



The potential of supplementing compound organic trace elements at lower levels in Chinese yellow-feathered broiler diets, Part I: Impacts on plasma biochemical parameters, antioxidant capacity, carcass traits, meat quality, and tissue mineral deposition

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ABSTRACT

This study was conducted to evaluate the effects of replacing inorganic trace minerals (ITM) with compound organic trace minerals (OTM) at lower levels on plasma biochemical parameters, antioxidant capacity, carcass traits, meat quality, and tissue mineral deposition in Chinese yellow-feathered broilers. A total of 960 one-day-old male broilers were randomly allocated to six treatment groups. The birds were fed with either the basal diets (negative control, NC), or diets supplemented with 1,000 mg/kg (positive control, PC), 300 mg/kg, and 500 mg/kg ITM or OTM for 53 d, respectively. The results showed that the alkaline phosphatase (ALP) activity of the OTM300 group was significantly higher than that of the NC, PC, and ITM300 groups ($P < 0.05$). Dietary OTM supplementation could significantly increase the serum concentrations of Fe and Cu, promote the deposition of Zn and Cu in breast muscle, and increase Zn content in the tibia of Chinese yellow-feathered broilers ($P < 0.05$). Furthermore, dietary OTM300 treatment could significantly increase plasma CAT and CuZn-SOD activities, as well as the CAT activity in the liver ($P < 0.05$). The liver GSH-Px activity of the OTM500 group were significantly higher than the other groups ($P < 0.05$). Moreover, the supplementation of dietary OTM could significantly increase the pH_{45min} of breast muscle, as well as decrease drip loss_{24h} and drip loss_{48h} of Chinese yellow-feathered broilers ($P < 0.05$). Furthermore, pH_{45min} was positively correlated with liver T-AOC activity and the concentrations of Zn, Fe, Cu, and Mn in breast muscle, while drip loss_{48h} was negatively correlated with liver T-AOC activity, plasma CAT and CuZn-SOD, as well as the concentration of Cu and Zn in breast muscle. Trace mineral sources or levels had no significant effect on the carcass traits of Chinese yellow-feathered broilers ($P > 0.05$). Compared with the ITM groups, OTM300 significantly increased the heart index of Chinese yellow-feathered broilers ($P < 0.05$). Dietary OTM upregulated the mRNA expression of TGF- β and downregulated the mRNA expression of IL-1 β in the spleen ($P < 0.05$). In conclusion, dietary supplementation with compound OTM at lower levels could promote the deposition of trace minerals in serum and tissues, enhance antioxidant capacity, and improve the meat quality of Chinese yellow-feathered broilers.

Introduction

With increasing demands of poultry consumption in modern society, chicken has become the most consumed poultry meat worldwide. This is due to its high quality protein and low caloric and fat content (Marmion

et al., 2021), which has led to the steady development of broiler production in recent years. Essential trace minerals, such as Fe, Zn, Cu, Mn, and Se, play indispensable roles in various biological processes as co-factors or enzyme activators, making them crucial for poultry nutrition and health (Hassan et al., 2020; Broom et al., 2021; Wan and Yin, 2023).

ORGANIC TRACE ELEMENTS FOR CHINESE BROILERS

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In past decades, inorganic trace minerals (ITM) were commonly provided in excessive amounts in poultry diets in order to meet the requirements for maintaining normal growth performance due to their poor stability, low bioavailability, and potential anti-nutritional effects (Frazzoli et al., 2015; Byrne and Murphy, 2022). However, this would not only increase the health risks in both animals and humans, but also threaten the quality and safety of animal products, and exacerbate environmental pollution (Frazzoli et al., 2015). Therefore, the effective and rational use of trace minerals has become a key strategy to ensure poultry health, improve meat safety, and reduce trace mineral emissions to protect the environment.

Increasing evidence has shown that organic trace minerals (OTM) have higher bioavailability than ITM and could be more easily absorbed and utilized in animal production (Byrne and Murphy, 2022). Importantly, there has been a tendency to apply chelated forms of micro-elements as an alternative to common mineral forms of micro-elements in poultry diets (Faghih-Mohammadi et al., 2022). For example, dietary organic forms of Zn in chelated methionine formed and significantly improved the meat quality of Ross 308 broilers when compared to their inorganic forms (Satek et al., 2020). Additionally, dietary supplementation with OTM (Mn, Zn, Fe, Cu, I, and Se) enhanced systemic and local immune responses and improved the antioxidant capacity of Ross broilers (Echeverry et al., 2016). In addition, a previous study has indicated that replacing the ITM premix containing Mn, Zn, Fe, Cu, Se, and Cr with OTM in Cobb 400 broiler diets could significantly improve growth performance, bone mineralization, and antioxidant capacity (Savaram Venkata et al., 2021). A recent study also found that the replacement of ITM with OTM at lower doses could promote the deposition of trace minerals in the tissues of 817 white-feathered broilers (Han et al., 2024). Interestingly, dietary supplementation of organic macro and trace minerals in slower-growing Ross 308 broiler breeders, but not faster-growing broiler breeders, increased the body weight, tibia development and trace mineral density of their offspring (Güz et al., 2022). Moreover, a previous study found that the total replacement of high levels of ITM with lower levels of OTM containing Fe, Cu, Mn, Zn as chelated mineral proteinates improved production performance and reduced fecal excretion in yellow-feathered broiler breeders (Wang et al., 2019a; Wang et al., 2019b). Notably, the above applications of OTM to replace ITM were mostly reported in white-feathered broilers or broiler breeders, with few reports concerning the yellow-feathered broilers.

Indeed, chelated micro elements can not only meet the trace mineral requirements for broiler breeders and their progeny more effectively, but also produce protein products at lower inputs, thus better ameliorating the disadvantages of ITM (Faghih-Mohammadi et al., 2022). Furthermore, chelated minerals have higher solubility, chemical stability and electrical neutrality, allowing them to maintain their structural integrity in the digestive tract and arrive at the absorptive site in the small intestine as intact molecules in their original form (Jacob et al., 2022). However, most previous research on the application of chelated minerals in broilers mainly involved single amino acid chelated OTM (Ma et al., 2012; Umar Yaqoob et al., 2020). Limited information was available concerning the potential effects of dietary supplementation with complex amino acid-chelated OTM at lower levels to replace the ITM in Chinese yellow-feathered broilers. Therefore, in our study, we selected a compound OTM premix containing Fe, Cu, Zn, and Mn chelated with a balanced complex of 18 amino acids derived from the hydrolysis of plant proteins, and aimed to evaluate dietary amino acid-chelated compound OTM at lower levels on plasma biochemical parameters, antioxidant capacity, carcass traits, meat quality, and tissue mineral deposition in Chinese yellow-feathered broilers.

Materials and methods

Animal management, diets, and experimental design

A total of 960 one-day-old healthy male broilers were provided from a commercial hatchery (Guangzhou Muyuan Poultry Industry Co., Ltd, China) and were randomly allocated to 6 treatment groups. Each group had 8 replicates with 20 birds per replicate. The experimental design was provided in Table 1. Water and feed were provided *ad libitum* throughout the trial. The composition and nutrient level of the basal diets (Table 2) were formulated according to the nutrient requirements of yellow chickens recommended by the Ministry of Agriculture and Rural Affairs of the People's Republic of China (2020).

The amino acid-chelated OTM premix of Fe, Cu, Zn, and Mn was provided as MINEXO FeTM, MINEXO CuTM, MINEXO ZnTM, and MINEXO MnTM (DeBon Bio-tech Co., Ltd., Hunan, China), which contained 1.8×10^5 mg/kg Fe, 1.8×10^5 mg/kg Cu, 1.6×10^5 mg/kg Zn, and 1.6×10^5 mg/kg Mn, respectively. The amino acids and trace minerals were chelated at a molar ratio of 2: 1 and the 18 types of amino acids were derived from hydrolyzed plant proteins. The measured values of Fe, Cu, Zn, and Mn in all experimental diets at both starter and grower-finisher stages are provided in Table 3. The natural levels of Zn, Fe, Cu, and Mn in the basal diets were measured as 36.92, 319.00, 6.03, and 60.27 mg/kg at the starter stage, and 34.41, 189.23, 4.21, and 104.93 mg/kg at grower-finisher stage, respectively. The experimental protocol of the animal study was reviewed and approved by the Animal Care and Use Committee of Foshan University (FOSU2022004).

Sample collection and measurements

At the end of the experiment (53 days), after a 12 h fasting period, two broilers close to the average BW from each replicate ($n = 8$) were randomly selected for blood collection (approximately 10 mL) from the wing vein. The blood was collected into evacuated heparinized tubes and vacuum tubes, then centrifuged at 3,000 rpm for 10 min at 4 °C to harvest the plasma and serum samples, which were stored at -80 °C for the determinations of plasma biochemical parameters and serum trace element concentrations. After blood collection, the broilers were slaughtered for sample collections.

The heart, liver, spleen, pancreas, thymus, and bursa of Fabricius were weighed individually to calculate the organ indexes. Dressing percentage, abdominal percentage, breast percentage, and thigh percentage were determined by weighing the carcass, abdominal fat, breast muscle and leg muscle, respectively, according to the previous method (Wang et al., 2021). The left side of breast muscle was used to evaluate meat quality. The liver and breast muscle samples were taken into 1.5 mL EP tubes to measure the antioxidant capacity. The spleen sample was collected to determine cytokine expression. All these tissue samples were snap-frozen in liquid nitrogen and stored at -80 °C until further analyses. Moreover, additional left portions of breast muscle, liver and tibia were collected and frozen in liquid nitrogen, and stored at -40 °C for the determination of tissue trace element concentrations in broilers.

Plasma biochemical parameters

The plasma biochemical parameters, including the concentrations of total protein (TP), albumin (ALB), uric acid (UA), glucose (GLU), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), as well as the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were determined using an automatic biochemistry analyzer (Selectra Pro XL, EliT-echGroup, Puteaux, France). All kits were purchased from Biosino Biotechnology & Science, Inc. (Beijing, China).

Table 1
Experimental design.

Item	Form	NC	PC	ITM300	ITM500	OTM300	OTM500
Fe	Organic sources Fe sulfate	0	65	7.5	12.5	7.5	12.5
Cu	Organic sources Cu sulfate	0	8	1.2	2	1.2	2
Zn	Organic sources Zn sulfate	0	70	12	20	12	20
Mn	Organic sources Mn sulfate	0	75	15	25	15	25
I	Ca(IO ₃) ₂	0.80	0.80	0.80	0.80	0.80	0.80
Se	Na ₂ SeO ₃	0.35	0.35	0.35	0.35	0.35	0.35

Table 2
Composition and nutrient levels of experimental diets (air-dry basis).

Items	1 to 21 days	22 to 53 days
Ingredient		
Corn	44.5	42.2
Soybean meal	33.5	27.7
Extruded soybean	5	0
Sorghum	10	20
Lard	1.5	6.4
Fish meal	1.3	0
CaHPO ₄	1.3	1.2
Limestone	1.4	1
L-Lys-HCl	0.25	0.24
DL-Met	0.23	0.19
L-Thr	0.05	0.05
Premix ¹	0.71	0.75
NaCl	0.12	0.15
Choline chloride	0.12	0.1
Antioxidants	0.01	0.01
Phytase	0.01	0.01
Total	100	100
Nutrient level ² (%)		
ME (kcal/kg)	2877	3173.48
CP	21.45	17.11
EE	4.87	8.85
Ca	1.00	0.75
P	0.64	0.56
Lys	1.39	1.06
Met	0.56	0.45
Thr	0.88	0.69

¹ The vitamin premix provided per kg diet: VA 12,000 IU, VD₃ 3,000 IU, VE 10 IU, VK₃ 2 mg, VB₁ 1 mg, VB₂ 3 mg, VB₆ 2 mg, VB₁₂ 0.01 mg, niacin 20 mg, calcium pantothenate 4 mg, folic acid 1 mg, biotin 0.05 mg, Fe 100 mg, Cu 20 mg, Mn 100 mg, Zn 80 mg, I 3 mg, Se 0.5 mg.

² Calculated values.

Table 3
Measured values of trace minerals in experimental diets (air-dry basis).

Item	NC	PC	ITM300	ITM500	OTM300	OTM500
1-21 d, mg/kg						
Zn	36.92	65.99	47.28	50.16	55.66	78.97
Fe	319.00	326.93	252.22	272.86	295.71	265.29
Cu	6.03	8.36	5.83	6.85	6.67	8.26
Mn	60.27	89.70	70.07	76.98	79.12	99.65
22-53 d, mg/kg						
Zn	34.41	74.06	47.90	52.79	48.53	54.69
Fe	189.23	200.06	195.42	197.43	194.23	180.67
Cu	4.21	8.19	5.28	5.52	5.37	5.68
Mn	104.93	146.29	123.94	126.81	116.52	118.07

Meat quality determinations

Meat color of breast muscle samples, including Lightness (L*), Redness (a*), and Yellowness (b*), was determined after slaughter using a colorimeter (CR-300, Konica Minolta, Tokyo, Japan) at time intervals of 45 min, 24 h, and 48 h. The pH values of breast muscle samples were

measured after slaughter using a pH meter (OPTO-STAR, R.Matthaus, Germany) at time intervals of 45 min, 24 h and 48 h. The drip loss was detected by weighing a meat cuboid (W1) with length × width × thickness (1 cm × 2 cm × 2 cm) after slaughter. Then, the breast muscle sample was hooked with a toothpick to make the muscle fiber face downward and was sealed into a plastic cup before being hung in the refrigerator at 4 °C for 24 h or 48 h. Finally, the weights of breast muscle samples at 24 h (W2) and 48 h (W3) were calculated separately and drip loss was calculated as follows: (%) = (W1 - W2/W3)/W1 × 100%. Each sample was measured twice and the average was used for subsequent analysis. The cooking loss was estimated by heating the samples at 98 °C for 20 min, followed by weighing after cooling to room temperature. The shear force of breast muscle samples (3 cm × 1 cm × 1 cm) was measured using a muscle tenderness meter (Instron model 4411, Instron Corp., Canton, MA). The intramuscular fat contents in breast muscle samples were extracted with petroleum ether Soxhlete for 12-16 h after being pretreated by vacuum freeze-drying for 48 h, according to the methods specified by the National Standards (GB 5009.6-2016) of the People's Republic of China.

Antioxidant capacity determinations

The frozen liver and breast muscle samples were homogenized using ice-cold saline (0.9%) and then centrifuged at 2,500 × g for 10 min at 4 °C to harvest the homogenates for further analyses. The antioxidant capacity determinations, including the activities of catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (T-AOC), and glutathione peroxidase (GSH-Px), as well as the copper zinc superoxide dismutase (CuZn-SOD) concentrations in the plasma samples and liver and breast muscle homogenates, were analyzed according to the instructions of corresponding commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Mineral concentrations in the serum and tissues

The liver and breast muscle samples were pretreated by vacuum freeze-drying (Alpha 1-2 LD, Christ, Germany) for 48 h and being ground to powder. The left tibia samples were crushed, defatted with petroleum ether for 24 h, and dried in an oven at 105 °C for 12 h followed by burning for 16 h in a muffle furnace preheated to 600 °C. The contents of Zn, Fe, Cu and Mn in serum, liver, breast muscle and tibia samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8300, PerkinElmer, Waltham, MA, USA), according to the previous methods described by Qiu et al., (2020).

RNA extraction, cDNA synthesis, and qPCR analysis for gene expression

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The quantity and quality of RNA were determined by using a DS-11 spectrophotometer (DeNovix, Wilmington, DE, USA) with A260/A280 1.8-2.0. The total RNA (1 µg) was used to synthesize cDNA using a reverse transcription kit

(Takara, Japan). Real-time qPCR was subsequently performed with iTaq™ Universal SYBR Green Supermix (Bio-Rad, USA) in a QuantStudio 3 Flex real-time system (Applied Biosystems Instruments, Thermo Fisher Scientific, San Jose, CA, USA). The amplification program comprised one cycle of 95 °C for 30 s, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. Relative mRNA expression levels were normalized to that of β -actin and calculated using the $2^{-\Delta\Delta CT}$ method. Primer sequences for all genes are provided in Table 4, which were designed using Primer Premier 5.0 software (Applied Biosystems, USA) and then synthesized by Ige Biotech Co. (Guangzhou, China).

Statistical analysis

The data was analyzed using one-way ANOVA analysis with the IBM SPSS Statistical 26.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests were used to determine statistically significant differences between treatment means. Differences were considered significantly expressed at a P -value of <0.05 and with a significant tendency at $0.05 \leq P < 0.10$. The results are presented as means and standard error of the means (SEM).

Results

Plasma biochemical parameters

The results of plasma biochemical indicators of Chinese yellow-feathered broilers are shown in Table 5. Dietary replacement of ITM with OTM at lower levels (OTM300) significantly increased the plasma ALP activity when compared to the NC group, PC group, and ITM300 group ($P < 0.05$). However, there were no significant differences in other plasma biochemical parameters after OTM treatments ($P > 0.05$).

Antioxidant capacity

As shown in Fig. 1, dietary supplementation of OTM could significantly increase the CAT activity in the plasma of Chinese yellow-feathered broilers ($P < 0.05$). The plasma CuZn-SOD activity in the OTM300 group was significantly increased when compared to the NC, ITM300, and ITM500 groups ($P < 0.05$). Furthermore, dietary OTM300 and OTM500 supplementation significantly increased the hepatic CAT activity when compared to the ITM treatment and NC group ($P < 0.05$), while the hepatic GSH-Px activity in the OTM500 group was significantly higher than the other groups ($P < 0.05$). However, there was no significant difference in antioxidant capacity of breast muscle among the groups of Chinese yellow-feathered broilers ($P > 0.05$).

Table 4

Primer sequences for quantitative real-time polymerase chain reaction.

Genes ^a	Primer sequences ^b	GenBank access number
TNF- α	F-GAGCGTTGACTTGGCTGTC R-AAGCAACAACCGCTATGCAC	GU230788.1
TGF- β	F-CGGGACGGATGAGAAGAA R-TCGGCGCTCCAGATGTAC	NT_176262.1
IL-1 β	F-ACTGGGCATCAAGGGCTACA R-GCTGTCCAGGCGGTAGAAGA	Y15006.1
IL-6	F-CTCCTCGCAATCTGAAGTC R-CCTCAGGTTCTTCCATAAAC	NM_204628
IL-8	F-GGCTTGCTAGGGGAAATGA R-AGCTGACTCTGACTAGGAACTGT	DQ393272.2
IL-10	F-ACCAGTCATCAGCAGAGCAT R-CCTCCTCATCAGCAGTACTC	NM_001004414.2
β -actin	F-ATGATATTGCTGCGCTCGTT R-TCTTCTGCGCCATACCAACC	AY550069

^a TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor- β ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10.

^b F forward; R, reverse.

Serum and tissue trace mineral deposition

As shown in Figs. 2 and 3, when compared to the ITM300 group, dietary supplementation at lower or medium levels of OTM significantly increased the concentrations of Fe and Cu in the serum of Chinese yellow-feathered broilers ($P < 0.05$). Furthermore, dietary OTM significantly increased the contents of Zn and Cu in breast muscle when compared to the NC and ITM300 groups, and the breast muscle Fe concentrations in the OTM500 group showed an increase when compared to the ITM300 group ($P < 0.05$). Meanwhile, dietary OTM significantly increased the Zn concentrations in the tibia of Chinese yellow-feathered broilers when compared to the other groups ($P < 0.05$). However, there were no significant differences in the concentrations of Zn, Fe, Cu, and Mn in the livers of Chinese yellow-feathered broilers among different groups ($P > 0.05$).

Carcass traits and meat quality

As shown in Table 6, there were no significant differences in dressing percentage, abdominal fat percentage, breast muscle percentage, and thigh percentage of Chinese yellow-feathered broilers among different groups ($P > 0.05$).

As shown in Table 7, dietary supplementation with lower or medium levels of OTM significantly increased the breast muscle pH_{45min} in Chinese yellow-feathered broilers in comparison to the NC and ITM300 groups ($P < 0.05$). There was no significance in the pH_{24h} or pH_{48h} of Chinese yellow-feathered broilers among groups ($P > 0.05$). Furthermore, the drip loss_{24h} in the OTM300 group of Chinese yellow-feathered broilers was significantly decreased when compared to the NC and ITM300 groups ($P < 0.05$). A significant decrease in drip loss_{48h} was detected in OTM300 or OTM500 groups when compared to the NC and ITM300 groups of Chinese yellow-feathered broilers ($P < 0.05$). However, the meat color, as demonstrated through the lightness (L*), redness (a*), and yellowness (b*), as well as the cooking loss, shear force and IMF did not differ among treatment groups ($P > 0.05$).

Spearman correlation analyses between meat quality and mineral deposition, or antioxidant parameters

Spearman correlation analysis was conducted to examine the relationship between meat quality and mineral deposition, or antioxidant parameters in Chinese yellow-feathered broilers. As shown in Fig. 4A, Fe and Mn concentrations of breast muscle showed a positive correlation with the activity of CAT in breast muscle ($P < 0.05$). For the spearman correlation analysis of meat quality and antioxidant parameters (Fig. 4B), the pH_{45min} of the breast muscle showed a positive correlation with T-AOC activity in liver ($P < 0.05$). The drip loss_{24h} of breast muscle showed a negative correlation with plasma CuZn-SOD activity ($P < 0.05$), while the drip loss_{48h} of breast muscle showed a negative correlation with the activity of CuZn-SOD and CAT in plasma, as well as T-AOC activity in the liver ($P < 0.05$). Moreover, as shown in Fig. 4C, the concentrations of Zn, Fe, Cu, and Mn in breast muscle showed a positive correlation with the pH_{45min} in breast muscle ($P < 0.05$), while the drip loss_{48h} of breast muscle showed a negative correlation with Zn and Cu concentrations of breast muscle ($P < 0.05$).

Organ indexes

As shown in Table 8, compared with ITM treatment, OTM500 significantly increased the heart index of Chinese yellow-feathered broilers ($P < 0.05$). Moreover, there was a tendency to increase the liver index, spleen index, and thymus index by the OTM500 group when compared to the ITM groups ($0.05 < P < 0.10$). However, there were no significant differences in the bursa of Fabricius and pancreas indexes among groups of Chinese yellow-feathered broilers ($P > 0.05$).

Table 5
Effects of organic trace elements on plasma biochemical parameters in Chinese yellow-feathered broilers.

Item	NC	PC	ITM300	ITM500	OTM300	OTM500	SEM	P-value
TP	30.1	31.6	32.2	32.0	31.8	32.4	0.363	0.502
ALB	19.3	20.5	19.8	19.0	19.7	19.5	0.306	0.839
UA	150	128	117	107	119	101	5.75	0.246
GLU	12.9	12.8	13.3	12.3	12.8	13.1	0.150	0.555
TG	0.328	0.308	0.371	0.335	0.372	0.386	0.020	0.874
TC	3.32	3.60	3.70	3.55	3.69	3.69	0.044	0.084
HDL	1.77	1.89	2.02	1.89	1.98	1.96	0.032	0.252
LDL	0.411	0.539	0.531	0.439	0.482	0.481	0.025	0.680
ALT	7.33	7.70	7.22	9.95	9.58	9.68	0.596	0.602
AST	195	212	199	204	223	213	2.96	0.065
ALP	1130 ^b	1363 ^b	1234 ^b	1604 ^{ab}	2021 ^a	1487 ^{ab}	92.1	0.046
LDH	698	665	601	600	699	667	20.9	0.589

¹ SEM, standard error of the mean (n = 8).

^{a,b} The means with no common superscripts within each row are significantly different ($P < 0.05$), $0.05 \leq P < 0.10$ indicates a significant trend. Abbreviations: TP, total protein; ALB, albumin; UA, uric acid; GLU, glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate hydrogenase.

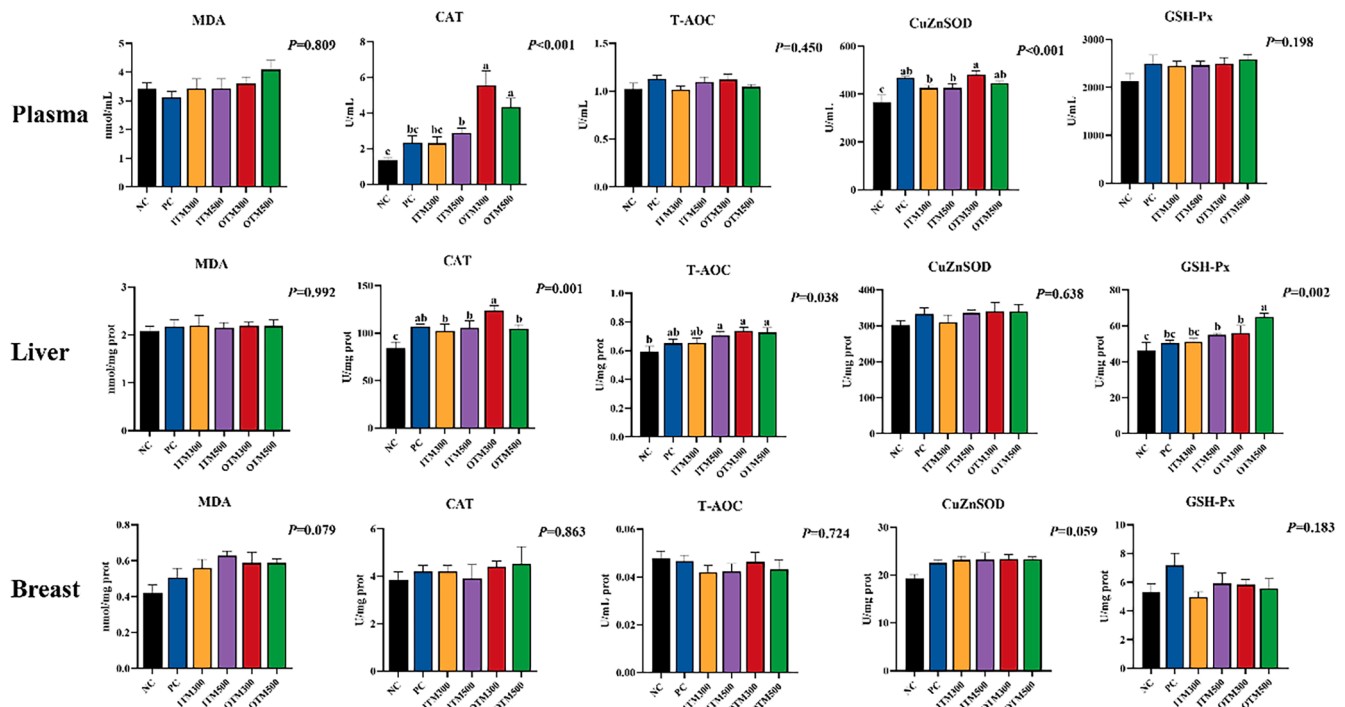


Fig. 1. Effects of organic trace elements on the antioxidative capacity of Chinese yellow-feathered broilers. Abbreviations: MDA, malondialdehyde; CAT, catalase; T-AOC, total antioxidant capacity; CuZn-SOD, copper-zinc superoxide dismutase; GSH-Px, glutathione peroxidase; NC, negative control fed diet without ITM or OTM premix; PC, positive control fed diet supplemented with ITM premix at 1,000 mg/kg; ITM300, control diet supplemented with ITM premix at 300 mg/kg; ITM500, control diet supplemented with ITM premix at 500 mg/kg; OTM300, control diet supplemented with OTM premix at 300 mg/kg; OTM500, control diet supplemented with OTM premix at 500 mg/kg.

Gene expression of cytokines in the spleen

As shown in Fig. 5, when compared to the NC and ITM300 groups, dietary OTM significantly upregulated the splenic mRNA expression of TGF- β , while downregulating the splenic mRNA of IL-1 β and IL-6 in Chinese yellow-feathered broilers ($P < 0.05$). However, there were no differences in IL-8, IL-10, and TNF- α mRNA expression in the spleens of Chinese yellow-feathered broilers ($P > 0.05$).

Discussion

China is one of the largest producers and consumers of chicken in the world. Chinese yellow-feathered broilers are recognized as a famous local breed and usually have superior disease resistance ability and

better meat flavor and taste than white-feathered broilers. Organic trace minerals (OTM) have stronger stability, and higher bioavailability characteristics than inorganic trace minerals (ITM), and have become a crucial strategy to ensure broiler health, emission reductions, and environment protection (Byrne and Murphy, 2022; Faghih-Mohammadi et al., 2022). The effectiveness of replacing OTM with ITM have been well demonstrated in finishing pigs (Xiong et al., 2023), laying hens (Yang et al., 2021), and white-feathered broilers or broiler breeders (Salek et al., 2020; Savaram Venkata et al., 2021; Güz et al., 2022). However, the potential of reducing dietary supplementation of trace minerals by chelated OTM in Chinese yellow-feathered broilers remained largely unclear. The present study found that dietary supplementation with low or medium levels of amino acid-chelated compound OTM of Fe, Cu, Mn, and Zn significantly increased the deposition of trace

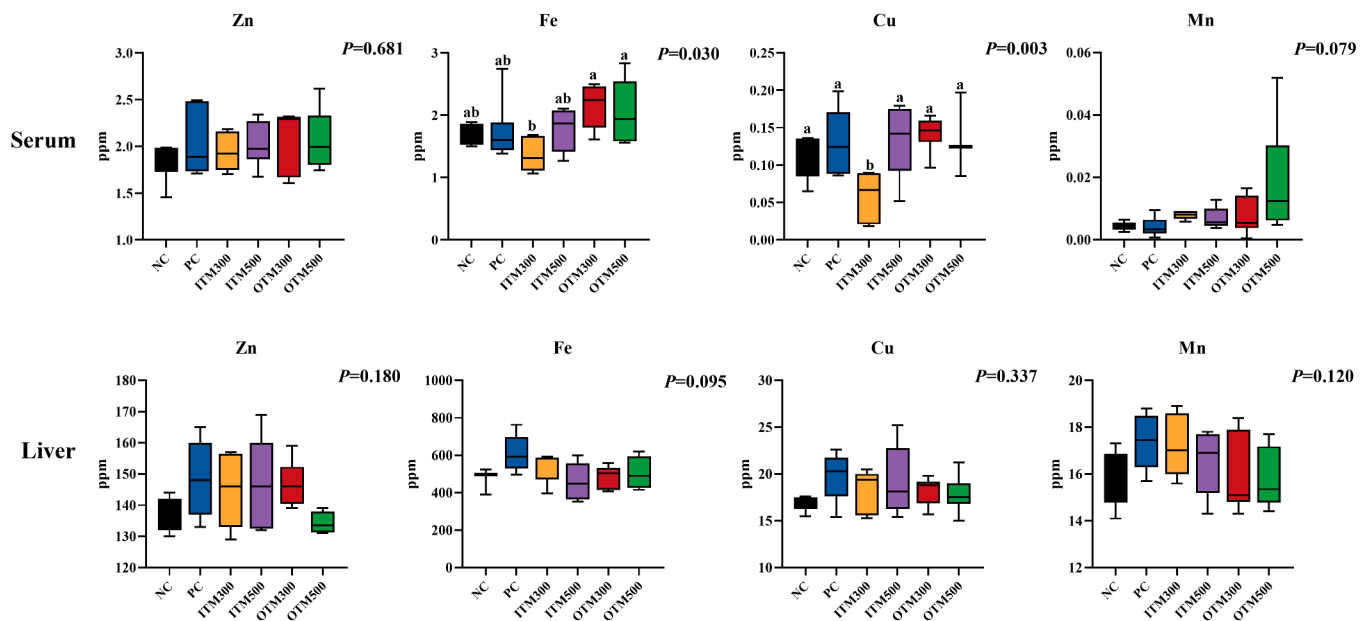


Fig. 2. Effects of organic trace elements on the deposition of trace minerals in serum and liver of Chinese yellow-feathered broilers. Abbreviations: NC, negative control fed diet without ITM or OTM premix; PC, positive control fed diet supplemented with ITM premix at 1,000 mg/kg; ITM300, control diet supplemented with ITM premix at 300 mg/kg; ITM500, control diet supplemented with ITM premix at 500 mg/kg; OTM300, control diet supplemented with OTM premix at 300 mg/kg; OTM500, control diet supplemented with OTM premix at 500 mg/kg.

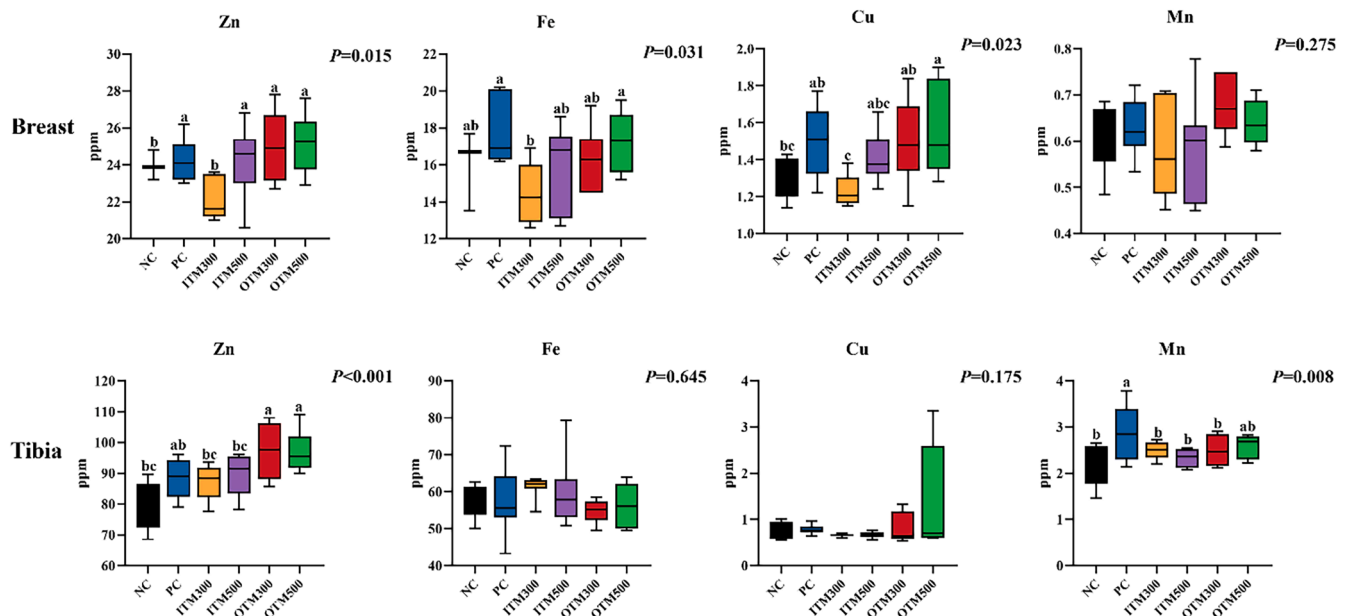


Fig. 3. Effects of organic trace elements on the deposition of trace minerals in breast muscle and tibia of Chinese yellow-feathered broilers. Abbreviations: NC, negative control fed diet without ITM or OTM premix; PC, positive control fed diet supplemented with ITM premix at 1,000 mg/kg; ITM300, control diet supplemented with ITM premix at 300 mg/kg; ITM500, control diet supplemented with ITM premix at 500 mg/kg; OTM300, control diet supplemented with OTM premix at 300 mg/kg; OTM500, control diet supplemented with OTM premix at 500 mg/kg.

minerals in serum and tissues, while also enhancing the antioxidant capacity. Therefore, it may help improve the meat quality of Chinese yellow-feathered broilers.

The concentrations of trace minerals in blood and tissues are important indicators to measure trace minerals bioavailability and distribution. Increasing evidence has shown that the replacement of ITM with OTM in equal amounts could increase the mineral deposition of different farm animals (Ma et al., 2012; Liu et al., 2016; Yin et al., 2022). For example, dietary comparable amounts of glycine chelated Fe instead of FeSO₄ significantly increased Fe concentrations in serum, liver, breast

muscle, and tibia of Ross broilers (Ma et al., 2012). Another study in growing-finishing pigs also found that dietary OTM could significantly improve the concentrations of liver Zn and heart Se (Liu et al., 2016). Notably, the lower inputs of compound OTM with higher tissue deposition are beneficial for promoting the practice of the One-Health concept in poultry production (Frazzoli et al., 2015). This has been confirmed in laying hens with dietary supplementation of 50% NRC-L (OHN) OTM (Zn, Fe, Cu, Mn and Se) could improve the concentrations of liver Fe and Se, kidney Cu and Se, and tibia Se (Yang et al., 2021). Similarly, a Cherry Valley duck study showed that dietary

Table 6
Effects of organic trace elements on the carcass traits in Chinese yellow feathered broilers.

Item	NC	PC	ITM300	ITM500	OTM300	OTM500	SEM	P-value
Dressing percentage, %	90.8	89.4	89.5	88.2	88.8	89.0	0.245	0.135
Abdominal fat percentage, %	3.48	4.24	3.72	3.83	4.25	3.50	0.150	0.506
Breast percentage, %	14.6	14.3	15.0	14.1	13.7	14.4	0.182	0.387
Thigh percentage, %	19.8	20.6	20.4	20.6	20.2	19.2	0.223	0.435

¹ SEM, standard error of the mean (n = 8).

^{a,b} The means with no common superscripts within each row are significantly different ($P < 0.05$), $0.05 \leq P < 0.10$ indicates a significant trend.

Dressing percentage (%) = carcass weight / pre-slaughter weight \times 100.

Table 7
Effects of organic trace elements on the meat quality in Chinese yellow-feathered broilers.

Item	NC	PC	ITM300	ITM500	OTM300	OTM500	SEM	P-value
Meat color								
L*	50.0	49.0	49.1	50.6	49.4	51.4	0.372	0.358
a*	1.30	1.57	1.24	1.02	1.66	1.23	0.080	0.211
b*	11.6	11.6	11.7	11.4	11.3	11.8	0.168	0.413
pH								
45 min	6.01 ^b	6.10 ^{ab}	5.96 ^b	6.08 ^{ab}	6.27 ^a	6.22 ^a	0.031	0.012
24 h	5.67	5.60	5.70	5.69	5.72	5.62	0.016	0.193
48 h	5.73	5.67	5.68	5.67	5.67	5.64	0.010	0.308
Drip loss								
24 h	2.12 ^a	1.79 ^{bc}	2.26 ^a	1.77 ^{bc}	1.71 ^c	1.95 ^{abc}	0.056	0.014
48 h	4.50 ^a	3.93 ^{ab}	4.48 ^a	3.97 ^{ab}	3.24 ^c	3.60 ^{bc}	0.104	<0.001
Cooking loss	0.275	0.249	0.261	0.264	0.255	0.268	0.004	0.472
Shear force	26.2	30.5	28.2	27.0	31.4	37.8	1.542	0.285
IMF, %	2.73	3.54	4.23	3.31	3.48	3.63	0.155	0.184

¹ SEM, standard error of the mean (n = 8).

^{ab} The means with no common superscripts within each row are significantly different ($P < 0.05$), $0.05 \leq P < 0.10$ indicates a significant trend.

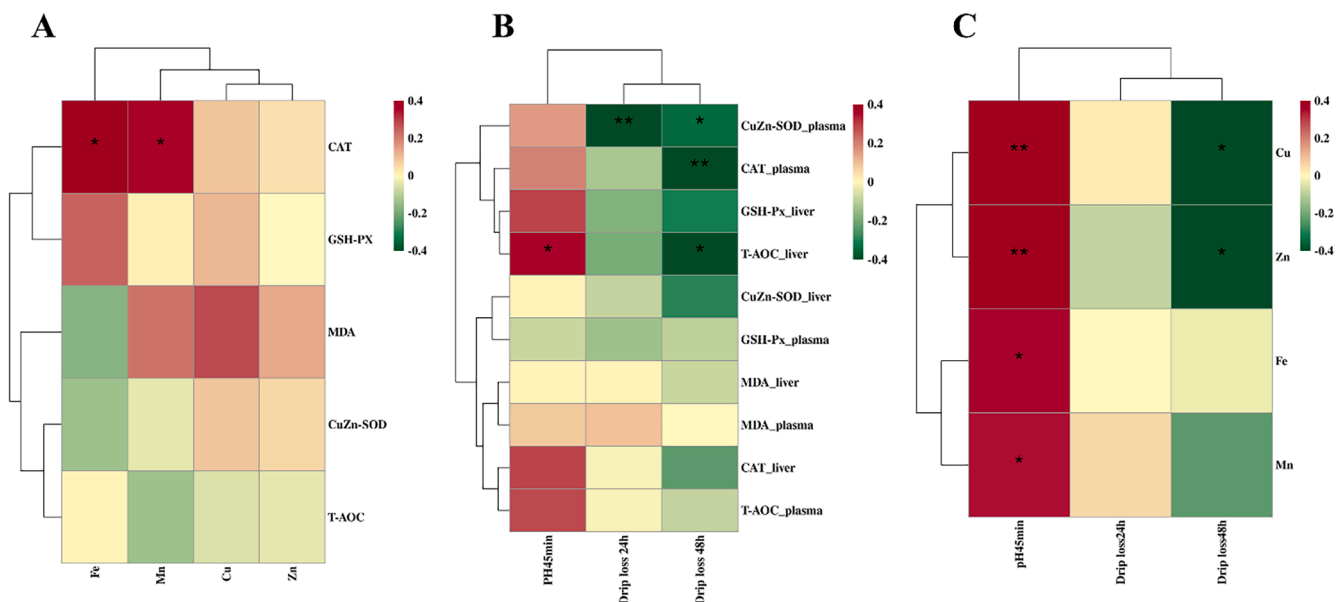


Fig. 4. Spearman correlation analysis between meat quality and mineral deposition, or antioxidant parameters of Chinese yellow-feathered broilers. (A) Correlation between mineral deposition of breast muscle and antioxidant parameters of breast muscle. (B) Correlation between meat quality and antioxidant parameters of plasma and liver. (C) Correlation between mineral deposition of breast muscle and meat quality. Abbreviations: CuZn-SOD, copper-zinc superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase. T-AOC, total antioxidant capacity; MDA, malondialdehyde.

supplementation with 500 mg/kg compound OTM of Se, Zn, Fe, Cu, Mn, and I showed comparable effects on serum, liver and muscle trace minerals deposition when compared to the supplementation of 1,000 mg/kg ITM (Yin et al., 2022). Moreover, complexed amino acid minerals (Zn, Mn, and Cu) could more effectively improve the deposition of trace minerals in the egg yolk and bone density than bis-glycinate chelated minerals in Lohmann White laying hens (Santos et al., 2023). Although

limited research concerning the replacement and reduction of ITM by amino acids chelated compound OTM has been conducted in Chinese yellow-feathered broilers, a previous study in yellow-feathered broiler breeders found that replacing 50% ITM (Cu, Zn, Fe, Mn, and Se) with OTM sources significantly increased trace minerals concentrations in serum, breast muscle, and heart (Wang et al., 2019a). Consistently, our current study found that dietary supplementation with low or medium

Table 8
Effects of organic trace elements on the organ index in Chinese yellow-feathered broilers.

Item	NC	PC	ITM300	ITM500	OTM300	OTM500	SEM	P-value
Bursa of Fabricius index, %	0.230	0.186	0.219	0.224	0.220	0.223	0.008	0.617
Liver index, %	1.75	1.59	1.56	1.61	1.59	1.70	0.021	0.065
Spleen index, %	0.180	0.151	0.158	0.124	0.141	0.146	0.005	0.068
Heart index, %	0.644 ^{ab}	0.610 ^{ab}	0.529 ^b	0.548 ^b	0.658 ^{ab}	0.715 ^a	0.019	0.041
Pancreas index, %	0.168	0.159	0.154	0.144	0.165	0.156	0.004	0.625
Thymus index, %	0.573	0.474	0.440	0.454	0.396	0.558	0.020	0.066

¹SEM, standard error of the mean (n = 8).

^{ab} The means with no common superscripts within each row are significantly different ($P < 0.05$), $0.05 \leq P < 0.10$ indicates a significant trend. Organ index (%) = Organ weight/ pre-slaughter weight \times 100.

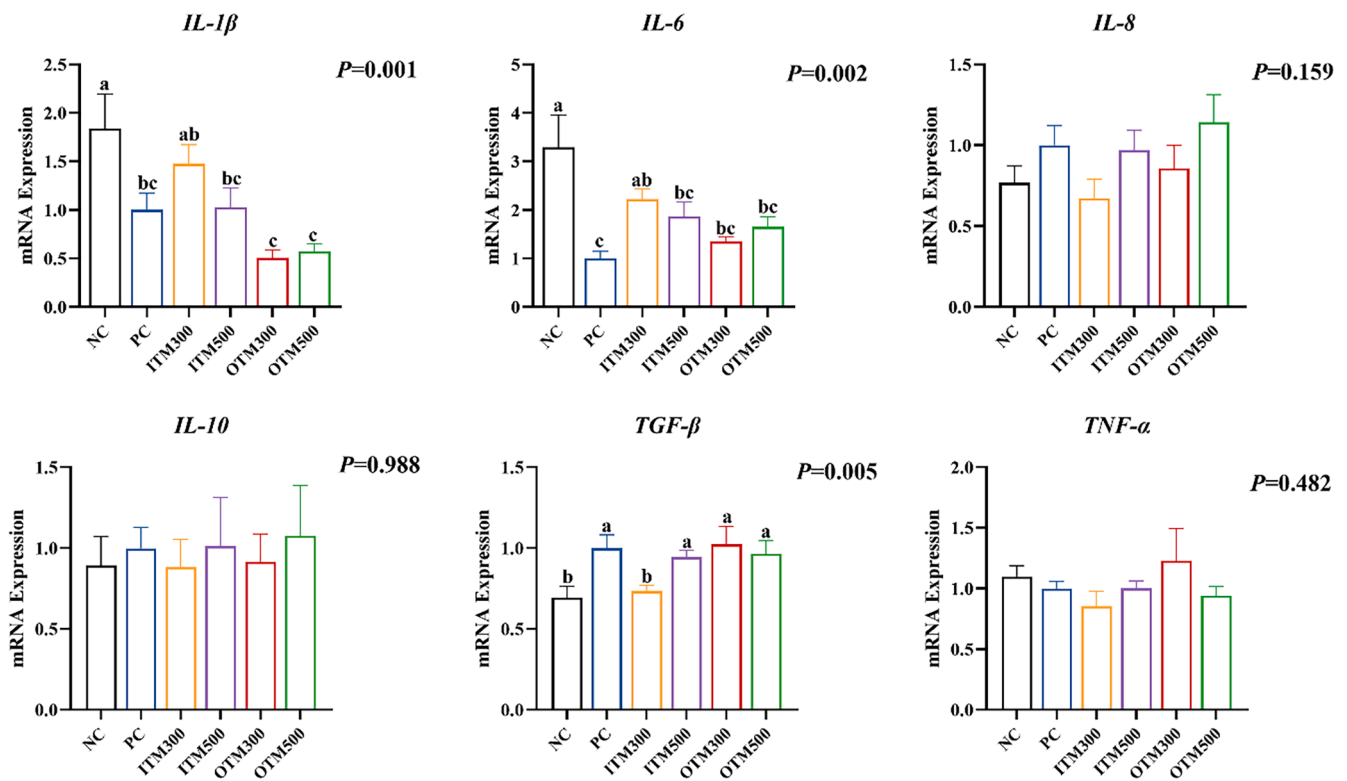


Fig. 5. Effects of organic trace elements on gene expression of cytokines in the spleen of Chinese yellow-feathered broilers. Abbreviations: IL-1 β , interleukin-1 β ; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor- β ; NC, negative control fed diet without ITM or OTM premix; PC, positive control fed diet supplemented with ITM premix at 1,000 mg/kg; ITM300, control diet supplemented with ITM premix at 300 mg/kg; ITM500, control diet supplemented with ITM premix at 500 mg/kg; OTM300, control diet supplemented with OTM premix at 300 mg/kg; OTM500, control diet supplemented with OTM premix at 500 mg/kg.

levels of amino acid-chelated compound OTM of Fe, Cu, Mn, and Zn increased the serum Fe and Cu concentrations, and enhanced the deposition of breast Zn, Fe and Cu, along with tibia Zn in Chinese yellow-feathered broilers. This might be attributed to the fact that amino acid-chelated trace minerals have a higher bioavailability due to their stronger solubility compared to ITM (Jacob et al., 2022). Furthermore, amino acid-chelated trace minerals are usually transported via the amino acid transporter mechanism, which ensures that the trace elements remain intact and are efficiently delivered to the small intestine for absorption, while also reducing antagonism between trace minerals (Gao et al., 2014; Sauer et al., 2017). Moreover, previous studies have found that dietary supplementation of OTM could significantly promote Zn deposition in the tibia of hens (Qiu et al., 2020), as well as broiler breeders and their offspring (Güz et al., 2022). Notably, Zn is a co-factor of various enzymes for bone mineralization to maintain bone growth and development (Bao and Choct, 2009). Furthermore, ALP activity is recognized as an important indicator of Zn absorption in poultry, and thus plays an important role in both bone mineralization and

metabolism (Idowu et al., 2011; Makris et al., 2023). Therefore, the observed increased plasma ALP activity of Chinese yellow-feathered broilers in the current study might be associated with the enhanced Zn deposition in the tibia. However, the present study failed to investigate the potential mechanism of amino acid-chelated compound OTM on trace mineral deposition and the interactions between ALP activity and trace mineral metabolism in Chinese yellow-feathered broilers.

The antioxidant defense system depends on antioxidant enzymes and substances that protect the body against oxidative damage caused by reactive oxygen species (Sies et al., 2017). Importantly, multiple studies have confirmed that trace minerals (Zn, Fe, Cu and Mn) have served as activators of antioxidant enzymes to regulate the oxidative stress (Woloncej et al., 2016; Ognik et al., 2018). Therefore, we hypothesized that the deposition of trace minerals in serum and tissues might further affect the antioxidant status of Chinese yellow-feathered broilers. As expected, we found that dietary supplementation of low or medium levels of compound OTM in equivalent amounts of ITM significantly increased plasma CAT and CuZn-SOD, as well as liver CAT, T-AOC, and

GSH-Px activities in Chinese yellow-feathered broilers. Our current findings also demonstrated a strong correlation between antioxidant capacity and notable alterations in trace mineral deposition in the serum and tissues of Chinese yellow-feathered broilers. Specially, the deposition of Fe and Mn in breast muscle were positively correlated with CAT activity of breast muscle. Indeed, Fe is of critical importance to maintain the CAT activity (Ma et al., 2016), which rapidly breaks down hydrogen peroxide and lessens its detrimental effects (Wolonciej et al., 2016). Additionally, Cu and Zn are vital components of CuZn-SOD, which eliminates free radicals in the body (Wolonciej et al., 2016). Thus, dietary supplementation with low or medium OTM could enhance the antioxidant capacity, at least partially, via the improvement of trace mineral deposition in Chinese yellow-feathered broilers. In the current study, we employed the compound OTM chelated by a balanced complex of 18 amino acids derived from the hydrolysis of plant proteins, which might display strong antioxidant capacity and work as antioxidant synergists (Monteiro and Paiva-Martins, 2022). Notably, previous studies have shown that dietary supplementation with low doses of small peptide-chelated OTM significantly increased serum GSH-Px and CAT activities alongside liver CAT and CuZn-SOD activities in 817 white feathered broilers (Kong et al., 2022). Similarly, the addition of moderate to low levels of complex glycine OTM increased liver T-AOC activity in broiler breeders (Umar Yaqoob et al., 2020). In addition, supplementing 30% amino acid-compound OTM instead of 100% ITM dramatically improved the muscle Mn-SOD activity in growing-finishing pigs (Xiong et al., 2023). The results of the current study were consistent with these previously reported results that dietary supplementation with lower amounts of amino acids or small peptide chelating trace minerals were more effective than ITM in increasing antioxidant capacity. This may be attributed to the natural potent antioxidant qualities of amino acids and their ability to react with free radicals in addition to their great solubility (Meucci and Mele, 1997; Vascotto and Tiribelli, 2015).

Carcass characteristics and meat quality are comprehensive and important economic indicators in broiler production, serving as critical factors that affect consumers' choices (Mir et al., 2017). The present study found that the trace mineral sources (organic vs. inorganic) had no significant differences in carcass characteristics of Chinese yellow-feathered broilers, which was consistent with previous studies conducted in Ross 308 broilers (Zhu et al., 2019) and 817 white broilers (Kong et al., 2022). Furthermore, previous studies have shown that dietary supplementation with amino acid-organically complexed minerals (Cu, Zn, and Mn) (Aksu et al., 2011) or organic Se as Se yeast (Rajashree et al., 2014) increased the lightness (L^* value), decreased redness (a^* value), and improved the water holding capacity of breast meat in Ross 308 broilers when compared to their inorganic forms. However, our results indicated that dietary supplementation with OTM at low levels significantly increased pH_{45min} and reduced drip loss_{24h} of breast muscle, which were consistent with previous studies conducted where dietary methionine-Zn had a beneficial effect on reducing drip loss of Ross 308 (Saek et al., 2020). The significant increase in the pH_{45min} of breast muscle could improve meat quality and delay the formation of PSE meat (Jiang et al., 2007; Wang et al., 2022). Furthermore, the pH value of the muscles was positively correlated with water-holding capacity (Qiao et al., 2001), while drip loss was negatively correlated with muscle water-holding capacity (Schäfer et al., 2002). This may be attributed to the principle that elevated muscle pH enhances its capacity to retain molecular water through increased protein binding, consequently minimizing water loss and enhancing water retention (De La Fuente et al., 2010). In the current study, additional Spearman analysis confirmed that the pH_{45min} of breast muscle was positively correlated with the T-AOC activity in the liver, while the drip loss_{48h} of breast muscle was negatively correlated with the activities of CuZn-SOD and CAT in the plasma and liver T-AOC activity. This indicates the close relationship between antioxidant capacity and meat quality in Chinese yellow-feathered broilers fed with OTM. The alterations in the antioxidant machinery within the body can lead to shifts in the anabolic

processes of lipids and proteins in cells, thus impacting the meat quality (Zhang et al., 2013; Herrera et al., 2021). Furthermore, we also found that the deposition of Zn, Fe, Cu, and Mn in breast muscle was positively correlated with pH_{45min} of breast muscle, while the deposition of Cu and Zn in breast muscle were negatively correlated with the drip loss_{48h} of breast muscle of Chinese yellow-feathered broilers after feeding the amino acids chelated OTM. This was inconsistent with the previous study stating that the chelating form of Fe could effectively reduce the occurrence of lipid oxidation and show positive influences on meat quality compared to its inorganic form (Huang and Ahn, 2019). Therefore, the enhanced trace mineral deposition and antioxidant capacity might help contribute to improve meat quality of Chinese yellow-feathered broilers when supplementing amino acid-chelated compound OTM of Fe, Cu, Mn, and Zn at lower doses in the diets. However, the exact mechanism through which amino acid-chelated compound trace minerals improve the meat quality of Chinese yellow-feathered broilers through enhancing tissue deposition and antioxidant capacity remains largely unknown and requires additional exploration.

Previous studies have shown that organic trace minerals have strong immunomodulatory effects and play important roles in the activation of the cellular and humoral immune responses, as well as the enhancement of resistance to infections (Jarosz et al., 2017; Biabani et al., 2024). The spleen is one of the major immune organs of broilers. Here, we found that dietary compound OTM supplementation at lower levels significantly upregulated the mRNA expression of TGF- β and downregulated the IL-1 β mRNA expression in the spleen of Chinese yellow-feathered broilers, indicating the potential of OTM for regulating immune function and health. Similarly, a previous study has shown that OTM supplementation significantly improves the organ indexes of the spleen and bursa of Fabricius in broilers, and upregulated the splenic IL-10 expression and IL-12p35 expression in bursa of Fabricius (Echeverry et al., 2016). However, the current study found that lower levels of compound OTM did not alter the immune organ indexes of the spleen, bursa of Fabricius, and thymus, but significantly improved heart index in Chinese yellow-feathered broilers compared with ITM treatment. A similar study in Arbor Acres broilers also reported that dietary organic Zn supplementation in low-protein diets had no significant effects of immune organ indexes (Dong et al., 2023). The discrepancies in the organ index results of these different studies might be due to the differences in broiler breeds, age, feeding conditions, and management. Nonetheless, the current study only explored certain aspects of splenic immune function and a lack of substantial evidence regarding the comprehensive effects of amino acid-chelated OTM on the immune system of Chinese yellow-feathered broilers remains. This should be strengthened in further investigations to uncover their dynamic roles and potential mechanism.

Conclusion

In conclusion, dietary supplementation with lower levels of amino acid-chelated compound OTM of Fe, Cu, Mn, and Zn could promote the tissue deposition of trace minerals, enhance antioxidant capacity and immune levels, and improve meat quality of Chinese yellow-feathered broilers. Furthermore, the increased antioxidant capacity and tissue mineral deposition may be closely related to the improvement of meat quality, most likely due to its increased bioavailability of amino acid-chelated OTM. The results of the current study may provide a scientific foundation for substituting common ITM with amino acid-chelated OTM supplements in the broiler industry, especially for Chinese local breeds. However, more studies are needed to reveal the underlying mechanism and regulatory molecular pathways concerning the physiological and biochemical impacts of amino acid-chelated compound OTM on Chinese yellow-feathered broilers.

Disclosures

The authors declare no conflicts of interest.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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