

Effect of inclusion of zinc-glycine chelate and zinc sulphate on live performance, immunity and lipid peroxidation in broilers

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ABSTRACT

Zinc (Zn) is a biologically significant trace mineral, serving as a cofactor in various metabolic functions. This study aimed to assess the relative effectiveness of organic and inorganic Zn on the growth performance, lipid peroxidation and humoral immune response in broiler chickens (Ross 308). A total of 450 d-old broilers were divided into five groups: the control group, a group receiving a basal diet with 50 mg/kg organic Zn (T1), another with 60 mg/kg organic Zn (T2), and two groups with basal diets supplemented with inorganic Zn at rates of 50 and 60 mg/kg zinc sulfate for the fourth (T3) and fifth (T4) groups, respectively. The experiment spanned 35 days. Results indicated that zootechnical characteristics such as weekly weight gain, feed intake, feed conversion ratio and dressing percentage were significantly higher ($P < 0.05$) in T1 compared to both the control and the inorganic Zn supplementation groups. Additionally, the antibody titre against Newcastle disease was significantly ($P < 0.05$) elevated, while malondialdehyde (MDA) levels were significantly ($P < 0.05$) lower in T1 and T2 compared to the control and the inorganic Zn supplementation groups. In conclusion, broilers supplemented with 50 mg/kg organic Zn exhibited significantly improved growth performance, reduced MDA levels, and enhanced humoral immune response.

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Introduction

In the realm of poultry nutrition, various feed additives are employed to enhance growth performance and overall health, as highlighted in studies by Ahmad et al. (2022), Mehmood et al. (2023), and Maqbool et al. (2023). Zinc (Zn) holds significance as a vital trace element, acting as a cofactor in essential physiological processes such as carbohydrate and energy metabolism, as well as protein synthesis (Star et al. 2012). Historical research by Roberson and Schaible (1958) initially recommended a Zn requirement of 30 mg/kg for chicks, a guideline later revised by the National Research Council (1994) to 40 mg/kg of feed. Salim et al. (2008) further demonstrated that Zn supplementation within the range of 30–50 mg/kg of feed positively influenced feed intake and efficiency in poultry.

The prolonged use of zinc (Zn) has demonstrated adverse effects on various bodily systems, including the respiratory, digestive, neural, and reproductive systems, as indicated by studies conducted by Chen et al. (2018) and Min et al. (2019). In the context of broiler nutrition, Zn supplementation typically involves the use of inorganic forms such as zinc sulfate, zinc oxide, and zinc chloride, as highlighted by Khan et al. (2023). However, recent findings suggest that the incorporation of organic forms of Zn, combined with amino acids, can significantly enhance Zn bioavailability, as reported in studies by

Salim et al. (2008), Star et al. (2012), Chen et al. (2018), and Khan et al. (2024).

Recently, there has been a trend among poultry producers to increase the levels of trace minerals, particularly organic zinc (Zn), to enhance the intestinal health of birds. This shift is attributed to the superior effectiveness of organic Zn in improving various aspects of broiler performance, such as intestinal histology, growth, antioxidant status and immune response as noted by Chand et al. (2020).

The literature on Zn supplementation presents conflicting reports, with some studies, such as those by Jahanian and Rasouli (2015), suggesting improved bioavailability of Zn in its organic form. However, others, including Cao et al. (2000, 2002), Huang et al. (2013), and Liu et al. (2013), found no significant difference in blood Zn concentration between organic and inorganic sources. These discrepancies are attributed to variations in the type and quality of organic Zn, as noted by Saeeda et al. (2023). Organic forms of Zn demonstrate greater relative bioavailability in comparison to inorganic sources, attributed to their distinctive absorption capacity and minimized interaction with other dietary components (Chand et al. 2020). In contrast, inorganic zinc forms stable and highly soluble compounds by binding with organic molecules, resulting in increased bioavailability (Chand et al. 2020). While most studies have focused on organic Zn combined with

methionine (Zn-Met), there is a noticeable scarcity of research on the effects of Zn-glycine (Zn-Gly) in broilers. Consequently, our study is designed to investigate and compare the impact of inorganic and organic Zn on growth performance, humoral immunity, and lipid peroxidation in broilers.

Materials and methods

All procedures in the experiment were prior approved by the local Committee on Ethics and Animal Welfare.

Birds husbandry and experimental design

In this experimental setup, 450 one-day-old broiler chicks of the Ross 308 breed were randomly assigned to five groups, each with five replicates. The control group received no supplementation, while the second and third groups were supplemented with organic Zn in the form of Zn-glycine at rates of 50 mg/kg (T1) and 60 mg/kg (T2), respectively. The fourth and fifth groups were supplemented with 50 and 60 mg/kg of zinc sulfate, denoted as T4 and T5, respectively.

During the initial week, considered as an adaptation period and had unrestricted access to feed and water. The feed composition adhered to the recommendations outlined by the NRC (1994), as detailed in Table 1. Temperature management involved maintaining 100°F for the first week, gradually reducing to 81°F by day 21. Continuous 24-hour lighting was avoided in accordance with welfare standards. Routine vaccinations were administered to protect the birds against infectious diseases.

Birds performance

Daily feed intake was recorded, and the chicks were initially weighed on the first day, followed by weekly assessments for weight gain calculation. Weight gain was calculated by subtracting the birds' initial weight from their final weight, while the feed conversion ratio (FCR) was derived by dividing the amount of consumed feed by the weight gained. On the 35th day of the experiment, five birds per replicate were

selected, weighed, slaughtered, and their internal organs were extracted and reweighed to determine the dressing percentage.

Blood collection

On the 35th day, a 2 ml blood sample was collected from the wing vein of two birds from each replicate. To obtain serum, the blood was centrifuged at 2500 rpm for 8 min. and stored at -20°C until further analysis.

Determination of Malondialdehyde (MDA; n Mol/ml)

To determine Malondialdehyde (MDA), the method outlined by Ohkawa et al. (1979) was employed. In brief, serum was combined with acetic acid (CH₃COOH 20%), thiobarbituric acid (0.8%), and sodium dodecyl sulfate (8.1%). The mixture was then heated on a water bath to 97°C for an hour and subsequently blended with n-butanol (1:15, v/v) and pyridine. Following this, the mixture underwent centrifugation at 3000 rpm for ten minutes. The upper organic layer was separated, and absorbance was measured at 352 nm using a spectrophotometer (IRMECO Model U2020).

Humoral immunity

Antibody titre against Newcastle disease (ND) virus was determined as described by Imtiaz et al. (2023).

Statistical analysis

The acquired data underwent statistical analysis utilizing the Complete Randomized Design. To discern mean differences, the Least Significant Difference Test at a 5% probability level was applied, following the methodology outlined by Steel and Torrie (1980). The statistical package Statistics 8.1 was employed for computer-based analysis.

Results

Table 2 presents the feed intake results for broiler chicks fed with various levels of organic and inorganic Zn. Weekly mean feed intake was significantly ($P < 0.05$) higher in T1 compared to the control and other treatments. Feed intake in T2 and T3 were similar for week 2 and 4. In the 5th week, T3 had higher values than T2. The lowest feed intake is shown for the control group. The effect of the supplementation of organic

Table 1. Diet composition and analysis of broilers.

Ingredients (%)	Starter (1–14 days)	Grower (15–22 days)	Finisher (22–35 days)
Yellow corn	57.5	58.0	59.0
Soybean (46%)	21.4	15.4	9.0
Canola meal	6.10	6.10	5.10
Vit.Premix	0.50	0.50	0.50
Vegetable oil	1.35	1.75	1.45
Limestone	0.54	0.50	0.50
sodium chloride	0.23	0.20	0.20
Na ₂ Co ₃	0.11	0.16	0.12
DL-Methionine	0.14	0.12	0.13
Analysis (%)			
Metabolizable energy (kcal/kg)	2990	3000	3120
Crude protein (%)	23.6	21.2	19.18
Fat (%)	4.71	5.45	5.83
Fiber (%)	3.61	3.64	3.55
Ca (%)	1.00	0.91	0.85
P (%)	0.75	0.66	0.62
Na, (%)	0.20	0.19	0.18
Lys (%)	1.34	1.16	1.09
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(NRC 1994).

Table 2. Mean \pm SE weekly feed consumption (g) in response to two forms of zinc in broiler.

Groups	Week 2	Week 3	Week 4	Week 5
Control	311.11 ^d \pm 2.15	564.34 ^e \pm 1.23	715.11 ^d \pm 2.85	912.31 ^e \pm 2.45
T1	342.13 ^a \pm 1.66	641.31 ^a \pm 1.48	781.33 ^a \pm 2.78	991.64 ^a \pm 1.66
T2	336.73 ^b \pm 1.33	637.66 ^b \pm 1.88	775.11 ^b \pm 2.67	978.36 ^c \pm 1.33
T3	335.57 ^b \pm 1.88	633.01 ^c \pm 2.33	771.27 ^b \pm 2.46	982.65 ^b \pm 1.66
T4	331.23 ^c \pm 1.88	618.68 ^d \pm 2.58	763.37 ^c \pm 2.25	946.32 ^d \pm 1.76

Different values in a column with same superscripts do not differ significantly ($P < 0.05$).

T1 and T2: organic Zn at the rate of 50 and 60 mg/kg; T3 and T4: inorganic Zn at the rate of 50 and 60 mg/kg.

Table 3. Mean \pm SE weekly weight gain (g) in response to two forms of zinc in broiler.

Groups	Week 2	Week 3	Week 4	Week 5
Control	226.33 ^d \pm 2.43	278.67 ^e \pm 1.18	351.00 ^e \pm 1.74	388.00 ^e \pm 1.16
T1	284.67 ^a \pm 1.84	368.33 ^a \pm 1.66	465.67 ^a \pm 1.21	498.33 ^a \pm 1.34
T2	274.33 ^b \pm 2.86	362.33 ^b \pm 1.88	451.33 ^b \pm 1.21	465.67 ^c \pm 1.35
T3	272.67 ^{bc} \pm 2.18	346.00 ^d \pm 1.15	434.00 ^c \pm 4.42	468.00 ^b \pm 1.56
T4	267.33 ^c \pm 2.41	352.33 ^c \pm 0.83	425.00 ^d \pm 1.53	447.50 ^d \pm 1.75

Different values in a column with same superscripts do not differ significantly ($P < 0.05$).

T1 and T2: organic Zn at the rate of 50 and 60 mg/kg; T3 and T4: inorganic Zn at the rate of 50 and 60 mg/kg.

Table 4. Mean \pm SE weekly feed conversion ratio in response to two forms of zinc in broiler.

Groups	Week 2	Week 3	Week 4	Week 5
Control	1.38 ^a \pm 0.21	2.00 ^a \pm 0.18	2.33 ^a \pm 0.13	2.36 ^a \pm 0.13
T1	1.21 ^c \pm 0.13	1.75 ^c \pm 0.14	1.66 ^c \pm 0.16	1.97 ^d \pm 0.11
T2	1.24 ^{bc} \pm 0.13	1.75 ^c \pm 0.13	1.71 ^c \pm 0.14	2.11 ^c \pm 0.11
T3	1.25 ^b \pm 0.11	1.82 ^b \pm 0.11	1.75 ^b \pm 0.11	2.11 ^c \pm 0.11
T4	1.26 ^b \pm 0.11	1.77 ^c \pm 0.18	1.78 ^b \pm 0.11	2.12 ^c \pm 0.11

Different values in a column with same superscripts do not differ significantly ($P < 0.05$).

T1 and T2: organic Zn at the rate of 50 and 60 mg/kg; T3 and T4: inorganic Zn at the rate of 50 and 60 mg/kg.

and inorganic Zn on body weight gain is given in Table 3. The results showed that weekly body weight was significantly ($P < 0.05$) higher in T1 compared to all other treatments. Like feed intake, body weight was also superior in birds treated with organic Zn compared to the inorganic Zn on weekly basis. The effect of the supplementation of Zn in organic and inorganic form on the FCR is presented in Table 4. The results revealed that FCR was the best in T1 in every week compared to the control and other treatments. The FCR was significantly ($P < 0.5$) higher in organic Zn supplemented compared to the inorganic Zn. Similarly, the dressing percentage was also significantly ($P < 0.05$) higher in T1 compared to the other groups (Table 5).

The effect of dietary treatments on antibody titre against ND and MDA is given in Table 5. The mean antibody titre was significantly ($P < 0.05$) higher in T1 followed by T2. No significant change was observed in T3 and T4. The MDA level was significantly ($P < 0.05$) lower in T1 and T2 compared to the other groups. The MDA level did not change significantly ($P < 0.05$) between T3 and T4. The MDA level also reduced significantly ($P < 0.05$) in organic Zn compared to inorganic Zn.

Table 5. Mean \pm SE dressing percentage, antibody titre against New Castle Disease virus and melanodialdehyde (MDA) in broiler chicks fed with two forms of zinc.

Group	Dressing percentage	Mean Antibody titre	MDA (nmole/mL)
Control	61.15 ^e \pm 1.45	4.92 ^d \pm 1.08	2.82 ^a \pm 0.13
T1	68.56 ^a \pm 1.06	6.87 ^a \pm 1.14	2.15 ^d \pm 0.12
T2	67.77 ^b \pm 1.15	6.52 ^b \pm 1.08	2.32 ^c \pm 0.11
T3	66.41 ^c \pm 1.31	6.11 ^c \pm 1.05	2.62 ^b \pm 0.09
T4	65.24 ^d \pm 1.34	5.92 ^c \pm 1.05	2.55 ^b \pm 0.08

Different values in a column with same superscripts do not differ significantly ($P < 0.05$).

T1: organic Zn at the rate of 50 mg/kg; T2: organic Zn at the rate of 60 mg/kg; T3: inorganic Zn at the rate of 50 mg/kg; T4: inorganic Zn at the rate of 60 mg/kg.

Discussion

In this study, the mean values of growth performance indicators in broilers demonstrated a significant enhancement with organic Zn compared to the inorganic counterpart. Moreover, the superiority of the organic Zn's effect was pronounced. The findings suggest a clear influence of Zn dosage and its source on broiler production performance. In recent years, the prevalence of utilizing Zn from organic sources has increased due to its higher bioavailability, as noted by Star et al. (2012). Zinc plays a vital role in avian digestive functions by facilitating the secretion of digestive enzymes, leading to improved nutrient digestibility and overall growth performance in birds (Chand et al. 2020).

Previous reports have indicated that organic Zn not only produces better skin quality in broilers but also has a subtle impact on overall production (Salim et al. 2012; Star et al. 2012; Yalçinkaya et al. 2012). The observed higher body weight, feed intake, and feed efficiency in our study may be attributed to increased secretion of digestive enzymes in response to Zn supplementation (Naz et al. 2016) and the enhanced bioavailability of Zn. The recognized superior bioavailability of organic or chelated forms of Zn, as compared to inorganic sources, is supported by their unique absorption process and limited interaction with other dietary components. Inorganic zinc tends to form stable compounds by binding with organic molecules, showcasing high solubility (Israr et al. 2021). It has been hypothesized that chelated minerals, particularly, exhibit enhanced absorption through the organic ligand pathway. Furthermore, improved villus dimensions, has been associated with organic and inorganic sources of Zn in broilers (Hu et al. 2013; Levkut et al. 2017; Shah et al. 2019), suggest a potential association between the enhanced growth performance and improved histomorphology of the intestines.

The current study reveals that supplementing both organic and inorganic Zn to the broiler diet leads to a reduction in the mean blood serum Malondialdehyde (MDA) concentration, with organic Zn showing a superior effect compared to its inorganic counterpart. Zinc plays a vital role in the antioxidant defense system by inhibiting the activity of free radicals Top of Form.

Additionally, it acts to prevent the formation of free radicals by other elements (Ebuehi and Akande 2008). The stabilizing effect of Zinc on lipid membranes and its ability to prevent lipid peroxidation by free radicals have been noted in previous studies (Naz et al. 2016). Zinc is an integral component of the avian antioxidant system. In normal conditions, the body produces a high level of reactive oxygen species, leading to chronic diseases and DNA damage. Antioxidant substances, including Zinc, play a vital role in reducing lipid peroxidation in the body (Khan 2011). Moreover, Zinc is a crucial component of important antioxidant enzymes, which defends the body against free radicals. The antioxidant effect of Zinc is thought to be exerted through the antagonism of redox-active transition metals or indirectly through the induction of other substances such as cysteine-rich proteins that act as antioxidants (Naz et al. 2016).

Our study uncovered that the supplementation of both organic and inorganic Zn led to an improvement in antibody

titres against the Newcastle Disease (ND) virus. Notably, the highest antibody titre was observed with organic Zn supplementation. Recent findings by Jarosz et al. (2017) align with our results, indicating that organic Zn enhances the expression of immunoglobulins (IgA and IgG) and stimulates the immune response, particularly in passive protection. Prior research has also highlighted an enhanced humoral immunity response under lower dietary Zn supplementation (Sunder et al. 2008). The postulation that Zn activation contributes to antibody production and increased engulfment of foreign materials has been suggested in previous studies (Khan et al. 2011).

Conclusion

The result of the present study revealed that supplementation of organic Zn at the level of 50 mg/kg improved the growth, antioxidant defense and humoral immunity in broiler.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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