In-ovo mineral supplementation: a comprehensive analysis of broiler bone morphometry and structural implications

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Abstract

This study aimed to investigate the impact of in-ovo micro (Cu, Mn, and Zn) and macro (Ca, P, and Mg) minerals feeding on various bone parameters, gait score, black bone syndrome, lameness, and curled toe paralysis in broiler chickens. The experiment was conducted using a completely randomized design with seven treatment groups, including a control group. Results revealed that in-ovo administration of Ca significantly influenced (P<0.05) tibia, femur, keel bone, and metatarsus morphometry. Femur bone, keel bone and metatarsus bone ash contents werehigher (P<0.05) in in-ovo Calcium administered birds, however, tibia ash was same across the groups. The Ca, P, Mg, Cu, Mn and Zn mineralization was higher (P<0.05) in respective in-ovo administered group. Moreover, in ovo Ca feeding positively influenced pododermatitis score, gait score, black bone syndrome score, lameness score, and curled toe paralysis score. In conclusion, in-ovo mineral supplementation, particularly with Ca, effectively enhanced broiler bone morphometry, bone ash and bone mineralization with positive impact on bone health and gait.

Keywords: *In-ovo* minerals feeding, bone morphometry, bone mineralization, skeletal problems

Introduction

Bone health in broiler chickens is a critical aspect for well-being and rapid growth as birds reared under intensive production systems. There are great chances of skeletal issues in rapid growth conditions; therefore, importance of proper nutrition in poultry industry is never ignored (Biesek et al., 2022). Calcium and phosphorus are the two primary minerals required for bone development, and magnesium, zinc, and copper improve the bone health by augmenting metabolic processes (Ciosek et al., 2021). Under such condition, designing a balanced feed by minerals fortification minimize the skeletal issues and retorted growth in broiler production (Angel, 2007).

In-ovo feeding of minerals, a technique involving the injection of minerals into the egg before it hatches, has shown potential impact on bone morphometry in broiler chickens. Post-hatch feeding of macro and micro-minerals potentially improve bone strength and mineral density in broilers (Yair et al., 2020). However, *in-ovo* minerals feeding effects on bone morphometry vary due to mineral type, amount/form of minerals fed, observation time and frequency of feeding (Abbasi et al., 2017). Beyond feeding practices, the genetics, and management also impact broiler bone health. Among previous studies, post-hatch broiler bone characteristics during in ovo feeding trials were observed (Oliveira et al., 2015; Yair et al., 2011; Yair et al., 2015; Bello et al., 2014)

However, effect of individual mineral in ovo feeding between days 12 to 18 of incubation is crucial as minerals involved in osteoclasts stimulation, bone growth and bone health in broilers. The long-term post-hatch studies are needed to assess the persistence of the observed benefits of minerals supplemented by in ovo feeding method and potential carry-over effects on bone health during the post-hatch period. Therefore, the current research was conducted to explore the carrying over effects of in ovo minerals (Ca, Mg, P, Cu, Mn and Zn) feeding at day 12 of incubation, on bone morphometry, mineralization and welfare issues of chicken broiler.

Materials and Methods

Experimental Design

This experiment used 700-day-old chicks that were produced after an in-ovo macro- (Mg, Ca, and P) and micro- (Zn, Mn, and Cu) mineral feeding trials on 12th day of incubation. Based on the kind of minerals feeding the chicks were split into seven groups: one served as control group (CON) with three macro minerals (Mg, Ca, and P) and three micro minerals (Zn, Mn, and Cu) treatment groups. Each groups had 100 chicks.

Housing Management

The floor, surrounding areas, and utensils were all meticulously cleaned and sanitized before to the chicks' arrival. The floor was covered with medium-sized wood-shaved litter, and feeders, drinks, brooding apparatus, and other accessories were added. In order to further sanitize the home, fumigation was carried out.

Following all conventional management procedures, the chicks were provided optimum housing requirements.

Bone morphometric variables

On the 42 day of age, eight birds (received in ovo minerals feeding on day 12 of incubation) per group (two per replicate, with average weight) were randomly selected and slaughtered by neck slitting. Tibia, femur, keel bone, and metatarsus bones were collected from the slaughtered birds. Adhering muscles and connective tissues were carefully removed by hand and the bones were dipped into boiling water for five minutes to eliminate any residual soft tissues. The following bone morphometric parameters were recorded:

The muscles and soft tissues were removed from the tibia, femur, keel, and metatarsus bones. Cleaned bones were weighed in grams using an electronic balance. Bone weight was calculated relative to body weight (g). Bone length (cm) was measured with a digital Vernier caliper between the ends of proximal and distal epiphyses. The bones were individually sealed in plastic bags, stored at -20°C for further analysis. Bone width measurements were conducted on respective bones using digital Vernier caliper. The proximal and distal width, as well as the mid-shaft width of the tibia, femur, keel, and metatarsus bones, were measured and recorded in cm.

Bone Ash

Selected bones from each group were kept in hot air oven and dried at 55°C for 5 hrs. The dried bones were weighed initially in crucibles, and the weight was recorded in grams using an electronic balance. The bones were placed in muffle furnace at 550°C for 2 hours until completely ashed. The ash weight was then compared to the original bone weight for calculation. The bone ash percentage was calculated by dividing the ash weight by the original bone weight sample and multiplying by 100.

Bone and gait deformities

Keel bone fractures manifest as calluses around the fracture site or sharp, unnatural deviations in the bone. The number of keel bone fractures was scored for each group and replicate and expressed as a percentage. Gait score is an estimation of locomotion deficiency based on the visual judgment of a broiler chicken's ability to walk on a known surface. Gait scores were observed at the 5th week of age for each replicate and group and scored as normal or abnormal. The gait score parameter was also measured as a percentage. Black bone syndrome causes darkening of the tissue adjacent to the bone due to injury, striking, or leakage of bone marrow contents. This parameter was observed by examining bones and tissues of randomly selected slaughtered birds from each group and replicate. Black bone syndrome was scored as normal or defective and measured as a percentage.

Lameness in broiler chickens is a primary welfare concern as it is considered painful due footpad dermatitis, excessive bird weight, bacterial infections, or the condition of the litter. Lameness was investigated as normal or defective. Lameness parameters were recorded as numbers in replicates of groups and measured as a percentage. Curled toe paralysis manifests as a condition where the chicken's toes curl inward, hindering their mobility owing to sciatic nerves damaged, poor growth, diarrhea, and weakness. Curled toe paralysis was investigated as normal or defective in replicates of groups. This parameter was recorded as a number in each replicate of the respective groups and measured as a percentage.

Bone Mineral Concentration

Bone ash of the tibia, femur, keel, and metatarsus bones was prepared in a muffle furnace at a temperature of 550°C. The prepared bone ash samples were weighed using an electronic balance. Bone mineralization was carried out by atomic absorption spectrophotometry after the digestion process of bones. One bone ash sample from each treatment replicate group was selected for the mineral concentration study. The samples were dissolved and digested following the US-EPA guidelines from 1986, and the amounts of minerals (Ca, P, Mg, Zn, Mn, and Cu) in each ash sample were assessed using atomic absorption methods.

Statistical Design

The data was tabulated and analysed by using JMP software of SAS. The data analysis method was used one-way analysis of variance (ANOVA). The significance level among the factors under study, as shown in various tables and figures, is indicated by the P-value <0.05.

Results

Bone Length, Width, Weight

The results regarding the effect of in ovo minerals feeding on tibia, femur, keel bone and metatarsus bone length, width and weight is presented as Fig.1. The tibia, femur, keel bone and metatarsus bone lengths were significantly ($P<0.05$) higher in birds given *in-ovo* Ca feeding (9.74cm), followed by rest of in ovo supplemented (P, Zn, Mg, Mn, Cu) and control groups (Fig. 1 A). Similarly, greater widths in tibia, femur, keel bone and metatarsus bones were in birds receiving *in-ovo* Ca feeding rather than Zn, P, Mg, Cu, Mn or control groups (Fig. 1 B). The birds receiving *in-ovo* Ca feeding exhibited a significantly ($P<0.05$) higher tibia, femur, keel bone and metatarsus bone compared to the control group as well as other in ovo supplemented groups (Fig. 1 C).

The results about the effect of in ovo minerals feeding on tibia, femur, keel bone and metatarsus bone length, width and weight is presented in Table 2. The tibia ash was similar across the control and in ovo supplemented mineral groups; however, femur, keel bone and metatarsus bone ash level were significantly higher in *in-ovo* Ca feeding group compared to groups.

The effect of *in-ovo* feeding of micro (Cu, Mn, and Zn) minerals on tibia, femur, keel bone and metatarsus bone mineralization is given in Table. The concentrations of Mg, P, Ca, Mn, Cu, and Zn in tibia, femur, keel bone and metatarsus of in ovo mineral supplemented groups had significantly higher levels than control group.

The impact of *in-ovo* feeding of macro and micro minerals on bone and mobility deformities is presented in Table. Based on classified of normal and defective keel bone fracture, non significant differences were observed in keel bone fracture scores as a result of *in-ovo* administration of inorganic salts of Ca, P, Mg, Cu, Mn, and Zn. Similar score was observed in black bone syndrome scores among groups of P, Ca, Mg, Cu, Mn, and Zn. No effect was observed in gait, lameness and curled toe paralysis score due to *in-ovo* feeding of inorganic salts of P, Ca, Mg, Cu, Mn, and Zn.

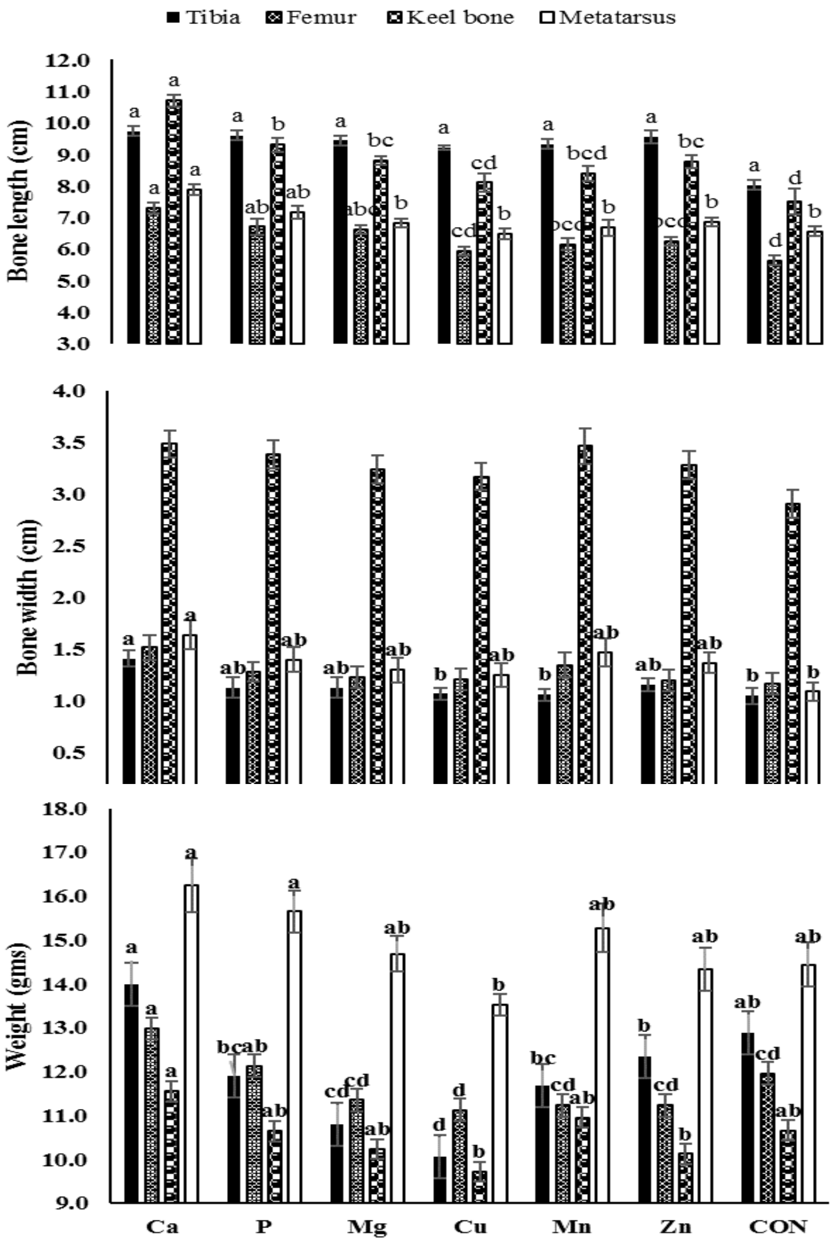

Figure 1. Effect of *in-ovo supplementation* of minerals on bone length, width and weight of broiler

Table 1. Effect of *in-ovo* supplementation of minerals on bone ash of broiler

Group	Bone ash (gms)			
	Tibia	Femur	Keel bone	Metatarsus
Ca	3.3	2.51[a]	3.23[a]	2.54[a]
P	3.2	2.13[ab]	2.89[ab]	2.28[ab]
Mg	3.1	2.02[b]	2.70[abc]	2.06[b]
Cu	2.9	1.98[b]	2.37[bc]	1.90[b]
Mn	3.1	2.27[ab]	2.61[abc]	2.17[ab]
Zn	3.0	2.06[b]	2.53[bc]	1.98[b]
Control	2.7	1.95[b]	2.16[c]	1.84[b]
SEM±	0.2	0.134	0.217	0.1471
P-value	0.25	0.001	0.0002	0.0002

Table 2 Effect of *in-ovo* minerals supplementation bone mineralization

Bone type	Groups	Minerals					
		Ca (mg/ml)	P (mg/ml)	Mg (mg/ml)	Cu (µg/ml)	Mn (µg/ml)	Zn (µg/ml)
Tibia	Control	0.24±0.02	0.23±0.00	0.19±0.00	0.15±0.01	0.17±0.01	0.20±0.00
	Treatment	0.31±0.02	0.28±0.00	0.23±0.00	0.22±0.00	0.24±0.01	0.25±0.00
	P-value	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001
Femur	Control	0.21±0.01	0.19±0.00	0.17±0.00	0.17±0.00	0.17±0.00	0.19±0.01
	Treatment	0.29±0.01	0.25±0.00	0.22±0.00	0.21±0.00	0.21±0.00	0.24±0.01
	P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Keel bone	Control	0.18±0.00	0.17±0.01	0.16±0.01	0.15±0.02	0.16±0.01	0.17±0.01
	Treatment	0.22±0.00	0.21±0.01	0.20±0.01	0.19±0.01	0.20±0.01	0.21±0.01
	P-value	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001
Metatarsus	Control	0.18±0.00	0.17±0.01	0.16±0.01	0.15±0.01	0.16±0.01	0.17±0.01
	Treatment	0.23±0.00	0.21±0.01	0.20±0.01	0.19±0.01	0.20±0.01	0.21±0.01
	P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 3 Effect of *in-ovo* minerals Supplementation on bone and mobility deformities

Groups	Bone deformities (%)		Mobility deformities (%)		
	Keel bone fracture	Black bone syndrome	Abnormal gait	Lameness	Curled toe paralysis
Ca	1	2	2	4	3
P	3	4	3	5	4
Mg	4	6	5	7	7
Cu	6	7	6	8	8
Mn	5	8	5	9	4
Zn	2	5	4	6	6

Control	5	8	5	8	7
χ^2-value	5.43	5.46	2.81	3.1	4.09
P-value	0.4896	0.486	0.8319	0.796	0.664

Discussion

The present study examines the influence of in ovo mineral feeding on bone health and welfare of broiler chickens. The inclusion of calcium (Ca) in the in ovo feeding regimen enhances various aspects of bone development, including length, weight, width, and mineralization when post-hatch birds are kept under optimized feeding and management systems (Das et al., 2021; Matuszewski et al., 2021; Salary et al., 2017). Studies demonstrating the positive effects of combining calcium with phosphorus (P), cholecalciferol (vitamin D3), and other minerals on bone strength and mineral deposition provide additional support (Trautvetter et al., 2014; Yair et al., 2015; Zhu et al., 2019).

In the present study, the concentration of essential minerals such as calcium, phosphorus, magnesium, copper, manganese, and zinc remains higher in the tibia bone, femur bone, keel bone, and metatarsus bone of birds until day 35 of growth (Oliveira et al., 2015; Yair et al., 2013, Yair et al., 2015). This suggests that in ovo feeding has an enduring effect on bone mineralization and demonstrates its potential as a long-term strategy to improve broiler performance and bone health during the crucial growing phase. Heffernan et al. (2019) and Tang et al. (2021) report that in addition to mineral supplementation, the combination of trace minerals with vitamins or amino acids has shown promising results in promoting bone mineralization. This demonstrates the significance of a holistic approach to in ovo nutrition, considering the synergistic effects of various nutrients on bone health.

Common welfare concerns in poultry production include bone fractures and improper gait, which can cause pain, tension, decreased feed intake and subsequent low weight gain. The current findings indicates that adequate macro and micro-mineral supplementation, including in ovo feeding of Ca, P, Cu, Mn, and Zn, can reduce the severity of keel bone fractures and improve the overall movement activities of broiler chickens (Puvadolpirod and Thaxton, 2000; Ebrahimi et al., 2018; Kraler et al., 2020). Similar findings were reported the positive influence of mineral supplementation, either alone or in combination with other compounds such as carbohydrates, amino acids, or vitamin D3, on addressing musculoskeletal issues in broilers (Nouri et al., 2018; Oikeh et al., 2019; de Carvalho et al., 2021; Waldenstedt, 2006; Saeed et al., 2017; Trautvetter et al., 2014). However, the assessment of welfare issues over restricted minerals feeding could reveals the importance of pre- and post-hatch minerals feeding in broiler.

In summary, the research on in ovo supplementation of macro and micro-minerals, particularly calcium, during early embryonic development has demonstrated significant effects on bone morphometry, mineralization, and strength in broiler chickens. This comprehensive nutritional approach positively impacts broiler performance throughout their growth phase. While in ovo feeding appears to have a limited impact on welfare issues, proper management practices play a crucial role in addressing bone-related deformities. The supplementation of minerals, alone or in combination with other essential compounds, further emphasizes the significance of a comprehensive nutritional approach in promoting optimal bone health and addressing welfare concerns in broiler chickens.

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