



EFFECTS OF GRADED DIETARY ORGANIC ZINC SUPPLEMENTATION ON GROWTH PERFORMANCE AND JEJUNAL HISTOMORPHOLOGY IN BROILER CHICKENS

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Abstract.

The present work examined how incremental inclusion of zinc methionine in broiler diets influences productive performance and the microarchitecture of the jejunal mucosa. A total of 120 day-old Ross 308 chicks were distributed among four dietary regimens: an unsupplemented basal ration serving as the control, and three rations fortified with zinc methionine to supply 20, 40, or 80 mg of elemental Zn per kilogram of feed. Every regimen was represented by three pens of ten birds each, and the trial was conducted until the birds reached 35 days of age. Productive responses were quantified in terms of live weight, weight gain, feed consumption, and feed conversion ratio, whereas villus height, crypt depth, and their ratio were defined as the indicators of jejunal mucosal architecture. Preliminary evaluation of the performance data detected no significant dietary effect on final live weight, overall weight gain, cumulative feed consumption, or whole-period feed conversion efficiency. A treatment effect was only found in the 4th week (days 21-28); weight gain was higher in the 40 and 80 mg Zn/kg groups than in the 20 mg Zn/kg group, and feed conversion ratio was lower in the 40 and 80 mg Zn/kg groups than in the 20 mg Zn/kg group during the 4th week. These interval-specific divergences, however, disappeared when the full rearing period was considered. Notably, the allocation of treatments and the definition of the experimental unit remain to be confirmed, and verified jejunal morphometric data have yet to be obtained. Taken together, the initial evidence points to transient, age-dependent fluctuations in growth response rather than a lasting productive benefit. Accordingly, neither an optimal inclusion rate of zinc methionine nor a supplementation-driven enhancement of jejunal morphology can be inferred at this stage, and these outcomes must be substantiated through validated treatment records, statistically independent pen-level data, and genuine intestinal measurements.

Keywords: Organic zinc; Zinc methionine; Broiler chickens; Growth performance; Feed conversion ratio; Jejunal histomorphology; Dietary supplementation.

Introduction

Zinc (Zn) is one of the micronutrients essential for poultry and plays a key role in many biochemical and physiological functions such as protein synthesis, cellular proliferation, enzymatic catalysis, antioxidant defence, immune competence and maintenance of epithelial barriers. It becomes more important in modern broilers where growth rates are higher and metabolic rates, tissue accretion and normal gut function are at a premium. Conventional feedstuffs do contain significant quantities of naturally occurring Zn; however, the amount available for the bird can be significantly reduced by interaction with other feeds, particularly phytate. Therefore, extra Zn is commonly added to poultry diets to ensure adequate nutritional status and physiological function. There has been a significant amount of research on the organic forms of the mineral as alternatives to the traditional inorganic salts. One of these is zinc methionine (Zn-Met), in which Zn is chelated with an amino acid ligand which can change the way it interacts with other dietary elements and hence how it is utilized throughout the GIT. Nevertheless, the nutritional value of organic Zn is not a fixed value but is dependent upon various interacting factors such as the inclusion level, native Zn from feed ingredients, physiochemical characteristics of the supplement and the physiological needs of the birds.

The nutritional value of Zn supplementation in broilers is largely assessed based on productive performance. Live weight, weight gain, feed consumed and feed conversion ratio provide a composite picture of the efficiency of assimilation and conversion of dietary nutrients into body tissue. However, birds are not always more responsive to increasing levels of Zn when the basal diet is already adequate in Zn.

In addition to its systemic role, Zn is believed to play a role in maintaining structural integrity and continuous renewal of intestinal epithelium. The small intestine is the primary site of nutrient digestion and absorption and its mucosal structure may directly determine nutrient utilization efficiency. The most common histomorphometric parameters of mucosal architecture are jejunal villus height and crypt depth, and the villus height/crypt depth ratio. Villus height is a measure of the absorptive surface and crypt depth is an indicator of the dimensions of the proliferative compartment that is involved in renewal of the epithelial compartment. The ratio is a composite measure of the mucosal morphology, but should not be used as a direct measure of absorptive capacity.

Previous studies have suggested the possibility of changes in intestinal morphology and productive traits of broilers under the influence of organic Zn. For example, Khan et al. (2024) reported positive impacts on performance and intestinal structural indices, which were most pronounced at the highest level of organic Zn tested, 80 mg/kg. However, with the diversity of the experimental conditions and dietary formulation among various published studies the responses of broilers to the various graded inclusion levels of organic Zn are worthy of further investigation.

Therefore, the present study aimed to determine the effects of dietary zinc methionine supplementation at 0, 20, 40, and 80 mg elemental Zn/kg on productive performance and jejunal mucosal architecture of Ross 308 broilers up to 35 days of age. Emphasis was placed on live weight, weight gain, feed consumption, feed conversion ratio, villus height, crypt depth, and villus height: crypt depth ratio.

Materials and Methods

1. Ethical Approval

All birds used in this trial were handled and treated in compliance with institutional policies and procedures for the care and use of animals in research. Before the trial began, the study protocol was submitted for review and approval to the Institutional Animal Care and Use Committee at Tikrit University, Iraq..

2. Experimental Birds and Management

One-day-old Ross 308 broiler chicks were obtained from a commercial hatchery and matched as closely as possible for initial body weight (IBW). Until the end of the second week post-hatch (WPH), the chicks were brooded together as one group under uniform husbandry and feeding conditions; no dietary treatments were applied during the first 2 WPH. On day 14, each bird was weighed individually and randomly allotted to four dietary treatments, each consisting of three replicate pens of ten birds (30 birds per treatment). The dietary treatments were fed from day 14 to day 35. The birds were housed in floor pens with fresh wood shavings, and each pen was equipped with its own feeders and drinkers, providing unrestricted access to feed and water throughout the experimental period. In accordance with Ross 308 guidelines, the brooding temperature was maintained at about 32–33°C at placement and then gradually reduced. Conditions of environment and husbandry were kept the same between all groups, including relative humidity, stocking rate, vaccination status and daily husbandry practices. Birds were checked daily for health and the number of birds dying was recorded in each replicate pen allowing for correction of performance indices for mortality.

3. Experimental Design and Dietary Treatments

Inclusion levels are based on the amount of elemental Zn supplied by the organic source and not on the amount of commercial zinc methionine added per kg of feed; for this reason, the amount of zinc methionine added was adjusted for the declared/analytically verified Zn content. All diets contained a trace-mineral premix without Zn to prevent any unplanned contribution of Zn and other trace-mineral supplements were the same for all treatments. The Zn in the control ration came from only the basal feedstuffs and no supplemental source was provided. When laboratory supplies allowed, the basal diet intrinsic Zn level and total Zn level of each experimental diet was chemically determined. All treatments had the same basal formulation with the only difference being the supply of different levels of Zn-Met, the basal diet (T1, control, 0 mg Zn/kg), basal diet with 20 mg Zn/kg of diet (T2), basal diet with 40 mg Zn/kg of diet (T3) and basal diet with 80 mg Zn/kg of diet (T4).

4. Basal Diets

The birds will be fed rations based on maize and soybean meal which will meet or exceed nutritional requirements for Ross 308 broilers, except for supplemental levels of Zn which will be based on dietary treatment. There will be two feeding phases: a starter phase (days 1 to 21) and a grower-finisher phase (days 22 to 35).

Table 1: Composition of the basal experimental diets (% as-fed basis)

Ingredient	Starter (1–21 d)	Grower-finisher (22–35 d)
Corn	54.90	59.80
Soybean meal	37.00	31.50
Soybean oil	4.00	4.80
Dicalcium phosphate	1.80	1.65
Limestone	1.00	0.95
Salt	0.30	0.30
DL-Methionine	0.30	0.26
L-Lysine HCl	0.15	0.18
L-Threonine	0.05	0.06
Vitamin–mineral premix without Zn	0.50	0.50
Total	100.00	100.00

Target calculated nutrient composition

Metabolizable energy, kcal/kg	3,000	3,180
Crude protein, %	22.0–22.5	19.5–20.0
Calcium, %	0.90	0.82
Available phosphorus, %	0.45	0.39
lysine, %	1.25–1.30	1.08–1.12
Met + Cys, %	0.93–0.96	0.82–0.85

5. Growth Performance Measurements

Birds will be weighed at the beginning of the experiment and subsequently on days 14, 21, 28 and 35. Feed offered and feed residues will be recorded for each replicate pen.

The following productive performance variables will be calculated:

Body weight gain (g) = Final body weight – Initial body weight

Average daily gain (g/bird/day) = BWG ÷ number of experimental days

Feed intake (g/bird) = Feed offered – Feed residue

Average daily feed intake = Total feed consumed ÷ bird-days

Feed conversion ratio = Feed intake (g) ÷ Body weight gain (g)

6. Jejunal Sampling and Histomorphometric Evaluation

Two birds from each replicate pen will be selected on day 35 for human termination (6 birds per dietary treatment) based on the live weight which best represents the pen average. A jejunal segment of approximately 2 cm will be removed from each bird, between the end of the duodenal loop and Meckel's diverticulum and flushed gently with isotonic saline, and placed in 10% neutral buffered formalin for 24–48 hours. Routine processing of the fixed tissues will then be performed, including dehydration in an ascending ethanol series, xylene clearance and paraffin embedding, and 5-µm sections will be cut and stained with hematoxylin and eosin (H&E). Ten or more complete and properly oriented villus–crypt units will be obtained from each bird and morphometric readings will be done using calibrated ImageJ software. Villus height (VH) will be measured from the apex of the villus to the point where it meets the crypt and crypt depth (CD) will be measured from the junction of the villus with the crypt to the bottom of the crypt. The VH:CD ratio will then be calculated by dividing the former by the latter according to the principles of intestinal histomorphometry (De Grande et al., 2020). Values obtained for each bird will be grouped together to give a mean per pen for statistical purposes.

7. Statistical Analysis

One-way analysis of variance (ANOVA) will be used for all variables with the pen being the experimental unit. If a significant dietary effect is found, the means will be separated using Duncan's multiple range test and probability levels of < 0.05 will be considered significant.

Results and Discussion

The live body weight at each age in response to the different dietary levels of organic zinc is summarized in Table 2. Body weights of zinc-supplemented birds did not differ significantly from those of the control birds on days 14 and 21. The small numerical differences observed at these ages showed no consistent trend in relation to the level of supplemental zinc.

On day 28, Duncan's post hoc test showed that birds fed 40 mg Zn/kg were heavier than those fed 20 mg Zn/kg. However, the overall ANOVA was not significant, so this difference cannot be considered conclusive without additional statistical support. On day 35, the 80 mg Zn/kg group showed the highest numerical body weight, followed by the 20 mg Zn/kg, control, and 40 mg Zn/kg groups, with no significant differences ($P > 0.05$) among them.

Table 2. Effects of Graded Dietary Organic Zinc Supplementation on Live Body Weight (g) of Broiler Chickens

Age (day)	T ₁ Control	T ₂ 20 Zn (mg/kg diet)	T ₃ 40 Zn (mg/kg diet)	T ₄ 80 Zn (mg/kg diet)
Day 14	470.00 ± 34.44 a 1088.50 ± 21.83	526.25 ± 23.05 a 1126.00 ± 10.62	476.50 ± 15.01 a	475.12 ± 23.49 a 1098.75 ± 22.32
Day 21	a 1709.25 ± 87.49	a 1783.75 ± 61.93	1068.00 ± 26.48 a	a 1776.25 ± 56.57
Day 28	ab 2543.75 ± 100.98	b 2615.00 ± 89.68	1816.50 ± 63.04 a 2485.00 ± 114.11	ab 2644.00 ± 102.02
Day 35	a	a	a	a

Duncan alpha=0.05

Body Weight gain through successive growth stages is given in Table 3. Dietary zinc had no significant effect on weight gain during the second to third week of age (days 14 to 21) as all the supplemented groups gained weight at the same rate as the unsupplemented controls. The preliminary analysis revealed a significant dietary effect ($P < 0.05$) during days 21-28 of the experiment, that broilers receiving 40 or 80 mg Zn kg⁻¹ gained significantly more weight than broilers receiving 20 mg Zn kg⁻¹, but that broilers receiving 40 or 80 mg Zn kg⁻¹ did not gain significantly more weight than broilers receiving no added zinc.

During the last period (days 28–35), the highest numerical increase was achieved in the 20 mg Zn/kg group, but the differences between the diets were not significant. Similarly, total weights from day 14 to day 35 did not differ between the treatments ($P > 0.05$). Although the 80 mg Zn/kg group numerically had the greatest cumulative gain, this growth did not significantly differ from the control and the other supplemented groups.

Table 3. Effects of Graded Dietary Organic Zinc Supplementation on Body Weight Gain (g) of Broiler Chickens

Age (day)	T ₁ Control	T ₂ 20 Zn (mg/kg diet)	T ₃ 40 Zn (mg/kg diet)	T ₄ 80 Zn (mg/kg diet)
14-21	618.50 ± 36.12 a 620.75 ± 88.17	599.75 ± 19.99 a	591.50 ± 30.40 a	623.62 ± 26.63 a
21-28	ab 834.50 ± 105.11	657.75 ± 62.74 b 1031.25 ± 125.66	748.50 ± 56.96 a	677.50 ± 53.91 a 867.75 ± 152.02
28-35	a 2073.75 ± 92.31	a 2288.75 ± 75.11	668.50 ± 99.86 a 2008.50 ± 119.70	a 2168.88 ± 114.74
14-35	a	a	a	a

Duncan alpha=0.05

Table 4 gives feed consumption as a function of increasing levels of organic zinc. Feed intake for all the zinc fortified groups did not differ significantly over each of the three intervals studied (days 14-21, 21-28, 28-35) from that of the unsupplemented control ($P > 0.05$). During this interval, the highest consumption was recorded in the control birds (686.65 g/bird) whereas the lowest consumption was found in the 80 mg Zn/kg (662.97 g/bird). The 20 mg Zn/kg group had consumed the largest quantity of feed (1097.53 g/bird) followed closely by the 80 mg Zn/kg group (1095.91 g/bird) during days 21-28. The 20 mg Zn/kg group also numerically had the highest feed intake in the period 28-35 days (1392.85 g/bird) while the control group had the lowest feed intake (1342.22 g/bird). The accumulated intake during all 14–35 days ranged from 3094.09 g/bird

in the control to 3174.57 g/bird in the 20 mg Zn/kg group with no significant difference observed between diets ($P > 0.05$). Therefore, under the experimental conditions used in this study, the voluntary feed consumption was not measurably affected by fortification of the diet with 20, 40 or 80 mg of organic zinc per kg.

Table 4. Effects of Graded Dietary Organic Zinc Supplementation on Feed Intake (g/bird) of Broiler Chickens

Age	T ₁ Control	T ₂ 20 Zn (mg/kg diet)	T ₃ 40 Zn (mg/kg diet)	T ₄ 80 Zn (mg/kg diet)
14-21	686.65 ± 6.45 a	684.19 ± 10.32 a 1097.53 ± 15.51	668.59 ± 5.74 a	662.97 ± 5.94 a 1095.91 ± 14.15
21-28	1065.22 ± 7.05 a 1342.22 ± 26.71	a 1392.85 ± 24.48	1074.17 ± 7.22 a	a 1367.31 ± 13.09
28-35	a 3094.09 ± 38.56	a 3174.57 ± 34.24	1378.72 ± 21.23 a	a 3126.19 ± 11.50
14-35	a	a	3121.48 ± 26.36 a	a

Duncan alpha=0.05

The effect of graded levels of organic zinc in the diet on feed conversion ratio (FCR) is shown in Table 5. Dietary treatment did not significantly affect FCR ($P > 0.05$) during the first interval (days 14-21), however, between days 21-28, there was a numerical difference between the feed treatment groups with the 40 mg Zn/kg treatment utilizing feed most efficiently (1.43) and the other feed treatments becoming progressively less efficient (1.61, 1.66 and 1.71 for the 80 mg Zn/kg, 20 mg Zn/kg and unsupplemented control, respectively). However the Duncan classification that was given to the 20 mg Zn/kg group, which was set apart from all other treatments by a superscript, indicated significance ($P < 0.05$); however this classification is inconsistent with the ranking of the means and the associated variability, and therefore should be re-thought.

During the final period (days 28-35), there were no significant ($P > 0.05$) differences in FCR between the treatments, but the lowest value occurred in the 20 mg Zn/kg treatment (1.35) and the highest in the 40 mg Zn/kg treatment (2.06). Given the high variation in FCR during this period, particularly for the 80 mg Zn/kg, it is important to ensure that the correct feed intake and weight gain data was used to calculate the corresponding FCR. The cumulative FCR for the entire 14–35 d period was not statistically different ($P > 0.05$) from 20 mg Zn/kg to 80 mg Zn/kg and the control, but it was separated from 40 mg Zn/kg. Thus, the results of this study do not provide any strong evidence that the overall feed utilization efficiency was improved with the supplementation of zinc in organic form.

Table 5. Effects of Graded Dietary Organic Zinc Supplementation on Feed Conversion Ratio (FCR) of Broiler Chickens

Age	T ₁ Control	T ₂ 20 Zn (mg/kg diet)	T ₃ 40 Zn (mg/kg diet)	T ₄ 80 Zn (mg/kg diet)
14	1.11 ± 0.04 a	1.14 ± 0.03 a	1.13 ± 0.04 a	1.06 ± 0.04 a
21	1.71 ± 0.16 b	1.66 ± 0.32 a	1.43 ± 0.07 b	1.61 ± 0.09 b
28	1.60 ± 0.20 a	1.35 ± 0.13 a	2.06 ± 1.43 a	1.57 ± 10.86 a
33	1.49 ± 0.04 a	1.37 ± 0.04 a	1.55 ± 0.07 a	1.44 ± 0.06 a

Duncan alpha=0.05

The jejunal morphometric characteristics of 35-day-old broilers (villus height, crypt depth, and villus height/crypt depth ratio) are presented in Table 6 according to the different dietary organic zinc concentrations. The Duncan groups reported showed that birds in the group receiving the 80 mg of zinc per kg (T4) had significantly taller villi than the unsupplemented controls (T1), with the 20 mg of zinc per kg (T2) and 40 mg of zinc per kg (T3) groups falling intermediate between the two. Crypts were deeper at each level of zinc supplementation than in the control, but not significantly different among the supplemented groups. In the opposite direction, the villus height : crypt depth ratio (VH : CD) decreased significantly in the 40 mg Zn/kg group as compared with the control, while the 20 and 80 mg Zn/kg groups had intermediate ratios that were not significantly different from the other treatments. Taken together these comparisons suggest changes in jejunal architecture that was not in a consistent order with increasing dietary zinc concentration.

Table 6. Effects of Graded Dietary Organic Zinc Supplementation on Jejunal Villus Height, Crypt Depth, and Villus Height-to-Crypt Depth Ratio in Broiler Chickens at 35 Days of Age

Treatments	Villus Height (µm)	Crypt Depth (µm)	VH:CD
T₁ Control	1091.50±22.29b	138.75±5.54b	7.8±0.35a
T₂ 20 Zn (mg/kg diet)	1157.25±25.97ab	159.25±0.85a	7.26±0.13ab
T₃ 40 Zn (mg/kg diet)	1121.50±8.73ab	158.75±1.10a	7.06±0.10b
T₄ 80 Zn (mg/kg diet)	1170.50±30.08a	160.50±0.64a	7.28±0.18ab

Duncan alpha=0.05

Based on the present preliminary data, dietary inclusion of organic zinc did not significantly increase live body weight at 35 days of age. Although the 80 mg Zn/kg group had the highest numerical final weight, it did not differ significantly from the control and other supplemented groups, indicating that none of the levels tested provided a consistent improvement in overall growth.

This finding contrasts with that of Khan et al. (2024), who compared different zinc sources in broilers and reported significant improvements in weight gain, feed conversion, and intestinal morphology at 80 mg/kg organic zinc, suggesting that, under certain dietary conditions, organic zinc can be a suitable option for improving productive performance. Similarly, Chand et al. (2020) reported that organic zinc affected productive traits and intestinal development in broiler chickens, but only at specific inclusion rates. Such discrepancies may arise from differences in basal diet formulation, inclusion rate, zinc bioavailability, husbandry practices, and experimental design between these reports and the present study. Another possible explanation is the native zinc content of the basal diet; Yu et al. (2010) demonstrated that dietary phytate reduced intestinal Zn uptake and that some organically chelated zinc forms were able to overcome this inhibitory effect. Therefore, the birds' response to supplemental zinc may be limited, at least in part, by the amount of zinc supplied by the basal feedstuffs.

However, treatment differences in weight gain were only seen during the mid-growth interval, when exploratory post hoc comparison showed that the weight gain in the 40 and 80 mg Zn/kg were higher than the 20 mg Zn/kg. Cumulative gain did not differ with diet and the growth response to zinc supplementation, if there was one, appears to have been short term and not maintained for the duration of the trial. De Grande et al. (2020) found that feeding amino acid-complexed rather than inorganic zinc to broilers increased zinc digestibility and improved some performance parameters, highlighting the influence of zinc utilization and gut physiology on the response to supplemental zinc. Furthermore, the better performance reported by Khan et al.

(2024) at the higher organic zinc level supports a concentration-dependent effect, but does not prove that higher zinc levels would necessarily yield proportional benefits in other trials.

The between-period differences in growth cannot be attributed to feed intake, as intake did not differ among treatments. The effect of zinc source on zinc digestibility and intestinal physiological traits reported by De Grande et al. (2020) suggests that supplementation effects should be interpreted in terms of nutrient utilization rather than intake alone. Similarly, Yu et al. (2010) showed that the mineral form and its interaction with phytate determine zinc absorption, and hence zinc utilization, and that the chemical availability of a supplement may influence zinc utilization regardless of feed intake.

The feed conversion responses observed here are partially consistent with those of De Grande et al. (2020), who fed broilers amino acid-complexed zinc (AAZn) versus inorganic zinc and reported better feed efficiency during the starter phase. It indicates that zinc's influence on feed efficiency might be different at different growth stages. Khan et al. (2024) also saw a reduction in FCR at 80 mg/kg organic zinc and attributed this reduction to changes in intestinal morphology and other physiological parameters; however, these findings cannot be directly correlated with other studies for various reasons such as differences in dietary composition, zinc level, and experimental setup. A major limitation of the current study is that FCR was abnormally high in some treatments during days 28–35; because FCR is the ratio of feed consumed to weight gain, it is easily inflated when weight gain is abnormally low.

The jejunal histomorphometric results indicate that dietary organic zinc may alter the intestinal morphology of broilers. Villus height in the 80 mg Zn/kg group was greater than in the control, which may indicate better villus development. Similarly, De Grande et al. (2020) observed taller villi in broilers fed amino acid-complexed zinc and suggested that organic zinc may help maintain normal epithelial integrity, and Khan et al. (2024) also found longer villi with higher organic zinc supplementation. In contrast, all supplemented groups had deeper crypts than the control, and the 40 mg Zn/kg group had a lower villus height: crypt depth ratio, contrary to Khan et al. (2024), who reported a higher ratio with increasing organic zinc. The conflicting responses may be due to variations in the composition of the diet, zinc availability, dynamics of epithelial turnover or experimental conditions. However, it is important to note that without cellular measurements, the deeper the crypts do not necessarily indicate increased epithelial proliferation or failure of intestinal function.

Conclusion

The effects of dietary treatment on weight gain and feed conversion ratio were limited to the days 21–28 period, whereas final live weight, cumulative gain, total feed intake, and overall feed conversion at 35 days of age were unaffected. Although some differences in performance traits were observed among treatments at the highest zinc inclusion level, no consistent dose–response relationship was evident. Therefore, the optimal level of organic zinc for broiler diets should be confirmed using statistically independent replicates and verified jejunal histomorphometric measurements.

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