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Exploring the Confluence of In-Ovo Mineral Supplementation and Hatching Attributes in Broiler Chick Progeny: A Symphony of Nutritional Enrichment and Broiler Chicks Development

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ABSTRACT

Embryonic nutrition is a key factor that influences broiler progeny performance with long-lasting nutritional adequacies or insufficiencies in the developmental phase. **Objective:** To explores the effects of in-ovo mineral supplementation or day in-ovo feeding on the hatching attributes and chick quality in broiler breeders. **Methods:** About 1400 hatching eggs of broiler breeders were selected, categorized into seven experimental groups: one control group, three macro mineral (P, Mg, and Ca) groups, and three micro-mineral (Zn, Mn, and Cu) groups. During the 12th and 18th day of incubation, eggs received in-ovo injections of their respective mineral (5% of the total mineral content in a 50g egg) solutions, prepared by dissolving the corresponding salts in deionized water. Post-injection, eggs were placed in the setter, and upon completion of incubation, hatching trays were removed to evaluate hatchability performance and chick quality parameters for each group. **Results:** The results showed that Zn, Ca, and Mg-supplemented eggs demonstrated the highest ($P<0.05$) hatchability rates for settable and fertile eggs at day 12 of in-ovo feeding. The chick weight, hatch window, and hatchling size were significantly influenced ($P<0.05$) by in-ovo feeding and the day of in-ovo feeding. However, no effect of in-ovo mineral feeding and day of in ovo feeding was observed on chick quality (chick grades, navel area, retracted yolk, and membrane score) and post-hatch chick activities (activity, down-appearance, vitality, eye score, and gait score). **Conclusions:** In conclusion, Zn, Ca, and Mg in ovo feeding improve the hatchability traits when administered at day 12 of incubation.

INTRODUCTION

Embryonic nutrition is a key factor that influences broiler progeny performance with long-lasting nutritional adequacies or insufficiencies in the developmental phase [1]. Like other dietary factors, adequate minerals in broiler-hatching eggs promote the skeletal, muscular, immune, and cardiovascular systems during embryo development

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[2]. Under natural circumstances, the mineral deposition occurs through ovarian transfer to yolk or oviduct contribution in albumin, shell, or shell membrane compartments [3]. These compartments fulfill the growth and development phases, particularly following day 18 of incubation, when functional abilities of the gut started because of the proliferation of enterocytes and intestinal crypts. At this time, amniotic fluid is consumed by the embryo orally first time, which is helpful for further intestinal tissue growth, maturation, and metabolism in the last phase of the prenatal and early post-hatch period [4]. Following the hatching of chicks, yolk residues are utilized as the main source of diet. From a growth, metabolism, and immune protection point of view, the abrupt shift from a yolk-based to an exogenous feed-based diet is crucial in hatched chicks. Maintaining essential nutrients while transporting hatched chicks from the hatchery to the farm is required because the concentration of macro and micro minerals is very low [5]. Under such conditions, the day-old chicks can easily be exposed to disease conditions with retarded growth. Beyond genetic selection for growth and the high metabolism of broilers, the proper diet provision at each growing phase plays a vital role. Nutrient shortage at the pre- and post-hatch stage leads to impaired musculoskeletal development; hence embryonic fortification by nutrients through in ovo technique is a potential strategy to mitigate such conditions. Previously, embryonic enrichment through in-ovo feeding with Cu, Fe, Mn, and Zn, phosphate, vitamin D3, and carbohydrates [6,7] enhanced the availability of supplemented minerals in the yolk for the embryo prior to hatch [8]. In ovo feeding of trace minerals have been utilized for various immunological, enzymatic, and metabolic processes [4, 5, 9-14]. However, utility of macro minerals and its influence on embryonic survival and growth has not yet been explored. In this context, this study aimed to investigate the impact of in-ovo micro- and macro-minerals feeding and day of incubation on the hatching traits of broiler breeders under prevailing environmental conditions of Pakistan.

METHODS

The study was carried out during March-April 2022 at Pir Mehar Ali Shah, Arid Agriculture University, Rawalpindi in the periphery of Islamabad (33.5637482, 73.2450181), Pakistan. A total of 1400 fertilized eggs were obtained on days 12 and 18 of incubation. The eggs were divided into treatment (n=1200) and control (n=200). The treatment group was subdivided into seven groups (n=200 each) based on the type of mineral in-ovo feeding. Three macros (magnesium; Mg, calcium; Ca, and phosphorous; P) and three micro (zinc; Zn, manganese; Mn, and copper; Cu) mineral groups were assigned for in-ovo inoculation.The selected macro (P, Mg, and Ca) and micro (Zn, Mn, and Cu) minerals (Sigma-Aldrich Chemicals USA) were dissolved in distilled water considering safety margins and osmolality to prevent embryonic toxicity. The solution was filtered to remove impurities and processed for sterilization to prevent bacterial contamination. The sample solutions of minerals for in-ovo feeding in broiler breeder hatching eggs were investigated for minerals availability through the digestibility process, and values were determined through atomic absorption spectrophotometer.

In ovo mineral feeding to hatching eggs

After obtaining the required mineral sample solutions through atomic absorption, the samples were introduced into hatching eggs at a hatchery. Initially, the selected hatching eggs were marked in egg trays, and in ovo, injections were performed in the air sac of eggs on the 12th day of incubation before transferring them to the hatcher. The mineral dosages (by weight) comprised 5% of the total mineral content in a large 50-gram egg. Each mineral solution was prepared by dissolving the respective salts in 1 ml of deionized water, and the required dosages were obtained in 200 µl for in-ovo injection. The dosages needed for each macro (Ca: 1.35 mg/egg, P: 4.77 mg/egg, and Mg: 0.3 mg/egg) and micro (Zn: 27 µg/egg, Mn: 0.95 µg/egg, and Cu: 2.55 µg/egg) mineral were injected to respective groups through the air cell of eggs using a small needle syringe [5]. Subsequently, the micro-hole was sealed with wax to minimize pathogen entry into the egg. Upon completing the in-ovo feeding process, the eggs were transferred to hatchers.

Observation of hatching traits

After incubation, the hatching trays were removed from the hatcher, and the following parameters were observed to assess hatchability performance and chick quality for each experimental group:

The hatchability of settable eggs was determined by calculating the number of chicks hatched relative to the total number of eggs set in the incubator.

$$\text{Hatchability of settable eggs} = \frac{\text{Total number of chicks hatched}}{\text{Total number of settable eggs}} \times 100$$

The hatchability of fertile eggs was measured by a total number of chicks hatched from the total number of fertile eggs or % the age of total chicks hatched from fertile eggs.

$$\text{Hatchability of fertile eggs} = \frac{\text{Total number of chicks hatched}}{\text{Total number of fertile eggs}} \times 100$$

The "hatch window" or "spread of hatch" refers to the time interval between the first and last chicks hatching. Monitoring the hatch window indicates whether chicks are being removed from the hatcher at the appropriate time. Hatched chicks that remain in the hatcher for extended periods may dehydrate and lose condition. The hatch

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window was observed in hours for each replicate group.

Determination of hatching traits

Chick quality parameters were measured using the Tona score method [15]. The chick's weight was measured utilizing an electrical weighing balance. The weight was taken randomly in grams from each replicate in groups. Hatchling length was determined by stretching the chick along a ruler and measuring the length from the beak to the end of the middle toe. Chick length measurements were taken randomly for each replicate group. Chicks were placed on their backs and observed to see how quickly they returned to their feet. An immediate return to their feet was considered strong, while a delayed return or remaining on their backs was considered weak. The activity was assessed by scoring as good or weak for each replicate in different groups and expressed as a percentage**.** Chicks were expected to be dry and clean, free from adhering dried yolk, shell, and membranes. Down and appearance were evaluated by scoring individual chicks and quantifying the results as a percentage distribution of clean and dry, wet, or wet and dirty chicks within each group. Following hatching, chick movement was observed and classified as good or bad. Gait scores were documented numerically within the replicates of respective groups and expressed as a percentage.

Statistical analyses

Data analysis was conducted using JMP software. A one-way analysis of variance (ANOVA) was applied to evaluate the data. To estimate significant effects among the variables of in-ovo feeding of mineral supplements on pre-hatch broiler chicken performance, the Least Significant Difference (LSD) test was applied across the groups.

RESULTS

The impact of in-ovo injection of macro (calcium (Ca), phosphorus (P), and magnesium (Mg) and micro (copper (Cu), manganese (Mn), and zinc (Zn) minerals and day of in-ovo feeding on the hatchability of settable eggs is illustrated in Table 1. The hatchability of settable eggs was significantly ($P<0.05$) influenced by the in-ovo administration of Ca and Mg in macro- and Zn in micro-minerals. The hatchability of fertile eggs was significantly ($p<0.05$) influenced by the in-ovo administration of Mg and Zn minerals. Compared to day 18 in ovo feeding, day 12 administration significantly ($p<0.05$) improved the hatchability of settable and fertile eggs; however, no interaction was found between the day of in ovo feeding and the type of macro- or micro-minerals in ovo feeding. The results presented in Table 1 indicate that chick weight was significantly ($P<0.05$) affected by the in-ovo feeding of Zn compared to Mn, Cu, and control groups. There were no significant ($P>0.05$) difference in chick weight by in-ovo feeding injected at 12^{th} and 18^{th}. The hatchling length was influenced ($P<0.05$) by the day and in ovo minerals feeding with a tendency in interaction. The hatch window was significantly ($p<0.05$) influenced by the day and in-ovo feeding treatment, but no interaction was observed between the studied factors. Internal or external pipped not hatched rate were non-significant by the in-ovo feeding treatments given at 12 and 18^{th} day of incubation.

Table 1: The effects of macro- or micro-minerals in ovo feeding and day of in ovo feeding on hatching traits of broiler breeder eggs

Main effects		Settable eggs hatchability (%)	Fertile eggs hatchability (%)	Hatch window (h)	Chick weight (gms)	Hatchling length (cm)	Internal pipped not hatched (%)	External pipped not hatched (%)
In ovo feeding (Day)	12	71.607a	86.621a	27.25b	38.46	18.30a	2.21	2.03
	18	70.214b	84.107b	29.25a	37.32	17.08b	2.53	2.32
In ovo macro and micro-minerals feeding (Treatment)	SEM	0.33	0.65	0.39	0.51	0.31	0.29	0.24
	Control	60.12e	79.87c	31.37a	35.37b	18.81ab	3.12	3.12
	Mg	76.87b	89.62ab	28.87abc	38.87ab	16.42bc	1.75	1.37
	Ca	72.75c	84.75bc	25.62c	38.87ab	20.81a	2.01	1.75
	P	67.12d	82.92c	27.12bc	38.0ab	16.57bc	2.62	2.12
	Cu	66.62d	84.37bc	29.12ab	35.25b	15.37c	1.87	1.87
	Mn	62.37e	81.87c	28.62abc	37.125b	17.56bc	2.37	2.37
	Zn	90.5a	94.12a	27.0bc	41.75a	18.31ab	2.87	2.62
	SEM	0.62	1.23	0.74	0.95	0.59	0.056	0.045
ANOVA								
Day		0.005	0.01	0.001	0.121	0.01	0.443	0.420
Treatment		0.001	0.001	0.0001	0.0001	0.0001	0.493	0.174
Day × Treatment		0.972	0.998	0.579	0.996	0.094	0.831	0.998

The results presented in Table 2 indicate that chick grades (A grade chicks were observed by weight and free from any abnormalities (ideal weight 38-45g), B grade chicks were observed by weight and some abnormalities and less activity (ideal weight below 38g), and C grade chicks were observed by weight which below 35g and have any abnormality, navel area (completely closed, not closed, not discolored, and discolored and open), retracted yolk score

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(normal or large), remaining yolk score (no yolk, small and large) and remaining membrane score (no membrane, small and large) were similar in hatched chick received macro or micro-minerals in-ovo feeding at day 12 and 18 of incubation.

Table 2: Effect of day of in ovo feeding and macro- or micro-minerals in ovo feeding on hatched chick quality traits

Treatment groups	Chick grades (%)			Navel area (%)			Retracted yolk score (%)		Remaining yolk score (%)			Remaining membrane score (%)		
	Grade A	Grade B	Grade C	Completely closed	Not closed and not discolored	Discolored and open	Normal	Large	No yolk	Small	Large	No membrane	Small	Large
Control	83	8	9	89	6	5	88	12	90	6	4	91.5	4.5	4
Ca	85	8.5	6.5	93	4	3	91.5	8.5	92	5.5	2.5	94	2	4
P	82.5	9	8.5	87.5	6.5	6	89	11	88.5	6	5.5	96	2	2
Mg	84.5	8.5	7	94	3.5	2.5	92	8	93.5	4.5	2	94.5	2.5	3
Cu	81.5	9.5	9	87	6	7	88	12	87.5	6.5	6	92	4	4
Mn	80	8.5	11.5	89	8.5	2.5	86	14	88.5	6	5.5	92	3	5
Zn	87	8.5	4.5	96	2.5	1.5	94	6	93.5	3.5	3	93	3.5	3.5
x^2 test	8.195			20.10			7.02		10.96			11.68		
P-value	0.7629			0.0652			0.3186		0.5322			0.4590		
Day of in ovo feeding														
Day 12	83.52	8.28	8.14	92.9	4.6	2.6	92.1	7.9	93.4	4.4	2.1	95.9	1.1	3.0
Day 18	84.57	8.0	7.43	88.7	6.0	5.3	87.4	12.6	87.6	6.4	6.0	90.7	5.0	4.3
X^2 test	5.333			8.632			9.333		11.000			2.667		
P-value	0.619			0.564			0.407		0.202			0.751		

The results of effects of in-ovo injection of macro (Ca, P and Mg) and micro (Cu, Mn and Zn) minerals and in ovo feeding day (12 and 18 day) on post-hatch chicks activities score indicate that a similar proportion of birds exhibited good and weak activities. Moreover, there was no effect of in ovo injection of macro and micro minerals or in ovo feeding day on the down appearance score of post-hatch chicks, and it had a clean and dry appearance rather than a wet or dirt wet appearance. The vitality and eye scores also remained similar in post-hatch chicks providing macro- or micro-minerals by in ovo feeding either at day 12 or 18 of incubation. No significant impact of in-ovo injection of macro and micro minerals on gait scores of post-hatch chicks was observed on day 12 or 18 of incubation (Table 3).

Table 2: Effect of day of in ovo feeding and macro- or micro-minerals in ovo feeding on hatched chick quality traits

Treatment groups	Activities score (%)		Down appearance score (%)			Vitality score (%)		Eyes score (%)		Legs score (%)		Legs score (%)	
	Good	Week	Clean and dry	Wet	Dirty and wet	Good	Week	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Control	88.5	11.5	90	5.5	4.5	88	12	90	10	88.5	11.5	87	13
Ca	91.5	8.5	94	2.5	3.5	94.5	5.5	93.5	6.5	96	4	95	5
P	92	8	90.5	4.5	5	89	11	92	8	93	7	90.5	9.5
Mg	93	7	95	3.5	1.5	90	10	91.5	8.5	91	9	91.5	8.5
Cu	87.5	12.5	89.5	7	3.5	86	14	90.5	9.5	88.5	11.5	92	8
Mn	86.5	13.5	89	7.5	3.5	89.5	10.5	91.5	8.5	94	6	90	10
Zn	94.5	5.5	95	3	2	89.5	10.5	94.5	5.5	91	9	92	8
x^2 test	6.94		8.37			7.48		7.77		6.62		8.13	
P-value	0.3261		0.7556			0.2789		0.2553		0.3577		0.2289	
Day of in ovo feeding													
Day 12	92.3	7.7	93.7	3.7	2.6	91.1	8.9	94.1	5.9	93.4	6.6	93.9	6.1
Day 18	88.7	11.3	90.0	5.9	4.1	87.9	12.1	89.7	10.3	90.0	10.0	88.4	11.6
X^2 test	7.333		7.533			7.200		8.000		8.954		10.000	
P-value	0.602		0.197			0.515		0.534		0.756		0.530	

DISCUSSION

In the present study, we investigated the impact of in-ovo macro- and micro-minerals supplementation on the hatchability and quality of broiler chick progeny. Our findings revealed that eggs injected with zinc (Zn) exhibited comparatively higher hatchability rates. This can be attributed to the increased efficiency of nanoparticle

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absorption and biological availability and enhanced interaction with other materials due to active surface area [16]. Adequate levels of zinc are essential for the overall performance of poultry, as it is involved in numerous biological systems and serves as a key component of many enzymes [17-19]. The application in-ovo feeding window influences the hatchability, hatch window, and hatchling length but has no effects on subsequent hatch health and activities. In contrast, Nowaczewski *et al.*, (2012) suggested that late-in incubation of vitamin C favors the hatchability attributes [20]. However, variation exists when different substances are used before chick development [3]. Our results align with previous studies that observed improved hatchability rates following in-ovo zinc supplementation [13, 21-23]. Similar outcomes were also reported in studies examining the effects of zinc supplementation in laying hen diets on hatching traits [24-26]. Variability in hatching parameters among studies can be attributed to differences in fertilized egg acquisition techniques. Furthermore, our findings are consistent with those of Ola Awais Hamza *et al.*, (2022) and Sahr *et al.*, (2020), who reported improved hatchability rates in eggs injected with Zn and Cu salts, as these minerals enhance the metabolic processes of embryogenesis due to active roles in physiological functions such as in improving reproduction, development of blood cells, immune system function, and bone development [27, 28] . Variations in the hatchability could be linked to injection volume, zinc concentration, injection time, and site of mineral deposition inside the egg [29]. However, Mg and Ca in ovo supplementation also showed promising results in terms of hatchability amongst the category of macro-mineral supplementation. To date, no published report is available about macro-minerals in ovo feeding in poultry; however, beneficial effects of dietary supplements of macro-minerals on post-hatch chick growth have been observed [30, 31]. Combined supplementation of Ca or Mg along with Zn in ovo feeding needs to investigate for embryonic development, hatchability, oxidative stress markers, and post-hatch growth of hatchlings. We observed a shorter and narrow hatch window duration in eggs injected with calcium (Ca) compared to other treatment groups. It indicates that narrow hatch windows allow the hatchlings early access to feed and water, ultimately maintaining the gut microbiota and subsequently improving the growth parameters [32]. This result is favorable as a shorter hatch window during vitamin E in ovo feeding is associated with the improved physical quality of broiler chicks, especially in incubators with single-hatching machine removals [33]. However, conflicting results have been reported in studies examining the effects of amino acid injection on hatch time [34], potentially due to differences in incubator conditions, fertile egg types, egg heterogeneity, and embryonic growth [35]. It is postulated that Ca in ovo feeding might have the potential to hasten embryonic growth, and the ultimate chick hatched out earlier compared to other chicks with compromised growth. In our study, chicks hatched from Zn-injected eggs exhibited the highest weight, and these findings are in agreement with those of several previous studies (21; 5; 22, 27; 13). Additionally, we observed the greatest hatchling length in chicks from Ca-injected eggs, consistent with Araujo et al. (2019), who reported improved chick quality following in-ovo vitamin E supplementation [36]. Current findings suggest that Ca in ovo supplementation is a potential way to enhance the hatchling length to attain better growth and earlier weight gain during the growth phase [37]. In addition, Ca-injected chicks exhibited the highest scores in activity, vitality, and gait categories, while magnesium (Mg)-injected chicks displayed the highest scores in down appearance. These findings resemble those of Nnanle *et al.*, (2017) and Al-Saeedi *et al.*, (2022), who observed improved chick characteristics following ovo zinc methionine injections [38, 39]. While dietary feeding, the unavailability of minerals, particularly Ca and P, affects the activities of chicks due to the production of defective feed, and chicks cannot consume the proper ratio of minerals [40]. Such deficiencies lead to skeletal and growth deformities, which can be ameliorated by in-ovo feeding during the late incubation stage. This study offers crucial insights into the effect of in-ovo mineral supplementation on the hatching characteristics and offspring quality of broiler breeders. However, it is essential to elucidate the specific mechanisms by which different minerals affect broiler chick hatchability, chick quality, and overall post-hatch development. This could involve investigating the metabolic and genetic alterations associated with supplementing various minerals. Additionally, the study can be expanded to evaluate the long-term effects of in-ovo mineral supplementation on mature bird growth, performance, and health. Investigating the optimal mineral supplementation levels for maximizing benefits while minimizing adverse effects would also be fascinating. In addition, it is necessary to examine the impact of combinatorial supplementation involving multiple minerals to identify potential synergistic or antagonistic effects. Lastly, the feasibility and economics of this method of mineral supplementation in large-scale poultry operations should be evaluated, which would contribute to its potential commercial application in enhancing the efficacy of poultry production.

CONCLUSIONS

It is concluded that Zn, Ca, and Mg in ovo feeding improve

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hatchability traits with no significant impact on post-hatch chick quality and physical activities. In ovo feeding at day 12 has comparatively better outcomes in terms of hatchability than at day 18 of incubation.

Authors Contribution

Conceptualization: IA
Methodology: NR
Formal analysis: IHL
Writing, review and editing: RRK, SS, ZA, FAK, NM, NR

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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