Effects of Different Levels of Organic Trace Minerals on Mineral Element Deposition and Excretion in Laying Hens (Postprint)

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Abstract

This experiment was conducted to investigate the effects of different levels of organic trace elements on mineral element deposition and excretion in laying hens. A total of 240 Hy-Line Brown laying hens aged 25 weeks were randomly allocated to 4 groups with 6 replicates per group and 10 hens per replicate. The control group diet was supplemented with inorganic trace elements (ITE) according to NRC (1994) recommendations, while diets of experimental groups 1, 2, and 3 were supplemented with organic trace elements (OTE) at 60%, 80%, and 100% of NRC (1994) recommendations, respectively. The experimental period lasted 49 days. The results showed: 1) Compared with the control group, blood copper and zinc contents in experimental group 1, and blood copper, zinc, manganese and pancreas zinc contents in experimental groups 2 and 3 were significantly increased ($P<0.05$). 2) Compared with the control group, egg zinc, manganese and iron contents in all experimental groups were significantly increased ($P<0.05$). 3) Compared with the control group, fecal copper contents in experimental groups 1 and 2 and fecal zinc and manganese contents in experimental group 1 were extremely significantly decreased ($P<0.01$); fecal manganese in experimental group 2 and fecal copper in experimental group 3 were significantly decreased ($P<0.05$); calcium and phosphorus contents in blood, liver, pancreas, tibia and eggs of all experimental groups showed no significant changes ($P>0.05$). In conclusion, organic trace elements can promote the deposition of iron, copper, zinc and manganese in the body and eggs of laying hens, reduce the excretion of corresponding elements, and supplementation at 60% of NRC (1994) recommendations showed the best effect.

Full Text

Effects of Different Levels of Organic Trace Elements on Mineral Element Deposition and Excretion in Laying Hens

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Abstract

This study investigated the effects of different levels of organic trace elements (OTE) on mineral element deposition and excretion in laying hens. Two hundred and forty 25-week-old Hy-Line Brown laying hens were randomly allocated into four groups with six replicates per group and ten hens per replicate. The control group received a diet supplemented with inorganic trace elements (ITE) according to NRC (1994) recommendations, while experimental groups 1, 2, and 3 received diets supplemented with OTE at 60%, 80%, and 100% of NRC (1994) recommendations, respectively. The 49-day trial yielded several key findings. First, compared with the control group, blood copper and zinc levels in group 1, and blood copper, zinc, manganese, and pancreatic zinc levels in groups 2 and 3 were significantly elevated ($P<0.05$). Second, all experimental groups showed significant increases in egg zinc, manganese, and iron content ($P<0.05$) relative to the control. Third, fecal copper levels in groups 1 and 2, and fecal zinc and manganese levels in group 1 were dramatically reduced ($P<0.01$), while fecal manganese in group 2 and fecal copper in group 3 showed significant decreases ($P<0.05$). Notably, calcium and phosphorus levels in blood, liver, pancreas, tibia, and eggs remained unchanged across all groups ($P>0.05$). These results demonstrate that OTE supplementation enhances iron, copper, zinc, and manganese deposition in both hen tissues and eggs while reducing their excretion, with the 60% NRC recommendation level proving most effective.

Keywords: trace elements; laying hens; deposition; excretion

1.1 Experimental Materials

Both inorganic and organic trace elements were provided by Henan Egdoo Biological Technology Co., Ltd. The specific types and specifications of trace elements in these preparations are detailed in Table 1 . Standard solutions of iron, copper, zinc, and manganese were obtained from the National Analysis and Testing Center for Iron and Steel (Central Iron and Steel Research Institute). The free element ratio, a measured value indicating the percentage of elemental mass dissolved in ultrapure water relative to total mass, is also provided for each preparation.

1.2 Experimental Animals and Basal Diet

The experiment utilized 240 25-week-old Hy-Line Brown laying hens with similar body weights. The basal diet, formulated primarily with corn and soybean meal without trace element supplementation, was designed according to NRC (1994) nutrient requirements for laying hens. Diet composition and nutrient levels are presented in Table 2 . The premix provided per kilogram of diet: vitamin A 7,500 IU, vitamin D 2,500 IU, vitamin E 35 mg, vitamin K 1 mg, vitamin B 1.5 mg, vitamin B 4 mg, vitamin B 2 mg, vitamin B 0.02 mg, nicotinic acid 30 mg, folic acid 0.55 mg, pantothenate 10 mg, biotin 0.16 mg, and choline chloride 420 mg. Crude protein was a measured value, while other nutrient levels were calculated.

1.3 Experimental Design and Management

The 240 hens were randomly divided into four groups with six replicates each containing ten birds. The control group received the basal diet supplemented with ITE at NRC (1994) recommended levels, while experimental groups 1, 2, and 3 received the basal diet supplemented with OTE at 60%, 80%, and 100% of NRC (1994) recommendations, respectively. Trace element additive amounts and measured contents in each diet are shown in Table 3 . The feeding trial was conducted at the Zhou Mountain Campus Experimental Farm of Henan University of Science and Technology. Hens were provided ad libitum access to feed and water via nipple drinkers under natural ventilation with 16 hours of daily lighting. Routine sanitation and disease prevention measures were implemented according to laying hen management standards. The experimental period lasted 49 days.

1.4.1 Sample Collection

Diet samples (200 g each) were collected using the quartering method, ground to pass through a 60-mesh sieve, and stored in sealed bags in a cool, dry place pending analysis. Fecal samples were continuously collected for seven days from two hens per replicate (12 hens per group) using the total collection method. Fresh fecal weight was recorded, and samples were dried to constant weight at 70°C. Dried feces were then quartered to obtain 50 g samples, ground to pass through a 60-mesh sieve, and stored in sealed bags in a dry location. Egg samples consisted of two eggs randomly selected per replicate (12 eggs per group), which were boiled and shelled, retaining only the albumen and yolk. On the day before trial conclusion, feed was withdrawn from all groups. The following day, one hen per replicate (six hens per group) was randomly selected for blood collection from the wing vein, followed by cervical dissection for collection of liver, pancreas, and tibia samples.

1.4.2 Sample Processing and Detection

Diet and fecal samples underwent wet digestion using the method reported by Xue et al. [12]. Blood trace elements (iron, copper, zinc, manganese) were extracted following the protocol of Zhang et al. [13]. Liver, pancreas, and egg samples were processed via wet digestion according to Yokoi et al. [14], while tibia samples were dry-ashed using the method described by Angel [15]. Digested samples were analyzed for iron, copper, zinc, and manganese content using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a, USA). Calcium content was determined according to GB/T 6436—2002, and phosphorus content was measured following GB/T 6437—1992.

1.5 Data Processing and Analysis

Raw data were processed using Excel 2007, with results expressed as "mean ± standard deviation." One-way ANOVA was performed using the SPSS 20.0 software package, and LSD tests were employed for post-hoc comparisons of significant differences.

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2 Results

2.1 Effects of Different OTE Levels on Blood Mineral Element Content in Laying Hens

As shown in Table 4 , blood copper and zinc levels in group 1, and blood copper, zinc, and manganese levels in groups 2 and 3 were significantly higher than those in the control group ($P<0.01$ for most comparisons; $P<0.05$ for some). Group 3 also showed significantly elevated blood manganese ($P<0.01$) and copper and zinc ($P<0.05$). While blood iron, calcium, and phosphorus levels increased to varying degrees across experimental groups, these differences were not statistically significant ($P>0.05$), and no significant differences were observed among experimental groups.

Note: In the same column, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), different lowercase letters indicate significant difference (P<0.05), and different uppercase letters indicate highly significant difference (P<0.01). This applies to all tables.

2.2 Effects of Different OTE Levels on Mineral Element Deposition in Liver, Pancreas, and Tibia

Table 5 reveals that liver iron, copper, zinc, and manganese levels increased to varying degrees in all experimental groups compared with the control, though differences were not significant ($P>0.05$). The highest values were observed in group 2 for liver iron and copper, and in group 3 for liver zinc and manganese.

No significant differences were detected in liver calcium or phosphorus content among groups (P>0.05).

Table 6 shows that pancreatic zinc content was significantly elevated in groups 2 and 3 compared with the control (P<0.05). Pancreatic iron, copper, and manganese levels increased across experimental groups but did not reach statistical significance (P>0.05), with no significant differences among experimental groups. Pancreatic calcium and phosphorus levels remained unaffected (P>0.05).

According to Table 7 , tibial iron, copper, zinc, and manganese levels increased to varying degrees in groups 1, 2, and 3, though differences were not significant (P>0.05). Tibial calcium and phosphorus content showed no significant variation among groups (P>0.05).

2.3 Effects of Different OTE Levels on Mineral Element Deposition in Eggs

Table 8 demonstrates that egg zinc content increased dramatically in group 1 (P<0.01) and significantly in group 1 for iron (P<0.05) compared with the control. Group 2 exhibited highly significant increases in egg iron and zinc (P<0.01) and a significant increase in manganese (P<0.05). Group 3 showed highly significant elevations in egg iron and zinc (P<0.01). Although egg copper content increased across experimental groups, differences were not significant (P>0.05). Egg calcium and phosphorus content remained unchanged across all treatments (P>0.05).

2.4 Effects of Different OTE Levels on Mineral Element Excretion in Laying Hens

Table 9 indicates that fecal iron, copper, zinc, and manganese levels decreased to varying degrees in groups 1, 2, and 3 compared with the control. Specifically, group 1 showed highly significant reductions in copper, zinc, and manganese (P<0.01), while group 2 exhibited highly significant decreases in copper (P<0.01) and significant reductions in zinc and manganese (P<0.05). Group 3 demonstrated a significant decrease in copper (P<0.05). Fecal calcium and phosphorus content did not differ significantly among groups (P>0.05).

3.1 Effects of Different OTE Levels on Mineral Element Deposition in Blood, Liver, Pancreas, and Tibia

The mineral element content in blood, liver, pancreas, and tibia serves as a primary indicator for evaluating the biological efficiency of dietary trace elements. Blood trace elements originate primarily from apoptosis, inter-tissue transport, and intestinal absorption, and normally remain relatively stable in circulation [16]. Consequently, alterations in blood trace element levels often reflect physiological abnormalities or dietary deficiencies/excesses. Li et al. [17] summarized extensive literature demonstrating significant correlations between serum trace element levels and 258 diseases. Since absorbed mineral elements are transported mainly via blood, free mineral element concentrations in blood are frequently used as sensitive indicators to evaluate dietary mineral absorption rates [18]. Tan et al. [19] reported that organic trace elements significantly increased serum copper, iron, and zinc levels in multiparous sows during early gestation compared with inorganic sources. The present study found that microbially synthesized OTE increased serum calcium, phosphorus, iron, copper, zinc, and manganese levels in laying hens, with significant elevations in serum zinc at the 60% level, serum copper, zinc, and manganese at the 80% level, and serum manganese at the 100% level compared with the control. These findings suggest superior absorption efficiency of microbially synthesized OTE over inorganic sources.

The liver represents the primary site for trace element metabolism and accumulation, exhibiting high sensitivity to dietary iron, copper, zinc, and manganese, with most absorbed trace elements entering the liver first [20]. The pancreas is the main metabolic organ for manganese and zinc, demonstrating high sensitivity to these elements and accumulating substantial amounts absorbed from the digestive tract [21-22]. Bone serves as a deposition site for calcium, phosphorus, manganese, and zinc, with tibial mineral content effectively reflecting overall mineral metabolism [23]. Compared with blood indicators, mineral levels in liver, pancreas, and tibia reflect longer-term physiological changes and dietary trace element biological efficiency. Wang et al. [22] demonstrated that organic zinc significantly increased zinc deposition in mink liver compared with inorganic zinc. Ao et al. [24] confirmed that organic zinc promoted zinc deposition in broiler tibia. Skivan et al. [25] reported that liver copper content increased significantly with increasing copper supplementation at levels below the EU standard of 35 mg/kg. Henry et al. [26] found that manganese methionine increased manganese content in lamb bone and liver compared with inorganic manganese sulfate. Sun et al. [27] showed that substituting organic copper, manganese, and zinc for inorganic sulfates significantly promoted copper and zinc deposition in laying hen liver and pancreas. The current results indicate that microbially synthesized OTE enhanced iron, copper, zinc, and manganese deposition in laying hen liver, pancreas, and tibia, with pancreatic zinc content at the 80% and 100% levels significantly exceeding the control. However, OTE supplementation did not significantly affect calcium or phosphorus levels in these tissues.

3.2 Effects of Different OTE Levels on Mineral Element Deposition in Eggs

Eggs represent important animal products and genetic resources, and increasing their mineral content can improve egg quality, fertilization rates, and human micronutrient supply [16,28]. When dietary mineral content or biological efficiency increases, corresponding egg mineral levels rise accordingly. Mabe et al. [29] found that supplementing laying hen diets with 60, 60, and 10 mg/kg of inorganic zinc, manganese, and copper significantly increased respective trace element levels in egg yolk. Studies on organic trace elements have demonstrated that organic forms of iron, copper, zinc, manganese, and selenium significantly elevate corresponding egg trace element concentrations [27,30-31]. However, Xue et al. [12] reported that supplementing diets with inorganic or organic trace elements at 25%-125% of NRC (1994) recommendations did not significantly promote manganese, copper, or zinc deposition in eggs as supplementation levels increased. The present study demonstrates that OTE enhanced iron, copper, zinc, and manganese deposition in eggs, with significant or highly significant increases in egg iron and zinc at the 60% level, egg iron, zinc, and manganese at the 80% level, and egg iron and zinc at the 100% level. These results indicate that OTE effectively promotes trace element deposition in eggs, particularly for iron, zinc, and manganese, without significantly affecting calcium or phosphorus deposition.

3.3 Effects of Different OTE Levels on Mineral Element Excretion in Laying Hens

Animal excreta represent a major environmental pollution source, with excessive trace element excretion constituting a primary contributor. Trace element excretion is closely related to both dietary supplementation levels [32] and biological availability [4]. Pierce et al. [33] demonstrated that supplementing pig and broiler diets with organic trace elements at 50% of NRC (1994) recommendations maintained normal performance while significantly reducing fecal trace element levels. Supplementing broiler diets at 25% of NRC standards decreased fecal copper, zinc, and iron by 75%, 50%, and 14%, respectively. Research in laying hens showed that at 25% of NRC recommendations, OTE increased manganese, iron, and selenium excretion compared with inorganic sources, whereas at 75% and 125% of NRC levels, inorganic trace elements resulted in higher manganese, iron, copper, and zinc excretion than OTE [12]. The current findings indicate that OTE supplementation reduced iron, copper, zinc, manganese, and calcium excretion in laying hens, with highly significant reductions in fecal copper, zinc, and manganese at the 60% supplementation level. As OTE supplementation levels increased, fecal trace element content also increased, suggesting that microbially synthesized OTE enhances trace element absorption and utilization, with appropriate reduction in supplementation effectively decreasing excretion.

These results demonstrate that OTE supplementation at reduced levels (60% and 80% of NRC recommendations) meets the trace element deposition requirements in blood, liver, pancreas, tibia, and eggs of laying hens while reducing excretion. Comparatively, the 60% NRC recommendation level was more effective than the 80% level in reducing fecal iron, copper, zinc, and manganese content.

Research on microbially synthesized OTE remains limited compared with chemically synthesized organic trace elements, and its mechanisms for promoting deposition and reducing excretion require further investigation. Unlike chemically synthesized trace elements with simple structures that may stress the digestive tract, the microbially synthesized OTE used in this trial aligns with animal digestive physiology and imposes minimal stress [34-35]. Studies have shown that microorganisms convert inorganic trace elements into protein-, amino acid-, and organic acid-bound forms, and can adsorb trace elements onto cell wall structures, thereby reducing binding by antinutritional factors like phytic acid in the digestive tract [36-37]. The measured free trace element content in the composite OTE prepared from lactic acid bacteria, yeast, and *Bacillus subtilis* fermentation was less than 25%, indicating that most elements existed in adsorbed or bound forms, ensuring biological availability. This likely explains the superior deposition efficiency and reduced excretion observed with OTE compared with inorganic sources.

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4 Conclusion

1. Compared with inorganic trace elements, organic trace elements enhanced iron, copper, zinc, and manganese deposition in laying hen blood, liver, pancreas, tibia, and eggs while reducing their excretion.
2. Supplementing diets with organic trace elements at 60% of NRC (1994) recommendations can maintain normal trace element deposition in laying hens while significantly reducing excretion.

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Note: Figure translations are in progress. See original paper for figures.