# Effect of Selenium on Performance, Egg Quality, Egg Selenium Content and Serum Antioxidant Capacity in Laying Hens

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## ABSTRACT

The objective of this work was to compare the effect of sodium selenite (SS) and selenium yeast (SY) on performance, egg quality, selenium concentration in eggs and serum antioxidant capacity in laying hens. Seven-hundred and twenty 21-week-old healthy Roman laying hens with a similar laying rate were randomly divided into 5 groups with 6 replicates of 24 hens. The experiment was designed by  $2 \times 2$  factorial arrangement with two sources of Se and two levels of Se. The hens in control group were fed a basal diet without adding exogenous selenium source, and the hens in other four groups were fed basal diets supplemented with SY and SS containing 0.3 ppm and 0.5 ppm, respectively. Pre-trial period lasted 7 d and experimental period lasted 42 d. The results showed that: compared with the control diets, 1) there was significant difference in average daily feed intake, and feed conversion rate (P < 0.05), but no difference in egg quality and laying rate (P > 0.05); 2) there was significant difference in serum GSH-Px and MDA (P < 0.05). No difference in SOD, CAT and T-AOC (P > 0.05). Compared with basal diets, adding selenium lead to increase serum GSH-Px and decrease MDA; 3) there was significant difference on egg selenium content after feeding different sources and levels of Se (P < 0.05). Compared with basal diets, adding 0.3 or 0.5 ppm SY can improve egg selenium content 89.5%, 139.2% respectively; adding 0.3 or 0.5 ppm SY in diets can improve egg selenium content 78.3%, 93.2% respectively. In conclusions, adding selenium increase laying hen antioxidant capability and egg selenium contents. We suggested that adding 0.5ppm SY in the diet of laying hens to produce Se-rich egg.

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Authors' Contribution
RF designed the experiments. HL,
QY, XT, CF and SC performed the
experiments and tested index. HL
worte the paper and anlyzed the data.
QY revised the paper.

Key words Selenium yeast, Sodium selenite, Laying hens, Performance, Serum antioxidant capacity, Egg quality, Egg selenium content

# INTRODUCTION

s one of the essential micro-elements, selenium (Se) Aplays several important physiological roles in many organisms. It is an antioxidant and increases reproduction, immune responses and thyroid hormone metabolism (Špela et al., 2017; Liu et al., 2017; Xu et al., 2017; Hoffmann et al., 2008; Safdari-Rostamabad et al., 2017; Choi et al., 2017; Ventura et al., 2017). Se intake is lower than the recommended daily allowance in human beings in most part of the world (Hefnawy et al., 2010; Tanguy et al., 2012). It is necessary to increase Se content in the common human food. Se-enriched eggs can be produced by adding Se additive in hen's diets. There are two major Se sources in feed additive, namely, inorganic Se (like sodium selenite, SS) and organic Se (like Se-Yeast, Se-Met, SeNPs and so on). However, the Japanese government banned sodium selenite use as an additive in 1993 because of its toxicity. Many researchers have focussed on the organic Se. Asadi et al. (2017) reported that organic Se showed higher efficacy to increase Se deposition in egg and to improve egg quality compared to other sources of Se. Han et al. (2017) reported the diet with equal amounts of the two sources of Se (SS and Se-Yeast) was more cost effective and affordable than a comparable amount of Seyeast to obtain the promising production performanceand nearly similar Se deposition. Qu et al. (2017) has found that adding 0.5ppm SeNPs can significantly increase serum calcium, but no difference on egg quality. They showed SeNPS plays an effective anti-oxidative protection from DON toxicity in laying hens, reduced DONs effect on egg production and blood calcium. Researchers have come to an agreement that organic Se is effective absorber and can be utilized by the body (Li et al., 2019; Han et al., 2017; Attia et al., 2010; Al-Rubaye et al., 2016; Li et al., 2004) and Se-Yeast is a superior additive compared with SS (Han et al., 2017; Prytkov et al., 2016).

The objective of this study was to evaluate the effects of Se-Yeast and sodium selenite (SS) and its levels in the diets of laying hens on productions, egg quality, antioxidant capacity and Se contents of eggs.

# MATERIAL AND METHODS

Design and animal assignment

This experiment was conducted on a commercial poultry farm. All animals care procedures were approved by the Committee of Laboratory Animal Management and

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Animal Welfare of Hunan Agricultural University (Ethics approval number 201607-5). Seven hundred and twenty healthy Roman laying hens around 21-week-old were selected with similar body size and laying rates. The hens were randomly divided into 5 groups. Each group consists of 6 replicates with 24 hens per replicate. The trial lasted for 42 d. Animals were fed 2 times per day (5:00 and 13:00).

## Animal management

Trial animals were housed 4 birds per cage in cage  $(45\text{cm} \times 45\text{cm} \times 45\text{cm})$ . Feed and water were provided *ad libitum* during the experiment. Disinfection and vaccination procedure were done according to layers feeding management manual.

#### Trial diet

A corn-soybean meal basal diet (Table I) was formulated to meet nutrient requirements suggested by NRC (1994). Se content of basal diet was analyzed before other treatment diets were produced and the content of Se in basal diet was 0.178 ppm. Adding 0.3ppm, 0.5ppm SY(Se> 0.3%, provided by LESAFFRE Co., Ltd.) or SS (Se> 1%, provided by Changsha Heyou Farming Co., Ltd.) in basal diet, respectively. The measured values of selenium content in experimental diets were 0.362, 0.572, 0.323, 0.533 ppm, respectively.

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

Ingredients	Contents (%)	Nutrition level	Contents	
Corn	61	ME/(MJ/kg) <sup>2</sup>	11.12	
Soybean meal	23	CP, %	15.9	
Limestone	8	Ca, %	3.5	
Rapeseed meal	3	AP, %	0.34	
Soybean oil	1	Lys, %	0.84	
Premix <sup>1</sup>	4	Met, %	0.33	
		Se (ppm)	0.178	

<sup>1)</sup> The premix provided the following per kg of diet: VA 7 715 IU, VD $_3$  2 755 IU, VE 8.8 IU, VK 2.2 mg, VB $_{12}$ 0.01 mg, VB $_2$ 4.41 mg, VB $_3$ 5.51 mg, VB 0.55 mg, nicotinic acid 19.8 mg, folic acid 0.28 mg, Mn 50 mg, Fe 25 mg, Cu 2.5 mg, Zn 50 mg, I 1.0 mg.

# Performance and egg quality

Feed intake and egg production of each replication was observed per day. Feed conversion rate was estimated as kilograms of food consumed per kilogram of eggs. On day 42 of the experiment per eggshell strength was measured by the Egg Force Reader; and yolk color and

Haugh unit were assayed by the Egg Analyzer. All of the equipment was from Orka Food Technology Co., Ltd (Ramat Ha Sharon, Israel).

## Blood sample collection

Two hens in each replicate were randomly selected for blood sample collection on d 42. Blood sample was collected from wing vein using vacuum tube, and then was settled at room temperature for 30 min, followed by centrifugation at 3000r/min for 10 min. Serum was collected into a 1.5 mL Eppendorf tube and stored at -20 ℃. The superoxide dismutase (SOD), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MAD) were measured by assay kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

#### Se concentration in egg

Three representative eggs per replicate were selected on 42 d. Egg yolk was separated from egg, dried at 65 °C for 12 h and ground for Se analysis. The mineral were diluted using deionized water to final volume of 25ml. Se was determined by inductively coupled plasma mass spectrometer (ICP-MS) according to Nóbrega et al. (1997).

## Statistical analysis

All data were analyzed using SAS software (version 9.4, 2013; SAS Institute Inc., Cary NC). Statistical analysis of results was made by one-way analysis of variance (ANOVA). Main effects of two factors (Se level and Se source) were evaluated by two-way ANOVA with replications. The significance level for all the analyses was set at P < 0.05.

# **RESULTS**

Performance and egg quality

There was significant difference in average daily feed intake, and feed conversion rate (P < 0.05), but no difference in egg quality and laying rate (P > 0.05). (Tables II and III).

# Serum antioxidant capacity

Effects of different selenium sources and levels on blood sample is shown in Table IV. There was significant difference in serum GSH-Px and MDA (P < 0.05). However, no difference in SOD, CAT and T-AOC (P > 0.05). There was a tendency that adding selenium can increase SOD, CAT and T-AOC. Compared with basal diets, adding selenium feed laying hens increased serum GSH-Px and decreased MDA. The percentage of GSH-Px

<sup>2)</sup> Calculated according to NRC (1994).

Table II. Effects of different selenium sources and levels on performance of laying hens.

Items	Basal diet	Se-Yeast		SS		P		
		0.3 ppm	0.5 ppm	0.3 ppm	0.5 ppm	Sources	Levels	Sources × Levels
Average daily feed intake (g/d)	116.15±2.25 <sup>A</sup>	113.99±0.23 <sup>B</sup>	113.72±0.32 <sup>B</sup>	115.40±3.45 <sup>A</sup>	113.66±0.23 <sup>B</sup>	0.491	0.014	0.576
Average egg weight (g)	55.29±0.61	55.46±0.31	55.30±0.61	54.48±0.84	54.67±0.52	0.013	0.353	0.154
Feed conversion	$2.26{\pm}0.05^{\mathrm{A}}$	$2.06\pm0.01^{B}$	$2.06 \pm 0.02^{\mathrm{B}}$	$2.12{\pm}0.04^{\rm AB}$	$2.08{\pm}0.02^{\rm B}$	0.020	< 0.001	0.082
Laying rate(%)	93.09±1.87	90.69±2.33	94.05±1.58	92.03±3.73	93.07±1.59	0.088	0.061	0.472

Note: In the same column, values without letter or with the same letter superscripts mean no significant difference (P > 0.05), while with different small letter superscripts mean significant difference (P < 0.05), and with different capital letter superscripts mean significant difference (P < 0.01). The same as below.

Table III. Effects of different selenium sources and levels on egg quality of laying hens.

Items	Basal diet	Se-Yeast		SS		P		
		0.3 ppm	0.5 ppm	0.3 ppm	0.5 ppm	Sources	Levels	Sources × Levels
Egg shape index	1.29±0.04	1.29±0.04	1.28±0.03	1.30±0.03	1.31±0.04	0.119	0.898	0.140
Eggshell thickness(mm)	$0.50\pm0.02$	$0.50\pm0.04$	$0.49\pm0.03$	$0.48 \pm 0.02$	$0.49 \pm 0.03$	0.187	0.944	0.352
Eggshell strength(kg/m²)	$5.18\pm0.53$	$4.98\pm0.60$	5.16±1.18	5.07±0.60	5.27±0.62	0.665	0.404	0.014
Yolk weight(g)	15.54±0.90	15.25±1.03	15.98±0.76	15.74±1.12	$15.45 \pm 0.73$	0.927	0.444	0.341
Yolk index	$0.40\pm0.03$	$0.39\pm0.04$	$0.40\pm0.05$	$0.38 \pm 0.06$	$0.41 \pm 0.05$	0.917	0.291	0.481
Albumen height(mm)	4.44±1.45	4.41±1.65	3.43±1.55	4.35±1.35	4.13±1.15	0.434	0.166	0.404
Haugh unit	67.29±11.82	72.35±6.88	66.56±7.50	67.75±10.35	62.18±12.14	0.177	0.840	0.156

Table IV. Effects of different selenium sources and levels on serum antioxidant of laying hens.

Items	Basal diet	Se-Yeast		SS		P		
		0.3 ppm	0.5 ppm	0.3 ppm	0.5 ppm	Sources	Levels	Sources × Levels
GSH-Px (U/ml)	1200.94± 216.31 <sup>D</sup>	1768.83± 151.33 <sup>BC</sup>	2083.50± 232.99 <sup>A</sup>	1628.36± 148.80 <sup>c</sup>	1885.86± 161.59 <sup>в</sup>	<0.001	<0.001	<0.001
SOD (U/ml)	154.85±10.65	153.98±10.48	161.20±4.44	158.46±4.06	157.11±16.15	0.941	0.627	0.788
CAT (U/ml)	4.31±0.58	$4.43 \pm 0.65$	4.45±0.82	4.39±0.81	4.41±0.54	0.895	0.892	0.997
MDA (nmol/L)	5.28±0.68 <sup>A</sup>	$4.66{\pm}0.74^{\rm AB}$	$4.20\pm0.82^{\rm B}$	$4.61\pm0.50^{AB}$	$4.51\pm0.79^{AB}$	0.692	< 0.001	0.156
T-AOC (U/ml)	5.69±0.41	$5.73 \pm 0.45$	$6.22 \pm 0.67$	5.72±0.42	6.01±0.86	0.692	0.125	0.462

CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

increased 47.3%, 73.6%, 35.7%, 57.1%, respectively after feeding 0.3 or 0.5 ppm SY or SS in diets, while the MDA decreased 11.7%, 20.5%, 12.7%, 14,6% respectively.

Egg selenium content

As showed in Figure 1, there were significant difference in egg selenium content after feeding different source and levels of Se (P < 0.05). Compared with basal diets, adding 0.3 or 0.5 ppm SY in diets improved egg

selenium content 89.5%, 139.2%, respectively, whereas adding 0.3 or 0.5 ppm SS in diets improved egg selenium content to 78.3% and 93.2%, respectively.

## **DISCUSSION**

Effect of selenium on laying performance or egg quality has bee one of the hottest for investigators. Some studies concluded that adding selenium increased laying

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performance and egg quality (Skrivan et al., 2006; Sahin et al., 2008), whereas others reported no effects on laying performance and egg quality after selenium supplement in diets (Gravena et al., 2011; Invernizzi et al., 2013). In this study, we found that adding selenium can decrease the laying performance and there is no influence on egg quality. The relationship between Se and laying performance might depend on many factors like animal species, feedstuff and composition, environment, Se levels and sources etc. The condition of production is different from the condition of the experiment where the temperature, humidity, and environmental pathogens are strictly controlled. That is to say, there are a series of stress factors in the experimental condition.

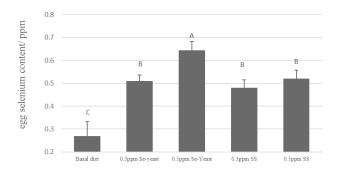


Fig. 1. Effects of different selenium sources and levels on egg Se content on d 42<sup>nd</sup> of laying hens.

GSH-Px is a Se-dependent enzyme that catalyzes the reduction of hydrogen peroxide and organic peroxides to water and the corresponding stable alcohol, thus inhibiting the formation of free radicals (Behne and Kyriakopoulos, 2001)

SOD is an important antioxidant enzyme in organisms and catalyzes the dismutation of superoxide anion to H<sub>2</sub>O<sub>2</sub> and molecular oxygen (Gaetani et al., 1996). MDA is one of the metabolic products of lipid peroxides, and the MDA level is negatively correlated with the GSH-Px activity (Ahmad et al., 2012).CAT exerts a dual function: (1) decomposition of H<sub>2</sub>O<sub>2</sub> to give H<sub>2</sub>O and O<sub>2</sub> (catalytic activity) and (2) oxidation of H donors (Aebi, 1984). Several studies have shown that selenium supplement increased antioxidant capability in livestock (Castillogodina et al., 2016; Gong et al., 2016; Manzanares et al., 2016; White et al., 2017). As shown in the results of our study, the combined supplementation of SS and SY proved to be successful in improving antioxidant capability, adding SY be more effective on serum antioxidant than SS. It was consistent with the results obtained in previous researches (Lee et al., 2014; Jing et al., 2015; Apsīte et al., 2012). Han et al. (2017) reported that the effects of SS and Se

yeast were approximately equal in promoting antioxidant capacity of laying hens, while Se yeast is easier to deposit into eggs and tissues. The diet with added equal amounts of the two sources of Se was more cost effective and affordable than a comparable amount of Se yeast to obtain the promising production performance and nearly similar Se deposition. In this paper, we found that adding selenium can increase egg selenium content and under the same level, adding SY was higher than SS. Se-rich eggs can be a good nutritional product to meet human need in order to achieve the daily recommended amount of selenium.

## **CONCLUSION**

In this study, we concluded that adding selenium can increase antioxidant capability and egg selenium contents of laying hens. Adding 0.5 ppm SY showed the strongest antioxidant capability and the highest egg selenium content and it was higher than another levels (0.3 ppm) and sources of SS.

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Statement of conflict of interests

The authors declare that there is no conflict of interest.

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