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Effects of supplementation of inorganic trace elements with organic trace elements chelated with hydroxy methionine on laying performance, egg quality, blood micronutrients, antioxidant capacity and immune function of laying ducks

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Introduction: This study aimed to investigate the effects of organic trace elements chelated with hydroxy methionine (OTE-HM) in diets, which substituted inorganic trace elements, on laying performance, egg quality, blood microelement content, antioxidant capacity and immune function of laying ducks.

Methods: A total of 300 healthy laying ducks at age of 30 wk were randomly divided into 5 treatments and 10 ducks per replicate. The treatments included a control group (CON) which was served with basal diet supplemented with 20 mg/kg Cu, 50 mg/kg Fe, 70 mg/kg Mn, and 70 mg/kg Zn in inorganic form, and 4 OTE-HM treated groups (OTE-HM25, OTE-HM50, OTE-HM75, OTE-HM100) which were served with basal diets supplemented with OTE-HM providing trace elements (combination of Cu, Fe, Mn, Zn) at 25%, 50%, 75% and 100% of the commercial levels, respectively.

Results: Results showed that substitution of inorganic trace elements with OTE-HM did not affect egg production, qualified egg rate, average egg weight, average daily egg mass, average daily feed intake, or feed per kg egg of laying ducks ($P > 0.05$). Dietary with OTE-HM did not influence eggshell strength, eggshell thickness, egg shape index, eggshell ratio, yolk ratio, albumen ratio, albumen height, and Haugh unit of the sampled eggs of ducks ($P > 0.05$), but increased the yolk color, compared with dietary with inorganic trace elements

(P< 0.01). Moreover, the blood content of Cu of the laying ducks was significantly increased by OTE-HM compared with that in CON (P< 0.001), but the other elements in laying duck blood were not different among treatments (P > 0.05). OTE-HM (75% and 100%) significantly increased serum activities of glutathione peroxidase and Cu-Zn superoxide dismutase, and decreased serum content of malonaldehyde of laying ducks compared with those in CON (P< 0.05). OTE-HM (50%, 75%, and 100%) significantly increased the serum contents of immunoglobulin G and immunoglobulin A of laying ducks compared with those in CON (P< 0.05).

Discussion: Collectively, replacing inorganic trace elements with 50% and 75% OTE-HM in diets did not influence the laying performance or egg quality, but improved trace element efficacy, antioxidant capacity and immune function of the laying ducks.

KEYWORDS

laying duck, organic trace element, laying performance, egg quality, antioxidant, immune

Introduction

Trace elements are defined as minerals that occur in the body in milligram per kilogram body weight or less amounts (Nielsen, 2003). As important as the main nutrients, the trace elements involve in the growth and development of poultry, including the nutritional metabolism, bone formulation, immune system, propagation, and so on (Richards et al., 2010). Functions of trace elements on poultry physiology were widely studied. Copper sulfate as Cu source in diet at 13.2-13.6 mg/kg could protect broilers from lipoperoxidation under the situation of heat stress, but high amounts of Cu might adversely affect broilers' performance (Sabry et al., 2021). Zn was reported improving carcass traits, meat quality, antioxidant capacity of Pekin duck (Wen et al., 2019b). Moreover, trace elements were essential to eggshell and eggshell membrane formation, because they acted as activators or components of key enzymes interacting with calcite minerals, and increased the eggshell strength and thickness by improving the ultrastructure of eggshell (Qiu et al., 2020).

In poultry farming industry, trace elements in inorganic sources were commonly used as additives in animal feed due to the low costs. Farmers tended to use these inorganic compounds over-dose to avoid problems of trace element deficiencies (Nys et al., 2018). However, a number of studies revealed the flaws of such method. Certain antagonisms were reported between trace elements as inorganic salts, such as sulphate Zn and Cu (Ao and Pierce, 2013). Furthermore, the bioavailability of trace elements in inorganic forms was low for animals (Liu et al., 2014), and the overuse of inorganic sources led to high levels of inorganic microelements in animal manure, which caused environment pollution (Zhang et al., 2022). In order to reduce the mineral excretion without jeopardizing the production performance of animals, trace elements in organic form were developed, in which the metal ion was chelated to two or more chelators, such as amino acids, protein digestions or protein products (Stanaćev et al., 2014). It was reported that organic trace elements (trace elements chelated with several amino acids) were more bioavailable than inorganic minerals salts so that it could be used at a lower rate in the feed, and still be able to improve eggshell quality, mineral deposition in the eggshell, antioxidant capacity and immune function of aged laying hens (Zhang et al., 2021). However, it was uncertain about the effects of organic trace elements chelated with a single type of amino acids, which were more available in the market than trace elements chelated with multiple types of amino acids, on the laying ducks. We hypothesized that replacing inorganic trace elements with lower rate of organic trace elements chelated with hydroxy methionine (OTE-HM) in diet could maintain, or even improve the health and production of laying ducks.

In this study, we aimed to inspect the effects of OTE-HM on laying performance, egg quality, blood microelements content, antioxidant capacity, and immune function of the laying ducks, and provide reference for the use of OTE-HM as new trace element source in duck feed.

Materials and methods

Birds and management

This study was conducted after the approval of the Institutional Animal Care and Use Committee at Hunan Institute of Animal Husbandry and Veterinary Science (Protocol No. AHVM20210502). A total of 300 healthy laying ducks (Youxian Shelduck, 30-week-old) with similar body weight and egg-laying rates were selected for a 9-week trial. The ducks were randomly divided into 5 treatments with 6 replicates and 10 ducks per replicate. The treatments included a control group (CON) which was served with basal diet supplemented with 20 mg/kg Cu, 50 mg/kg Fe, 70 mg/kg Mn, and 70 mg/kg Zn in inorganic form, and 4 OTE-HM treated groups (OTE-HM25, OTE-HM50, OTE-HM75, OTE-HM100) which were served with basal diets supplemented with OTE-HM providing trace elements (combination of Cu, Fe, Mn, Zn) at 25%, 50%, 75% and 100% of the commercial levels, respectively. Basal diets were formulated according to the recommendation for laying ducks by GB/T 41189-2021, 2021 (State Administration for Market regulation of China, Standardization Administration of China, 2021), and the compositions and calculated nutrient levels were listed in Table 1. The OTE-HM was provided by Changsha XJ-bio Co. Ltd (Hunan, China), in which the trace elements chelated with hydroxy methionine at 1:1 mole, and the hydroxy methionine content was about 50%. In the formulations of the experimental diets, the feed ingredient of *DL*-methionine was reduced according to the supplementation of OTE-HM, to make sure the total methionine in the diets were equal for all treatments.

Ducks were housed in three-dimensional cages measuring 35 cm × 40 cm × 35 cm (1 duck per cage) with free access to water and feed. Immunization and sanitation were conducted regularly. Lighting, temperature, and humidity were set according to the conventional standards of commercial laying duck farms.

Laying performance

During the experiment period, eggs produced were collected daily. The number and weight of total eggs produced, the number of unqualified eggs (eggs with soft shell, broken shell, and irregulated shape) and the amount of feed intake were measured and recorded daily on replication basis. Egg production, qualified egg rate, average egg weight, average daily egg mass, average daily feed intake, feed per kg egg were calculated at the end of experiment as follow:

$$\text{Egg production} = \text{number of total eggs} \div 63(\text{experiment days}) \div 10(\text{number of laying ducks}) \times 100\,\%$$

$$\text{Qualified egg rate} = \text{number of qualified eggs} \div \text{number of total eggs} \times 100\,\%$$

$$\text{Average egg weight} = \text{weight of total eggs} \div \text{number of total eggs}$$

$$\text{Average daily egg weight} = \text{average egg weight} \div 63(\text{experiment days})$$

TABLE 1 Ingredients and nutrient composition of the basal diet (dry matter basis, %).

Ingredients		Nutrient levels[1]	
Corn	48.55	Metabolic energy, MJ/kg	10.89
Soybean meal	26.32	Crude protein (CP)	18.00
Rapeseed meal	2.00	Calcium (Ca)	3.52
Wheat middling	11.70	Total phosphorus (TP)	0.63
$CaHPO_4$	1.00	Available phosphorus (AP)	0.35
Salt	0.30	Lysine	0.90
L-Lysine HCl (78.5%)	0.10	Methionine	0.42
DL-Methionine (98.5%)	0.13	Methionine and cystine	0.74
Limestone	8.90		
Premix[2]	1.00		
Total	100.00		

[1]The nutrient levels were calculated values.
[2]The premix without trace minerals provided the following nutrients per kg diet: vitamin A 12,000 IU; vitamin D3 2,500 IU; vitamin E 20 mg; vitamin K3 3 mg; vitamin B1 3 mg; vitamin B2 8 mg; vitamin B6 7 mg; vitamin B12 0.03 mg; D-pantothenic acid 20 mg; nicotinic acid 50 mg; biotin 0.1 mg; folic acid 1.5 mg; selenium 0.16 mg, iodine 0.6 mg.

$$\text{Average daily feed intake} = \text{total feed intake} \div 63(\text{experiment days}) \div 10(\text{number of laying ducks})$$

$$\text{Feed per kg egg} = \text{total feed intake} \div \text{total egg weight} \times 1000\ \text{g/kg}$$

Egg quality

At the end of experimental trail, 4 eggs were randomly retrieved from each replication for the egg quality evaluation. Egg shape index was determined as a ratio of short-to-long axis using electronic vernier caliper (DL91150, Ningbo, China). Albumen, yolk and shell weights for each sampled eggs were measured individually to calculate the percentage contents of egg components. Albumen height, Haugh units (HU), and yolk color were determined by an egg multitester (EMT-7300, Loimaa, Finland). The eggshell thickness were measured as the average of the thicknesses at sharp end, blunt end, and the equator end of the eggshell after removing the interrior membrance by an eggshell thickness gauge (ESTG-1, Herzliya, Israel). Eggshell strength was measured by an egg force reader (EFR-01, Herzliya, Israel).

Trace element concentration in blood

At the end of 9 w of the experimental trial, one duck from each replication (6 ducks per group) was randomly selected for blood sampling. Blood was collected from the wing vein, and kept in regular vacuum blood collection tubes and tubes with anticoagulant. Blood samples in tubes with anticoagulant were diluted with de-ionized water (1:40), then were aspirated in to the atomic absorption spectrophotometers (ContrAA 700, Jena, Germany) for the estimation of trace element (Cu, Fe, Mn, Zn, Ca, Mg) concentrations.

Antioxidative and immunological parameters in serum

Blood samples were kept in the regular tubes for 2 h and then centrifuged at 3,000×*g* for 10 min to obtain serum. The antioxidative parameters included activities of glutathione peroxidase (GSH-Px), Mn-superoxide dismutase (MnSOD), Cu/Zn-superoxide dismutase (CuZnSOD), and concentration of malonaldehyde (MDA) in serum. The immunological parameters included concentrations of complement protein 4 (C4), immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA) in serum. Those parameters were determined by commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with an automated fluorescence instrument (Multiskan™ SkyHigh, Waltham, US).

Statistical analysis

One-way ANOVA followed by Duncan's multiple range test were used to test the significant mean differences among treatments after examination of the homogeneity of variance. All data analysis were performed in SPSS statistical program (SPSS25, Armonk, United States). The results were presented as means, and the variability was expressed as SEM. A probability of $P< 0.05$ was considered significant.

Results

Laying performance

As shown in Table 2, the laying performance associated parameters including egg production ($P = 0.95$), qualified egg rate ($P = 0.63$), average egg weight ($P = 0.56$), average daily egg mass ($P = 0.97$), average daily feed intake ($P = 0.72$), and feed per kg egg ($P = 0.69$) of the experimental laying ducks were not significantly different among 5 treatments.

TABLE 2 Effects of experimental diets on laying performance of laying ducks[1].

Item	Treatments					SEM	*P* value
	CON	OTE-HM25	OTE-HM50	OTE-HM75	OTE-HM100		
Egg production, %	79.51	80.97	82.23	79.63	83.22	4.09	0.95
Qualified egg rate, %	99.59	99.73	99.55	98.47	99.72	0.29	0.63
Average egg weight, g	65.74	65.58	66.42	67.06	65.52	0.33	0.56
Average daily egg mass, g/layer	52.34	53.03	54.58	53.44	54.49	1.10	0.97
Average daily feed intake, g/layer	155.94	158.52	155.96	156.02	157.54	2.50	0.72
Feed per kg egg, g	3034.48	3025.14	2872.95	3117.77	2891.59	59.52	0.69

[1]Data were mean of six replications (each replicate 10 laying ducks) per treatment.

TABLE 3 Effects of experimental diets on egg quality of laying ducks[1].

Item	Treatments					SEM	*P* value
	CON	OTE-HM25	OTE-HM50	OTE-HM75	OTE-HM100		
Eggshell strength, N	41.91	52.27	46.24	43.60	48.79	1.48	0.18
Eggshell thickness, mm	0.36	0.35	0.35	0.34	0.35	<0.01	0.56
Egg shape index	1.34	1.37	1.38	1.35	1.37	<0.01	0.08
Eggshell ratio, %	11.27	11.23	10.89	10.91	11.38	0.09	0.32
Yolk ratio, %	31.06	31.46	32.11	32.01	31.13	0.25	0.56
Albumen ratio, %	57.68	57.31	57.01	57.08	57.49	0.25	0.91
Albumen height, mm	6.38	3.77	5.79	6.12	6.66	0.16	0.28
Yolk color	2.00[b]	2.50[a]	2.42[a]	2.67[a]	2.00[b]	0.07	<0.01
Haugh units	76.89	78.44	72.15	73.90	76.29	1.17	0.47

[a,b]Values with different superscripts in the same row are significantly different (P< 0.05).
[1]Data were mean of six replications (each replicate 12 eggs) per treatment.

Egg quality

As shown in Table 3, eggshell strength (*P* = 0.18), eggshell thickness (*P* = 0.56), egg shape index (*P* = 0.08), eggshell ratio (*P* = 0.32), yolk ratio (*P* = 0.56), albumen ratio (*P* = 0.91), albumen height (*P* = 0.28), and HU (*P* = 0.47) of the sample eggs were not significantly different among 5 treatments. However, the yolk color of eggs in OTE-HM25, OTE-HM50, and OTE-HM75 was significantly higher than that in CON and OTE-HM100 (*P*< 0.01).

Trace element concentration in blood

The trace element concentrations in blood of experimental ducks were listed in Table 4. Compared with the CON, the concentration of blood Cu in all OTE-HM treated ducks was significantly higher (*P*< 0.01). But the concentrations of Fe (*P* = 0.82), Mn (*P* = 0.45), Zn (*P* = 0.71), Ca (*P* = 0.16), and Mg (*P* = 0.43) in blood of the ducks were similar among all 5 treatments.

Antioxidative cytokine level in serum

As shown in Table 5, serum activity of GSH-Px in all OTE-HM-fed ducks was significantly higher than that in CON (*P*< 0.01), but no differences were noticed among OTE-HM-treated groups. Activity of CuZnSOD in OTE-HM50, OTE-HM75, and OTE-HM100 was significantly higher than that in CON, and activity of CuZnSOD in OTE-HM100 was significantly higher than that in OTE-HM25 (*P*< 0.01). Concentration of MDA in OTE-HM75 and OTE-HM100 was significantly lower than that in CON (*P* = 0.04).

Immunological cytokine level in serum

The immunological cytokine levels in serum of experimental ducks were showed in Table 6. Serum IgG concentration was significantly higher in OTE-HM50, OTE-HM75, and OTE-HM100 compared with that in CON; and IgG concentration

TABLE 4 Effects of experimental diets on trace element concentration in blood of laying ducks[1].

Item	Treatments					SEM	*P* value
	CON	OTE-HM25	OTE-HM50	OTE-HM75	OTE-HM100		
Cu, umol/L	14.85[b]	22.58[a]	25.22[a]	22.70[a]	22.87[a]	0.89	<0.01
Fe, mmol/L	5.77	6.20	6.05	6.27	6.33	0.16	0.82
Mn, μg/L	108.05	86.67	123.73	129.62	125.73	8.17	0.45
Zn, umol/L	133.20	134.38	150.92	130.80	151.18	5.93	0.71
Ca, mmol/L	3.93	3.74	4.98	3.68	4.70	0.21	0.16
Mg, mmol/L	2.62	2.67	2.72	2.52	2.81	0.05	0.43

[a,b]Values with different superscripts in the same row are significantly different (P< 0.05).
[1]Data were mean of six replications (each replicate 10 laying ducks) per treatment.

TABLE 5 Effects of experimental diets on antioxidative cytokine levels in serum of laying ducks[1].

Item	Treatments					SEM	*P* value
	CON	OTE-HM25	OTE-HM50	OTE-HM75	OTE-HM100		
Glutathione peroxidase, U/ml	2.57[b]	3.23[a]	3.15[a]	3.23[a]	3.41[a]	0.08	<0.01
Manganese superoxide dismutase, U/ml	113.57	116.84	127.73	125.25	133.09	2.88	0.18
Copper/zinc superoxide dismutase, U/ml	78.07[c]	89.73[bc]	103.75[ab]	101.30[ab]	120.62[a]	3.93	<0.01
Malondialdehyde, nmol/L	2.01[a]	1.95[ab]	1.92[ab]	1.60[b]	1.62[b]	0.06	0.04

[a-c]Values with different superscripts in the same row are significantly different (P< 0.05).
[1]Data were mean of six replications (each replicate 10 laying ducks) per treatment.

in OTE-HM100 was significantly higher than that in OTE-HM25 (P = 0.01). IgA concentration was significantly higher in OTE-HM50, OTE-HM75, and OTE-HM100 compared with that in CON and OTE-HM25 (P< 0.01).

Discussion

The importance of adequate trace elements to the performance and egg quality of laying birds was proved in numerous literatures (Mabe et al., 2003; Gheisari et al., 2011; Kim et al., 2013; Jiang et al., 2021; Yang et al., 2021). However, the overuse of trace elements in the form of inorganic mineral not only deteriorated the health of laying birds, but also increased the excretion of minerals into the soil and water, causing environmental pollution (Mézes et al., 2012). OTE-HM as an organic form of trace elements provided higher bioavailability and bio-efficacy than traditional inorganic form (Sun et al., 2010; Meng et al., 2021), that made it an ideal substitute for inorganic minerals in feed. In the present study, we studied the effects of OTE-HM that provided trace elements at similar or lower rates of recommendation on the laying performance of laying ducks. Results showed that compared with inorganic Cu, Fe, Mn, Zn at recommended concentrations (20, 50, 70, and 70 mg/kg, respectively), dietary supplementation with OTE-HM at lower (25%, 50%, and 75%) or full (100%) rate of recommendation did not change the laying performance associated parameters, such as egg production, qualified egg rate, average egg weight, average daily egg mass, or average daily egg mass. It suggested that inorganic trace elements (Cu, Fe, Mn, and Zn) in feed of laying ducks could be substitute with similar or lower rate of OTE-HM, without scarifying the laying performance. This result was similar to a previous study that dietary supplementation of hydroxy methionine-chelated minerals (Zn, Cu, Mn) replacing inorganic minerals did not change the laying performance of 96-wk-old ISA brown laying hens (Lim and Paik, 2003). Zhang et al. (2021) also stated that low levels of amino acid-chelated trace elements (20%, 30%, and 50% of inorganic trace elements levels) in diets did not influence the laying performance of 57-wk-old laying hens compared with the ones fed with inorganic trace elements.

After knowing the effects of OTE-HM on the laying performance, we further checked the influence of OTE-HM on the egg quality of laying ducks. In the present study, no differences in eggshell, yolk, and albumen ratios, eggshell strength and thickness, egg shape index, or Haugh units were found between inorganic trace element or OTE-HM-treated groups, suggesting substitution of inorganic trace elements by low or full rate of OTE-HM in diet had no harmful influences on above egg quality parameters. Moreover, lower rates of OTE-HM (25%, 50%, and 75%) in diets significantly increased the yolk color compared with the one in inorganic trace element-treated groups (P = 0.002), showing OTE-HM in diets might have effects on improving yolk color of duck eggs. Li et al. (2018) also found that dietary Mn-methionine could improve yolk color of 53-wk-old laying hens after 4 wk of feeding. Lim and Paik (2003) reported similar results that replacement of inorganic Zn, Mn, and Cu by methionine-chelated minerals in diets did not influence the albumin height, Haugh unit and eggshell thickness, but improved the eggshell strength, which was partially consistent with our result. Several studies also showed

TABLE 6 Effects of experimental diets on immunological cytokine levels in serum of laying ducks[1].

Item	Treatments					SEM	*P* value
	CON	OTE-HM25	OTE-HM50	OTE-HM75	OTE-HM100		
Complement protein 4, µg/ml	24.67	30.93	23.75	28.32	29.94	1.12	0.16
Immunoglobulin M, µg/ml	133.66	126.75	133.95	113.25	129.40	3.62	0.38
Immunoglobulin G, µg/ml	370.15[c]	409.56[bc]	466.72[ab]	447.66[ab]	477.26[a]	11.48	0.01
Immunoglobulin A, µg/ml	37.66[b]	41.05[b]	55.51[a]	61.76[a]	61.41[a]	2.23	<0.01

[a-c]Values with different superscripts in the same row are significantly different (P< 0.05).
[1]Data were mean of six replications (each replicate 10 laying ducks) per treatment.

that lower rates of proteinated trace elements and methionine hydroxyl analog chelated Zn could improve eggshell quality in aged laying hens by improving the eggshell ultrastructure during the late laying period (Min et al., 2018; Qiu et al., 2020). The possible explanations of the fluctuations on certain egg quality related parameters could be the differences in age of the experimental birds. As getting older, the laying birds' metabolic abilities for minerals and other nutrients decreased, so that the egg quality related parameters changed (Esfahani et al., 2020).

Inorganic minerals were commonly used in poultry production as trace elements sources. But during the passage through the digestive tract, the ions from soluble inorganic trace elements could potentially be combined and excreted with other dietary components, making them less available for the animals (Yang et al., 2021). In contrast, organic trace minerals were stable and not ionized before absorption, and they could hardly be precipitated or adsorbed by precipitants, such as phytic acid, phosphoric acid, and oxalic acid, after passing the digestive tract (Liu et al., 2014). Moreover, proteinated minerals were transported and absorbed as amino acids, which attributed to higher absorption and less excretion compared with inorganic trace elements (Singh et al., 2015). In the present study, we investigated the concentrations of Cu, Fe, Mn, Zn, Mg and Ca in laying duck bloods. Results showed that lower (25%, 50%, and 75%) or equal rate of OTE-HM significantly increased the blood concentration of Cu in laying ducks compared with the ones treated with dietary inorganic trace element ($P< 0.01$). Blood concentrations of Fe, Mn, Zn, Ca, and Mg in ducks in OTE-HM50 and OTE-HM100 were higher than the ones in CON, but without statistical significances ($P > 0.05$). These results indicated that OTE-HM had better availabilities than the trace elements in inorganic form, especially Cu. Similar results were reported previously that hydroxy methionine-chelated Cu significantly increased the Cu concentrations in the plasm of broilers and in the serum of late-phase laying hens compared with the ones fed with $CuSO_4$ (Yenice et al., 2015; Wen et al., 2019a). Cu as a component of numerous enzymes and proteins, was involved in cellular respiration, antioxidant activity, iron transport, pigmentation, and connective tissue development (Broom et al., 2021). And it was possible that higher availability of hydroxy methionine-chelated Cu maintained the laying performance and egg quality of the laying ducks, even under the condition of feeding low rates of OTE-HM (Mabe et al., 2003; Güçlü et al., 2008).

Laying ducks in commercial farms were kept in high productive performance. As growing older, the oxidative stress, immunological stress, and lipid peroxidation became severer, which in turn decreased the health condition, laying performance, and egg quality of the laying ducks (Jian et al., 2021). Previous studies stated that chelate compounds-based mineral supplement in diet could improve the oxidative status and immune function of laying ducks (Wang et al., 2019; Zhang et al., 2021; Ghasemi et al., 2022). In the present study, oxidative status associated parameters were measured, including serum activities of GSH-Px, MgSOD, and CuZnSOD, as well as serum content of MDA. Similar with previous studies, OTE-HM at lower rate (25% to 75%) or same rate as inorganic trace element supplemented in diets significantly increased the activities of antioxidative enzymes, and reduced the content of MDA in serum ($P< 0.05$), which supported the conclusion that OTE-HM improved the antioxidative capacity and alleviated the antioxidative stress of the laying ducks. GSH-Px, MgSOD, and CuZnSOD were common antioxidative enzymes in poultry, which helped removing excessive oxidative substances and maintaining the homeostasis of the antioxidation system of the organism (Surai et al., 2019). MDA was the product of oxidative substance degrading the polyunsaturated lipids in the organism, and was considered as a common biomarker of oxidative stress (Fu et al., 2013). It was well-demonstrated that Cu, Fe, Mn, and Zn were important components of some oxidoreductases, as well as crucial antioxidant substances in laying birds (Liu et al., 2019). Therefore, the higher availabilities of Cu, Fe, Mn, and Zn in OTE-HM might contribute to better antioxidative capacity by activating the antioxidative enzymes, and eventually decreasing the content of MDA in the laying ducks.

IgA and IgG were the main immunoglobulins in birds, which were secreted by B cells, serving as the antibodies against invaded antigens (Sharma, 1999). The comparisons of immune status associated parameters in laying ducks' serum showed that OTE-HM at different rates (50%, 75%, and 100% of commercial rate for trace elements supplementation) increased the contents of IgG and IgA, suggesting improvements in immune capability. Similar results were reported previously. Wu et al. (2019) indicated that dietary methionine-chelated Cu increased the serum contents of immunoglobulins in broilers compared with the ones fed with inorganic Cu. Manangi et al. (2015) reported that trace minerals in chelated form improved the immune responses to antigenic challenge in 63-wk-old laying hens compared with the ones fed the minerals as sulfates. Another study by Zhang et al. (2021) also showed that low levels of methionine-chelated trace elements (20%, 30%, and 50%) improved immune functions of aged laying hens by increasing the contents of immunoglobulins and decreasing the contents of proinflammatory cytokines in serum. Trace elements could increase the immunoglobulins contents in the body's inflammatory response, who played important roles in stabilization of the immune defense system (Bao and Choct, 2009). It was highly possible that OTE-HM provided the trace elements for birds in a more efficient way than inorganic trace elements which helped improving the immune function of laying ducks (Świątkiewicz et al., 2014).

Conclusion

In conclusion, feeding laying ducks with OTE-HM which provided Cu, Fe, Mn, Zn at similar or even lower rate than recommendation had analogous effects on laying performance and egg quality as the inorganic trace elements did. In addition, OTE-HM increased the egg yolk color, trace elements contents in blood, antioxidative capacity, and immune function of laying ducks compared with the one fed with inorganic trace elements. It was practical to substitute the inorganic trace elements in feed with OTE-HM to diminish the excretion of minerals to the environment. Considering the results of the present study, the proper supplementation rate of OTE-HM in laying ducks' feed were 50% to 75% as commercial rate for trace elements.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee at Hunan Institute of Animal Husbandry and Veterinary Science (Protocol No. AHVM20210502).

Author contributions

Conceptualization, QD; methodology, JC; validation, SW; formal analysis, HW; investigation, CL; resources, XH; data curation, XZ; writing—original draft preparation, YZ; writing—review and editing, YL; visualization, PD; supervision, QD; project administration, QD; funding acquisition, QD. All authors contributed to the article and approved the submitted version.

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Conflict of interest

YZ, JC, SW, HW and QD were employed by Changsha Xingjia Bio-Engineering Co., Ltd during the time that the experiment was conducted and the paper was composed.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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