Effects of Dietary Supplementation with Different Selenium Sources on Production Performance and Antioxidant Capacity of Laying Hens: Postprint

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Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment investigated the effects of dietary supplementation with sodium selenite, selenium yeast, selenomethionine, and nano-selenium on production performance, egg quality, plasma antioxidant capacity, and egg selenium content in laying hens, aiming to provide a theoretical basis for the rational use of selenium in laying hen diets. A total of 540 healthy Hy-Line Gray laying hens at 18 weeks of age with similar laying rates were selected and randomly divided into 5 groups with 6 replicates per group and 18 hens per replicate. The control group was fed a basal diet without selenium supplementation (total selenium content 0.08 mg/kg), while the other 4 groups were supplemented with 0.30 mg/kg selenium from sodium selenite, selenium yeast, selenomethionine, and nano-selenium, respectively (measured dietary selenium contents were 0.37, 0.38, 0.34, and 0.41 mg/kg). The experiment consisted of a 1-week preliminary period and an 8-week formal experimental period. The results showed: 1) Different selenium sources had no significant effects on production performance or egg quality in laying hens ($P>0.05$). 2) Compared with the control group, dietary supplementation with 0.30 mg/kg of the four selenium sources all significantly increased plasma glutathione peroxidase (GSH-Px) activity ($P<0.05$). At the end of week 4, the nano-selenium group had the highest GSH-Px activity; at the end of week 8, the selenium yeast and nano-selenium groups had higher GSH-Px activity. Compared with the control group, dietary supplementation with the four selenium sources all increased plasma total antioxidant capacity (T-AOC), and the nano-selenium group was significantly higher than the other groups at both week 4 and week 8 ($P<0.05$). The four selenium sources had no significant effects on plasma superoxide dismutase (SOD) activity or malondialdehyde (MDA) content ($P>0.05$). 3) Compared with the control group,

supplementation of the four selenium sources in the basal diet all significantly increased egg selenium content (P<0.05), with the selenium yeast group being significantly higher than the other three groups (P<0.05). In conclusion, supplementation of the four selenium sources in the basal diet had no significant effects on production performance or egg quality in laying hens; all four selenium sources could significantly increase plasma GSH-Px activity and T-AOC, with selenium yeast and nano-selenium showing better effects; compared with sodium selenite, selenomethionine, and nano-selenium, selenium yeast was more effective in increasing egg selenium content.

Full Text

Effects of Dietary Supplementation of Different Selenium Sources on Production Performance and Antioxidant Capacity of Laying Hens

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Abstract: This experiment investigated the effects of dietary supplementation with sodium selenite, selenium yeast, selenium methionine, and nano-selenium on production performance, egg quality, plasma antioxidant capacity, and egg selenium content in laying hens to provide a theoretical basis for the rational use of selenium in laying hen diets. Five hundred forty healthy 18-week-old Hy-Line Grey laying hens with similar laying rates were randomly allocated into 5 groups with 6 replicates of 18 hens each. The control group was fed a basal diet without selenium supplementation (total selenium content 0.08 mg/kg), while the other four groups received the basal diet supplemented with 0.30 mg/kg selenium from sodium selenite, selenium yeast, selenium methionine, or nano-selenium (analyzed dietary selenium contents of 0.37, 0.38, 0.34, and 0.41 mg/kg, respectively). The experiment consisted of a 1-week preliminary period and an 8-week formal trial period. The results showed: 1) Different selenium sources had no significant effects on production performance or egg quality (P>0.05). 2) Compared with the control group, dietary supplementation with 0.30 mg/kg of the four selenium sources significantly increased plasma glutathione peroxidase (GSH-Px) activity (P<0.05). At the end of week 4, the nano-selenium group showed the highest GSH-Px activity; at week 8, both the selenium yeast and nano-selenium groups exhibited higher GSH-Px activity. Dietary supplementation with the four selenium sources also increased plasma total antioxidant capacity (T-AOC), with the nano-selenium group being significantly higher than other groups at both week 4 and week 8 (P<0.05). No significant effects were observed on plasma superoxide dismutase (SOD) activity or malondialdehyde (MDA) content among the four selenium sources (P>0.05). 3) Compared with

the control group, supplementation with the four selenium sources in the basal diet significantly increased egg selenium content ($P<0.05$), with the selenium yeast group being significantly higher than the other three groups ($P<0.05$). In conclusion, supplementation with the four selenium sources in the basal diet had no significant effects on production performance or egg quality of laying hens. All four selenium sources significantly improved plasma GSH-Px activity and T-AOC, with selenium yeast and nano-selenium showing better effects. Compared with sodium selenite, selenium methionine, and nano-selenium, selenium yeast was more effective in increasing egg selenium content.

Keywords: selenium sources; laying hens; antioxidant capacity; egg selenium content

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Selenium is an essential trace element for animals and humans. As a component of the active center of glutathione peroxidase (GSH-Px), selenium can regulate redox status and endocrine systems, enhance immunity, and improve health [1]. Thyroid hormone 5 -deiodinase is also a selenium-containing enzyme, and selenium plays an important role in regulating thyroid hormone metabolism [2]. Selenium exerts physiological functions in the form of selenoproteins in the body. Recent studies have shown that there are 25 selenoproteins, which are crucial for slowing and preventing oxidative damage [3]. Selenium distribution has obvious regional characteristics, and selenium content in feed varies greatly. In practical production, sodium selenite is commonly used for supplementation, but it has high toxicity and low bioavailability. With the discovery of more selenoenzymes and their corresponding biological functions, consumer enthusiasm for selenium-enriched foods has continued to grow. Selenium-enriched eggs are a good source of selenium supplementation, and developing selenium sources that can maintain health while being efficiently deposited in eggs has become a research hotspot in recent years. In addition to sodium selenite, current selenium sources on the market include selenium yeast, selenium methionine, nano-selenium, and selenium-enriched plants. Studies have shown that dietary supplementation with 0.30 mg/kg selenium from sodium selenite and selenium yeast did not affect production performance or egg quality of laying hens, but egg selenium content was higher in the selenium yeast group [4]. L-selenomethionine showed higher deposition efficiency in eggs than selenium yeast and sodium selenite [5]. Selenium yeast and nano-selenium improved production performance and egg quality in quails while increasing egg selenium content, with nano-selenium showing better effects [6]. Both selenium yeast and selenium-enriched alfalfa improved production performance in laying hens, with selenium yeast showing higher deposition efficiency in eggs [7]. Research indicates that common selenium sources can effectively increase egg selenium content, but their effects on production performance and egg quality have been reported inconsistently, and systematic studies on nano-selenium application in laying hens are lacking. Therefore, this study investigated the effects of selenium yeast, selenium methionine, nano-selenium, and sodium selenite on production performance, egg

quality, plasma antioxidant capacity, and egg selenium content in laying hens under controlled conditions eliminating differences in breed, age, and nutritional level, to provide a theoretical basis for rational selenium use in laying hen diets.

1.1 Experimental Materials and Animals

The selenium sources used in the experiment are shown in Table 1 . The experimental animals were 18-week-old healthy Hy-Line Grey laying hens with similar laying rates.

1.2 Experimental Design

Five hundred forty 18-week-old healthy Hy-Line Grey laying hens with similar laying rates were randomly divided into 5 groups with 6 replicates of 18 hens each. The control group was fed a basal diet without selenium supplementation, while the other four groups were fed the basal diet supplemented with 0.30 mg/kg selenium from sodium selenite, selenium yeast, selenium methionine, or nano-selenium, respectively. The dietary selenium contents were 0.08, 0.37, 0.38, 0.34, and 0.41 mg/kg, respectively.

1.3 Experimental Diets and Management

The basal diet was formulated according to NRC (1994). The composition and nutrient levels of the basal diet are shown in Table 2 . The hens were housed in 4-tier cage systems with 3 hens per cage, allowed free access to feed and water, and exposed to natural light plus artificial lighting (16 h/d). The relative humidity was maintained at 50%-60% with natural ventilation combined with longitudinal negative pressure ventilation. Manure was removed twice daily, and disinfection was performed weekly with routine epidemic prevention and immunization. The experiment included a 1-week preliminary period and an 8-week formal trial period.

1.4.1 Production Performance

Eggs were collected daily at 09:00. Egg weight, number of eggs, and numbers of soft-shelled, broken, or abnormally shaped eggs were recorded per replicate to calculate average egg weight, average daily egg mass, and laying rate. Feed consumption was measured and weighed weekly per replicate to calculate average daily feed intake and feed-to-egg ratio.

1.4.2 Egg Quality

At the end of week 8, five eggs were collected from each replicate for egg quality determination. A SONOVA Egg AnalyzerTM (Orka Technology Ltd.) was used to measure albumen height, Haugh unit, and yolk color. Eggshell strength was measured using an Egg Force Reader (Orka Technology Ltd.), eggshell thickness with an Egg Shell Thickness Gauge (Orka Technology Ltd.), and egg shape index

with an Egg index reader (Fujihira Industry Co., Ltd.). For egg component analysis, whole egg and shell weights were measured, then yolk and albumen were separated and weighed to determine the proportions of shell, albumen, and yolk.

1.4.3 Antioxidant Capacity Determination

At the end of week 4 and week 8, one hen was randomly selected from each replicate, and blood was collected from the wing vein after fasting. Plasma was prepared by centrifugation at 3,600 r/min for 10 min, aliquoted, and stored at -20°C. Plasma superoxide dismutase (SOD) activity was determined by xanthine oxidase method, malondialdehyde (MDA) content by thiobarbituric acid method, and GSH-Px activity and total antioxidant capacity (T-AOC) by colorimetric method using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4.4 Selenium Content Determination

Selenium content in diets and whole egg liquid was determined by hydride generation-atomic fluorescence spectrometry (according to GB 5009.93–2010). Diet samples were mixed and ground. At the end of week 8, two eggs were collected per replicate, weighed, shelled, and the egg liquid was mixed and freeze-dried. Approximately 2 g of prepared sample was placed in a 250 mL conical flask with a stopper, and 10.0 mL of mixed acid (perchloric acid and nitric acid at a 1:9 volume ratio) and several glass beads were added. After overnight cold digestion, the sample was heated on an electric hot plate (digestion temperature $ $180°C) with timely addition of mixed acid. When the solution became clear and colorless with white smoke appearing, heating continued until about 2 mL remained. After cooling, 5 mL of 6 mol/mL hydrochloric acid was added and heating continued until the solution became clear and white with white smoke. After cooling, the solution was transferred to a 50 mL volumetric flask, diluted to volume, and mixed. An aliquot of the digested sample was transferred to a 25 mL volumetric flask, 1 mL of 10% (mass concentration) potassium ferricyanide solution was added, and the solution was diluted with 3 mol/L hydrochloric acid. Ultrapure water, reagents, and selenium standard reference material (GBW8551) were used as blank and standard controls, and selenium content was determined by atomic fluorescence spectrometer.

1.5 Data Processing and Calculation

Experimental data were analyzed by one-way ANOVA using SPSS 19.0, and Duncan' s multiple comparison test was used for post-hoc analysis. Significance was set at $P<0.05$, and results were expressed as "mean ± standard deviation" .

2.1 Effects of Dietary Supplementation of Different Selenium Sources on Production Performance of Laying Hens

As shown in Table 3 , there were no significant differences in laying rate, average egg weight, average daily feed intake, feed-to-egg ratio, or average daily egg mass between the selenium-supplemented groups and the control group at any stage ($P>0.05$). Dietary supplementation with different selenium sources tended to increase laying rate, average egg weight, and average daily egg mass while improving feed-to-egg ratio, but these differences were not significant ($P>0.05$).

2.2 Effects of Dietary Supplementation of Different Selenium Sources on Egg Quality of Laying Hens

As shown in Table 4 , there were no significant differences in eggshell thickness, eggshell strength, egg shape index, or albumen height among groups ($P>0.05$). Compared with the control group, selenium supplementation slightly increased eggshell thickness and eggshell strength. No significant differences were observed in Haugh unit among groups ($P>0.05$), with all values above 80 indicating good egg quality. No significant differences were found in yolk color among groups ($P>0.05$), though yolk color was deepened in selenium-supplemented groups, particularly in the nano-selenium group. No significant differences were observed in yolk percentage, eggshell percentage, or albumen percentage among groups ($P>0.05$), with eggshell percentage slightly increased in selenium-supplemented groups.

2.3 Effects of Dietary Supplementation of Different Selenium Sources on Plasma Antioxidant Capacity of Laying Hens

As shown in Table 5 , dietary supplementation with different selenium sources significantly increased plasma GSH-Px activity compared with the control group ($P<0.05$). At week 4, the nano-selenium group showed the highest GSH-Px activity, significantly higher than other groups ($P<0.05$). At week 8, the selenium yeast and nano-selenium groups showed significantly higher GSH-Px activity than other groups ($P<0.05$), with all groups except the selenium yeast group showing a slight decrease. At week 4, plasma T-AOC was increased in all selenium-supplemented groups compared with the control group, with the selenium yeast, selenium methionine, and nano-selenium groups being significantly higher than the control and sodium selenite groups ($P<0.05$), and the nano-selenium group showing the highest value. At week 8, plasma T-AOC was increased in all selenium-supplemented groups compared with the control group, with the selenium yeast, selenium methionine, and nano-selenium groups being significantly higher than the control group ($P<0.05$), and the nano-selenium group showing the highest value. No significant differences were observed in plasma SOD activity among groups at any stage ($P>0.05$), though SOD activity was higher in selenium-supplemented groups than in the control group, with this effect being more pronounced at week 8 and the selenium yeast group showing the highest SOD activity at all stages. No significant differences were

observed in plasma MDA content among groups at any stage (P>0.05), though MDA content was lower in selenium-supplemented groups than in the control group. These results indicate that dietary supplementation with different selenium sources can improve the antioxidant status of laying hens, with nano-selenium and selenium yeast showing better effects.

2.4 Effects of Dietary Supplementation of Different Selenium Sources on Egg Selenium Content

As shown in Figure 1 [Figure 1: see original paper], egg selenium content was significantly increased in all selenium-supplemented groups compared with the control group (P<0.05). The sodium selenite, selenium yeast, selenium methionine, and nano-selenium groups showed increases of 168.60%, 269.30%, 163.22%, and 169.10%, respectively, compared with the control group, with the selenium yeast group showing the greatest increase and being significantly higher than the sodium selenite, selenium methionine, and nano-selenium groups (P<0.05). These results indicate that dietary supplementation with different selenium sources significantly affects egg selenium content, with selenium yeast being the most effective for deposition.

3.1 Effects of Dietary Supplementation of Different Selenium Sources on Production Performance of Laying Hens

As an essential trace element for animals and humans, selenium is an important component of many antioxidant enzymes, deiodinases, and selenoproteins that regulate growth, affect metabolism, enhance immunity, and improve reproductive performance. This study showed that dietary supplementation with 0.30 mg/kg selenium from sodium selenite, selenium yeast, selenium methionine, or nano-selenium had no significant effects on production performance of laying hens during the experimental period, which is consistent with findings by Delezie et al. [5], Jlali et al. [8], Utterback et al. [9], and Cai et al. [4]. However, some studies have reported that dietary supplementation with selenium yeast can improve production performance in laying hens [10]. Production performance of laying hens is affected by breed, age, dietary selenium content, and selenium source, with different sensitivity to nutrients during the initial laying period versus the late laying period. The aforementioned studies used 80-week-old molted hens, whereas this experiment used hens in the transition period from initial laying to peak production, when the antioxidant system, metabolic level, and immunity may be at optimal status, resulting in minimal influence of selenium source and level on production performance. Additionally, research has shown that even when external selenium supply is insufficient, hens mobilize stored selenium in the body to maintain health [11]. The experimental period in this study was 8 weeks, and the control group was fed a basal diet containing 0.08 mg/kg selenium without showing selenium deficiency symptoms. This may be because the hens mobilized stored body selenium during the experimental period, or because the experimental period was relatively short, as Latshaw

et al. [12] reported that decreased laying rate occurred only after 3 months of feeding a basal diet containing 0.03 mg/kg selenium.

3.2 Effects of Dietary Supplementation of Different Selenium Sources on Egg Quality of Laying Hens

Many factors affect egg quality, including breed, laying age, nutritional level, rearing system, disease, and storage time, with breed and laying age being particularly important factors. This study showed that dietary supplementation with 0.30 mg/kg selenium from the four sources did not significantly affect egg quality, and albumen height and Haugh unit were in good condition. Studies have shown that dietary supplementation with sodium selenite and selenium yeast had no significant effects on routine egg quality parameters [13], consistent with the present results. Pavlović et al. [14] reported that dietary supplementation with sodium selenite or selenium yeast had no significant effects on eggshell quality, eggshell thickness, eggshell strength, or egg shape index, suggesting that while selenium supplementation may alter eggshell membrane secretion and synthesis (potentially negatively affecting eggshell quality), it may also protect eggshell quality by enhancing antioxidant capacity. Thus, producing selenium-enriched eggs through dietary selenium supplementation does not adversely affect egg quality.

3.3 Effects of Dietary Supplementation of Different Selenium Sources on Plasma Antioxidant Capacity of Laying Hens

The antioxidant capacity of the body's defense system is closely related to health status. GSH-Px is a widely distributed enzyme that catalyzes hydrogen peroxide decomposition and is an important component of the antioxidant defense system. It specifically catalyzes the reduction of hydrogen peroxide by reduced glutathione, protecting the integrity of cell membrane structure and function. This study showed that dietary supplementation with the four selenium sources significantly increased GSH-Px activity in laying hens, consistent with findings by Jing et al. [15], Qu et al. [6], and Wang et al. [16]. Selenium is an essential component of GSH-Px, and GSH-Px activity is closely related to selenium status in the body [17]. Therefore, the increased GSH-Px activity may be due to increased selenium intake from dietary supplementation, which elevated blood selenium levels [18] and consequently increased plasma GSH-Px activity. This study showed that plasma SOD activity and MDA content were not affected by selenium source, consistent with findings by Pan et al. [19].

T-AOC is a comprehensive indicator of antioxidant capacity. Dietary supplementation with selenium yeast and sodium selenite can significantly increase plasma T-AOC [19]. This study showed that the three selenium sources other than sodium selenite significantly increased plasma T-AOC, while sodium selenite only slightly increased T-AOC. The increase in T-AOC may be related to increased GSH-Px activity after selenium supplementation. GSH-Px works together with SOD and catalase to remove O_2^- and H_2O_2, participating in

the first line of antioxidant defense, and also participates in the second line of defense by reducing hydroperoxides, making it significant for the antioxidant enzyme system.

This study demonstrated that different selenium sources have different capacities to improve antioxidant status, with nano-selenium and selenium yeast showing better effects. Nano-selenium has efficient absorption, strong adsorption capacity, and can directly scavenge free radicals in the body [20]. As an organic selenium source, selenium yeast can more easily enter the body and exert effects. Their different metabolic pathways in the body may be the main reason for the differences in antioxidant capacity.

3.4 Effects of Dietary Supplementation of Different Selenium Sources on Egg Selenium Content

The clinical benefits of selenium, including anticancer effects [21], prevention of childhood asthma [22], protection of cardiovascular and cerebrovascular systems [23], and immunity enhancement [1], have increased consumer enthusiasm for selenium-enriched foods. Eggs are an important part of the human diet, and their selenium content is highly regulable [24], making selenium-enriched eggs an important medium for selenium supplementation. Therefore, finding a selenium source that can be efficiently deposited in eggs is of great significance.

This study showed that dietary supplementation with 0.30 mg/kg selenium from the four sources significantly increased selenium deposition in eggs, consistent with reports by Cai et al. [4] and Delezie et al. [5]. Moreover, egg selenium content in the selenium yeast group was significantly higher than in the sodium selenite, selenium methionine, and nano-selenium groups. The deposition efficiency of selenium yeast in eggs was higher than that of sodium selenite, consistent with previous studies [4,13,18,25], indicating that organic selenium from selenium yeast can enter the body more rapidly and in greater amounts than sodium selenite. The application of nano-selenium in laying hens is relatively limited, and comparisons with other selenium sources have not been reported. Qu et al. [6] showed that nano-selenium had better deposition effects in quail eggs than selenium yeast. This study demonstrated that selenium yeast had higher deposition efficiency in eggs of Hy-Line Grey laying hens during the early laying period than nano-selenium, which may be related to different mineral deposition capacities among poultry species. Although previous studies have shown that selenium methionine is the main form of organic selenium in selenium yeast [26], this study found that selenium yeast resulted in significantly greater selenium deposition in eggs than selenium methionine. Richie et al. [27] reported that selenium yeast was more effective than selenium methionine in reducing oxidative stress levels and could be deposited more in target organs, though the specific reasons remain unclear.

Sevcikova et al. [28] fed different forms of selenium to goats during pregnancy and lactation and found that selenium yeast was more effective than other or-

ganic selenium sources in increasing tissue selenium content in lambs at weaning. However, a 7-month supranutritional feeding study in aged beagle dogs showed no differences between selenium yeast and selenium methionine in prostate tissue selenium content or other parameters, although previous reports indicated that selenium yeast, but not selenium methionine, reduced prostate cancer [29]. Thus, more research is needed to draw definitive conclusions about the effects of different selenium sources on tissue selenium deposition.

Conclusions: 1. Dietary supplementation with 0.30 mg/kg selenium from sodium selenite, selenium yeast, selenium methionine, or nano-selenium had no significant effects on production performance or egg quality of 18- to 26-week-old Hy-Line Grey laying hens. 2. All four selenium sources improved antioxidant status to some extent, with nano-selenium and selenium yeast showing better effects. 3. Among the four selenium sources, selenium yeast showed the highest deposition efficiency in eggs.

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