basal diet (**Table 1**) was formulated to meet the recommendations for laying hens of the National Research Council (*12*) with regard to the requirements of all nutrients except Se. Dietary Se addition was based on calculated levels for each source. Selenium from SS (analytical grade, 45.5% Se content, Tianjin Chemical Reagent Co. Ltd., China) and SY (Sel-Plex, 1000 μg/g Se content, Alltech, Inc., Nicholasville, KY) was added at 0.2, 0.5, and 1.0 mg/kg into the basal diet to make each treatment, respectively. The basal diet without Se supplementation served as the control. Before the start of the experiment, all hens were fed a basal diet for 2 weeks. During the 28 day experiment, feed and water were provided ad libitum. The experiment was conducted in the spring (from April to May).

Sample Collection and Preparation. Two eggs per replicate from each treatment were collected on days 0, 7, 14, 21, and 28 at random and stored at 4 °C until analysis. Each individual egg sample was weighed and broken. The shell and shell membrane were discarded. Each liquid egg was homogenized with an electric blender at chilled conditions and collected into chilled 10 mL plastic tubes for the determination of Se. Blood samples of two hens per replicate from each treatment were collected on days 0, 14, and 28. Five milliliters of blood was drawn from the main wing vein from each hen, collected into chilled heparinized tubes, and stored at −20 °C until analysis between 09:00 and 10:00 h. Before blood sampling, the hens were not fed. At the end of the experiment, two hens per replicate from each treatment were slaughtered at random. Samples of about 10 g of breast muscle, 10 g of cardiac muscle, and whole liver, spleen, and kidney from each hen were collected and stored at −20 °C until analysis. For each individual hen sample, aliquots (2.0 g wet weight basis) for blood and the above-mentioned tissues were weighed and homogenized by the same method as the liquid eggs, respectively. All samples were marked with their treatments, replicate cage number, and sampling date.

Selenium Assay. A AF-610A atomic fluorescence (AF) spectrometer (Ruili Analysis Instrument Co., Beijing), with an electrically ignited concentric quartz tube atomizer, was employed for the Se assay. All glassware was cleaned prior to use by washing with soap and water and then soaking in 10% (v/v) nitric acid overnight. They were then rinsed with 2% (v/v) nitric acid, triple-rinsed with ultrapure water (resistance 18 MΩ cm), and allowed to dry.

All reagents used were of analytical grade or higher. Perchloric acid ($HClO_4$), nitric acid (HNO_3), hydrochloric acid (HCl), sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH), and potassium ferricyanide [$k_3Fe(CN)_6$] were purchased from Sinopharm Chemical Reagent Co. Ltd. (SCRC) (Shanghai, China). Selenium stock standard solutions of SS [GBW(E)080215] were provided by the National Research Center for Standard Materials (Beijing, China).

The Se assay for the hen samples was performed by hydride generation atomic fluorescence spectrometry (HG-AFS) (*13*). Briefly, the homogenized sample aliquots (1.0 g wet weight basis) for blood, eggs, and tissues were weighed accurately and dissolved with 2 mL of HNO_3 and transferred to a Kjeldahl flask. After 1 h of predigestion at room temperature, 2 mL of $HClO_4$ was added and heated on the galvanothermy board until the appearance of white fumes. Then, the samples were evaporated almost to dryness, transferred to a volumetric flask, and made up to a final volume of 25 mL with ultrapure water. At the same time, ultrapure water and a certified reference material for Se, GBW 08551 pork liver (Food Detection Science Institute, Ministry of Commerce, Beijing, China), digested by the same method, served as the blank and the standard control, respectively. Before the samples were injected into the HCl carrier, 2 mL of the prepared sample was transferred to volumetric tube with 1 mL of 10% $k_3Fe(CN)_6$ and made up to a final volume of 25 mL with 3 mol/L HCl. Then, the treated sample was injected into the HCl carrier. After it was merged with the $NaBH_4$ stream, the volatile hydride was formed and swept out of the gas−liquid separator by an argon stream into a chemically generated hydrogen diffusion flame. The flame was maintained by the excess of hydrogen produced in the reaction between $NaBH_4$ and HCl. The hydride was then atomized in the flame, and the atoms were detected by fluorescence spectrometry.

Under the optimal concentrations of HCl (3 mol/L) and $NaBH_4$ (2%, w/v, dissolved by 0.5% NaOH solutions) and the flow rate (800 mL/min) of argon, the quantitative determinations were performed with plotting calibration curves by Se standard solutions of SS. The regression equations for the calibrated curves were constructed for Se analysis, $y = 9.49x - 1.37$, and a relative coefficient of 0.9999 was obtained. The detection limits were 0.7 ng/mL for Se, while the relative standard deviations of 12 replicate determinations were 2.1% for Se. In addition, the diet samples for different treatments were analyzed for Se concentration with the same method.

Statistical Analyses. The data were analyzed using the one-way analysis of variance (ANOVA) to compare means and the multivariate of general linear model (GLM) procedure of the SPSS 13.0 for Windows. All data were expressed as means ± standard error (SE). Treatment contrasts for Se sources and levels including the non-Se-fortified treatment group were evaluated with difference analysis using one-way ANOVA. The Post Hoc and Option of multivariate of GLM were conducted to examine the main effects of three factors (Se source, Se level, and/or the length of experiment) and interaction effects between the factors on Se concentrations in blood and eggs and tissue Se deposition. The least significant difference (LSD) test of multiple comparisons was used to determine differences between means. Differences were considered as significant at $P < 0.05$ for all tests used.

RESULTS AND DISCUSSION

Actual Se Level in Diet of Each Treatment. The Se content of each treatment is reported in **Table 2**. The result showed

Table 2. Se Sources and Levels Supplemented in Diets

		Se level (mg/kg)		
treatment	Se source	supplemental	calculated	actual
1		0.0		0.150 ± 0.010
2	SS	0.2	0.35	0.347 ± 0.009
3	SS	0.5	0.65	0.652 ± 0.018
4	SS	1.0	1.15	1.157 ± 0.008
5	SY	0.2	0.35	0.353 ± 0.007
6	SY	0.5	0.65	0.648 ± 0.012
7	SY	1.0	1.15	1.145 ± 0.016

that calculated Se levels in the experiment were confirmed by analysis. Hence, the results of the present study were not affected by differences between actual Se levels and calculated Se levels.

Whole-Egg Se Concentrations. Egg Se concentrations of each treatment are presented in **Table 3**. The statistics results are as follows: Se source effect, $p < 0.01$; Se level effect, $p < 0.01$; length of experiment effect, $p < 0.01$; Se source × level effect, $p < 0.01$; Se source × length of experiment effect, $p < 0.01$; Se level × length of experiment effect, $p < 0.01$; and Se source × level × length of experiment effect, $p < 0.01$.

As compared with the control, Se supplementation from either Se source significantly increased ($p < 0.01$) Se concentrations in eggs, which is consistent with the results reported by Cantor et al. (*14*), Latshaw et al. (*15*), and Hassan (*16*). Irrespective of the supplemental Se level, Se concentrations were significantly higher ($p < 0.01$) in eggs from hens fed SY than eggs from hens fed SS (**Figure 1**). The results are in agreement with the results reported by Cantor et al. (*7*), Paton et al. (*8*), Utterback et al. (*9*), and Payne et al. (*10*). Irrespective of the supplemental Se source, egg Se concentrations were also gradually increased with an increase of the supplemental Se level. Paton et al. (*8*), Payne et al. (*10*), and Athanasios et al. (*17*) also reported that egg Se concentrations increased as the dietary Se level increased.

In addition, egg Se concentrations of different Se treatments on day 28 in the present experiment were significantly higher ($p < 0.05$) than those of corresponding treatments on days 7, 14, and 21. The results suggested that dietary Se was gradually transferred into eggs with the extension of experiment duration. Echevarria et al. (*18*) reported that Se concentrations in tissues of sheep increased ($p < 0.01$) as time advanced. Our results in hens were similar to the results in other animals.

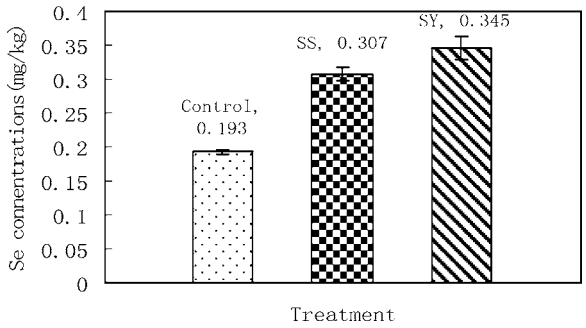

Figure 1. Effect of Se source in hen diets on the overall average egg Se concentrations (mg/kg, wet weight basis). Columns with different designs are significantly different ($p < 0.05$). The value above the column is the overall average egg Se concentrations regardless of Se level, calculated from the egg Se concentrations for each collection period for each source through the 28 day experiment. Egg Se concentrations data are means of five replicates, two eggs per replicate for each collection period.

Whole-Blood Se Concentrations. The factors including source ($p < 0.01$), level ($p < 0.01$), length of experiment ($p < 0.01$), source × level ($p < 0.01$), and level × length of experiment ($p < 0.05$) had a significant effect on whole-blood Se concentrations (**Table 4**). Selenium supplementation from either Se source significantly increased ($p < 0.01$) the average Se concentrations in whole-blood as dietary Se levels increased. Our results in hens are similar to the results in cows and pigs reported by other researchers. Ortman et al. (*19*) reported that Se concentration of whole blood in cow increased ($p < 0.05$) in all of the supplemented groups during the period of supplementation. Gunter et al. (*20*) reported that cows supplemented with Se had greater ($p < 0.01$) whole-blood Se concentrations. Kim et al. (*21*) reported that plasma and blood cell Se in growing−finishing pigs increased ($p < 0.01$) at each period as dietary Se levels increased.

Irrespective of the supplemental Se level, SS significantly increased ($p < 0.01$) the average Se concentrations in whole blood in comparison with SY (**Figure 2**). At each supplemental Se level, Se concentrations in blood from hens fed SS also were higher than those of hens fed SY. The present results in hens disagree with those of Ortman et al. (*19*), Gunter et al. (*20*),

Table 3. Effect of Se Source and Level in Hen Diets on the Egg Se Concentrations[a] (mg/kg, Wet Weight Basis)[b]

			on days of Se treatment				
treatment	Se source	supplemental Se level (mg/kg)	7	14	21	28	average[c]
1		0.0	0.187 ± 0.009 a	0.194 ± 0.005 a	0.201 ± 0.005 a	0.192 ± 0.005 a	0.193 ± 0.003 a
2	SS	0.2	0.213 ± 0.014 a	0.219 ± 0.011 a	0.233 ± 0.014 ab	0.337 ± 0.007 ef	0.256 ± 0.014 b
3	SS	0.5	0.260 ± 0.003 cd	0.262 ± 0.005 bcd	0.291 ± 0.011 d	0.354 ± 0.009 f	0.295 ± 0.010 bd
4	SS	1.0	0.316 ± 0.017 e	0.337 ± 0.009 ef	0.373 ± 0.019 f	0.439 ± 0.024 g	0.371 ± 0.015 c
5	SY	0.2	0.185 ± 0.009 a	0.232 ± 0.005 ab	0.275 ± 0.013 cd	0.358 ± 0.017 f	0.268 ± 0.018 b
6	SY	0.5	0.187 ± 0.013 a	0.305 ± 0.016 e	0.349 ± 0.008 f	0.445 ± 0.030 g	0.329 ± 0.025 cd
7	SY	1.0	0.307 ± 0.008 e	0.363 ± 0.008 f	0.466 ± 0.012 g	0.578 ± 0.026 h	0.437 ± 0.028 e

Se source	$P = 0.000$
Se level	$P = 0.002$
the length of experiment	$P = 0.000$
Se source × Se level	$P = 0.000$
Se source × the length of experiment	$P = 0.000$
Se level × the length of experiment	$P = 0.000$
Se source × Se level × the length of experiment	$P = 0.010$

[a] Egg Se concentrations are means of five replicates, two eggs per replicate for each collection period. [b] Means within a column with different letters are significantly different ($p < 0.05$). [c] The value in this column is the average egg Se concentrations calculated from the egg Se concentrations for each collection period for each treatment through the 28 day experiment. Egg Se concentrations data are means of five replicates, two eggs per replicate.

Table 4. Effect of Se Source and Level in Hen Diets on Whole-Blood Se Concentrations[a] of Hen (mg/kg, Wet Weight Basis)[b]

treatment	Se source	supplemental Se level (mg/kg)	on days of Se treatment 14	on days of Se treatment 28	average[c]
1	0	0.0	0.131 ± 0.005 a	0.148 ± 0.013 a	0.139 ± 0.007 a
2	SS	0.2	0.185 ± 0.005 b	0.221 ± 0.014 c	0.203 ± 0.010 b
3	SS	0.5	0.218 ± 0.011 c	0.268 ± 0.006 d	0.243 ± 0.011 c
4	SS	1.0	0.262 ± 0.013d	0.339 ± 0.018 e	0.300 ± 0.018 d
5	SY	0.2	0.162 ± 0.007 b	0.182 ± 0.005 b	0.172 ± 0.005 b
6	SY	0.5	0.182 ± 0.009 b	0.223 ± 0.016 c	0.202 ± 0.012 b
7	SY	1.0	0.216 ± 0.070 c	0.274 ± 0.010 d	0.245 ± 0.012 c

Se source	$P = 0.000$
Se level	$P = 0.000$
the length of experiment	$P = 0.000$
Se source × Se level	$P = 0.006$
Se source × the length of experiment	$P = 0.314$
Se level × the length of experiment	$P = 0.012$
Se source × Se level × the length of experiment	$P = 0.929$

[a] Whole-blood Se concentrations data are means of five replicates, two blood samples per replicate for each collection period. [b] Means within a column with different letters are significantly different ($p < 0.05$). [c] The value in this column is the average whole-blood Se concentrations calculated from the whole-blood Se concentrations for each collection period for each treatment through the 28 day experiment.

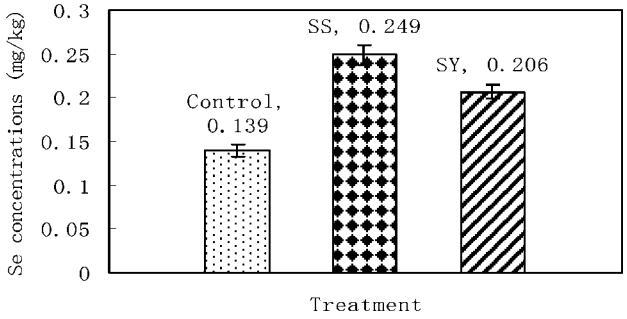

Figure 2. Effect of Se source in hen diets on overall whole-blood Se concentrations of hens (mg/kg, wet weight basis). Columns with different designs are significantly different ($p < 0.05$). The value above the column is the overall average whole-blood Se concentrations regardless of Se level, calculated from the whole-blood Se concentrations for each collection period for each source through the 28 day experiment. Whole-blood Se concentrations data are means of five replicates, two blood samples per replicate for each collection period.

Kim et al. (*21*), and Pehrson et al. (*22*) who reported that SY as an organic Se source was much more effective than inorganic SS in increasing the whole-blood Se concentrations in cows and pigs. We propose that this discrepancy may be because (i) different species were used (hens vs cows or pigs) or (ii) different sites of Se deposition. During the period, the total amount of absorbed Se from either Se source is determined, but organic Se is mainly deposited in egg, milk, and body tissues (*16*, *19*−*26*), whereas inorganic Se remains in the blood. When Se from inorganic Se sources exceeds the nutritional or production needs, excess inorganic Se is excreted via the urine (*26*, *27*).

When SS was offered, there was a Se level × length of experiment interaction effect ($p < 0.01$) on blood Se concentrations of hens. At each dietary Se supplemental level, blood Se concentrations were significantly higher ($p < 0.05$) on day 28 than on day 14, whereas the addition of Se from SY only at 0.5 and 1.0 mg/kg led to a significant increase ($p < 0.05$) in blood Se concentrations. Our results in hens suggested that dietary Se was gradually transferred to the blood of hens, and then, blood Se concentrations increased as the experimental time advanced. The results in hens are similar to those in sheep reported by Echevarria et al. (*18*) and in broiler chicks reported by Tarla et al. (*28*).

Tissue Se Concentrations. Tissue Se concentrations of each treatment are presented in **Table 5**. The addition of either SS or SY increased ($p < 0.05$) Se concentrations in liver, kidney, spleen, and cardiac and breast muscles of laying hens in comparison with the control. Both Se source and Se level significantly ($p < 0.01$) influenced Se concentrations in the above-mentioned tissues, and there was a Se source × Se level interaction effect on the Se concentrations in cardiac muscle ($p < 0.05$) and breast muscle ($p < 0.01$). With an increase in the supplemental Se level from either Se source, the Se concentrations in kidney, liver, spleen, and cardiac and breast muscles had a significant increase ($p < 0.05$), except for Se concentrations in breast muscle, when SS was added. Hassan (*16*) reported that the addition of Se from barley Se caused a significant increase in tissue (liver, heart, and muscle) Se concentrations in hens in comparison with those observed for the hens not receiving Se supplementation. Echevarria et al. (*18*) reported that added dietary SS increased Se linearly ($p < 0.01$) in liver and kidney of sheep. Tarla et al. (*28*) reported that liver and kidney Se concentrations in broiler chicks increased ($p < 0.01$) with an increase of dietary inorganic Se concentration. Mahan et al. (*29*) reported that the higher Se level (0.3 mg/kg) increase gilt and progeny tissue (liver, loin, pancreas, and other muscles) Se concentrations, but the organic SY source increases the tissue Se content of these animals over that when SS is provided.

In addition, the kidney Se concentrations reached the highest when Se was added at 0.5 mg/kg, whereas when Se was added at 1.0 mg/kg, kidney Se concentrations had no significant difference in comparison with Se added at 0.5 mg/kg in our experiment. The result suggested that there was a limit for kidney to deposit Se within a certain range of added Se amount.

Regardless of level, the Se concentrations in liver, spleen, and cardiac and breast muscles of hens fed SY were higher than those of hens fed SS (**Table 6**), whereas the opposite in kidney was observed, which suggested that more Se from SS was excreted by kidney than Se from SY. The effect of Se source on Se distribution in kidney is similar to that of Se source on Se distribution in whole blood in our experiment, which implies that there is a certain relationship between Se concentrations in the kidney and the whole blood. This relationship may be

Table 5. Effect of Se Source and Level in Hen Diets on Tissue Se Deposition of Hen[a]

treatment	Se source	supplemental Se level (mg/kg)	Se concentrations[b] (mg/kg, wet weight basis) liver	kidney	spleen	cardiac muscle	breast muscle
1	0	0	0.582 ± 0.007 a	0.551 ± 0.013a	0.534 ± 0.011 a	0.307 ± 0.009 a	0.135 ± 0.003 a
2	SS	0.2	0.612 ± 0.005 b	0.763 ± 0.018 b	0.605 ± 0.011 b	0.328 ± 0.008 b	0.137 ± 0.005 a
3	SS	0.5	0.634 ± 0.003 c	0.863 ± 0.022c	0.655 ± 0.010 c	0.365 ± 0.006 c	0.140 ± 0.003ab
4	SS	1.0	0.690 ± 0.009 d	0.826 ± 0.006cd	0.761 ± 0.007 d	0.440 ± 0.006 d	0.149 ± 0.003 b
5	SY	0.2	0.623 ± 0.009 bc	0.700 ± 0.019 e	0.641 ± 0.010 c	0.363 ± 0.007 c	0.149 ± 0.004 b
6	SY	0.5	0.661 ± 0.009 e	0.782 ± 0.015bdf	0.702 ± 0.004 e	0.391 ± 0.005 e	0.161 ± 0.003 c
7	SY	1.0	0.722 ± 0.005f	0.775 ± 0.016 bf	0.788 ± 0.006 f	0.477 ± 0.004f	0.182 ± 0.004 d
Se source			$P = 0.002$	$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.002$
Se level			$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.000$	$P = 0.000$
Se source × Se level			$P = 0.150$	$P = 0.100$	$P = 0.080$	$P = 0.048$	$P = 0.001$

[a] Means within a column with different letters are significantly different ($p < 0.05$). [b] Se concentrations data are means of five replicates; two hens per replicate were slaughtered on day 28 of the experiment, and then, tissues were sampled.

Table 6. Effect of Se Source in Hen Diets on Tissue Se Concentrations[a] of Hen (mg/kg, Wet Weight Basis)[b]

Se source	liver	kidney	spleen	cardiac muscle	breast muscle
	0.582 ± 0.007 a	0.551 ± 0.013 A	0.534 ± 0.011 a	0.307 ± 0.009 a	0.135 ± 0.003 A
SS	0.645 ± 0.011 b	0.817 ± 0.015 B	0.674 ± 0.020 b	0.378 ± 0.015 b	0.142 ± 0.003 B
SY	0.669 ± 0.014 b	0.753 ± 0.014 C	0.711 ± 0.019 c	0.410 ± 0.015 b	0.164 ± 0.005 C

[a] Se concentrations data are means of samples in each source regardless of Se level; two hens per replicate were slaughtered on day 28 of the experiment, and then, tissues were sampled. [b] Means within a column with different lower case letters are significantly different ($p < 0.05$); with different capital letters, are significantly different ($P < 0.01$).

explained as follows: Kidney contains abundant capillary vessels, and these capillary vessels are filled with blood. When Se absorption from inorganic Se source exceeds the nutritional or production need, excessive inorganic Se is excreted through the urinary route. So, kidney Se content reflects the amount of Se deposited in kidney and Se eliminated from the body via the urine.

The present study proved that the addition of commercial SY or SS significantly increased ($p < 0.05$) Se concentrations in liver, kidney, spleen, and cardiac and breast muscles of laying hens in comparison with the control. SY increased more Se concentrations in liver, spleen ($p < 0.05$), and cardiac and breast muscles ($p < 0.05$) of laying hens than SS did. The results have an important practical significance in hens' Se supplementation from SY for humans as laying hen meat and viscera are used as foods in China.

In China, Se levels in many kind of food are low and a typical balanced diet may not provide the recommended dietary intake of Se for adult males and females, respectively. In 2000, the Nutrition Council of China promulgated the Referenced Dietary Nutrient Intake of Chinese. The adequate intake of Se for infants is 15−20 μg/day, and for people over 14 years of age, it is 50 μg/day. The tolerable upper intake levels are 360 and 400 μg/day for youths (14−18 years of age) and those over 18 years of age, respectively (*30*). In the present study, a 60−65 g egg, 50 g of liver, and 100 g of muscle from hens fed SY would provide approximately 21−23, 34, and 29 μg of Se, respectively.

In conclusion, the addition of 0.2 mg/kg of Se or more from either SS or SY increased the overall average whole-egg Se concentrations, average whole-blood Se concentrations, and Se concentrations in liver, kidney, spleen, and cardiac and breast muscles of hens in comparison with the control. Both Se sources and levels significantly ($p < 0.05$) influenced Se concentrations in whole-egg, whole-blood, and the above-mentioned tissues of laying hens. There was a more significant increase in the Se concentrations in egg ($p < 0.01$), spleen ($p < 0.05$), and breast muscle ($p < 0.01$) and a decrease ($p < 0.01$) in whole blood and kidney from hens fed SY than those from hens fed SS. The order of Se distribution in hen tissue and egg is liver > kidney > spleen > cardiac muscle > egg > blood > breast muscle. These results indicate that meat and eggs from hens fed SY are potential sources of Se for humans.

ABBREVIATIONS USED

AF, atomic fluorescence; ANOVA, analysis of variance; CP, crude protein; DM, dry matter; GLM, general linear model; HG-AFS, hydride generation atomic fluorescence spectrometry; HCl, hydrochloric acid; LSD, least significant difference; ME, metabolism energy; MJ, megajoule; HNO_3, nitric acid; $HClO_4$, perchloric acid; $k_3Fe(CN)_6$, potassium ferricyanide; RCB, randomized complete block; Se, selenium; SY, Se-enriched yeast; SCRC, Sinopharm Chemical Reagent Co. Ltd.; SS, sodium selenite; $NaBH_4$, sodium borohydride; NaOH, sodium hydroxide; SE, standard error.

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