

Article

Not peer-reviewed version

Influence of Trace Mineral Sources and Levels on Growth Performance, Carcass Traits, Bone Characteristics, Oxidative Stress, and Immunity of Broiler

<u>Tassanee Trairatapiwan</u>, <u>Rachakris Lertpatarakomol</u>*, <u>Sucheera Chotikatum</u>, <u>Achara Lukkananukool</u>, <u>Jamlong Mitchaothai</u>

Posted Date: 7 July 2025

doi: 10.20944/preprints202507.0437.v1

Keywords: inorganic minerals; organic mineral; trace mineral; broiler



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Influence of Trace Mineral Sources and Levels on Growth Performance, Carcass Traits, Bone Characteristics, Oxidative Stress, and Immunity of Broiler

Tassanee Trairatapiwan ¹, Rachakris Lertpatarakomol ^{1,*} Sucheera Chotikatum ¹, Achara Lukkananukool ² and Jamlong Mitchaothai ³

- ¹ Faculty of Veterinary Medicine, Mahanakorn University of Technology (MUT), Bangkok 10530, Thailand
- ² Department of Animal Production Technology and Fisheries, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand
- Office of Administrative Interdisciplinary Program on Agricultural Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand
- * Correspondence: rachakris@mut.ac.th; Tel.: +66-891272611

Simple Summary

Trace minerals are essential for optimal growth and health in broiler chickens. However, they are often added to poultry diets at high levels in the form of low-cost inorganic compounds that are poorly absorbed. This study investigated whether low levels of highly bioavailable organic trace minerals could support broiler health and performance while reducing environmental mineral excretion. Broilers were assigned to diets containing either commercial levels of inorganic trace minerals, low levels of organic trace minerals, or low levels of inorganic trace minerals. Birds receiving low levels of organic trace minerals exhibited improved feed conversion ratios during the starter phase and showed increased fillet and thigh muscle yields. Bone development, oxidative stress markers, and humoral immune responses were not affected by either the source or inclusion level of dietary trace minerals. Notably, reducing the inclusion level of organic trace minerals did not compromise broiler growth or health, indicating more efficient mineral utilization. Similarly, reducing inorganic trace mineral supplementation did not impair performance, suggesting that current commercial inclusion levels may exceed physiological requirements. These findings suggest that incorporating low levels of organic trace minerals in broiler diets can sustain productivity while promoting more sustainable and environmentally responsible poultry production practices.

Abstract

This study investigated the effects of different trace mineral sources and inclusion levels on broiler growth performance, carcass characteristics, bone (tibia) characteristics, oxidative stress markers (superoxide dismutase, SOD, malondialdehyde, MDA), and immune response (serum IgG). A total of 384 one-day-old Ross 308 chicks were randomly assigned to three dietary treatments: (1) commercial-level inorganic trace minerals (ILI), (2) low-level organic trace minerals (LLO), and (3) low-level inorganic trace minerals (LLI). Each treatment consisted of eight replicates with 16 birds per replicate, and diets were provided in two phases: starter (days 1–21) and grower (days 22–35). The results showed that LLO group demonstrated a significantly improved feed conversion ratio (FCR) during the starter phase (P < 0.05) and exhibited higher fillet and thigh muscle yields (P < 0.05), while other carcass traits, pH, and drip loss remained unaffected. Tibia breaking strength at day 35 was highest in the ILI group (P < 0.05). No significant differences were observed in oxidative stress markers or IgG levels among groups. Overall, a low level of organic trace minerals sustained broiler performance and improved select traits, whereas reduced inorganic levels were less effective, supporting the potential of organic sources for sustainable poultry production.

Keywords: inorganic minerals; organic mineral; trace mineral; broiler

1. Introduction

Trace minerals, though required in small quantities, are essential for maintaining optimal physiological, enzymatic, and immune functions in animals. Elements such as zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), and iron (Fe) are critical cofactors for a variety of biological processes, including growth, bone development, oxidative balance, and immunological responses [1–3]. In commercial poultry production, achieving precise trace mineral nutrition is vital to support performance, health, and product quality [4–6].

Conventional broiler diets often rely on inorganic trace mineral (ITMs) sources, such as sulfates, oxides, and carbonates, due to their low cost and ease of formulation. However, these forms are generally characterized by poor bioavailability, leading to their over-supplementation to meet metabolic needs. In addition, Thailand's hot and humid climate increases the risk of mycotoxin contamination in feed ingredients. To mitigate these risks and ensure adequate nutrient supply under such challenging conditions, animal feed manufacturers often supplement diets with vitamins and trace minerals at levels exceeding the recommendations of research institutions by 100 to 500% as a safety margin. This excessive use contributes to mineral antagonism, oxidative stress, and elevated excretion rates, thereby posing risks to animal health and environmental sustainability [5–8].

Organic trace minerals (OTMs), which involve chelation or complexation with amino acids, peptides, or organic acids, offer a more bioavailable and stable alternative to traditional inorganic forms. Numerous studies have demonstrated that OTMs enhance mineral absorption, improve growth performance, booster immune responses, and reduce fecal mineral output in broilers [7–13]. Moreover, OTMs support the principles of precision nutrition and sustainable animal production by enabling reduced dietary inclusion levels without compromising productivity [10–12]. Therefore, this study aims to evaluate the effects of trace mineral sources and levels on growth performance, carcass traits, bone characteristics, oxidative stress, and immune response of broiler chicken.

2. Materials and Methods

2.1 Animal Cares

The present experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Mahanakorn University of Technology (ACUC-MUT-2024/009).

2.2 Animals, Diets, and Experimental Design

This study was conducted at Faculty of Veterinary Medicine, Mahanakorn University of Technology. All chicks were raised in 6 x 10 m² evaporative cooling system house. A total of 384 Ross 308, one-day-old broiler chickens, were randomly distributed into twenty-four 1 x 1.2 cm² metal mesh pallet cages with rice husk as litter material cages as completely randomized experimental design (CRD) into 3 treatments with 8 replicates of 16 birds in each. The experimental treatments consisted of Control group: commercial levels of ITMs (ILI; Zn 100 ppm; Cu 15 ppm; Fe 100 ppm; Mn 80 ppm; Se 0.2 ppm; I 3 ppm), replace trace minerals with low levels of OTMs (LLO; Zn 30 ppm; Cu 4 ppm; Fe 11 ppm; Mn 30 ppm; Se 0.225 ppm; I 3 ppm), and low levels of ITMs (LLI; Zn 30 ppm; Cu 4 ppm; Fe 11 ppm; Mn 30 ppm; Se 0.225 ppm; I 3 ppm)

The dietary periods were divided in two phases; starter diet (1-21 days) and grower diet (22-35 days) which are 22% CP; 3,100 Kcal ME/kg and 20% CP; 3,150 Kcal ME/kg, respectively according to the recommendation of NRC (1994) [14]. All diets were pellet feed. All birds were given *ad libitum* access to feed and drinking water. The composition of the basal diets showed in Table 1. Routine medication, vaccination, and husbandry practices were administered. Growth performance data were collected and analyzed for accumulated periods of 1 to 21, 22 to 35, and 1 to 35 days of age. The

body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of all treatments were analyzed.

Table 1. Composition of the basal diets.

Ingredients (%)	Starter (1 – 21 days)	Grower (22 – 35 days)
Corn	48.35	51.87
Soybean meal 44%	32.57	29.30
Full fat soybean	10.00	7.50
Soybean oil	4.80	5.73
Lysine	0.12	0.16
Methionine	0.25	0.31
Limestone	1.44	1.26
Mono-calcium phosphate (MCP)	1.47	1.36
Choline chloride 60%	0.05	0.06
Salt	0.44	0.45
Premix*	0.50	0.50
Calculated nutrient analysis		
Metabolizable energy (ME, kcal/kg)	3,100.00	3,150.00
Crude protein (%)	22.00	20.00
Calcium (%)	0.90	0.80
Total phosphorus (%)	0.70	0.65
Available phosphorus (%)	0.46	0.42
Total Lysine (%)	1.30	1.20
Total Methionine (%)	0.58	0.61

^{*}Composition per kg: Vit A 10,000 IU, Vit D $_3$ 3,000 IU, Vit E 50 IU, Vit K $_3$ 3 mg, Vit B $_1$ 4 mg, Vit B $_2$ 7.5 mg, Niacin (B $_3$) 50 mg, Pantothenic acid (B $_5$) 15 mg, Vit B $_6$ 5 mg, Vit B $_1$ 25 μ g, Folic acid 1 mg, Biotin 100 μ g; Control: Zn 100 ppm, Cu15 ppm, Fe 100 ppm, Mn 80 ppm, Se 0.2 ppm, I 3 ppm; ILI: Zn 30 ppm, Cu4 ppm, Fe 11 ppm, Mn 30 ppm, Se 0.225 ppm, I 3 ppm, Cr 0.15 ppm; LLI: Zn 30 ppm, Fe 11 ppm, Mn 30 ppm, Se 0.225 ppm, I 3 ppm.

2.3 Carcass Characteristics and Meat Quality

At 35 days of age, carcass yield was determined using two birds per replicate pen. Birds were subjected to a 6-hour pre-slaughter fasting period, individually identified, and manually slaughtered. The slaughter process included bleeding, scalding, and feather removal, after which carcasses were stored overnight at 2±2°C. For carcass yield calculation, the cold carcass weight including feet, head, and offal was recorded and expressed as a percentage of the live body weight measured prior to slaughter. Yields of primal cuts (including bone and skin) were calculated based on the live weight and included the entire head and neck, breast, fillet, thigh, drumstick, wing, and ribs. The heart, gizzard, and liver were also weighed and expressed relative to live body weight.

The ultimate pH of the breast muscle was measured 24 hours post-mortem using a pH meter (Eutech Instruments ECPH70042GS), which was calibrated with standard buffers of pH 4.0 and 6.9. Approximately 10 g of ground breast meat was mixed with 100 ml of distilled water, blended at high speed for 30 seconds, and immediately transferred to a 250 ml glass beaker. The electrode was then inserted to record pH. For drip loss, breast muscle samples were weighed, sealed in zip-lock plastic bags, stored at 2±2°C for 24 hours, and reweighed to calculate drip loss as a percentage of initial weight.

2.4 Bone Characteristics

Two samples of the right tibia from each broiler chicken were collected to evaluate fresh weight. The samples were then dried at room temperature for 24 hours and weighed using a digital scale. Bone dimensions were measured using a digital Vernier caliper, including the length from the proximal to the distal end and the width at the narrowest and widest points. Each bone was sectioned longitudinally to measure the diameter of the diaphysis and the medullary canal. The samples were further dried in a hot air oven at 138°C until a constant weight was achieved, then cooled in a desiccator before weighing. Bone ash content was determined by incinerating the samples at 600°C for 4–6 hours, following the AOAC (2012) method [15].

Two samples of the left tibia from each broiler were used to measure bone hardness using a hardness tester (LLOYD Instruments, model LR5K), equipped with a 500 N load cell. The test was performed at a distance of 40 mm from the platform with an indenter speed of 5 mm per minute.

2.5 Determinations of Oxidative Stress and Humeral Immunity

In this study, oxidative stress and humoral immunity in broiler chickens were assessed by measuring superoxide dismutase (SOD) activity, malondialdehyde (MDA) concentration, and total serum IgG antibody levels. SOD activity (expressed as inhibition rate %) was determined using the S311 SOD Assay Kit – WST (Dojindo Laboratories, Tokyo, Japan), while MDA concentration was measured using the M496 MDA Assay Kit (Dojindo Laboratories, Tokyo, Japan). Total IgG levels were quantified using the MBS260043 Chicken IgG ELISA Kit (MyBioSource, Inc., San Diego, USA).

2.6 Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). When a significant treatment effect was observed (P < 0.05), means were separated using Duncan's New Multiple Range Test.

3. Results

3.1. Growth Performance

The effects of trace mineral sources and levels on broiler growth performance are presented in Table 2. During the starter phase (1–21 days), the LLO group exhibited a significantly lower FCR than the ILI and LLI groups (P < 0.05). However, no significant differences (P > 0.05) were observed among treatments in FI, BWG, or FCR over the entire rearing period (1–35 days). Additionally, there were no significant performance differences between the ILI and LLI groups (P > 0.05).

Table 2. Influence of trace mineral sources and levels on growth performance of broiler.

Parameters	Groups			SEM	P-value
	ILI	LLO	LLI		
1-21 days					
Initial weight (g/bird)	48.87	48.87	48.65	0.08	0.50
BWG (g/bird)	963.39	982.63	963.43	6.05	0.34
FI (g/bird)	1,216.20	1,204.37	1,208.24	5.91	0.72
FCR	1.26^a	1.23 ^b	1.26a	0.01	0.02
Mortality (%)	1.56	0.00	0.00	0.36	0.12
22-35 days					
BWG (g/bird)	1,289.65	1,316.58	1,329.03	17.17	0.65
FI (g/bird)	2,191.26	2,228.20	2,157.18	19.62	0.35
FCR	1.71	1.70	1.62	0.02	0.15

Mortality (%)	0.00	0.00	0.00	0.00	1.00
1-35 days					
BWG (g/bird)	2,253.04	2,299.21	2,292.46	19.43	0.60
FI (g/bird)	3,407.46	3,432.56	3,365.42	22.07	0.47
FCR	1.51	1.50	1.47	0.01	0.21
Mortality (%)	1.56	0.00	0.00	0.36	0.12

 $^{^{}a,b}$ Means within the same row with different superscripts are significantly different (P < 0.05). SEM, standard error of the mean; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; ILI, commercial levels of ITMs; LLO, low levels of OTMs; LLI, low levels of ITMs.

3.2 Carcass Characteristics and Meat Quality

As shown in Table 3, fillet and thigh percentages were significantly higher (P < 0.05) in the LLO group compared to the ILI group. However, no significant differences (P > 0.05) were observed among treatments for the head and neck, wing, breast, drumstick, leg and feet, ribs, internal organs, pH, or drip loss.

Table 3. Influence of trace mineral sources and levels on carcass characteristics and meat quality of broiler.

Parameters		Groups		SEM	P-value
	ILI	LLO	LLI		
Live weight (g/bird)	2,577.94	2,595.63	2,619.75	38.81	0.91
Plucked weight (g/bird)	2,322.47	2,337.88	2,357.56	34.79	0.92
Carcass weight (%)	90.07	90.11	89.98	0.15	0.94
Relative weight of pri	mal cut (% of	live weight)			
Head & neck (%)	5.19	5.59	5.56	0.13	0.38
Wing (%)	7.35	7.94	7.68	0.16	0.31
Breast (%)	18.58	19.42	17.71	0.33	0.10
Fillet (%)	3.45b	3.86a	3.52b	0.07	0.03
Thigh (%)	10.52a	11.99 ^b	11.18^{ab}	0.23	0.02
Drumstick (%)	9.05	9.13	9.34	0.10	0.50
Leg & feet (%)	3.51	3.65	3.50	0.05	0.37
Ribs (%)	18.18	16.56	17.70	0.43	0.30
Internal organs (% of	live weight)				
Heart (%)	0.48	0.49	0.49	0.01	0.93
Liver (%)	2.28	2.25	2.34	0.06	0.84
Gizzard (%)	1.44	1.42	1.50	0.04	0.63
Meat quality					
Ultimate pH	5.95	5.96	5.94	0.00	0.94
Drip loss (%)	1.40	1.37	1.45	0.03	0.09

 $^{^{}a,b}$ Means within the same row with different superscripts are significantly different (P < 0.05). SEM, standard error of the mean; ILI, commercial levels of ITMs; LLO, low levels of OTMs; LLI, low levels of ITMs.

3.3 Bone Characteristics

Table 4 showed that tibia length, diameter, weight, and ash content did not differ significantly (P > 0.05) among treatments. However, tibia breaking strength on day 35 was significantly higher (P < 0.05) in the ILI group compared to the LLO and LLI groups.

Table 4. Influence of trace mineral sources and levels on carcass characteristics and meat quality of broiler.

Parameters	Groups			SEM	P-value
	ILI	LLO	LLI		
21 days					
Fresh weight (mg)	5,754.47	6,137.59	6,054.38	113.69	0.37
Ash (%)	41.16	40.28	38.80	0.51	0.16
Length (mm)	76.71	76.45	76.45	0.38	0.96
Weight : length	74.85	80.25	79.16	1.27	0.19
Breaking strength (N)	196.69	197.10	192.82	4.06	0.90
Small outer diameter (mm)	7.03	7.09	7.15	0.07	0.82
Large outer diameter (mm)	19.20	19.84	19.87	0.14	0.10
35 days					
Fresh weight (mg)	13,913.04	14,355.36	13,714.88	368.55	0.78
Ash (%)	39.16	37.02	40.70	0.84	0.21
Length (mm)	101.42	101.87	101.43	1.17	0.99
Weight : length	136.52	139.90	133.98	2.46	0.63
Breaking strength (N)	406.40a	345.01 ^b	342.62 ^b	11.00	0.02
Small outer diameter (mm)	9.03	8.78	8.98	0.11	0.64
Large outer diameter (mm)	26.18	26.15	25.66	0.20	0.49

 $^{^{}a,b}$ Means within the same row with different superscripts are significantly different (P < 0.05). SEM, standard error of the mean; ILI, commercial levels of ITMs; LLO, low levels of OTMs; LLI, low levels of ITMs.

3.4 Oxidative Stress and Immunity

According to Table 5, trace mineral source and level had no significant effect (P > 0.05) on oxidative stress markers (SOD and MDA) or serum IgG antibody levels.

Table 5. Influence of trace mineral sources and levels on lipid oxidation and humeral immunity of broiler.

Parameters		Groups			P-value
	ILI	LLO	LLI	-	
Superoxide dismutase a	activity (SOD in	hibition rate %	(o)		
21 days	65.76	66.00	58.76	0.42	0.61
35 days	61.94	65.62	67.68	0.59	0.99
Malondialdehyde (MD	A, nmol/ml)				
21 days	6.39	6.15	6.34	0.10	0.31
35 days	7.88	7.52	6.77	0.08	0.70
Total IgG antibodie	es				
(ng/mL)					
35 days	103.51	106.40	100.09	1.23	0.91

SEM, standard error of the mean; ILI, commercial levels of ITMs; LLO, low levels of OTMs; LLI, low levels of ITMs.

4. Discussion

4.1 Growth Performance

In a study examining the effects of various trace mineral sources on broiler performance, it was found that during the starter phase (1–21 days), grower phase (22–35 days), and the entire rearing period (1–35 days), using OTMs at levels equivalent to the reduced levels of ITMs commonly used in commercial broiler diets (LLO) did not negatively impact FI, BWG, FCR, or mortality. In fact, broilers fed OTMs at reduced levels during the starter phase exhibited improved FCR. These findings are consistent with those of Nollet et al. [16], who reported that supplementing OTMs at levels lower than current inorganic recommendations did not adversely affect broiler growth performance across all rearing phases and showed a trend toward improved FCR in the early phase (1–14 days) compared to ITMs (P = 0.06). This suggests that OTMs can be effectively used at lower inclusion rates in broiler feed formulations.

The present study also evaluated the effect of reducing ITM levels to those commonly used in commercial diets (LLI). When compared to OTMs at the same reduced level (LLO), broilers receiving OTMs demonstrated better FCR during the early phase, further supporting the findings of Nollet et al. [16]. While OTMs improved early growth efficiency, they did not influence performance during the grower phase or over the entire period, differing from the findings of M'Sadeq et al. [17], who observed improved FCR at the end of the rearing period when using OTMs, even at reduced levels. However, the current findings are in line with those of Franklin et al. [11] and Núñez et al. [18], who reported no significant differences in broiler performance when equal levels of OTMs and ITMs were used throughout the growth cycle.

These inconsistencies suggest that the effects of trace mineral sources on growth performance may vary. This aligns with the conclusion of Świątkiewicz et al. [19], who noted that the response to OTMs in poultry diets is not always consistent, particularly regarding growth performance. Variability may arise from several factors, including the type of trace minerals used (organic vs. inorganic), mineral inclusion levels, feed formulation, and animal-related factors.

Additionally, reducing ITMs to the lower levels typical of commercial diets (LLI) did not negatively affect growth performance at any stage, indicating that current industry recommendations may exceed actual broiler requirements. This is likely due to the inclusion of broad safety margins in commercial formulations. These findings support the possibility of reducing ITM inclusion rates without compromising growth performance, in agreement with Franklin et al. [11], who found that reducing dietary inorganic Zn, Mn, and Cu had no negative effect on broiler performance, as dietary supply remained sufficient. Furthermore, mineral levels in commercial premixes are often much higher than the animals' requirements, leading to excess excretion and environmental pollution [10,12].

4.2. Carcass Characteristics and Meat Quality

In this study, fillet and thigh percentages were significantly higher (P < 0.05) in broilers receiving low levels of OTMs (LLO) compared to those receiving commercial levels of ITMs (ILI), suggesting enhanced muscle development associated with organic mineral supplementation. Organic minerals, often in the form of chelates or proteinates, are bound to organic ligands such as amino acids, peptides, or proteins. This binding alters the physicochemical properties of the minerals, improving their stability and bioavailability within the gastrointestinal tract. The neutral or slightly positive net charge of these complexes reduces antagonistic interactions with other dietary components and prevents the formation of insoluble complexes, which are common with inorganic minerals [1,5]. As a result, OTMs are absorbed more efficiently through specific amino acid or peptide transport systems, bypassing the less selective and competitive pathways used by inorganic minerals [20,21].



The superior bioavailability of OTMs can enhance metabolic functions, including protein synthesis, enzyme activity, and antioxidant defense, all of which are critical for muscle accretion and tissue development [17,23]. Specifically, trace elements such as Zn and Mn play vital roles in protein metabolism, collagen synthesis, and cellular proliferation, which may explain the observed increases in fillet and thigh yields [7,9,24].

Moreover, several studies have demonstrated that reduced inclusion levels of OTMs, compared to traditional inorganic sources, can maintain or even improve performance and carcass traits due to their higher utilization efficiency [10,16]. Additionally, previous studies have demonstrated that organic zinc supplementation does not influence the basic chemical composition or pH of broiler meat [22]. This not only promotes better nutrient conversion into lean tissue but also supports precision nutrition by minimizing excess mineral excretion and reducing environmental impact.

4.3. Bone Characteristics

The current study revealed that most measured bone parameters, including tibia length, diameter, ash content, and weight, were not significantly affected by the different treatments (P > 0.05). These findings align with those of M'Sadeq et al. [17], who reported that reducing OTMs to levels below those recommended for ITMs did not negatively impact tibia size, ash percentage, or breaking strength in 38-day-old broilers. The lack of substantial variation suggests that, even at reduced inclusion levels, trace minerals—whether organic or inorganic may meet the mineral requirements necessary for bone development in modern broiler genotypes [4].

Specifically, using OTMs at levels equivalent to reduced ITM inclusion (LLO) did not affect bone size, ash content, or strength at both 21 and 35 days of age. These results further support the findings of M'Sadeq et al. [17], indicating that OTMs can be used at lower levels without compromising bone development.

The limited impact of trace mineral form and concentration on bone characteristics likely stems from the distinct roles played by macro and trace minerals in skeletal physiology. Calcium (Ca) and phosphorus (P) are the principal mineral components of bone and are required in relatively large amounts to ensure proper mineralization and structural integrity [25–27]. These macro minerals form the hydroxyapatite matrix, the primary structural component of bone. In contrast, trace minerals such as Zn, Mn, and Cu function mainly as enzyme cofactors involved in cartilage formation, collagen synthesis, and bone remodeling, and are therefore required in much smaller quantities [1,24,28].

Interestingly, tibia breaking strength at day 35 was significantly higher in the ILI group compared to the LLO and LLI groups (P < 0.05), suggesting a potential transient benefit of higher ITM supplementation on bone robustness under certain conditions. This may be attributed to greater mineral availability or enhanced enzymatic support during rapid growth. However, the absence of consistent differences across other bone parameters and time points suggests that this effect is likely marginal or context-dependent.

Overall, broilers receiving the low levels of OTMs exhibited bone development comparable to those receiving full or reduced levels of ITMs. This supports the idea that OTMs, due to their superior bioavailability and gastrointestinal stability, can fulfill physiological mineral requirements at lower inclusion levels [16,23]. While macro minerals remain the primary determinants of bone development, trace minerals appear to play a supportive role that can be met even at reduced levels, particularly when supplied in highly bioavailable organic forms. These results reinforce the feasibility of using OTMs at lower inclusion rates without impairing bone integrity, aligning with the principles of precision nutrition and environmentally sustainable feeding practices.

4.4. Oxidation Stress and Immunity

The results indicated that the source and level of trace minerals had no significant effect (P > 0.05) on oxidative stress or immunity. These findings are consistent with those of Vieira et al. [10], who similarly reported no significant differences in MDA concentrations in breast muscle and SOD activity in cardiac tissue of broilers fed varying levels and sources of trace minerals.



Lipid oxidation in muscle tissue, primarily assessed through MDA levels, serves as a key marker of oxidative stress and meat quality deterioration. SOD, an antioxidant enzyme that neutralizes superoxide radicals, is an important indicator of antioxidant defense mechanisms. The lack of variation in these parameters suggests that both OTMs and ITMs, even at reduced inclusion levels, are sufficient to maintain redox homeostasis under standard production conditions. This is particularly relevant because trace minerals such as Zn, Cu, and Mn act as essential cofactors for antioxidant enzymes including SOD and catalase [2,7].

This study also found no significant differences in total IgG antibody levels among dietary treatments (P > 0.05, Table 5). These findings are in agreement with previous studies reporting that replacing ITMs with OTMs maintained an adequate immune response in broilers [17,18,29]. However, Jain et al. [30] observed that supplementation with organic Zn, Se, and Cr enhanced humoral immune response and upregulated chTLR4 gene expression in the bursa and spleen of broilers.

Importantly, this study used dietary trace mineral levels (Cu 4 mg/kg, Mn 30 mg/kg, Zn 30 mg/kg) that were lower than NRC (1994) [14] recommendations (Cu 8 mg/kg, Mn 60 mg/kg, Zn 40 mg/kg), yet no adverse effects on growth performance, carcass traits, oxidative stability, or immunity were observed. These findings support growing evidence that broiler mineral requirements may be overestimated, particularly when highly bioavailable organic forms are used. OTMs exhibit superior stability in the gastrointestinal tract and greater absorption efficiency than their inorganic counterparts [1,5], allowing for reduced dietary inclusion without compromising physiological functions.

Moreover, lowering dietary trace mineral inclusion has important implications for environmental sustainability. Excessive supplementation, especially with inorganic minerals, leads to increased fecal mineral excretion and contributes to soil and water contamination [16,23]. Using organic minerals at lower levels not only maintains broiler performance and meat quality but also reduces environmental risks associated with mineral waste.

5. Conclusions

This study demonstrates that the use of OTMs at levels lower than those of commercial ITMs does not compromise growth performance, carcass traits, bone characteristics, oxidative stress, or immune response in broiler chickens. During the starter phase (1–21 days), OTMs significantly improved FCR compared to both standard and reduced levels of ITMs, indicating superior bioavailability. While carcass traits such as fillet and thigh yields were enhanced with OTMs, no significant differences were observed in bone parameters, oxidative stress markers, or humoral immunity across treatments. Notably, reducing ITMs to levels below current commercial standards also maintained broiler performance, suggesting that existing inclusion rates may exceed actual physiological requirements. These findings support the strategic use of OTMs to promote more sustainable poultry production by improving mineral utilization efficiency and reducing environmental mineral excretion, without compromising bird health or productivity.

Author Contributions: Conceptualization, T.T., R.L. and S.C.; methodology, T.T. and R.L.; software, R.L.; validation, T.T., A.L. and J.M.; formal analysis, R.L. and S.C.; investigation, T.T., R.L. and S.C.; resources, T.T. and R.L.; data curation, T.T. and R.L.; writing—original draft preparation, T.T. and R.L.; writing—review and editing, T.T., R.L., S.C., A.L. and J.M.; visualization, T.T., R.L., S.C., A.L. and J.M.; supervision, T.T. and R.L.; project administration, T.T. and R.L.; funding acquisition, R.L. All authors have read and agreed to the published version of the manuscript.

Funding: The funds for this study were provided by Alltech Biotechnology Co. Ltd. (Thailand) and Mahanakorn University of Technology.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee of Mahanakorn University of Technology (approval no. ACUC-MUT-2024/009).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the corresponding author upon request.

Acknowledgments: The authors wish to acknowledge the personnel involved in this study for their assistance in supporting and conducting various tests. During the preparation of this work, ChatGPT was used to help correct the English. The authors have reviewed and edited the output and take full responsibility for the content of this publication."

Conflicts of Interest: The authors declare no conflicts of interest.

References

- 1. Bao, Y.; Choct, M., Iji, P., Bruerton, K. Trace mineral interactions in broiler chicken diets. *Br Poult Sci* **2010**, 51, 109–117. https://doi.org/10.1080/00071660903571904
- 2. Surai, P.F.; Fisinin, V.I. Selenium in poultry breeder nutrition: An update. *Anim Feed Sci Technol* **2014**, 191, 1–15. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2014.02.005
- 3. Surai, P.F.; Kochish, I.I.; Fisinin, V.I.; Velichko, O.A. Selenium in poultry nutrition: from sodium selenite to organic selenium sources. *J Poult Sci* **2018**, 2, 79–93. https://doi.org/https://doi.org/10.2141/jpsa.0170132
- 4. Güz, B.C.; de Jong, I.C.; Bol, U.E.; Kemp, B.; van Krimpen, M.; Molenaar, R.; van den Brand, H. Effects of organic macro and trace minerals in fast and slower growing broiler breeders' diet on offspring growth performance and tibia characteristics. *Poult Sci* 2022, 101. https://doi.org/10.1016/j.psj.2021.101647
- 5. Byrne, L.; Murphy, R.A. Relative bioavailability of trace minerals in production animal nutrition: A Review, *Animals (Basel)* **2022**, 4;12(15), 1981. https://doi.org/10.3390/ani12151981
- Brugger, D.; Wagner, B.; Windisch, W.M.; Schenkel, H.; Schulz, K.; Südekum, K.H.; Berk, A.; Pieper, R.; Kowalczyk, J.; Spolders, M. Review: Bioavailability of trace elements in farm animals: definition and practical considerations for improved assessment of efficacy and safety, *Animal* 2022, 16(8), 100598. https://doi.org/10.1016/j.animal.2022.100598
- Liu, Z.H.; Lu, L.; Wang, R.L.; Lei, H.L.; Li, S.F.; Zhang, L.Y.; Luo, X.G. Effects of supplemental zinc source and level on antioxidant ability and fat metabolism-related enzymes of broilers1. *Poult Sci* 2015, 94, 2686– 2694. https://doi.org/10.3382/ps/pev251
- 8. Alharbi, K.; Asnayanti, A.; Do, A.D.T.; Perera, R.; Al-Mitib, L.; Shwani, A.; Rebollo, M.A.; Kidd, M.T.; Alrubaye, A.A.K. Identifying dietary timing of organic trace minerals to reduce the incidence of osteomyelitis lameness in broiler chickens using the aerosol transmission model. *Animals* **2024**, 14. https://doi.org/10.3390/ani14111526
- 9. Huang, Y.L.; Lu, L.; Luo, X.G.; Liu, B. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. *Poult Sci* **2007**, 86, 2582–2589. https://doi.org/10.3382/ps.2007-00088
- 10. Vieira, R.; Ferket, P.; Malheiros, R.; Hannas, M.; Crivellari, R.; Moraes, V.; Elliott, S. Feeding low dietary levels of organic trace minerals improves broiler performance and reduces excretion of minerals in litter. *Br Poult Sci* **2020**, 61, 574–582. https://doi.org/10.1080/00071668.2020.1764908
- 11. Franklin, S.B.; Young, M.B.; Ciacciariello, M. The impact of different sources of zinc, manganese, and copper on broiler performance and excreta output. *Animals* **2022**, 12. https://doi.org/10.3390/ani12091067
- 12. Ribeiro, M.V.; Bittencourt, L.C.; Hermes, R.G.; Rönnau, M.; Rorig, A.I.; Lima, F.K.; Fernandes, J.I.M. Mineral source and vitamin level in broiler diets: Effects on performance, yield, and meat quality. *Braz J Poult Sci* **2020**, 22, 1–14. https://doi.org/10.1590/1806-9061-2017-0718
- 13. Mishra, A.; Caesar, D.D.; Pradesh Pashu-Chikitsa, M.; Vishwavidyalaya, V.; Jain, A.K.; Kumar Jain, A.; Shakkarpude, J.; Mourya, A.; Baghel, R.; Sharma, R.K. Can different concentration of chelated and inorganic trace minerals (Zn, Se and Cr) be an effective supplement for better production performance and carcass traits in broilers? *J Entomol Zool Stud* 2020, 8, 197–204.
- 14. NRC. Nutrient Requirement of Poultry. National Academies Press, Washington, DC, 1994.
- 15. AOAC. Official Methods of Analysis. 20th edition. Association of Official Analytic Chemists. Washington, DC, 2012.



- 16. Nollet, L.; Van Der Klis, J.D.; Lensing, M.; Spring, P. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. *J Appl Poultry Res* **2007**, 16, 592–597. https://doi.org/10.3382/japr.2006-00115
- 17. M'Sadeq, S.A.; Wu, S.B.; Choct, M.; Swick, R.A. Influence of trace mineral sources on broiler performance, lymphoid organ weights, apparent digestibility, and bone mineralization. *Poult Sci* **2018**, 97, 3176–3182. https://doi.org/10.3382/ps/pey197
- 18. Núñez, R.; Elliott, S.; Riboty, R. The effect of dietary supplementation of organic trace minerals on performance, mineral retention, lymphoid organs and antibody titres of broilers. *J Appl Anim Nutr* **2023**, 11, 23–32. https://doi.org/10.3920/JAAN2022.0002
- 19. Świaętkiewicz, S.; Arczewska-WŁosek, A.; Józefiak, D. The efficacy of organic minerals in poultry nutrition: Review and implications of recent studies. *World Poult Sci J* **2014**, 70(3), 475-486. https://doi.org/10.1017/S0043933914000531
- 20. Ashmead, H. The absorption and metabolism iron amino acid chelate. Arch Latinoam Nutr 2001, 51, 13–21.
- 21. Wedekind, K.J.; Hortin, A.E.; Baker, D.H.; Wedekind, K.J.; Baker, D. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. *J Anim Sci* **1992**, 70(1), 178-87. https://doi: 10.2527/1992.701178x
- Winiarska-Mieczan, A.; Kwiecień, M.; Purwin, C.; Jachimowicz-Rogowska, K.; Borsuk-Stanulewicz, M.;
 Pogorzelska-Przybyłek, P.; Kiczorowska, B. Fatty acid profile and dietary value of thigh meat of broiler chickens receiving mineral or organic forms of Zn. *Animals* 2024, 14. https://doi.org/10.3390/ani14081156
- 23. Leeson, S.; Summers, J.D. Nutrition of the Chicken. University Books, 2001.
- 24. Underwood, E.J.; Suttle, N.F. The Mineral Nutrition of Livestock. CABI Pub, 1999.
- 25. Perry, T.W.; Cullison, A.E.; Lowrey, R.S. Feeds & Feeding. Prentice Hall, 2003.
- 26. Church, D.C.; Pond, W.G. Basic Animal Nutrition and Feeding. Wiley, 1988.
- 27. van der Eijk, J.A.J.; Bakker, J.; Güz, B.C.; van Krimpen, M.M.; Molenaar, R.; van den Brand, H.; de Jong, I.C. Providing organic macro minerals and an elevated platform improved tibia characteristics, and increased locomotion and performance of fast- and slower-growing broilers. *Poult Sci* **2022**, 101. https://doi.org/10.1016/j.psj.2022.101973
- 28. Frandson, R.D.; Wilke, W.L.; Fails, A.D. Anatomy and Physiology of Farm Animals. Wiley-Blackwell, 2016.
- 29. Azad, S.K.; Shariatmadari, F.; Torshizi, M.A.K.; Chiba, L. Comparative effect of zinc concentration and sources on growth performance, accumulation in tissues, tibia status, mineral excretion and immunity of broiler chickens. *Braz J Poult Sci* **2020**, 22, 1–10. https://doi.org/10.1590/1806-9061-2019-1245
- 30. Jain, A.K.; Mishra, A.; Singh, A.P.; Patel, P.; Sheikh, A.A.; Chandraker, T.R.; Vandre, R. Effects of different concentration of organic and inorganic trace minerals (zinc, selenium, and chromium) supplementation on expression of chTLR4 gene and humoral immune response in broilers. *Vet World* **2021**, 14, 1093–1101. https://doi.org/10.14202/vetworld.2021.1093-1101

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.