Effects of Different Levels of Inorganic and Organic Complexed Trace Minerals on Plasma Antioxidant Capacity in Laying Hens (Postprint)

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Abstract

This experiment aimed to investigate the effects of different levels of inorganic and organic complex trace elements on the plasma antioxidant capacity of laying hens. A total of 990 22-week-old Jinghong No. 1 laying hens were selected and randomly divided into 11 groups, with 6 replicates per group and 15 hens per replicate. Group 1 served as the control group, and the experimental groups were supplemented with different levels of inorganic (groups 2-6) or organic complex trace elements (groups 7-11) in the diet at 25%, 50%, 75%, 100%, and 125% of the NRC (1994) recommended requirements, respectively. The supplementation levels of manganese (Mn), iron (Fe), zinc (Zn), and selenium (Se) followed the NRC (1994) standards for laying hens, while the copper (Cu) supplementation level followed the NRC (1994) standard for broilers. The experimental period lasted for 24 weeks. The results showed: 1) Supplementation with inorganic complex trace elements at 75%, 100%, and 125% of the NRC recommended requirements extremely significantly increased plasma total superoxide dismutase (T-SOD) activity in laying hens ($P<0.01$); supplementation with inorganic complex trace elements at 100% and 125% of the NRC recommended requirements extremely significantly increased plasma glutathione peroxidase (GSH-Px) activity ($P<0.01$); supplementation with inorganic complex trace elements at 125% of the NRC recommended requirements extremely significantly increased plasma total antioxidant capacity (T-AOC) at weeks 8 and 24 of the experiment ($P<0.01$); supplementation with inorganic complex trace elements at 75%, 100%, and 125% of the NRC recommended requirements extremely significantly decreased plasma malondialdehyde (MDA) content at week 16 of the experiment ($P<0.01$). 2) Supplementation with different levels of organic complex trace elements extremely significantly increased plasma T-AOC at week 8, plasma T-SOD activity at weeks 4, 8, and 16, and plasma GSH-Px activity at week 16 of the experiment ($P<0.01$); supplementation with organic complex trace elements at 50%, 75%, 100%, and 125% of the NRC recommended requirements extremely significantly decreased plasma MDA content at weeks 4 and 16 of the experiment ($P<0.01$). 3) At the 75% level of the NRC recommended requirements, plasma T-AOC in the organic group was significantly higher than that in the inorganic group at week 8 of the experiment ($P<0.05$); at weeks 16 and 24 of the experiment, plasma GSH-Px activity in the organic group was significantly higher than that in the inorganic group ($P<0.05$). At the 125% level of the NRC recommended requirements, plasma T-SOD activity in the organic group was significantly higher than that in the inorganic group at week 16 of the experiment ($P<0.05$). The experiment demonstrated that supplementation with inorganic complex trace elements at $ $75% of the NRC recommended requirements in the diet of laying hens was beneficial for improving plasma antioxidant capacity; supplementation with organic complex trace elements at 25% and 50% of the NRC recommended requirements in the diet of laying hens was beneficial for improving plasma antioxidant capacity; at the 75% and 125% levels of the NRC recommended requirements, organic complex trace elements were superior to inorganic complex trace elements in improving the antioxidant capacity of laying hens.

Full Text

Effects of Inorganic and Organic Complex Trace Elements at Different Levels on the Plasma Antioxidant Activities of Laying Hens

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Abstract

This experiment was conducted to study the effects of inorganic and organic complex trace elements at different levels on the plasma antioxidant activities of laying hens. Nine hundred and ninety 22-week-old Beijing Red No.1 laying hens were randomly allocated to 11 groups with 6 replicates per group and 15 hens per replicate. Group 1 served as the control. Experimental groups were fed basal diets supplemented with inorganic (groups 2-6) and organic complex trace elements (groups 7-11) at 25%, 50%, 75%, 100%, and 125% of NRC (1994) recommendations, respectively. Manganese (Mn), iron (Fe), zinc (Zn), and selenium (Se) supplementation levels followed the NRC (1994) standards for laying hens, while copper (Cu) followed the NRC (1994) standards for broilers. The experiment lasted 24 weeks.

The results showed: 1) Inorganic complex trace elements at 75%, 100%, and 125% of NRC recommendations significantly increased plasma total superoxide dismutase (T-SOD) activity ($P<0.01$); inorganic complex trace elements at

100% and 125% significantly increased plasma glutathione peroxidase (GSH-Px) activity ($P<0.01$); inorganic complex trace elements at 125% significantly increased plasma total antioxidant capacity (T-AOC) at weeks 8 and 24 ($P<0.01$); and inorganic complex trace elements at 75%, 100%, and 125% significantly decreased plasma malondialdehyde (MDA) content at week 16 ($P<0.01$). 2) Organic complex trace elements at all levels significantly increased plasma T-AOC at week 8, T-SOD activity at weeks 4, 8, and 16, and GSH-Px activity at week 16 ($P<0.01$); organic complex trace elements at 50%, 75%, 100%, and 125% significantly decreased plasma MDA content at weeks 4 and 16 ($P<0.01$). 3) At 75% of NRC recommendations, the organic group showed significantly higher plasma T-AOC at week 8 and GSH-Px activity at weeks 16 and 24 compared to the inorganic group ($P<0.05$). At 125% of NRC recommendations, the organic group showed significantly higher plasma T-SOD activity at week 16 ($P<0.05$).

In conclusion, supplementing laying hen diets with inorganic complex trace elements above 75% of NRC recommendations, or with organic complex trace elements at 25% and 50% of NRC recommendations, is beneficial for improving plasma antioxidant capacity. At 75% and 125% of NRC recommendations, organic complex trace elements demonstrate superior effects on antioxidant capacity compared to inorganic forms.

Key words: inorganic trace element; organic trace element; laying hens; antioxidant activities

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Trace elements are essential components and active centers of various enzymes, regulating or activating enzyme activities, controlling metabolic processes of nutrients such as energy and protein in animals, and modulating endocrine functions. Zinc (Zn), copper (Cu), and manganese (Mn) are active components of superoxide dismutase (SOD), and these trace elements also affect the production of non-enzymatic antioxidant proteins (such as glutathione and ceruloplasmin). Rotruck et al. [1] discovered in 1973 that selenium (Se) is an essential component of glutathione peroxidase (GSH-Px), thereby revealing the antioxidant function of Se. The nutritional status of Zn, Cu, and Mn in animals affects the body' s antioxidant capacity. Total antioxidant capacity (T-AOC) is a comprehensive indicator measuring the body' s antioxidant capacity, representing the sum of antioxidant enzyme systems and antioxidant substance systems. Malondialdehyde (MDA) is the primary product of free radicals and lipid peroxidation, and its level in blood indirectly reflects the severity of free radical attack on body cells. Total superoxide dismutase (T-SOD) is widely distributed in tissue cells throughout the body, and its activity indirectly reflects the body' s ability to scavenge free radicals while serving as one indicator for assessing the nutritional status of Zn, Cu, and Mn.

GSH-Px specifically catalyzes the reduction reaction of reduced glutathione on peroxides, with Se serving as the active center of this enzyme. When animals are deficient in Se, the enzyme' s activity decreases, leading to reduced antioxidant capacity [1-2]. In summary, the nutritional status of trace elements plays a crucial role in animal antioxidant capacity, and both the dosage and form of complex trace element supplementation are important factors affecting antioxidant capacity. Therefore, this experiment investigated the effects of supplementing laying hen diets with different levels of inorganic and organic complex trace elements on plasma T-AOC, MDA content, and T-SOD and GSH-Px activities, providing experimental evidence for rational determination of complex trace element supplementation levels.

1. Materials and Methods

1.1 Experimental Materials

The types and specifications of inorganic and organic trace elements used in the experiment are shown in Table 1.

1.2 Experimental Animals and Basal Diet

Nine hundred and ninety 22-week-old Beijing Red No.1 laying hens were used as experimental animals. The basal diet was formulated with corn, soybean meal, and cottonseed meal as main ingredients without trace element supplementation. Nutrient levels were determined according to NRC (1994) requirements for laying hens. The composition and nutrient levels of the basal diet are shown in Table 2 .

1.3 Experimental Design and Management

Nine hundred and ninety 22-week-old Beijing Red No.1 laying hens were randomly divided into 11 groups with 6 replicates per group and 15 hens per replicate. Group 1 served as the control group receiving no complex trace elements [Mn, Fe, Cu, Zn, Se]. Groups 2-6 received inorganic complex trace elements, while groups 7-11 received organic complex trace elements, each at 25%, 50%, 75%, 100%, and 125% of NRC (1994) recommendations. Mn, Fe, Zn, and Se supplementation followed NRC (1994) standards for laying hens, while Cu followed NRC (1994) standards for broilers. The supplemental levels and measured values of trace elements in each group’ s diet are shown in Table 3 . The experimental period lasted 24 weeks.

The feeding trial was conducted at the Changping Experimental Base of the Institute of Animal Science, Chinese Academy of Agricultural Sciences. A three-tier cage system was used with 3 hens per cage. Feed was provided three times daily with ad libitum access to feed and water. Lighting was controlled at 16 hours per day using a lighting program controller, with automatic temperature control, heating, and ventilation.

1.4 Sample Collection and Analysis

1.4.1 Dietary Trace Element Content Approximately 1 kg of diet samples were collected using the quartering method to obtain about 500 g. Samples were ground to pass through a 40-mesh sieve, sealed in bags, and stored in a cool, dry place for analysis. Sample pretreatment was performed using the CEM high-throughput closed digestion system (CEM-MARS 5). Diet samples (0.10-0.15 g) were weighed into digestion tubes, pre-digested with 6 mL nitric acid for 1 hour, then 2 mL hydrogen peroxide was added. After 30 minutes of reaction, digestion tube caps were loosened to release gas, then tightened. Tubes were placed symmetrically on the turntable, which was installed in the instrument chamber. After editing the method, the start/pause button initiated the digestion program. Upon completion, digestion tubes were removed and placed on an electric digester for acid evaporation for 45-50 minutes, then cooled and diluted 500-fold with ultrapure water. Processed sample solutions were analyzed for Mn, Fe, Cu, Zn, and Se content using inductively coupled plasma mass spectrometry (Agilent 7700 ICP-MS).

1.4.2 Antioxidant Capacity Indicators During the experiment, blood was collected via venipuncture from 2 randomly selected hens per replicate every 4 weeks. Plasma was separated and stored for analysis. Plasma T-AOC, MDA content, and T-SOD and GSH-Px activities were measured using a double-beam UV-visible spectrophotometer (TU-1901). Assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, and measurements were performed strictly according to the manufacturer' s instructions.

1.5 Data Processing and Analysis

The effects of different levels of inorganic or organic complex trace elements on plasma antioxidant capacity were analyzed using the ANOVA procedure in SAS 9.2 software. Duncan' s multiple range test was used for intergroup comparisons. The effects of inorganic versus organic complex trace elements at the same level were analyzed using t-tests. $P<0.05$ was considered statistically significant. Results are expressed as means ± standard deviation (SD).

2. Results

2.1 Effects of Different Levels of Inorganic Complex Trace Elements on Plasma Antioxidant Capacity of Laying Hens

As shown in Table 4 , when complex trace elements were added in inorganic form, compared with the control group, inorganic complex trace elements at 25%, 50%, 75%, and 125% of NRC recommendations significantly increased plasma T-AOC at week 8 ($P<0.01$), and at 125% significantly increased plasma T-AOC at week 24 ($P<0.01$). Inorganic complex trace elements at 75%, 100%, and 125% significantly decreased plasma MDA content at week 16 ($P<0.01$). Inorganic complex trace elements at 50%, 75%, 100%, and 125% significantly increased plasma T-SOD activity at weeks 8 and 16 ($P<0.01$), while at 75%, 100%, and 125% significantly increased plasma T-SOD activity at weeks 4 and 24 ($P<0.01$). Inorganic complex trace elements at 75%, 100%, and 125% significantly increased plasma GSH-Px activity at weeks 8 and 16 ($P<0.01$), and at 100% and 125% significantly increased plasma GSH-Px activity at weeks 4 and 24 ($P<0.01$).

2.2 Effects of Different Levels of Organic Complex Trace Elements on Plasma Antioxidant Capacity of Laying Hens

As shown in Table 5 , when complex trace elements were added in organic form, compared with the control group, organic complex trace elements at all levels significantly increased plasma T-AOC at week 8 ($P<0.01$), and at 100% significantly increased plasma T-AOC at week 16 ($P<0.05$). Organic complex trace elements at all levels significantly decreased plasma MDA content at week 4 ($P<0.01$), and at 50%, 75%, 100%, and 125% significantly decreased plasma MDA content at week 16 ($P<0.01$). At all experimental periods, organic complex trace elements at all levels significantly increased plasma T-SOD activity (except at 25% at week 24) ($P<0.01$). Organic complex trace elements at 75%, 100%, and 125% significantly increased plasma GSH-Px activity at week 4 ($P<0.01$), at all levels significantly increased plasma GSH-Px activity at week 16 ($P<0.01$), and at 100% and 125% significantly increased plasma GSH-Px activity at week 24 ($P<0.01$).

2.3 Effects of Inorganic and Organic Complex Trace Elements at the Same Level on Plasma Antioxidant Capacity of Laying Hens

As shown in Table 6 , at 75% of NRC recommendations, the organic group showed significantly higher plasma T-AOC at week 8 and GSH-Px activity at weeks 16 and 24 compared to the inorganic group ($P<0.05$). At 125% of NRC recommendations, the organic group showed significantly higher plasma T-SOD activity at week 16 compared to the inorganic group ($P<0.05$). At other supplementation levels, no significant differences were observed between inorganic and organic groups in T-AOC, MDA content, or T-SOD and GSH-Px activities ($P>0.05$).

3. Discussion

3.1 Effects of Different Levels of Inorganic Complex Trace Elements on Plasma Antioxidant Capacity of Laying Hens

T-AOC is a comprehensive indicator of antioxidant capacity. When free radicals attack polyunsaturated fatty acids in biological membranes, they initiate lipid peroxidation, producing lipid peroxides whose accumulation causes irreversible tissue damage [3]. MDA is the primary product of free radicals and lipid peroxidation, and its blood level indirectly reflects the severity of free radical attack on cells. GSH-Px and T-SOD constitute the body’ s antioxidant stress barrier

[4]. T-SOD is widely distributed in tissue cells and protects cells from damage by scavenging oxygen free radicals; its activity indirectly reflects the body’ s free radical scavenging ability and serves as an indicator of Mn, Cu, and Zn nutritional status. GSH-Px specifically catalyzes the reduction of peroxides by reduced glutathione, converting peroxides into non-toxic products and thereby protecting cell membrane structure and function from peroxide damage. As Se is the active center of this enzyme, its content directly affects enzyme activity [5].

Studies by Gao et al. [6] and Wang Hongyang et al. [7] demonstrated that dietary Zn supplementation significantly increased serum copper-zinc superoxide dismutase (CuZn-SOD) activity in animals, with activity increasing as dietary Cu supplementation increased. Wang Shumei et al. [8] found that manganese superoxide dismutase (Mn-SOD) activity in duck serum increased significantly with increasing dietary Mn levels. Pan Cuiling et al. [9] reported that GSH-Px activity in laying hen blood increased with increasing dietary Se levels. Our results showed that inorganic complex trace elements at 75%, 100%, and 125% of NRC recommendations significantly increased plasma T-SOD activity, and at 100% and 125% significantly increased plasma GSH-Px activity, consistent with these previous findings. Additionally, inorganic complex trace elements at 125% significantly increased plasma T-AOC at weeks 8 and 24, and at 75%, 100%, and 125% significantly decreased plasma MDA content at week 16. These results indicate that supplementing laying hen diets with inorganic complex trace elements above 75% of NRC recommendations is beneficial for improving plasma antioxidant capacity.

3.2 Effects of Different Levels of Organic Complex Trace Elements on Plasma Antioxidant Capacity of Laying Hens

Research reports on the effects of organic complex trace elements on plasma antioxidant capacity indicate that amino acid-chelated trace elements significantly increased liver T-SOD activity and serum GSH-Px activity while decreasing MDA content in growing-finishing pigs [10]. Our results demonstrated that organic complex trace elements at all levels significantly increased plasma T-AOC at week 8, T-SOD activity at weeks 4, 8, and 16, and GSH-Px activity at week 16. Organic complex trace elements at 50%, 75%, 100%, and 125% significantly decreased plasma MDA content at weeks 4 and 16, generally consistent with previous results. These findings indicate that supplementing laying hen diets with organic complex trace elements at 25% and 50% of NRC recommendations is beneficial for improving plasma antioxidant capacity.

3.3 Comparison of Inorganic and Organic Complex Trace Elements at the Same Level on Plasma Antioxidant Capacity of Laying Hens

Our results showed that at 75% of NRC recommendations, the organic group exhibited significantly higher plasma T-AOC at week 8 and GSH-Px activity at weeks 16 and 24 compared to the inorganic group. At 125% of NRC recommendations, the organic group showed significantly higher plasma T-SOD activity at week 16. This is attributed to the metabolic superiority of organic trace elements in vivo [11], which are absorbed intact, thereby reducing catalytic oxidation with easily oxidized nutrients, enhancing the activity of certain digestive enzymes, facilitating transport after absorption, and directly acting on target organs to participate in biochemical reactions [12]. Consequently, organic trace elements offer greater advantages than inorganic forms in improving antioxidant capacity. In summary, when trace element supplementation levels are low, inorganic and organic complex trace elements show comparable effects; however, as supplementation levels increase, organic complex trace elements demonstrate superior effects, suggesting that high-level supplementation reduces absorption efficiency of inorganic trace elements but does not significantly affect organic forms. Therefore, at 75% and 125% of NRC recommendations, organic complex trace elements are more effective than inorganic forms in improving plasma antioxidant capacity in laying hens.

1. When supplemented as inorganic complex trace elements, levels above 75% of NRC recommendations in laying hen diets are beneficial for improving plasma antioxidant capacity.
2. When supplemented as organic complex trace elements, levels at 25% and 50% of NRC recommendations in laying hen diets are beneficial for improving plasma antioxidant capacity.
3. Compared at the same supplementation levels, organic complex trace elements demonstrate superior effects on antioxidant capacity in laying hens at 75% and 125% of NRC recommendations compared to inorganic forms.

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Table 1 The varieties and specifications of inorganic and organic trace elements

Table 2 Composition and nutrient levels of the basal diet (air-dry basis)

Table 3 Supplemental levels and measured values of trace elements in diets

Table 4 Effects of inorganic complex trace elements at different levels on the plasma antioxidant activities of laying hens

Table 5 Effects of organic complex trace elements at different levels on the plasma antioxidant activities of laying hens

Table 6 Effects of inorganic and organic complex trace elements on the plasma antioxidant activities of laying hens