Effects of Organic Trace Elements on Production Performance, Serum Antioxidant Indices, Trace Element Content in Egg Yolk, and Trace Element Emission Reduction in Laying Hens Postprint

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

Abstract

This experiment aimed to investigate the effects of combined use of small peptide chelated iron, copper, manganese, zinc and nano-selenium at levels lower than conventional inorganic trace mineral supplementation in premixes on laying hen performance, serum antioxidant indices, trace mineral content in egg yolk, and trace mineral emission reduction. A total of 225 healthy Hy-Line Brown laying hens at 50 weeks of age with similar body weight were selected and randomly divided into 5 groups with 5 replicates per group and 9 hens per replicate. The basal diet was supplemented with feed-grade inorganic trace minerals at 6 mg/kg copper, 75 mg/kg iron, 60 mg/kg zinc, 60 mg/kg manganese, and 0.3 mg/kg selenium as the inorganic group; the experimental groups were 70% organic group, 60% organic group, 50% organic group, and 40% organic group, with trace mineral supplementation levels at 70%, 60%, 50%, and 40% of the inorganic group, respectively. The pre-trial period was 10 days, and the formal trial period was 42 days. The results showed: 1) Compared with the inorganic group, the 60% organic group significantly increased laying rate ($P<0.05$) and significantly decreased feed-to-egg ratio ($P<0.05$), while there were no significant differences in egg quality among all groups ($P>0.05$). 2) Compared with the inorganic group, the 70% organic group significantly increased serum glutathione peroxidase (GSH-Px) activity ($P<0.05$); all organic groups significantly increased serum total antioxidant capacity (T-AOC) ($P<0.05$); and the 70% and 60% organic groups significantly decreased serum malondialdehyde (MDA) content ($P<0.05$). 3) There were no significant differences in copper, manganese, and iron contents in egg yolk among all groups ($P>0.05$); compared with the inorganic group, the 60% organic group significantly increased egg yolk zinc content ($P<0.05$), while the 50% and 40% organic groups significantly increased egg yolk zinc content (P<0.05) but significantly decreased selenium content (P<0.05). 4) The contents of copper, manganese, and zinc in feces of all organic groups were significantly lower than those of the inorganic group (P<0.05). Based on comprehensive consideration, to ensure normal production and physiological functions of laying hens at 50-56 weeks of age, supplementation of small peptide chelated iron, copper, manganese, zinc and nano-selenium at 60% of conventional inorganic trace mineral supplementation levels in premixes showed better effects.

Full Text

Effects of Organic Trace Minerals on Performance, Serum Antioxidant Indexes, Trace Mineral Contents in Egg Yolk, and Trace Mineral Emission Reduction of Laying Hens

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Abstract: This study investigated the effects of organic trace minerals (oTM) —specifically small peptide-chelated iron, copper, manganese, zinc, and nano-selenium—at levels lower than conventional inorganic trace mineral (iTM) supplementation rates in premix on the production performance, serum antioxidant indexes, trace mineral contents in egg yolk, and trace mineral emission reduction in laying hens. A total of 225 healthy Hy-Line brown layers at 50 weeks of age with similar body weight were randomly divided into 5 groups with 5 replicates per group and 9 hens per replicate. The inorganic group received a basal diet supplemented with feed-grade inorganic trace minerals at 6 mg/kg copper, 75 mg/kg iron, 60 mg/kg zinc, 60 mg/kg manganese, and 0.3 mg/kg selenium. The organic groups received the basal diet supplemented with the combination of small peptide-chelated iron, copper, manganese, zinc, and nano-selenium at 70%, 60%, 50%, and 40% of the inorganic group' s supplementation levels, respectively. The pre-experimental period lasted 10 days, and the experimental period lasted 42 days.

The results showed: (1) Compared with the inorganic group, the 60% organic group significantly increased laying rate (P<0.05) and significantly decreased feed-to-egg ratio (P<0.05), while no significant differences were observed in egg quality among all groups (P>0.05). (2) Compared with the inorganic group, the 70% organic group significantly increased serum glutathione peroxidase (GSH-Px) activity (P<0.05), all organic groups significantly increased serum total antioxidant capacity (T-AOC) (P<0.05), and the 70% and 60% organic groups significantly decreased serum malondialdehyde (MDA) content ($P<0.05$). (3) No significant differences were found in copper, manganese, or iron contents in egg yolk among groups ($P>0.05$). However, compared with the inorganic group, the 60% organic group significantly increased zinc content in egg yolk by 22.92% ($P<0.05$), while the 50% and 40% organic groups significantly increased zinc content ($P<0.05$) but significantly decreased selenium content ($P<0.05$). (4) Copper, manganese, and zinc contents in feces were all significantly lower in the organic groups compared with the inorganic group ($P<0.05$).

In conclusion, to maintain normal physiological function and production in 50- to 56-week-old laying hens, supplementation with oTM (small peptide-chelated iron, copper, manganese, zinc, and nano-selenium) at 60% of the conventional inorganic trace mineral supplementation level in premix provides optimal results.

Keywords: organic trace minerals; laying hens; performance; antioxidant; emission reduction

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Currently, the trace mineral requirements for chickens still refer to the NRC (1994) standards, yet significant progress has been made in chicken breed development and nutritional requirement research. Investigating the use of low-dose organic trace minerals is important for effective resource utilization. Boruta et al. [**?**] reported that feeding laying hens organic trace minerals at 8%, 13%, and 33% of NRC (1994) standards resulted in similar laying rates compared to 100% NRC (1994) levels of inorganic salts. Peric et al. [**?**] found that adding organic trace minerals copper, manganese, iron, and zinc at 30% of NRC (1994) standards to broiler diets (1-42 days) maintained normal production performance. Rao et al. [**?**] demonstrated that low-dose organic trace minerals did not affect growth performance or antioxidant capacity in broilers. These findings suggest that utilizing the high absorption characteristics of organic trace minerals to reduce mineral usage in animals is potentially feasible. However, no studies have simultaneously reduced all five trace minerals to investigate the effects of low-dose organic trace minerals on laying hens. Therefore, this study primarily explored the effects of combined use of small peptide-chelated iron, copper, manganese, zinc, and nano-selenium at levels below conventional premix inorganic trace mineral supplementation rates on laying hen production performance, serum antioxidant indexes, trace mineral contents in egg yolk, and trace mineral emission reduction, and discussed the appropriate level for reducing trace mineral usage to provide theoretical reference for scientific application of organic trace minerals in laying hen production.

1.1 Experimental Materials

Feed-grade inorganic trace minerals used were: $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot H_2O$, $ZnSO_4 \cdot H_2O$, $MnSO_4 \cdot H_2O$, and Na_2SeO_3. Small peptide-chelated trace minerals and nano-selenium were commercial products with the following contents:

zinc 15%, manganese 15%, copper 10%, iron 12%, and selenium 1‰. Nano-selenium had an average particle size of 50-100 nm as observed by transmission electron microscopy.

1.2 Experimental Animals and Diet Composition

Healthy Hy-Line brown layers at 50 weeks of age were selected for this experiment and provided by Hunan Sanjian Feed Co., Ltd. The experimental diets were formulated according to NRC (1994) and the "Feeding Standard of Chickens" (NY/T33-2004) for laying hens during the laying period. The diet composition and nutrient levels are shown in Table 1 .

Table 1 Composition and Nutrient Levels of the Basal Diet (Air-Dry Basis)
%

Item	Content
Ingredients	
Corn	
Soybean meal	
Cottonseed meal	
Rapeseed meal	
Corn protein meal	
Wheat middling	
Feather meal	
Limestone	
$CaHPO_4$	
NaCl	
Soybean oil	
Premix[1])	
Total	
Nutrient Levels[2])	
Metabolic energy (MJ/kg)	
Crude protein (CP)	
Calcium (Ca)	
Available phosphorus (AP)	
Lysine (Lys)	
Methionine (Met)	
Methionine + Cysteine (Met+Cys)	

[1]) The premix provided the following per kg of diet: VA 13,500 IU, VD_3 3,000 IU, VE 22.5 mg, VK 3.0 mg, VB_1 3.0 mg, VB_2 7.5 mg, VB_6 3.0 mg, VB_{12} 0.22 mg, calcium pantothenate 15.0 mg, nicotinic acid 30.0 mg, folic acid 1.5 mg, biotin 0.12 mg, choline chloride 400 mg, I (as potassium iodide) 0.3 mg.
[2]) Nutrient levels were calculated values.

1.3 Experimental Design and Management

This study employed a single-factor experimental design. A total of 225 healthy 50-week-old laying hens with similar body weight and normal feed intake were randomly divided into 5 groups with 5 replicates per group and 9 hens per replicate. Based on NRC (1994) standards and actual domestic laying hen feeding practices, the inorganic group received a basal diet supplemented with feed-grade inorganic trace minerals at 6 mg/kg copper ($CuSO_4 \cdot 5H_2O$), 75 mg/kg iron ($FeSO_4 \cdot H_2O$), 60 mg/kg zinc ($ZnSO_4 \cdot H_2O$), 60 mg/kg manganese ($MnSO_4 \cdot H_2O$), and 0.3 mg/kg selenium (Na_2SeO_3). The organic groups (70%, 60%, 50%, and 40% organic groups) received the basal diet supplemented with the combination of small peptide-chelated iron, copper, manganese, zinc, and nano-selenium at 70%, 60%, 50%, and 40% of the inorganic group' s trace mineral levels, respectively. The pre-experimental period lasted 10 days, during which the flocks were adjusted to ensure no significant differences in feed consumption, laying rate, or egg weight among groups. The experimental period lasted 42 days.

The experiment was conducted at the laying hen farm of Hunan Sanjian Feed Co., Ltd. The chicken house was semi-open, and experimental hens were housed in three-tier step cages (upper, middle, lower) with 3 hens per cage and 3 cages per replicate. All groups were managed under identical conditions: fed twice daily (09:00, 15:00), eggs collected twice daily (11:30, 16:30), with free access to feed and water. Daily lighting duration was 16 h, combining natural and artificial light. The house was cleaned weekly and disinfected by spray with hens present.

1.4 Sample Collection and Index Determination

1.4.1 Sample Collection and Processing On day 42 of the experiment at 18:00, all eggs laid that day were collected and labeled with date and group. Fifteen eggs per replicate per group were selected for egg quality determination. Another ten eggs per replicate were collected, and yolks were stored at -20 °C for trace mineral content analysis.

At 08:00 on the first day after the experimental period, blood samples were collected from the wing vein. Ten hens per replicate per group were randomly selected, and 5 mL of blood was slowly injected into tubes. After standing for 30 min, samples were centrifuged at 3,000 r/min for 10 min. The supernatant (0.5-1.0 mL) was transferred to 1.5 mL centrifuge tubes, labeled with group and date, and stored at -20 °C for serum antioxidant index determination.

On day 42 of the experimental period, fecal samples were continuously collected for 3 days from 3 randomly selected hens per replicate per group, sealed in bags, labeled, and stored at -20 °C. Approximately 150 g of each sample was dried at 65 °C, rehumidified for 24 h, weighed, crushed, and prepared as air-dried fecal samples for trace mineral content determination.

1.4.2 Laying Hen Production Performance During the experimental period, daily feed intake, number of eggs laid, and total egg weight were recorded per replicate to calculate average egg weight, laying rate, daily egg production, average daily feed intake, and feed-to-egg ratio.

1.4.3 Egg Quality Egg shape index (length/width) was measured using digital calipers. Egg weight, yolk color, albumen height, and Haugh unit were determined using an egg quality analyzer (EA-01, ORKA, Israel). Eggshell thickness (average of blunt end, pointed end, and middle) was measured using an eggshell thickness gauge (NFN-380, FHK, Japan). Eggshell strength was determined using an eggshell force gauge (EFR-01, ORKA, Israel).

1.4.4 Serum Antioxidant Indexes Serum total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD) activity, and malondialdehyde (MDA) content were determined using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4.5 Trace Mineral Content Trace mineral content was determined by flame atomic absorption spectrometry, referencing GB/T 5009.92-2003, GB/T 5009.92-2003, GB/T 9695.3-2009, and Zhang et al. [**?**].

Yolk processing: 1.0-1.5 g of homogenized yolk sample was placed in a 150 mL conical flask, mixed with 15 mL of mixed acid [$V(HNO_3)$:$V(HClO_4)$ = 9:1], covered with film, and digested overnight. The next day, the flask was heated on a hot plate until the solution became clear and colorless with white smoke (volume ~2 mL). After cooling, 5 mL HCl (6 mol/L) was added and heating continued until clear and colorless with white smoke, then diluted to 100 mL in a volumetric flask. Blank tests were performed simultaneously.

Fecal sample pretreatment used dry ashing. Approximately 3 g of fecal sample was weighed into a crucible, mixed with 10 mL dilute HCl [V(HCl):$V(H_2O)$ = 1:3], carefully heated to dryness on an electric furnace, then ashed in a muffle furnace at low temperature until smokeless, followed by 550 °C until ashing was complete. After cooling, a small amount of water was added to moisten, followed by 15 mL dilute HCl [V(HCl):$V(H_2O)$ = 1:3] and 1-2 mL mixed acid [$V(HNO_3)$:$V(HClO_4)$ = 9:1], heated to boil for 3-5 min, filtered, and diluted to 100 mL in a volumetric flask.

Standard solutions were prepared and measured according to flame atomic absorption instrument operation, with standard curves established before each measurement. For magnesium and manganese determination, 1-2 mL of 10% $SrCl_2$ solution was added to eliminate interference.

1.5 Statistical Analysis

Experimental data were analyzed using SAS 9.0 software for one-way ANOVA, followed by Duncan' s multiple comparison tests. Data among groups were expressed as mean ± standard deviation, with P<0.05 considered statistically significant.

2.1 Effects of Different Levels of Organic Trace Minerals on Laying Hen Performance

As shown in Table 2 , compared with the inorganic group, the 60% organic group significantly increased laying rate by 8.72% (P<0.05), while other organic groups showed no significant changes (P>0.05). No significant differences were observed among groups in average daily feed intake, average egg weight, or daily egg production (P>0.05). Except for the 40% organic group, all organic groups had lower feed-to-egg ratios than the inorganic group, with the 60% organic group being significantly lower (P<0.05) by 3.88%.

Table 2 Effects of Different Levels of Organic Trace Minerals on Performance of Laying Hens (n=5)

Item	Inorganic Group	70% Organic	60% Organic	50% Organic	40% Organic	P-value
Laying rate (%)	79.71$\pm$8.31^b	80.91±6.42^b	86.66±5.73^a	82.63±5.92^b	79.48±8.91^b	
Average egg weight (g)	60.01±1.88	60.				
to-egg ratio	2.32±0.15^{ab}	2.27±0.15^{bc}	2.23±0.16^c	2.24±0.13^{bc}	2.37±0.18^a	
Average daily feed intake (g/d)	1					

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Different Levels of Organic Trace Minerals on Egg Quality of Laying Hens

As shown in Table 3 , no significant differences were observed among groups in eggshell strength, eggshell proportion, eggshell thickness, egg shape index, albumen height, Haugh unit, or yolk color (P>0.05).

Table 3 Effects of Different Levels of Organic Trace Minerals on Egg Quality of Laying Hens

Item	Inorganic Group	70% Organic	60% Organic	50% Organic	40% Organic	P-value
Eggshell strength (N)	39.06±7.25	37.67±5.52	39.51±6.60	40.31±6.54	39.66±5.48	
Eggshell proportion (	±0.78	10.00±0.78	10.			

2.3 Effects of Different Levels of Organic Trace Minerals on Serum Antioxidant Indexes of Laying Hens

As shown in Table 4 , compared with the inorganic group, serum GSH-Px activity in the 70% organic group significantly increased by 16.82% (P<0.05), while the 60%, 50%, and 40% organic groups decreased by 10.67%, 9.20%, and 17.02%, respectively (P<0.05). No significant differences were observed in serum T-SOD activity among groups (P>0.05). Compared with the inorganic group, serum T-AOC in organic groups significantly increased by 170.11%, 133.15%, 126.09%, and 134.24%, respectively (P<0.05). Serum MDA content was significantly decreased in the 70% and 60% organic groups (P<0.05) by 28.65% and 27.79%, respectively.

Table 4 Effects of Different Levels of Organic Trace Minerals on Serum Antioxidant Indexes of Laying Hens

Item	Inorganic Group	70% Organic	60% Organic	50% Organic	40% Organic	P-value
GSH-Px (mU/mL)	770.18±73.09^b	899.75±57.35^a	687.95±60.42^c	699.29±57.20^c	639.12±70.91^{cd}	<0.001
T-SOD (U/mL)	95.55±3.31	92.00±6.02	99.03±6.55	98.84±6.44	101.31±3.39	
T-AOC (U/mL)	1.84±0.13^b	4.97±0.85^a	4.29±0.54^a	5.05±1.07^b	4.16±0.52^a	<0.001
MDA (mmol/mL)	6.98±1.44^a	4.98±1.24^b	5.61±1.62^{ab}	4.31±0.76^a	6.61±1.84	

2.4 Effects of Different Levels of Organic Trace Minerals on Trace Mineral Contents in Egg Yolk

As shown in Table 5 , dietary supplementation with different levels of organic trace minerals had no significant effect on copper, manganese, or iron contents in egg yolk (P>0.05). However, compared with the inorganic group, the 60% organic group significantly increased zinc content in egg yolk by 22.92% (P<0.05). Meanwhile, the 50% and 40% organic groups significantly increased zinc content (P<0.05) but significantly decreased selenium content (P<0.05).

Table 5 Effects of Different Levels of Organic Trace Minerals on Mineral Elements in Egg Yolk (Wet Weight Basis)

Item	Inorganic Group	70% Organic	60% Organic	50% Organic	40% Organic	P-value
Copper (mg/kg)	2.84$±0.22|2.74±0.51|2.67±0.30|2.64±0.43|2.57±0.42|||Manganese(mg/kg)|0.81±0.19|0.81±0.17|0.86± <0.001 0.001||Iron(mg/kg)|67.71±5.42|69.50±8.77|66.06±9.75|61.67±7.48|63.79±7.51|||Selenium(μg/kg)|285.					

2.5 Effects of Different Levels of Organic Trace Minerals on Trace Mineral Contents in Feces

As shown in Table 6 , except for iron content, copper, manganese, and zinc contents in feces were all significantly lower in organic groups compared with the inorganic group (P<0.05), decreasing with reduced supplementation levels. Compared with the inorganic group, copper content in feces decreased by 49.47%, 57.29%, 62.16%, and 64.42%; manganese content decreased by 28.32%, 36.91%, 40.00%, and 42.26%; and zinc content decreased by 22.33%, 24.71%, 26.56%, and 28.29% in the organic groups, respectively.

Table 6 Effects of Different Levels of Organic Trace Minerals on Mineral Elements in Manure (Dry Weight Basis)

Item	Inorganic Group	70% Organic	60% Organic	50% Organic	40% Organic	P-value
Copper (mg/kg)	57.37$±7.42^a\|28.99±8.39^b\|24.50±4.14^b\|21.71±2.58^b\|20.41±3.54^b\| < 0.001\|\|Manganese(mg/kg)\|254.48±19.36^a\|182.42±20.27^b\|160.54±17.42^b\|152.68±17.92^b\|146.94±19.32^b\| 0.001\|\|Zinc(mg/kg)\|369.49±44.28^a\|286.97±29.71^b\|278.18±31.09^b\|271.34±27.59^b\|264.96±31.69^b| < 0.001||Iron(mg/kg)|472.74±49.36|447.66±49.64|448.12±48.69|440.67±48.26|445.79±$48.22					

3.1 Effects of Different Levels of Organic Trace Minerals on Laying Hen Performance

Gheisari et al. [?] found that amino acid complexes at 50%-75% of NRC (1994) recommended levels for zinc, copper, and manganese could maintain normal laying rates in hens, and compared with sulfate forms at 60, 75, and 7 mg/kg for zinc, manganese, and copper, amino acid complex forms at 40, 40, and 7 mg/kg significantly reduced feed-to-egg ratio. Payne et al. [?] reported that low-level yeast selenium (0.15 mg/kg) showed no difference in production performance compared with higher levels (0.30 mg/kg) in Hy-Line W-36 hens. Abdallah et al. [?] concluded that the sole addition of organic trace minerals to broiler diets had no significant effect on performance, but adding peptide-chelated zinc and manganese improved body weight, feed conversion efficiency, and tibia ash. Our results indicate that combined use of low-dose small peptide chelates of iron, copper, manganese, zinc, and nano-selenium can also meet normal production needs of laying hens, with the 60% organic group significantly improving laying rate and reducing feed-to-egg ratio. This may be related to the absorption mechanisms of small peptide-chelated trace minerals and nano-selenium, whose high efficiency may enhance trace mineral absorption to maintain normal production requirements. Qiao et al. [?] suggested that based on the absorption and transport mechanisms of small peptides, trace mineral chelates using small peptides as primary ligands may be transported as intact molecules, thereby improving trace mineral bioavailability. Nano-selenium has a more efficient interaction interface with animal cell membranes, increasing mucosal permeability to promote absorption, and forms nano-emulsions in the intestine that improve selenium absorption [?]. The type of organic trace mineral (protein chelate, amino acid chelate, metal polysaccharide complex), substitution ratio, different combinations, and hen age stage all affect laying rate. In this study, the 60% organic group had significantly higher laying rate than the 70% organic group, possibly because small peptide-chelated trace mineral supplementation exceeded the optimal level, providing no additional promotion of egg production.

3.2 Effects of Different Levels of Organic Trace Minerals on Egg Quality

External egg quality is primarily determined by eggshell quality, with copper, zinc, and manganese having significant effects [?]. Long et al. [?] found that using hydroxy-methionine chelated manganese and zinc in laying hen diets, compared with inorganic sources, showed no significant changes in eggshell thickness or proportion when trace mineral supplementation was reduced by 50%. Gheisari et al. [?] reported that adding amino acid complex forms of zinc, manganese, and copper at 20.0, 20.0, 3.5 mg/kg and 40, 40, 7 mg/kg showed no significant differences in eggshell proportion, thickness, or strength compared with sulfate forms at 60, 75, 7 mg/kg. Our results demonstrate that combined use of low-dose small peptide chelates and nano-selenium had no negative effects on eggshell quality, possibly because low-dose supplementation enhanced calcium and phosphorus absorption. Yang [?] noted that eggshell thickness, strength, and smoothness are closely related to calcium and phosphorus absorption, and trace minerals may improve eggshell quality by increasing calcium utilization. Mabe et al. [?] suggested trace minerals may improve eggshell strength by promoting early fusion during initial shell formation, while Zamani et al. [?] further proposed that trace minerals may affect eggshell quality by influencing key enzymes in shell and shell membrane formation or directly affecting calcium crystal structure formation.

Regarding internal egg quality, this study focused on albumen height, yolk color, and Haugh unit. Fernandes et al. [?] found that organic zinc, manganese, and zinc at lower levels than the control inorganic minerals had no effect on egg quality, similar to our results showing low-dose trace minerals maintained internal egg quality. Gheisari et al. [?] reported that low-dose methionine copper, manganese, and zinc complexes did not reduce internal egg quality and significantly improved Haugh unit. Zhao [?] reported that replacing inorganic trace minerals with 80% complex methionine iron, copper, manganese, zinc, and selenium resulted in higher Haugh units than the inorganic sulfate group. In our study, Haugh units were similar between low and high organic groups, possibly related to selenium levels, as Tian [?] reported high selenium doses could cause significant changes in egg shape index, eggshell strength, albumen height, and eggshell proportion.

3.3 Effects of Different Levels of Organic Trace Minerals on Serum Antioxidant Indexes

In this study, compared with the inorganic group, the 60%, 50%, and 40% organic groups all significantly reduced serum GSH-Px activity. Guo et al. [?] found that adding 0.1, 0.3, 0.5, and 0.7 mg/kg methionine selenium to 1-day-old broiler diets showed no significant differences in performance, but serum GSH-Px activity decreased with reduced selenium supplementation. Xu [?] reported that serum GSH-Px activity increased with increasing nano-selenium levels, with the 0.2 mg/kg group being significantly higher than the 0.1 mg/kg group. Other studies have shown that zinc deficiency or low zinc reduces active GSH-Px content, increases lipid peroxidation, and consumes more GSH-Px, leading to decreased activity [?]. In our study, the 70% organic group significantly increased serum GSH-Px activity, possibly because the reduction in trace minerals was modest and the high absorption efficiency of organic trace minerals enhanced serum GSH-Px activity. No significant differences in serum T-SOD activity were observed between the inorganic and low-dose organic groups. T-SOD reflects both manganese SOD (MnSOD) and copper-zinc SOD (CuZn-SOD), indicating that low-dose small peptide-chelated copper, manganese, and zinc can maintain MnSOD and CuZn-SOD activities. Sun et al. [?] showed that adding 50 mg/kg yeast copper to piglet diets had the same antioxidant enzyme activity as 250 mg/kg $CuSO_4$. All low-dose organic groups in this study significantly increased serum T-AOC, and the 70% and 60% organic groups significantly reduced serum MDA content, suggesting low-level organic trace minerals may increase antioxidant enzyme activity and antioxidant protein synthesis, though the mechanisms require further investigation.

3.4 Effects of Different Levels of Organic Trace Minerals on Trace Mineral Contents in Egg Yolk

Our results show that low-dose organic trace minerals had no significant effect on copper, manganese, or iron contents in egg yolk but affected zinc and selenium contents. Zhang et al. [?] demonstrated that when trace mineral ratios are appropriate, lower supplementation levels can also increase egg trace mineral contents, consistent with our findings that the 70% and 60% organic groups did not significantly reduce trace mineral contents. In this study, the 40% and 50% organic groups reduced selenium content but increased zinc content in egg yolk, possibly due to antagonism between zinc and other trace minerals. Skrivan et al. [?] reported that yolk zinc and copper contents were significantly affected by zinc-copper antagonism. The reduced selenium content may be related to selenium supplementation levels, as Xu [?], Qu et al. [?], and Cai et al. [?]

showed that low-selenium diets reduce selenium content in animal tissues.

3.5 Effects of Different Levels of Organic Trace Minerals on Trace Mineral Contents in Feces

Research shows that low-dose organic trace minerals, due to better absorption, can reduce trace mineral excretion in feces and alleviate environmental pollution pressure from livestock production. Bao et al. [?] reported that supplementing 4 mg copper and 40 mg iron, manganese, and zinc as protein-chelated organic trace minerals met normal broiler growth requirements, and low-dose organic trace minerals avoided high mineral excretion. Leeson and Caston [?] found that using protein-chelated trace minerals at 100%, 80%, 60%, 40%, and 20% of inorganic trace mineral levels (control: 60, 77, 5.0, and 85 mg/kg zinc, manganese, copper, and iron) in 17-42 day broiler diets significantly reduced trace mineral excretion, with the 20% group reducing manganese, zinc, and copper emissions by 52%, 38%, and 21%, respectively, while iron excretion reduction was not significant, demonstrating the superiority of organic trace minerals in reducing excreted mineral concentrations. Our results also show that low-dose organic trace minerals reduced manganese, zinc, and copper contents in feces but not iron. Some studies indicate that mineral element excretion in pig feces is independent of iron source, with excretion decreasing as supplementation decreases regardless of iron source [?].

Under the conditions of this study, combined use of small peptide-chelated iron, copper, manganese, zinc, and nano-selenium at levels below conventional premix inorganic trace mineral supplementation rates met the normal production and physiological needs of 50-56 week-old laying hens and effectively reduced trace mineral emissions. Considering all factors, supplementation at 60% of the inorganic trace mineral level provided optimal results.

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