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Full Length Article

Effect of Selenium-Enriched Yeast and Nano-Selenium in Daily Diet on Selenium Content in *Gallus domestiaus* Eggs

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Abstract

The effect of adding selenium-enriched yeast and nano-selenium at different concentrations in daily diets on the content of selenium in the *Gallus domestiaus* eggs was investigated. Healthy *Gallus domestiaus* aged 96 weeks were randomly divided into seven groups and fed a basic daily diet or one supplemented with selenium-enriched yeast or nano-selenium at doses of 0.5, 0.8, or 1.1 mg/kg. The chickens were fed for 30 days in the trial period. Eggs were collected at about 10, 20, and 30 days. At 10 and 20 days, there were significantly higher levels of selenium in albumen, yolk, and whole egg in the groups that received selenium-enriched yeast compared to the control group or groups that received only nano-selenium ($P < 0.05$). At 20 days, with addition of selenium-enriched yeast, there was increased selenium in albumen and whole egg. The content of selenium in the albumen increased with increased nano-selenium added, but the content in yolk and whole egg initially increased and then declined. At 30 days, there was significantly higher selenium in yolk in the groups that received selenium-enriched yeast at doses of 0.8 and 1.1 mg/kg compared to levels in the control group and the nano-selenium groups ($P < 0.05$). No significant effect on the selenium levels was observed in albumen, yolk, or whole egg in the groups that received nano-selenium. The results indicate that daily supplementation with selenium-enriched yeast can significantly elevate the selenium level in albumen, yolk, and whole egg, but no significant effect on selenium content in eggs was observed for groups supplemented with nano-selenium.

Keywords: Content of selenium; Eggs; *Gallus domestiaus*; Selenium-enriched yeast; Nano-selenium

Introduction

Selenium is one of the 14 essential trace elements in animals (Qudoos *et al.*, 2017). It is an important component of glutathione peroxidase (GSH-Px) and participates in the metabolic regulation of many important hormones, such as androgen, insulin and thyroid hormone (Zhan *et al.,* 2011; Guo *et al.,* 2017). Deficiency of selenium in egg-laying chickens leads to a decrease in immunity and egg production (Qu *et al.,* 2014a). Some studies have shown that supplementation of daily diets with either inorganic selenium or organic selenium may significantly elevate the content of selenium in eggs but that organic selenium in eggs may precipitate better (Hu *et al.,* 2015). The study reported that adding selenium-enriched yeast to the diet of layer chickens significantly increases the content of selenium in egg albumen and yolk (Rajashree *et al.,* 2014). Previous study reported that adding selenium-enriched malt can significantly increase the content of selenium in both albumen and yolk, with a larger effect on the selenium content in yolk (Li *et al.,* 2001). Nano-selenium can increase the permeability of mucous membrane, further improve the capacity of the intestine to absorb nano-selenium and promote sedimentation of selenium in tissues and organs due to its advantages such as small particle sizes, large specific surface area *etc*. (Liao *et al.,* 2010).

Organic selenium is a key focus of current research on animal nutritional science due to its high utilization rate, low toxicity, and high biological safety compared to inorganic selenium (Surai and Fisinin, 2016). Selenium-enriched yeast is a high-quality organic selenium source containing about 20% selenocysteine (SeCys) and about 70% selenomethionine (SeMet), with bioavailability far higher than that of inorganic selenium. Many studies have demonstrated that selenium-enriched yeast is more easily absorbed in animals. Additionally, selenium-enriched yeast may be stored in the body for a short period of time, avoiding recurrent selenium deficiency after meeting the current biological demand for selenium (Yin *et al.,* 2012). Nano-selenium is a zerovalent reducing selenium with particles that are smaller than 100 nm. Nano-selenium is more widely used than selenomethionine, selenium yeast, or sodium selenite, due to its low toxicity (Liu *et al.,* 2007). In production, the performance, quality and bioactive

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substance content of laying hens are affected by many factors (Duru *et al.,* 2017; Gevrekçi and Takma, 2018).

The goal of this work was to determine the effects of consuming a selenium-enriched diet on selenium levels in eggs. Selenium-enriched yeast and nano-selenium were tested as supplements to the daily diet of *Gallus domestiaus*, and we evaluated the effects on the content of selenium in albumen, yolk, and whole egg. The results provide guidance for selection of the appropriate doses of inorganic selenium and nano-selenium, and can serve as the theoretical and practical basis for effective production of selenium-enriched eggs.

Materials and Methods

Test Material and Reagents

Selenium-enriched yeast (Selenium Enriched Yeast, SY, containing 2000 mg/kg selenium) was purchased from Angel Yeast Co., Ltd. Nano-selenium (containing 10000 mg/kg selenium) was purchased from Guangdong Xingteng Biotechnology Co., Ltd. The basic fodder (containing 0.412 mg/kg selenium) was purchased from the first Affiliated Factory of Tianjin Hengjilide Biotechnology Development Co., Ltd.

The standard stock solution of selenium (containing 100 μg/mL selenium) was purchased from the China National Institute of Metrology. Potassium borohydride (KBH_4) and potassium hydroxide (KOH) were analytically pure reagents and were purchased from Booute Chemical Trade Co., Ltd. $HClO_4$, HNO_3, and HCl were all highly pure reagents and were purchased from the Tianjin Fengchuan Chemical Reagent Technology Co., Ltd.

Apparatus and Equipment

The PF6-2 non-dispersive atomic fluorescence spectrophotometer used for assays was purchased from the Beijing Persee General Apparatus Co., Ltd. The high-performance hollow cathode selenium was from the Beijing General Research Institute for Nonferrous Metals. The DHG-9246A electric constant-temperature air-blower, drying oven was purchased from the Shanghai Jinghong Experimental Equipment Co., Ltd. The micro-control digital electric hot plate was from Beijing LabTech Apparatus Co., Ltd. The FB223 electronic analytical balance was from Shanghai Shunning Hengping Scientific Apparatus Co. Ltd., Shangai, China.

Test Design

The experiment was conducted in the Breeding Base for *G. domestiaus* of Tianjin Jinwa Agricultural Technology Development (Tianjin) Co. Ltd., from October to December 2017. A total of 280 healthy *G. domestiaus*, aged 96 weeks, were randomly selected and divided into seven groups with four replications for each group, with 10 chickens for each replication. The chickens were maintained in a free-range environment, and fed and drank ad libitum. The chickens in Group A were fed the basic daily rations. The chickens in Groups B, C, and D were fed the basic rations, with selenium-enriched yeast added at doses of 0.5, 0.8, and 1.1 mg/kg respectively. The chickens in Groups E, F, and G were fed the basic rations with nano-selenium added at doses of 0.5, 0.8 and 1.1 mg/kg, respectively. The specific composition of the diet is described (Table 1).

Measuring Indicators and Methods

At about 10, 20, and 30 days of the test, three eggs were randomly taken from each replication in each group and stored at 4°C for later use. Using the method of hydride generation-atomic fluorescence spectrometry was used to measure the contents of selenium in albumen, yolk, and whole egg.

Data Analysis

With the S.P.S.S. 17.0 software, one-way ANOVA analysis was employed for multiple comparisons between mean values and significance was assessed. All data in the test were expressed as mean values ± standard deviation. A difference was considered significant when $P < 0.05$.

Results

Effects of Selenium-enriched Yeast and Nano-selenium on the Content of Selenium in Albumen

Compared with the control group, the addition of selenium-enriched yeast to the daily diet significantly increased the content of selenium in albumen ($P < 0.05$), but nano-selenium showed no significant effects on selenium levels in albumen ($P < 0.05$). At 10 days, the selenium levels in albumen in Groups B, C, and D were significantly higher than those in other test groups ($P < 0.05$). There were no significant differences in the selenium level between Group C and Group D ($P > 0.05$) but the amounts of selenium in the two groups were significantly higher than that in Group B ($P < 0.05$). There were no significant differences in selenium content for the control group and the groups with nano-selenium added ($P > 0.05$). At 20 and 30 days, there were no significant difference in the selenium content for the groups with nano-selenium added and the control group ($P > 0.05$) but the contents of selenium in the two groups were significantly lower than that in the groups with selenium-enriched yeast added ($P < 0.05$). With increased dose of selenium-enriched yeast, there were significant differences in the selenium content in albumen between the groups with selenium-enriched yeast added ($P < 0.05$) (Table 2).

Table 1: Composition of the basal diet

Main ingredients	Percentage content (%)	Main ingredients	Percentage content (%)
Corn	59.5	Bran	2.0
Soybean meal	25.0	Stone powder	8.5
Premixture*	5.0		

Note: *The premixture provides the following nutrients per kilogram of fodder: Vitamin A 30000 IU, Vitamin B_1 20 mg, Vitamin B_2 40 mg, Vitamin B_6 60 mg, Vitamin D_3 800 IU, Vitamin E 20 mg, Vitamin K_3 400 mg, Fe 0.7 g, Cu 0.008 g, manganese 0.3 g, zinc 0.8 g, iodine 2 mg, selenium 2 mg, calcium 5.1%, choline chloride 7 g, sodium chloride 1.8%, methionine 2%, phosphorus 1.5%, and water 10%

Table 2: Effects of selenium enriched yeast and nano-selenium on selenium content of egg white (mg/kg)

Bird groups	Group and dose (mg/kg)	10 d	20 d	30 d
Control group	A(0)	$0.089^{c} \pm 0.018$	$0.088^{d} \pm 0.011$	$0.113^{d} \pm 0.014$
Selenium enriched yeast group	B(0.5)	$0.302^{b} \pm 0.025$	$0.367^{c} \pm 0.032$	$0.446^{c} \pm 0.038$
	C(0.8)	$0.433^{a} \pm 0.031$	$0.468^{b} \pm 0.026$	$0.620^{b} \pm 0.032$
	D(1.1)	$0.482^{a} \pm 0.041$	$0.644^{a} \pm 0.061$	$0.766^{a} \pm 0.060$
Nano-selenium group	E(0.5)	$0.081^{c} \pm 0.007$	$0.109^{d} \pm 0.014$	$0.149^{d} \pm 0.022$
	F(0.8)	$0.080^{c} \pm 0.008$	$0.113^{d} \pm 0.010$	$0.155^{d} \pm 0.024$
	G(1.1)	$0.079^{c} \pm 0.005$	$0.126^{d} \pm 0.018$	$0.151^{d} \pm 0.021$

Note: In the same column, values with different small letter superscripts indicate significant difference ($P < 0.05$), the same small letter superscripts indicate no significant difference between values ($P > 0.05$). The same applies below

Effects of Selenium-enriched Yeast and Nano-selenium on the Content of Selenium in Yolk

Adding selenium-enriched yeast can significantly increase the content of selenium in yolk ($P < 0.05$) but there were no significant differences in the content of selenium in yolk between the groups with nano-selenium added and the control group ($P > 0.05$). At 10 d, the selenium levels in yolk in Groups B, C, and D were significantly higher than those in other test groups ($P < 0.05$). The amount of selenium in Group C was significantly higher than that in Group B ($P < 0.05$), but there were no significant differences between the two groups and Group D ($P > 0.05$). There were no significant differences in the content of selenium in yolk between the groups with nano-selenium added and the control group ($P > 0.05$) but the amounts in the groups with nano-selenium added were significantly lower than in the groups with selenium-enriched yeast added ($P < 0.05$). At 20d, the contents of selenium in the groups with selenium-enriched yeast added were significantly higher than that in the control group and the groups with nano-selenium added ($P < 0.05$), but there were no significant differences in the selenium content between the groups with nano-selenium added and the control group ($P > 0.05$). At 30d, the content of selenium in yolk in Group D was highest and significantly higher than that in other groups ($P < 0.05$). There were no significant differences in the selenium content in yolk between Group B and Group C ($P > 0.05$) but the content of selenium in either of these two groups was significantly higher than that in any of the groups with nano-selenium added ($P < 0.05$). There were no significant differences in the content of selenium in yolk between the groups with nano-selenium added and the control group ($P > 0.05$) (Table 3).

Effects of Selenium-enriched Yeast and Nano-selenium on the Content of Selenium in whole Egg

Adding selenium-enriched yeast can significantly increase the content of selenium in whole egg ($P < 0.05$), but there were no significant differences in the content of selenium in the whole egg between the groups with nano-selenium added and the control group ($P > 0.05$). At 10 d, the selenium level in whole egg for each of the groups supplemented with selenium-enriched yeast was significantly higher than that in the control group and each of the groups with nano-selenium added ($P < 0.05$). There were no significant differences in the selenium levels in whole egg between Group C and Group D, but the content of selenium in whole egg in either of the two groups was significantly higher than that in Group B ($P < 0.05$). There were no significant differences in the selenium content in whole egg between the groups with nano-selenium added and the control group ($P > 0.05$). At 20 d and 30 d, the selenium in whole egg in each of the groups with selenium-enriched yeast was significantly higher than that that received the diet supplemented with nano-selenium. There were no significant differences in content of selenium in whole egg between Group B and Group C but the content of selenium in whole egg in either of the two groups was significantly lower than that in Group D ($P < 0.05$). There were no significant differences in the content of selenium in whole egg between each of the groups with nano-selenium added and the control group ($P > 0.05$) (Table 4).

Discussion

Currently, there is significant interest in comparing the applications of selenium-enriched yeast, nano-selenium, and sodium selenite in animal husbandry. Many studies have

Table 3: Effects of selenium enriched yeast and nano-selenium on selenium content of egg yolk (mg/kg)

Bird groups	Group and Dose (mg/kg)	10 d	20 d	30 d
Control group	A(0)	$0.565^{c}\pm0.029$	$0.626^{b}\pm0.037$	$0.794^{cd}\pm0.067$
Selenium enriched yeast group	B(0.5)	$0.722^{b}\pm0.043$	$0.956^{a}\pm0.065$	$0.992^{bc}\pm0.117$
	C(0.8)	$0.847^{a}\pm0.033$	$1.011^{a}\pm0.036$	$1.119^{b}\pm0.060$
	D(1.1)	$0.780^{ab}\pm0.038$	$1.002^{a}\pm0.059$	$1.377^{a}\pm1.120$
Nano-selenium group	E(0.5)	$0.515^{c}\pm0.032$	$0.556^{b}\pm0.033$	$0.724^{d}\pm0.033$
	F(0.8)	$0.535^{c}\pm0.030$	$0.617^{b}\pm0.032$	$0.718^{d}\pm0.032$
	G(1.1)	$0.506^{c}\pm0.029$	$0.590^{b}\pm0.027$	$0.739^{d}\pm0.044$

Table 4: Effects of selenium enriched yeast and nano-selenium on selenium content of total egg (mg/kg)

Bird groups	Group and Dose (mg/kg)	10 d	20 d	30 d
Control group	A(0)	$0.322^{c}\pm0.018$	$0.334^{c}\pm0.023$	$0.400^{c}\pm0.031$
Selenium enriched yeast group	B(0.5)	$0.552^{b}\pm0.032$	$0.690^{b}\pm0.050$	$0.749^{b}\pm0.064$
	C(0.8)	$0.680^{a}\pm0.032$	$0.732^{b}\pm0.030$	$0.883^{b}\pm0.034$
	D(1.1)	$0.721^{a}\pm0.048$	$0.897^{a}\pm0.078$	$1.144^{a}\pm0.078$
Nano-selenium group	E(0.5)	$0.297^{c}\pm0.024$	$0.398^{c}\pm0.041$	$0.427^{c}\pm0.040$
	F(0.8)	$0.347^{c}\pm0.029$	$0.405^{c}\pm0.047$	$0.449^{c}\pm0.044$
	G(1.1)	$0.303^{c}\pm0.017$	$0.328^{c}\pm0.010$	$0.545^{c}\pm0.069$

shown that significantly higher bioavailabilities of selenium in selenium-enriched yeast and the nano-selenium compared to that in sodium selenite (Čobanová *et al.,* 2011; Li *et al.,* 2017). However, there has been no analysis of the effects of selenium-enriched yeast and nano-selenium on the content of selenium in the eggs of *G. domestiaus*.

Added selenium-enriched yeast or nano-selenium to the diet for quails in the later egg producing period (Qu *et al.,* 2014b). The results indicated that both types of addition significantly increased the content of selenium in eggs, and a higher level of selenium in eggs was observed for the group that received nano-selenium compared to the level in the eggs of the group that received selenium-enriched yeast at the same dosage. Administered radiolabeled nano-selenium to meat chickens by oral administration and intravenous injection (Hu *et al.,* 2012). The results indicated a significantly higher absorption of selenium in the liver for meat chickens that received nano-selenium compared to the absorption for chickens that received the same dose of sodium selenite. This suggests higher absorption rate and precipitation rate for nano-selenium within the body of meat chickens compared to the rates sodium selenite. By adding different selenium sources to the diet for goats, found that the levels of selenium in the liver and kidney of goats in the groups that received nano-selenium were significantly higher than the levels in the groups that received selenium-enriched yeast or sodium selenite (Shi *et al.,* 2011). The contents of selenium in the liver and kidney of the goats supplemented with nano-selenium were 3.12 and 4.29 μg/g, respectively (Shi *et al.,* 2011). These findings indicated that addition of selenium-enriched yeast to the daily diet can significantly increase the contents of selenium in albumen, yolk, and whole egg, but addition of nano-selenium in the daily diet has no significant effects on the contents of selenium in albumen, yolk, and whole egg. The difference in these results and our results may be to different environmental conditions. Here, the test was conducted in cold winter, and the layer chickens were aged 96 weeks. Both environmental and age factors may affect the ability of layer chickens to absorb selenium.

A study found that compared to the control group, the amounts of selenium in albumen and yolk increased by 15.0 and 20.5 mg/kg, respectively, for a dose of 0.3 mg/kg, and 15.9 and 21.6 mg/kg, respectively, for a dose of 0.5 mg/kg after 10 weeks of supplementation with selenium-enriched yeast (Rajashree *et al.,* 2014). Adding selenium-enriched yeast to the diet for layer chickens at a dose of 0.3 mg/kg significantly increased the content of selenium in yolk (Skrivan *et al.,* 2008), and the adding nano-selenium to the diet for layer pigeons at doses of 0.4 and 0.5 mg/kg significantly increased the content of selenium in yolk at 21 d and 42 d (Yang *et al.,* 2015). The research findings of this study found dose-dependent increased selenium in albumen, whole egg, and the yolk at 30d are increasing gradually with selenium-enriched yeast supplementation. The content of selenium in the yolk at first increased and then decreased at 10 d and 20 d. At 30 d, the contents of selenium in albumen and yolk in the group with nano-selenium added increased, but the differences were not significant. This indicates that compared with nano-selenium, selenium-enriched yeast of the same dose may increase the content of selenium in albumen and whole egg in a short period of time. However, the effects of long-term feeding of chickens with selenium-enriched yeast and nano-selenium on the content of selenium in eggs should be studied over a longer time period.

The research showed that adding hydroponic selenium-enriched cabbage (HPSeKS) in the daily diet can significantly increase the content of selenium in albumen and yolk, with the highest increases in selenium in yolk (Chinrasri *et al.,* 2013; Chantiratikul *et al.,* 2018). The report showed that adding selenium-enriched malt can significantly increase the content of selenium in albumen and yolk, with a more significant increase in the content of selenium in yolk (Li *et al.,* 2001). Some studies have demonstrated that adding nano-selenium to the daily diet at a dose of 0.40 mg/kg results in the highest amount of selenium precipitated in whole eggs of layer chickens. The amount of selenium precipitated in albumen in the group that received nano-selenium at a dose ranging from 0.25 to 0.4 mg/kg was 3–4 times that in the control group, and the amount of selenium in the yolk was 4–5 times that in the control group (Radwan *et al.,* 2015). The content of selenium in yolk is significantly higher than that in albumen possibly due to differences in the mechanism of precipitation of selenium. The yolk protein is mainly synthesized in the liver. Most selenium ingested by layer chickens is transported to the liver for synthesis of selenium protein, and enters the yolk together with partial selenium protein. The albumen protein is mainly synthesized in the oviduct. Selenomethionine may directly participate in the synthesis of albumen protein, but the amount of selenium deposited in other forms may be small (Zhao *et al.,* 2008). The addition of selenium-enriched yeast in the daily diet can significantly increase the content of selenium in albumen and yolk, and the level of selenium in the diet is closely associated with the content of selenium in yolk. In contrast, adding nano-selenium in the daily diet showed no significant effects on the content of selenium in albumen and yolk.

Added HPSeKS in the daily diet for layer chickens at doses of 5 and 10 mg/kg and observed no adverse effects (Chantiratikul *et al.,* 2016). When nano-selenium was added in the daily diet at a dose of 113 mg/kg, 7.5 times the dose of sodium selenite, selenium toxicity reactions occurred (Gao *et al.,* 2000). Added nano-selenium in the daily diet for layer chickens at doses of 5.0 and 10.0 mg/kg. At 30 d of feeding, nano-selenium at either dose significantly increased the content of selenium in the liver of layer chickens. At 60 d, nano-selenium at either dose significantly increased the content of selenium in whole blood. However, there were no significant differences in the content of selenium in the liver of layer chickens between the test groups and the control group (Zhu *et al.,* 2010). The highest content of selenium in the treatment groups was 1.5 mg/kg. Selenium supplementation should not be so high that there are adverse effects, such as decreased productivity, environment pollution, or poisoning of layer chickens. The test results showed that feeding layer chickens selenium-enriched yeast at a dose of 1.1 mg/kg can significantly increase the content of selenium in albumen, yolk, and whole egg, but no significant changes in selenium levels were detected in albumen, yolk, or whole egg in response to supplementation with nano-selenium. In addition, no clinical toxic symptoms were observed in the layer chickens in any of the test groups, indicating no adverse effects of supplementation.

Conclusion

The results indicated that adding selenium-enriched yeast in the daily diet can significantly increase the contents of selenium in albumen, yolk, and whole egg of *G. domestiaus*, but adding nano-selenium in the daily diet has no significant effects on the contents of selenium in albumen, yolk, and whole egg of *G. domestiaus*.

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