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INVESTIGATING THE IMPACT OF IN-OVO MINERAL SUPPLEMENTATION ON POST-HATCH GROWTH AND DEVELOPMENT IN BROILER CHICKENS

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Abstract

In-ovo mineral feeding is a technique used in poultry farming, where essential minerals are injected directly into the fertilized eggs of birds during the incubation process. This method is employed to provide developing embryos with vital minerals, such as calcium, phosphorus, and trace elements like zinc and selenium, to support their growth and development. The injected minerals are absorbed by the developing chick through the egg's amniotic fluid, helping to ensure that the chicks have a better mineral status when they hatch, potentially leading to improved health and performance. This technique is typically performed under controlled and sanitary conditions to enhance the overall well-being of the poultry flock. The present study aimed to evaluate the impact of in-ovo mineral feeding on the growth performance, nutrient absorption, and welfare indicators of broiler chickens. A total of 700 chicks, hatched from an in-ovo feeding trial, were utilized and divided into two treatment (n=600) and control (n=100) groups. The treatment group was further divided into six sub-groups based on macro (magnesium; Mg, calcium; Ca, and phosphorous; P) and micro (Zinc; Zn, manganese; Mn, and copper; Cu) minerals. In-ovo Mg feeding significantly increased feed intake (3294 g/b, P<0.05), while in-ovo Zn feeding enhanced body weight gain (2111.25 g/b, P<0.05), FCR (1.53, P<0.05), and European broiler index (371.86, P<0.05). In-ovo mineral feeding also improved nutrient absorption and digestibility, with the highest values recorded in Zn-treated birds for dry matter digestibility (77.70±0.65%, P<0.05) and protein digestibility (83.13±0.65%, P<0.05). Welfare indicators, such as pododermatitis score (98% in no lesion category, P<0.05) and hock burn/swollen joints score (96% in good category, P<0.05), showed better results in the Ca and Zn-treated groups, respectively. These findings suggest that in-ovo mineral feeding, particularly Zn, can optimize broiler chicken performance, nutrient absorption, and welfare, thus providing a potential strategy to enhance poultry production efficiency.

Keywords: *Digestibility, Growth performance, In-ovo minerals feeding, Mineral absorption/retention, Welfare issues*

INTRODUCTION

In-ovo feeding is an innovative and efficacious approach for administering essential nutrients and bioactive compounds to the developing embryo within the egg (1). The macro and micro-minerals (Zn, Cu, Mn, Mg, Ca, and P) are critical for the healthy development of broiler chicks. In-ovo feeding of minerals enhances post-hatch body weight, feed conversion ratio, meat yield (Uni et al., 2015) and improves immune function, and reduces disease incidence in broiler chickens (2).

Provision of adequate dietary trace and macro-minerals improve the skeletal system, immune system, gut health, and cardiovascular system and in turn reduce the mortality rate in growing broiler (3-5). The role of Ca and P in body growth and skeletal development or metabolic processes (6), Mg in energy metabolism and neuromuscular function (7), Mn for bone formation, enzyme function, and antioxidant protection (8), Cu for various enzymatic reactions, iron metabolism, and connective tissue formation (9), Fe in cellular oxidative energy metabolism (10), Mn in bone growth and embryonic development and Zn involvement in the immune system (11), is crucial in poultry production.

As minerals reserves deplete in egg yolk during the late window of incubation; therefore, in ovo minerals feeding technique provides an additional allowance for growing embryos and growing jump for chicks after hatching (12). In ovo feeding of minerals is getting popularity to enhance the metabolism, growth, and immune function in broiler embryos and post-hatched birds if stress factors i.e. mineral deficiency, disease, environmental, physical stress, etc., are encountered (13). Given the significance of mineral feeding on the growth performance of broilers, this study was designed to investigate the effect of *in-ovo* feeding of micro-(Zn, Mn, and Cu) and macro-(Mg, Ca, and P) minerals on the post-hatch performance of broiler chickens.

MATERIALS AND METHODS

IN-OVO FEEDING OF MINERALS

A total of 700-day-old chicks, hatched from an in-ovo feeding of micro-(Zn, Mn, and Cu) and macro-(Mg, Ca, and P) minerals trials at day 12 of incubation, were used in this experiment. The chicks were divided into a control group (n=100) and a treatment group (n=600). The treatment group was further subdivided into six groups (n=100 each), based on the type of mineral in-ovo feeding, consisting of three macro minerals (Mg, Ca, and P) and three micro minerals (Zn, Mn, and Cu).

POULTRY HOUSE MANAGEMENT

Before the arrival of the chicks, the farm was thoroughly cleaned and disinfected, including the floor, premises, and utensils. A wood-shaved litter with medium-sized particles was spread on the floor, and brooding equipment, feeders, drinkers, and other necessary equipment were installed. Fumigation was performed to disinfect the house further.

Upon the chicks' arrival at the farm, the predetermined temperature and humidity index was maintained for the two-week brooding period, and all standard management practices were followed.

GROWTH OF BIRDS

The growth performance of broiler birds during the starter (0-21 days) and finisher (22-35 days) rearing periods, was observed using the following parameters. The chicks were weighed weekly using an electronic balance, and their final weight gains were compared to the control group. The formulated diets were weighed and recorded daily. The remaining feed was noted and weighed at the end of each week. Feed intake was calculated by subtracting the refused feed from the offered feed and dividing the total consumed feed by the number of chicks per replicate.

Feed intake (gm) = feed offered (gm) – Feed refused (gm)

The feed conversion ratio was calculated weekly as feed intake per kg divided by body weight gain in kg.

FCR = feed intake / total live body weight gain

This factor standardizes technical results, considering feed conversion, mortality, and daily weight gain. The European Broiler Index compares technical results but does not automatically imply economic comparability.

(Average grams gained/day × survival rate (%) / FCR × 10

The mortality rate was calculated by totaling the number of deaths, dividing by the number of chickens in the house on that day, and multiplying by 100.

POST-HATCH MINERAL RETENTION IN CHICKS

After 35 days of age, eight birds were randomly selected from each replicate of their respective groups, confined to separate compartments, and their faeces were collected and preserved. The faecal samples were then subjected to atomic absorption to determine individual minerals in samples taken from their respective groups, and the results were interpreted.

POST-HATCH NUTRIENTS DIGESTIBILITY IN CHICKS

At the end of the experiment, eight chickens from each group were separately placed in metabolic cages for faeces collection. Faecal samples were collected for three consecutive days and brought to the laboratory for further analysis. Using standard procedures and formulas, the samples were dried, ground, and analyzed for dry matter content, crude protein, ether extraction (fat content), and ash content.

The dry matter content of the faecal sample was concluded by the method as defined by AOAC (2005). Fresh faeces samples were collected, dried in a hot air oven, and then ground and processed. Dry matter after processing was calculated from the following formula.

$$\text{Dry matter (\%)} = W2\text{-}W3/W2\text{-}W1 \times 100$$

Crude protein content was evaluated, allowing the procedure. The sample was digested with the help of a Micro-Kjeldhal digester. The fresh faeces sample were collected, dried in a hot air oven, grind, and then processed. After processing, protein% was calculated by the following formula:

Nitrogen (%) = 1.4 (V1-V2) × regularity of HCL /weight of sample carried x volume of reduced ×250

The soxhlet isolation component was used to determine the total fat as described. Soxhlet Extraction was set with a reflux condenser and distillation flask. Fresh faeces samples were collected, dried in a hot air oven, grind, and then processed. After processing, fat% was calculated by the given formula,

$$\text{Fat (\%)} = W1\text{-}W2/W3 \times 100$$

Ash content was used to determine the ash percentage %, as a muffle furnace procedure. Fresh mill samples were collected, dried in a hot air oven, and then ground and processed. After processing the ash material, it was concluded by utilizing the following formula.

Ash (%) = Weight of ash sample / Weight of sample take off ×100.

WELFARE ISSUES IN POST-HATCH BROILER CHICKS

The pododermatitis /bumble foot lesions scoring on the skin of the foot pad were investigated at the age of the 5^{th} week. Pododermatitis lesion scoring was scored in each replicate of respective groups. The feet of each bird were examined according to the base of severity as no lesion, mild lesion, more lesion, and severe. After scoring, pododermatitis was measured as %age.

Hock burn was investigated at the age of 5^{th} week. Hock burn scoring was scored in birds of each replicate of respective groups. The bird's hock joint was observed according to score based on severity as good, bad, very bad, and worst. The Hock burn scoring was measured as %age.

STATISTICAL DESIGN

Data were analyzed using JMP software. One-way analysis of variance (ANOVA) was employed for data analysis. The LSD test was applied among the groups to estimate significant effects among the variables of in-ovo feeding of mineral supplements on post-hatch broiler chicken performance.

RESULTS

GROWTH PERFORMANCE OF BROILER CHICKS HATCHED OUT FOLLOWING IN OVO FEEDING OF MACRO- AND MICRO-MINERALS

The result of in-ovo feeding of macro (Ca, P, and Mg) and micro (Cu, Mn, and Zn) minerals on broiler overall weight gain, feed intake, and FCR is presented in Table I. The feed intake was the same across the birds hatched out after in-ovo feeding of micro- and macro-minerals when reared either on starter (0 to 21 days) or finisher (22 to 35 days) feed. In contrast, average feed intake was significantly higher ($P<0.05$) in birds hatched out after ovo feeding of Zn, Ca, P, and Mg than the rest of the groups. The weight gain was the same among birds of different groups during 0 to 21 of the growth period The feed intake, w eight gain, and FCR were the same between the groups when post-hatch chicks were reared on starter feed (0 to 21 days). A significant ($P<0.05$) decreasing trend of weight gain was observed during the 22 to 35 days of the

Table I. The ingredients and nutrient composition of starter and finisher feed offered to broiler chicks

Starter ration (0-21 days)		
Ingredients	**Treatment**	**Control**
Maize	55.550	55.550
Soyabean meal 44%	30.880	30.880
Poultry (BP) meal	3.000	3.000
Lysine sulphate	0.540	0.540
Methionine	0.310	0.310
Salt	0.340	0.340
L threonine	0.150	0.150
Vegetable oil	1.800	0.000
Phytase	0.010	0.010
Vitamin premix	0.050	0.050
Mineral premix	-	0.050
Choline chloride (70%)	0.070	0.070
Toxin binder	0.050	0.050
Wheat bran	4.700	4.700
L. isoleucine	0.040	0.040
Carrier (rice polish)	0.010	0.010
Nutrients		
Moisture %	12	12
Net energy (Kcal/kg)	2900	2900
Crude protein (%)	21.5	21.5
Lysine (Av.) (%)	1.2	1.2
Meth+Cys (Av.) (%)	0.9	0.9
Threo (Av.) (%)	0.84	0.84
Valine (Av.) (%)	0.9	0.9
Iso (Av.) (%)	0.81	0.81
Sodium (%)	0.18	0.18
Chlorides (%)	0.26	0.26
Ash (%)	3	3
Ether extract (%)	4.75	4.75

Finisher ration (21-42 days)		
Ingredients	**Treatment**	**Control**
Maize	57.150	57.150
Soyabean meal 44%	29.000	29.000
Poultry (BP) meal	3.000	3.000
Lysine sulphate	0.350	0.350
DL methionine	0.260	0.260
Salt	0.350	0.350
L threonine	0.050	0.050
Wheat bran	4.600	4.600
Vegetable oil	3.000	0.000
Phytase	0.010	0.010
2Vitamin premix	0.050	0.050
Choline chloride (70%)	0.070	0.070
Minerals Premix	-	0.050
Toxin binder	0.050	0.050
Carrier (rice polish)	0.010	0.010
Nutrients		
Moisture %	12	12
Net energy (Kcal/kg)	3000	3000
Crude protein (%)	20.5	20.5
Lysine (Av.) (%)	1.05	1.05
Meth+Cys (Av.) (%)	0.82	0.82
Threo (Av.) (%)	0.7	0.7
Valine (Av.) (%)	0.86	0.86
Iso (Av.) (%)	0.74	0.74
Sodium (%)	0.18	0.18
Chlorides (%)	0.26	0.26
Ash (%)	2.8	2.8
Ether extract (%)	6	6

growth period in chicks hatched out from in-ovo feeding of Cu, Mn, Zn, Ca, Mg, P, and control, respectively. The average weight was significantly higher (P<0.05) in Zn followed by Mg, and Ca group birds hatched out after in-ovo feeding. The average FCR and FCR between 0-21 days was similar in birds hatched out after in-ovo feeding; however, a significantly higher FCR was observed in control and P groups birds compared to other treatment group birds hatched out after in-ovo feeding. The European broiler index was significantly (P<0.05) different in birds receiving in-ovo Zn and Mg feeding compared to the rest of the groups (Fig. 1).

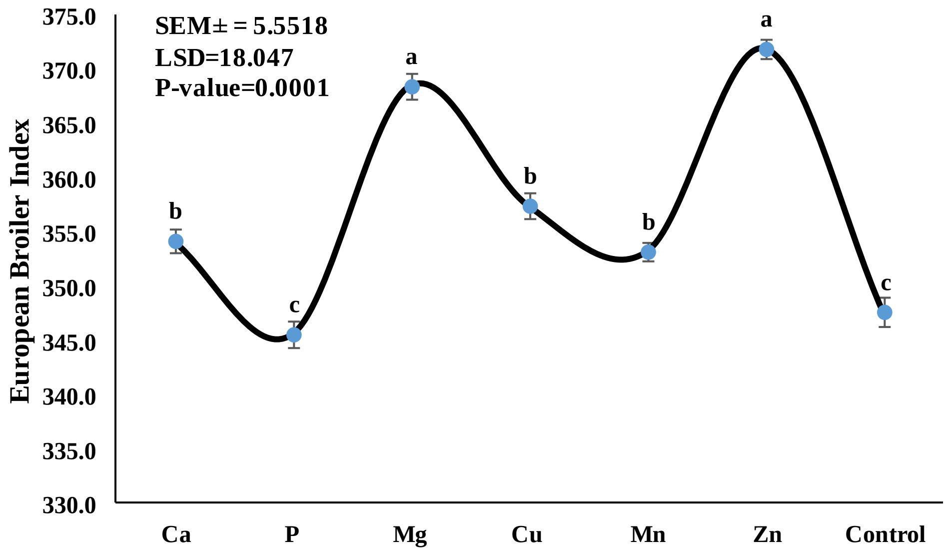

Fig. 1. Effect of in-ovo feeding of macro (Ca, P, and Mg) and micro (Cu, Mn, and Zn) minerals on European broiler index of post-hatch broiler progeny. *Different superscripts (a, b, c) indicates significant (p<0.05) difference across the groups

EFFECT OF *IN-OVO* FEEDING ON MINERALS ABSORPTION/RETENTION

The effect of in-ovo feeding of macro (Ca, P, and Mg) and micro (Cu, Mn, and Zn) minerals on the absorption/retention of minerals has been illustrated in Fig. 2. The absorption of Ca, P, and Mg in the treatment groups was significantly (P<0.05) higher than in the non-treated (control) group. Similarly, the absorption of Cu, Mn, and Zn treated groups was significantly (P<0.05) different than the control group.

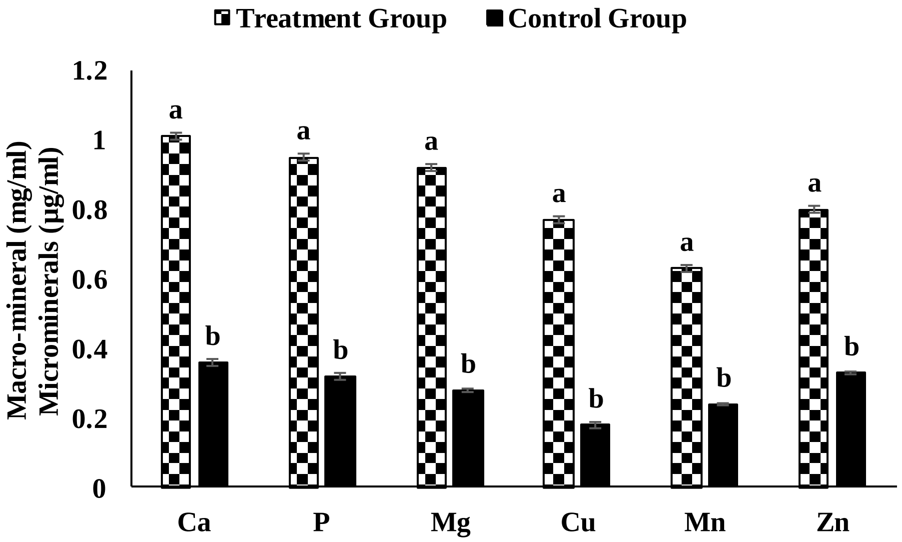

Fig. 2. Effect of *in-ovo* feeding of minerals on absorption/retention of macro minerals (mg/ml) and micro minerals (µg/ml). *Different superscripts (a, b) indicates significant (p<0.05) difference between the treatment and control groups

EFFECT OF *IN-OVO* FEEDING ON NUTRIENTS DIGESTIBILITY

The data presented in Table II showed the results of the effect of in-ovo feeding of minerals on nutrient digestibility. Digestibility of dry matter was significantly (p<0.05) affected by in-ovo feeding of minerals and maximum dry matter digestibility was determined in birds given in-ovo Ca or Mg in macro mineral and Zn and Cu in micro mineral feeding. A similar pattern of protein digestion was observed in in-ovo feeding of macro- or micro-minerals. In fat digestibility, the birds given in-ovo P feeding had maximum values compared to other macro- or micro-mineral feeding groups. However, ash digestibility was not affected by the in-ovo feeding of minerals.

Table II. Effect of *in-ovo* feeding of macro (Ca, P, and Mg) and micro (Cu, Mn, and Zn) minerals on nutrients digestibility of broiler birds (%)

Groups	Dry matter (Unit)	Protein (Unit)	Fat (Unit)	Ash (Unit)
Ca	75.60±0.70^{a}	82.40±0.11^{a}	74.40±1.10^{ab}	3.46±0.17
P	74.20±0.23^{ab}	81.20±0.41^{ab}	78.60±0.70^{a}	3.53±0.17
Mg	76.26±1.15^{a}	81.80±0.30^{a}	73.13±0.37^{b}	3.43±0.12
Cu	74.60±0.94^{a}	81.73±0.99^{a}	71.86±0.74^{b}	3.56±0.20
Mn	71.06±0.35^{bc}	81.00±0.41^{ab}	70.06±0.92^{b}	3.73±0.29
Zn	77.70±0.65^{a}	83.13±0.65^{a}	74.66±0.29^{ab}	3.13±0.40
Control	70.40±0.61^{c}	79.13±0.29^{b}	73.66±1.91^{b}	3.86±0.48
SEM±	1.0289	0.7483	1.4165	0.4133
LSD @ 0.05	3.5154	2.5567	4.8393	1.4119
P-value	0.0001	0.0035	0.0013	0.6990

EFFECT OF *IN-OVO* FEEDING ON WELFARE ISSUES

The results of in-ovo feeding macro (Ca, P, and Mg) and micro (Cu, Mn, and Zn) minerals effects on mortality, pododermatitis/bumblefoot score, black bone syndrome, curled toe paralysis, lameness, gait score, and hock burn/hock joints swollen score is presented in Table III. The results showed that the mortality ratio of birds was non-significant either provided administered macro- (Ca, P, and Mg) or micro-minerals (Cu, Mn, and Zn) through in-ovo feeding. The pododermatitis scores and black bone syndrome were not affected in birds hatched out by the in-ovo administration of macro (Ca, P, and Mg) and micro-

Table III. Welfare issues in broiler progeny hatched-out following *in-ovo* feeding mineral feeding

Groups	Mortality (%)	Pododermatits (%)				Black bone syndrome (%)		Curled toe paralysis (%)		Lameness (%)		Hock burn (%)				Gait score (%)	
		No lesion	Mild	More	Severe	Normal	Defected	Normal	Defected	Normal	Defected	Good	Bad	Very bad	Worst	Normal	Abnormal
Ca	5	98	2	0	0	98	2	97	3	96	4	95	3	2	0	98	2
P	5.25	97	2	1	0	96	4	95	4	95	5	94	4	2	0	97	3
Mg	4.62	95	2	3	0	94	6	93	7	93	7	93	4	3	0	95	5
Cu	5.57	92	3	3	2	93	7	92	8	92	8	91	5	3	1	94	6
Mn	5.67	91	5	3	1	92	8	96	4	91	9	92	2	1	1	93	5
Zn	3.53	96	2	2	0	95	5	94	6	94	6	96	3	1	0	96	4
Control	5.64	93	3	3	1	92	8	93	7	92	8	93	4	3	0	95	5
χ^2 test	5.3				13.81		5.46		4.09		3.10				8.99		2.81
P-value	0.5466				0.7413		0.4860		0.6640		0.7960				0.9599		0.8319

minerals (Cu, Mn, and Zn). Similarly, the curled toe paralysis, lameness, and hock burn/hock joints swollen scores were also similar in the experimental birds either administered macro- (Ca, P, and Mg) or micro-minerals (Cu, Mn, and Zn) through in ovo feeding. The gait score also remained unchanged in birds hatched out following the administration of macro- (Ca, P, and Mg) or micro-minerals (Cu, Mn, and Zn) throughout ovo feeding.

DISCUSSION

The present study demonstrates that in-ovo feeding of macro- and micro-minerals can have an impact on broiler chickens growth performance, mineral absorption, and nutrient digestibility. The performance variable showed that ovo feeding by Ca, Mg, P, and Zn had improved the feed intake in post-hatched birds. The current promising results align with previous findings where Zn was provided through in-ovo technique and subsequent post-hatch performance (14-17); however, the post-hatch performance of birds hatched-out macro-minerals is not comparable due unavailability of similar literature. The current results indicate that in-ovo feeding of minerals is involved in enhancing growth performance, mineral absorption, and nutrient digestibility in broiler chickens. Tako et al. (2005) reported a significant improvement in growth performance and immune response in broilers after in-ovo feeding of Zn, Mn, and Cu, consistent with our findings but Mn and Cu supplementation had no influence in this study which might be linked to dose selection, day of in-ovo feeding and housing management (18). Attainment of maximum weight gain along minimum FCR during the finisher phase in the birds hatched-out following Cu and Zn in-ovo feeding indicates the beneficial impact of trace minerals over the growth variables. The overall weight gain with optimum FCR in broiler hatched out following Zn in-ovo feeding clearly describe the obvious potential carrying-over effects of Zn in the growth of broiler birds.

The nutrient digestion and absorption have occurred in small intestine plays which are completely developed and functional at hatch. Chicks feed intake shifts from endogenous to exogenous nutrients is a critical phase that greatly depends on the functional small intestine and pancreatic activities. Hence post-hatch pancreatic and intestinal histogenesis in a continuous development manner is necessary for optimal during in the next 6 to 8 weeks of age. Previously luminal digestion activity is enhanced in post-hatched birds after ovo supplementation of certain bioactive substances in the broiler (19). The present results indicate promising nutrient digestibility in post-hatch birds after ovo feeding of Zn followed by Mg, Ca, P, and Cu. The provision of in-ovo minerals might improve the small intestine development and functionality which in turn improves the feed intake and weight gain. It is observed that Zn-compromised birds have shortened and narrow villi that lead to malfunctioning of the small intestine (9); therefore, the in-ovo feeding of Zn at advanced stages could be an option to improve intestinal development and functioning. Moreover, the Ca and P have a participatory role in prolonging the retention time of feed ingredients and continuing the peristalsis in birds (20) Ravindran and Abdollahi, 2021). The provision of macro-minerals in-ovo feeding seems a better option in post-hatch birds to improve nutrient digestibility by feed retention. However, the estimation of isomorph of the intestine and pancreatic enzyme activities in post-hatch birds at a different level of growth, need to be elucidated by the involved mechanism.

The studies documenting the retention coefficients for macro and micro-minerals, in-ovo feeding studies, in poultry are scanty (4). To the authors' knowledge, no previous data was published in post-hatch birds, following micro or macro-mineral supplementation. There are significant differences in excreta minerals retention all the birds hatched out after ovo feeding of macro and micro-minerals which explained the high availability and low retention of minerals. Under such conditions, a grower diet fortified with phytase or organic source minerals could be an option to enhance the utility of minerals in-ovo fed post-hatched broilers.

The mortality rate in broilers is one of the basic welfare indicators that depict the health status of the flock. In the present, no significant difference was observed in mortality rate across the groups which could be linked to better house management, proper vaccination, and nutritional management. The least mortality rate was seen in the Zn-supplemented group which shows the immunomodulation, growth-promoting, and gut health effects of Zn. A recent study revealed that supplementation of Zn and Vitamin E had significant effect on egg production, quality and hatchability reread under different dietary treatments (21).

Regarding the welfare aspects of in-ovo fed post-hatched birds, no change was observed in pododermatitis, black bone syndrome, curled toe paralysis, lameness, hock burn, and gait score. This is in contrast to the findings of other studies that reported reduced incidences of pododermatitis (22) and hock burn (23, 24) in broilers receiving in-ovo minerals. However, some studies also support our findings, reporting no significant impact of in-ovo mineral feeding on welfare indicators (16, 25). The skeletal problems affect broiler welfare due to reduced access to feed and water owing to lameness, low walking ability, and pain (12). As a concern for economics and welfare, it is focused to minimize the skeletal problems in broilers and reduce mortality (26). The welfare issue could be greater and more severe when broilers are suffers from pain following footpad lesions due to bacterial infection (27), moist litter, low nutrition, and genetic susceptibility (28).

The present study supports and expands upon the current literature on in-ovo mineral feeding and its potential to enhance growth performance, mineral absorption, and nutrient digestibility in broiler chickens. Nevertheless, when interpreting the results of different studies, it is essential to consider the multifactorial nature of broiler performance and welfare, including factors such as genetics, management practices, and environmental conditions.

CONCLUSION

In conclusion, the findings of this study suggest that in-ovo feeding of minerals, particularly Zn, has a significant positive impact on the growth and development of broiler chickens. Furthermore, the digestibility of nutrients was affected by the in-ovo feeding of minerals. The absorption of several essential minerals, including Ca, P, Mg, Cu, Mn, and Zn, was improved in the treated group compared to the non-treated control group. However, the effect of in-ovo feeding on welfare issues was not linked to the present study.

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