



Comparative Evaluation of Organic and Inorganic Zinc Supplementation on Biochemical and Hematological Profiles in Broiler Chickens

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Abstract Zinc is important for poultry metabolism, immunity, and growth at trace levels. The purpose of this study was to assess the effect of organic and inorganic zinc supplementation in poultry's diet on biochemical and hematological parameters. Thus, 300 one-day Ross 308 chicks were used in the experiment. The chicks were assigned in groups of one hundred for each treatment. The control treatment received no zinc supplementation and the first treatment (T1) received 250 mg with organic zinc acetate and the second treatment (T2) received 250 mg of inorganic zinc oxide. The experiment lasted 6 weeks with controlled conditions with food and water provided ad libitum. On the 42nd day of the experiment, blood samples were taken and analyzed for glucose (G), total proteins (Tp), triglycerides (Tg), cholesterol (C), uric acid (UA), creatinine (C), liver enzymes (ALT, AST, ALP), and hematological parameters (RBC, WBC, Hb, PCV). With the help of one-way and two-way ANOVA and Duncan's test, it was established that organic zinc supplementation improved the total proteins, ALP, RBC, and Hb count while also effectively controlling blood glucose, cholesterol, triglycerides, and uric acid levels relative to control. Inorganic zinc produced moderate improvement in these indices. The biochemical stability and organic zinc group's clear and controlled results showed a 12–15% enhancement and a 10–14% improvement in the organic zinc group compared to the control group. Results further confirm the excellent physiological effectiveness and bioavailability of organic zinc, justifying its addition to commercial broiler diets to improve health and productivity.

Keywords: Biochemical, Broiler, Hematological, Organic zinc, Supplementation

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Introduction The primary focus of modern broiler production is to create affordable and innovative techniques that facilitate the chickens' development while enhancing their health. The importance of zinc in the functioning and the integrity of tissue of enzymes and other biological antioxidants is highlighted by numerous researchers (1-6). The Zinc status conditioning of immune readiness and lipid metabolism is also crucial. Organic zinc reduced malondialdehyde and increased the antioxidant status in broilers indicating that oxidative damage was minimized (1,5). Zinc glycinate chelate improved and growth and modulated gut microbes and gut barrier integrity that supports host defense (6). Organic zinc added to the diet of broilers lead to relatively greater body-weight gain and feed efficiency compared to those that used inorganic sources at the same inclusion levels (7). Combinations with other trace elements also optimize glucose use and protein synthesis critical for carcass yield and resiliency (5). Stress of both the

environment and management amplify the requirement for effectively utilizable minerals. Under heat stress conditions, zinc supplementation preserved gut morphology, reduced inflammation, and improved overall performance (8). The supporting of low crude protein diet growth with appropriate zinc sources were carried out without adequate protein and let to favorable blood profile, indicating staged mineral form can alleviate restrictive diet (9). Therefore, targeted, formulated diets containing bioavailable zinc can significantly decrease the levels of crude protein and the amount of nitrogen excreted, without the cost of animal welfare and environmental sustainability (1,8,9). Hematological and biochemical markers provide objective and reliable assessments to gauge the effectiveness of zinc. In controlled trials, nano-zinc and chelated sources showed an increase in the parameters RBC and WBC and Hb and improved markers of liver health as compared to inorganic salts (3,10). Such



alterations are in concert with improved serum antioxidant status and improved nutrient utilization (1,3,6). Collectively, the results justify the use of organically complexed or more sophisticated forms of zinc when the aim is to improve the structure of the gastrointestinal tract, the immune system, and the overall physiology of the body while maintaining optimum growth (1–10).

Materials and methods

Ethical approval

The current study procedures were approved by the College of Veterinary Medicine, University of AlQadisiyah (proposal Ref.No.1278 Date:9/8/2022)

Experimental Design

The subjects of the research were three hundred one-day-old Ross 308 broiler chicks. Immediately, brooder chicks were weighed then randomly allocated into three groups (100 each). Each group underwent the same procedures for six weeks, with Group 1 serving as the control. This group was fed a predetermined basal diet, while Group 2 (T1) received 250 mg of organic zinc acetate tiamnekozit, and Group 3 (T2) received 250 mg of inorganic zinc oxide. All the chicks were housed in a fully controlled environment with the same light, temperature, and ventilation. Throughout the experiment, all birds were fed and watered without limitations. All subjects endured two separate feeding stages: the starter phase (day 1–21) and the finisher phase (day 22–42). All the diets were made and sold according to the nutrient recommendations set forth by the NRC (1994). Furthermore, to ensure local and practical field relevance, all necessary local market feed ingredients were procured. To eliminate germs, the poultry hall was cleaned and disinfected with commercial grade disinfectants prior to the baby chicks arriving. Each day, waterers and feeders were cleaned. Proper bedding with dry sawdust was used. Ventilation was controlled to ammonia levels as well. All the birds were watched for strange behavior and sudden increases in feed intake and sudden death. The guideline of Aviagen (2018) was utilized to establish the management of the flocks' vaccination routine and vaccination policy. During the first week the average temperature was kept at 32 °C and then gradually decreased to 24 °C. The lighting schedule was organized so that it reduced the stress of the birds and promoted normal feeding behavior. Such operational factors ensured that stable experimental conditions were achieved and stress conditions of the environment were to be minimal.

Sample Collection and Hematological Analysis

At day 42, blood collections were conducted via blood sampling by wing vein puncture on 25 birds per group. Plain tubes were used for serum biochemistry while hematology relied on EDTA tubes. The samples were

subjected to a 15-minute centrifugation cycle at 5000rpm. The serum was stored at –18 degrees Celsius pending subsequent testing. The fully blood counted parameters were the red blood cells (RBCs), the white blood cells (WBCs), the total blood hemoglobin, and the pack cell volume (PCV). The Natt and Herrick diluent was used to determine the concentration and then counted to measure both the red blood cells (RBCs) and white blood cells (WBCs) (1952). The hemoglobin was determined as cyanmethemoglobin at 540 nm using Drabkin' spear and a hemoglobin filter. The pack cell volume was determined using the microhematocrit centrifugation (Archer, 1965). The values were determined by standard calculations and expressed as units. The collection procedure was done in a likeness to reduce enzymatic degradation and all tests were conducted in pairs for reliability. The monitored parameters show the effects of zinc on erythropoiesis, immune status and blood physiology as a whole.

Biochemical and Enzymatic Analysis

The biochemical analyses of the serum focused on determining the concentration of glucose, total protein, triglycerides, uric acid, creatinine, cholesterol, and liver enzymes such as ALT, AST, and ALP. The guidelines provided by the manufacturers were followed as commercial diagnostic kits provided by the Bio Research Company (Baghdad, Iraq) were utilized. Uric acid levels were determined by the Jaffe method as described by Henry et al. (1982). The determination of the total concentration of cholesterol described by Franey & Elias (1968) and other authors of the accepted procedures followed a colorimetric method with the use of ferric chloride and sulfuric acid and the resulting optical density was measured on a spectrophotometer at 560 nm. Total proteins were determined by the biuret method and glucose and triglycerides were determined enzymatically. Creative levels were determined by the Jaffe reaction. The biochemical tests were executed in duplicates, and the biochemical tests were controlled with internal quality standards. Each biochemical test was equipped with a standard curve for calibration as well as internal quality standards. These tests served as primary indicators for oxidative stress balance, kidney and liver function and for specific metabolic status markers which provided information on the physiological impact of zinc supplementation.

Statistical Methods

The GLM procedure in SPSS v 22 was used for one way ANOVA analyses. Duncan's test was used to compute means to groups up to $p \leq 0.05$. The means along with Smith's error of the mean are reported. The data was checked for normal distribution prior to testing. To evaluate systemic responses to zinc, biochemical and hematological parameters were correlated. This analysis

quantified the differences in treatment and physiological responses to zinc. It provided a relative organic and inorganic source comparison. The mean percentage improvement of critical profiles was used to gauge

Results

Biochemical Parameters

With respect to organic and inorganic zinc supplementation, changes in the biochemical indices of the chickens was evident in the last stage of the 6 week trial. The birds in the organic zinc group were able to attain higher serum total protein values and lower glucose, triglycerides and cholesterol levels as compared to the control group (T1) ($P \leq 0.05$). The lower cholesterol and triglyceride levels points towards enhanced lipid metabolism and liver function. Metabolic stress in the control group was evident as they had the highest glucose and cholesterol levels (220.86 ± 0.15 mg/dL) and (114.64 ± 0.28 mg/dL) respectively. Birds exposed to the organic zinc, on the other hand, managed to lower their levels of glucose (213.82 ± 0.16 mg/dL) and cholesterol (97.18 ± 0.17 mg/dL). The ability of organic zinc to balance lipid and carbohydrate pathways were clearly more effective. Inorganic zinc also aided in the improvement of these parameters, but to a lesser degree, confirming lower than expected bioavailability. The protein and uric acid values suggest that the zinc fed groups were able to better retain nitrogen in the body and reduced the rate of protein catabolism. Among the groups, organic zinc carried the highest protein levels (4.84 ± 0.02 g/dL) while uric acid levels were lower (3.92 ± 0.06 mg/dL) indicative of more effective protein sparing. There was also a minor decline in creatinine levels which was indicative of normal kidney function. The most biochemically balanced profile was recorded from the group fed organic zinc. The group fed inorganic zinc continued to display intermediate levels. In any case, the improvement in the biochemical traits seems to indicate that organic zinc is metabolically more efficient as a result of being more readily absorbed

biological effectiveness. All data were run for multiple iterations for coherent results within the physiological and nutritional framework.

and exercising greater antioxidant action (Figure 1 and Table 1).

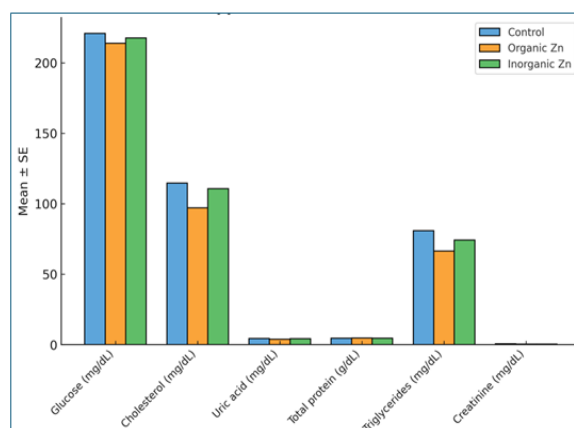


Figure 1: The figure evaluates biochemical indices of glucose, cholesterol, uric acid, total protein, triglycerides, and creatinine in the control, organic, and inorganic zinc groups of broiler chickens. For each parameter, the bar shows the mean value \pm standard error on day 42 of the trial.

Table 1: Effect of organic and inorganic zinc supplementation on biochemical parameters of broiler chickens at day 42. This table shows the mean \pm SE values of glucose, cholesterol, uric acid, total protein, triglycerides, and creatinine across the groups. Organic zinc supplementation significantly ($p \leq 0.05$) enhanced protein and lipid metabolism and attenuation of glucose and uric acid relative to control.

Parameter (unit)	Control	Organic Zn (T1)	Inorganic Zn (T2)	\pm SE
Glucose (mg/dL)	220.86	213.82	217.66	± 0.15
Cholesterol (mg/dL)	114.64	97.18	110.62	± 0.16
Uric acid (mg/dL)	4.48	3.92	4.18	± 0.03
Total protein (g/dL)	4.50	4.84	4.62	± 0.02
Triglycerides (mg/dL)	80.84	66.44	74.16	± 0.09
Creatinine (mg/dL)	0.52	0.44	0.50	± 0.004

Liver Enzyme Activity

per the study. While the serum ALT and AST levels were markedly higher in the control group (23.66 ± 0.09 U/L and 4.48 ± 0.03 U/L, respectively), control group still demonstrated mild liver strain. Birds on

Zinc supplementation observed during the study used more zinc which is protective liver enzyme balance organic zinc showed the lowest ALT (21.44 ± 0.08 U/L) and AST (3.92 ± 0.06 U/L) levels which entirely demonstrates diminished liver stress and improved metabolic equilibrium. The group fed on inorganic zinc

(T2) showed intermediate values, suggesting some degree of protection. The higher ALP value in the organic zinc-fed birds, which is 24.34 ± 0.39 U/L, indicated a higher level of ALP associated with stronger bone mineralization and greater enzyme activity because of zinc which serves as a cofactor. The data highlights the protective factors correct methods of organic zinc use approximates towards liver balance enzymatically which is zinc's. The protective ALT and AST levels confirm less leakage of hepatic cells and optimal ALP activity correspond more tissue metabolism bone and enzyme. All the differences in the chest concerning the organic zinc values remained significantly different ($p \leq 0.05$). Results and discussions other biochemical evidence suggest that more organic zinc is most efficient and bio-available, protective from metabolic strain organ, and unbalanced enzymatically is also sustained (figure 2, and Table 2).

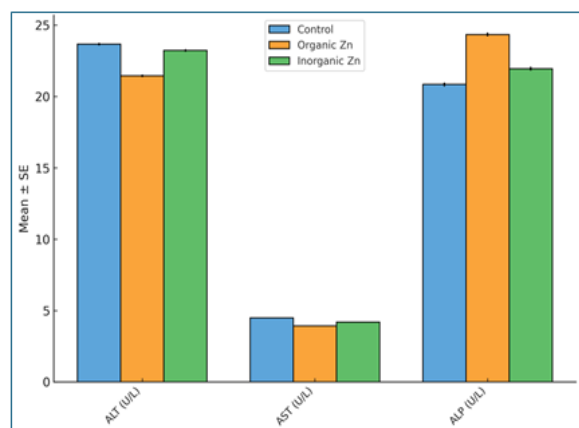


Figure 2: Liver enzyme assessment related to Zinc Supplementation. This figure reflects the changes in liver enzyme activities of broiler chickens after Zinc supplementation. Each bar in the figure represents the mean enzyme activity \pm standard error for each group. The control group indicates the greatest acute condition of the liver, denoted by the highest concentrations of ALT and AST.

Table 2: Activities of liver enzymes (ALT, AST, ALP) in broiler chickens after supplementation of organic and inorganic zinc. The organic zinc proved to be more beneficial to the liver and more efficient in mineral utilization by the body, as indicated by lower ALT and AST and higher ALP activities in comparison to both inorganic zinc and control.

Enzyme (U/L)	Control	Organic Zn (T1)	Inorganic Zn (T2)	\pm SE
Alanine transaminase (ALT)	23.66	21.44	23.22	± 0.09
Aspartate aminotransferase (AST)	4.48	3.92	4.18	± 0.03
Alkaline phosphatase (ALP)	20.84	24.34	21.94	± 0.13

Hematological Parameters, Further Studies

Hematological records, particularly those detailing supplementation of various forms of zinc, indicated marked improvement compared to the control within the supplemented groups, more so for the organic zinc form. Blood cell components such as red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and packed cell volume (PCV) were, in all zinc supplemented instances, significantly higher ($p \leq 0.05$) than control. Recorded mean RBC values indicated dominance in the organic form, $33.66 \pm 0.04 \times 10^6/\mu\text{L}$, followed shortly after by the inorganic zinc group, $33.28 \pm 0.05 \times 10^6/\mu\text{L}$. The result showcases improvement in oxygen transport and cell production. Hemoglobin concentration also improved where the organic group transcended 7.04 ± 0.02 g/dL, and reached 7.26 ± 0.04 g/dL while the inorganic group reached 7.12 ± 0.03 g/dL, compared to control. WBC count increased, albeit slightly, within the organic zinc group compared to control and $23.2 \pm 0.03 \times 10^3/\mu\text{L}$ ($23.2 \pm 0.03 \times 10^3/\mu\text{L}$ in inorganic zinc) relative to $23.08 \pm 0.03 \times 10^3/\mu\text{L}$ in control suggesting the possibility of improved immune responses. The PCV values followed this pattern too suggesting further improvement in the volume of blood and oxygen-carrying efficiency. The conclusion from the. Results show that zinc supplementation, especially of organic form, offers significant improvement by way of enhancing erythropoietic activity, immunity, and physiological immunity. Further corroboration of the importance of zinc as an enzymatic activator necessary for the synthesis of DNA and the subsequent synthesis of enzymes involved in cell proliferation is the enhancement of the cellular blood constituents (Table 3).

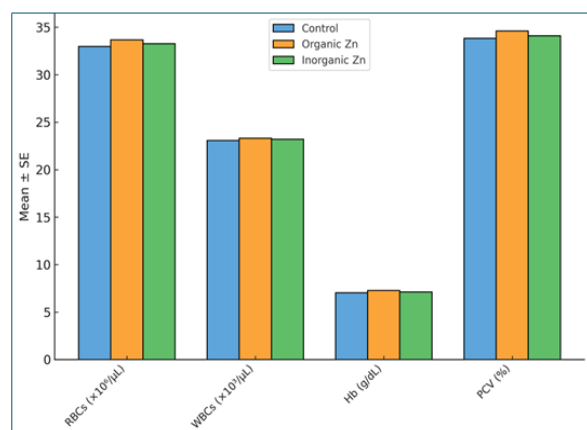


Figure 3: Demonstrates the impact of hematological parameters oil treatment hematological parameters oil treatment hematological parameters oil treatment having zinc supplementation and organic and inorganic control. Each of the figures of the treatments-received samples are shown below the bars of the graph and represent the mean 25 blood samples taken to conduct the treatment for the treatment control group. Table 3 Assessment the impact zinc supplementation and zinc organic and inorganic control to the treatment broiler chickens of the parameters meant by to the blood examination made the values of Control Treatment Z. Organic treatment to the analyzed indicate an additional value for the treatment from 12.05 to 15. 18 to control and greater than 80 to 85 observed zinc.

Parameter	Control	Organic Zn (T1)	Inorganic Zn (T2)	±SE
RBCs (×10 ⁶ /μL)	32.98	33.66	33.28	±0.04
WBCs (×10 ³ /μL)	23.08	23.30	23.20	±0.03
Hemoglobin (g/dL)	7.04	7.26	7.12	±0.03
Packed cell volume (%)	33.82	34.62	34.10	±0.05

Discussion

Organic zinc raised serum proteins concentrating on decreasing glucose, triglycerides, cholesterol, and uric acid. Such patterns suggest more efficient nutrient utilization and fewer metabolic costs. In broilers, glycine chelate zinc similarly shifted biochemistry, improving growth and antioxidant status (11,12). Organic zinc at the same dose zinc oxide or sulfate had superior performance on lipids and growth and (13). Nano-zinc, and these references suggest, shows similar or more vigorous gains with blood counts and some serum markers and signifies more rapid uptake and delivery (14,15). These patterns correspond with the current biochemical findings and

suggest the form and copper matrix positively influence the physiology (11–15). The behavior of the biochemical parameters under examination also exhibited protective tendencies. Organic zinc decreased ALT and AST; however, ALP remained normal and elevated. Trace elements in coated or chelated form diminished oxidative stress while stabilizing hepatic indicators in practical diets (12). Direct comparisons also demonstrated lower transaminases and improved redox balance associated with organic formats (11,13). In chickens fed zinc glycine versus zinc sulfate, proteomic data reflected changes in metabolism and stress-control proteins, supporting enzyme normalization (16–18). Phytase enhanced zinc release and utilization without enzyme toxicity, consistent with safe ALP elevation (19). In summary, the hepatic response is consistent with previous controlled studies linking bioavailable zinc to improved hepatic cellular integrity (11–13,16,19). Hematology improved with zinc supplementation, with the strongest changes in organic zinc. Broilers administered chelated zinc or zinc nanoparticles demonstrated enhanced RBC indices and increased immune cells (11,14,15). A clinical study integrating organic zinc with functional additives also enhanced pivotal serum markers and immunity (18). Meta-analysis and multi-trial data corroborate that advanced zinc sources elevate hematological values more than conventional salts (15). Evidence like these align with the current findings. They suggest increased erythropoiesis and enhanced immune preparedness associated with more effective zinc provisioning (11,14,15,18). The use of coated or organic zinc increases the effective uptake of antagonism across intestinal membranes and improves antioxidant systems and gut barrier integrity and Access (12,13). Work in broilers and other poultry populations demonstrates improved villus structure, barrier tightness, and microbiome balance with zinc glycine chelate (11,17). These effects are consistent with decreased lipid peroxidation and enhanced protein synthesis, which aligns with elevated total protein and decreased lipids reported here (11-13,17). ALP trends also correlate with sufficiency of zinc, since zinc-dependent ALP increases with gold and decreases with stress relief, whilst increase with stress relief, while ALT and AST fall with stress relief on the liver (11-13,16). Intestinal zinc and performance levels often correlate and the strong context protective organic or coated zinc trace minerals are supportive under heat stress or dietary restrictions (12). Phytases often are positive supporters of zinc, while other strategies reduce the need for high supplemental levels (19). More recent trials with low organic inclusion have achieved better growth than high inorganic inclusion and mineral excretion, which aids in sustainability (12,20). Results obtained are in conjunction least with the current study and advance



practical strategies for dietary zinc refinement for optimal productivity and health (12,19,20).

Conclusion

Improved biochemical and hematological profiles were observed with organic zinc inclusion in broiler chickens. These include lower ALT, AST, uric acid, triglycerides, cholesterol, and ALT levels. The Total Protein, ALP, RBC, Hb, and PCV counts showed significant improvement. Inorganic zinc had some moderate benefits. The data points to improved nutrient utilization, liver function, and erythropoiesis with organic zinc. The adoption of organic zinc in practical diets may also improve performance and welfare while reducing metabolic constraints.

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Conflict of Interest

No conflict of interest is declared by the authors.

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