Original Research Article

ISSN (Print) 2786-0272 ISSN (Online) 2786-0280

Effects of in ovo zinc and curcumin nanoparticles feeding on hatchability, gut histomorphometric changes, and economic profile in broiler hatchlings

Asmaa H. Abdel-Kareem [1], Hassan A. Abdel-Rahman [2], Mohammed S. Abdel-Hafez [3], Ibrahim S. Zahran [4], Ahmed F. Abdalla [5] Mohammed A. Mahmoud [1]

[1]Department of physiology, Faculty of Veterinary Medicine, New Valley University, New Valley, Egypt.
[2]Department of physiology, Faculty of Veterinary Medicine, University of Sadat City 32897, Egypt.
[3]Department of poultry diseases, Faculty of Veterinary Medicine, New Valley University, New Valley, Egypt.
[4]Department of physiology, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt.
[5]Department of pathology and clinical pathology, Faculty of Veterinary Medicine, New Valley University, New Valley, Egypt.
*Corresponding author: asmaa.hussein@vet.nvu.edu.eg
Received at: 2024-11-18 Accepted at: 2024-11-30

ABSTRACT: Several nutritional and health management approaches are endlessly applied for optimizing the wellbeing and performance of day-old chicks. One of the promising techniques is nanoparticles (NPs) on ovo-feeding with zinc oxide (ZnO) and curcumin (Cur), with proven adding benefits for the poultry health. This makes sense beyond the use and examination of their nanoparticles for in ovo inoculation (IO). Although limited study, examined the combined effects of both zinc and curcumin nanoparticles in ovo feeding. This study aims to examine the possible combined effects of both zinc and curcumin nanoparticles by IO on the hatched broiler chicks physiological parameters, immune status, and gut health. Six hundred fertilized eggs (Ross 308) were randomly allocated into five groups with 4 replicates per group in a completely randomized design (n = 120). The 1st group (G1) was kept as negative control without injection (G1), while the second one (G2) was IO with vehicle (sterile distilled water-DW). The remaining eggs were IO with Cur-NPs (G3), ZnO-NPs (G4), and both of NPs (G5). Current work outcome results have shown a significant ($p < 0.05$) increase of heterophil counts in G4 and G5 compared to other tested groups. The intestinal villus height and width showed an important increase in G4 and G5 in relation to other ones. The present study concluded that zinc nanoparticles through IO has a positive effect on the day-old chick's physiological properties. As well as the gut health and integrity, that may positively affect the growth performance of the chick.

KEYWORDS: zinc oxide, curcumin, nanoparticles, *in ovo* inoculation, broilers eggs, hatchability traits, intestinal histomorphometric changes, economic profile.

1. Introduction

In the dynamic realm of poultry science, advancements in nutrition and health management strategies are crucial for optimizing the well-being and performance of day-old chicks [1]. Several approaches are applied directly to fertilized eggs as sanitation, optimal incubation parameters. Early in ovo inoculation is firstly used for studying and handling Marek's disease. Since then, this technology has been developed and used for experimental research work. By using this technique, researchers have managed to deliver several bioactive substances. Several challenges were theirs, like, for example, volume, concentration of the inoculated substances, site, route, and day of inoculation [2]. Further developments have been added through the following years to the initial introduced method; nowadays, the technique developed by El-Sabrout [3] has become the most extensively used method for in ovo injection. Nano-minerals effectively interact with inorganic and organic substances due to their increased surface area, high catalytic effect, and strong adsorbing ability. Nanominerals have the ability to cross the small intestine and further distribute into animals circulation and internal body organs[4]. Zinc is an important trace element that promotes growth and regulates immunity [5]. It is required for more than 300 enzymes; it is involved in different carbohydrate, lipid, protein, and nucleic acid metabolic processes. It plays a vital role in physiological processes in animals and birds bodies. Particularly, it acts as a free radical scavenger; this makes

NVVJ., Vol. 5, Issue (1), 2025

it an mportant element helping the organism to withstand stressors such as heat stress [6]. Curcumin is a carotenoid xanthophyll isolated from turmeric [7]. It is one of the most commonly commercially accepted and used natural dietary carotenoids in poultry feed; it has a vast range of therapeutic uses and activities, like antioxidant, antibacterial, immunomodulatory, anti-inflammatory, antimutagenic, and anti-cancer properties [8]. A known issue about curcumin reduced bioavailability because of its low absorption rate as well as the short plasma half-life due to fast metabolism, this service for its rapid elimination from the body, though using nanoparticles is a suggested approach for improving curcumin absorption and bioavailability [9]. One promising avenue of research involves the utilization of nano-sized particles, particularly those of zinc and curcumin, to enhance the efficacy of IO [10]. In recent years, this innovative approach has garnered attention for its potential to modulate physiological parameters and induce beneficial changes in the intestinal environment of newly hatched chicks [11]. Zinc, an essential trace element, plays a pivotal role in various physiological processes, including immune function, growth, and development [12]. Similarly, curcumin, a bioactive compound derived from turmeric, possesses potent antioxidant and anti-inflammatory properties [13]. The downsides of conventional supplementation methods have prompted researchers to explore IO as a precise and efficient delivery system for these nano-sized particles [14]. As we embark on this exploration, it becomes imperative to understand the intricate mechanisms that govern the absorption and utilization of these nanoparticles in the early stages of chick development [15]. Additionally, assessing changes in intestinal morphology and composition and immunological responses will provide valuable insights into the long-term implications of IO with zinc and curcumin nanoparticles [16] . Previous works investigated the rule of zinc and curcumin nanoparticles on ovo-inoculation. However, there is a lack of knowledge around the use of both curcumin and zinc nanoparticles together on the day old chicks physiological parameters, as well as the possible effect on intestinal villi development. This study goes in depth to investigate the interplay between zinc and curcumin nanoparticles administered through IO and the resultant impact on key physiological parameters of day-old chicks.

The current study hypothesized that in ovo feeding of CurNps and/or ZnNps may possess a positive impact on the day-old chick physiological parameters, immune response, and gut health that may influence growth. We aspire to contribute to the development of sustainable and effective strategies for enhancing the physiological well-being of day-old chicks, ultimately fostering healthier, more resilient poultry populations and enhance the economic profits.

2. Materials and Methods

2.1. Experimental design

The present study was involved in studying the effect of in ovo injection (IO) of zinc oxide nanoparticles and curcumin nanoparticles on hatchability of offspring broilers, growth performance, intestinal morphometric study, antioxidant parameters, and immunity of chicken broilers under optimum temperature (OT) and high temperature stress (HT). Six hundreds fertilized eggs with a similar weight of Ross 308 were randomly allocated into five treatments with four replicates per treatment in a completely randomized design (n = 120). The first treatment (G1) was not injected (intact control), and the second treatment (G2) was IO with sterile distilled water (DW as vehicle- 0.05 ml/egg). The 3rd treatment (G3) was IO with CurNPs (4.0 mg in 0.05 ml DW/egg- according to Heidary et al [17]. The 4th one (G4) was IO with ZnONPs (0.0006 mg or 6x10-4) in 0.06 ml DW/egg- according to Biria et al[18]. The 5th treatment (G5) was IO with both nanoparticles [0.025 ml (4.0 mg) + 0.030 ml ZnONPs (0.0006 mg)/egg].

2.2. Preparation of zinc and curcumin nano forms and their characterizations

Zinc oxide nanoparticles were synthesized by the sol-gel method as described by Mahdavi and Talesh [19]. Briefly, zinc oxide sol was produced by adding 0.15 molar zinc acetate into methanol. Then, the homogeneous solution of the mixture was stirred for 90 minutes by a magnetic stirrer. Diaphanous sol changed to white color by adding 1.5 molar sodium hydroxide, and the pH of the solution was tuned to 10. The milky white solution was stirred again for 60 minutes. In the next step, the sol was centrifuged for 20 minutes at 10000 rpm. A two-step washing with a 60-40 mixture of ethanol and water was used to remove completely organic materials from the surface. The residue was dried for 2 h at 60°C and 200°C, respectively, and a white powder was obtained. Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione] powder (95% purity). Synthesis of Cur-NPs was performed according to the procedure described by Pandit and Zeugolis [20]. Briefly, 100 mg of curcumin was dissolved in 20 ml of dichloromethane, and then one ml of this solution was added in a drop-wise manner to 50 ml of boiling water. After ultra-sonication (50 kHz) for 30 min, the mixture was stirred for 20 min at 800 rpm until obtaining the orange-colored precipitate. Subsequently, the supernatant was removed and the pellet was collected to use as a dietary supplement. In brief, a drop of Cur-NPs solution was located on carbon-coated copper grids and spotted with infrared light until it got dried and powdered. Curcumin nanoparticles were loaded on the specimen holder, and the TEM micrograph was taken by HRTEM-EDS and JEOL-JEM-1200. Furthermore, the crystalline structure of synthesized CurNPs was characterized using the X-ray diffraction method (XRD). The diffraction patterns of CurNPs dried powder were recorded by a Europe 600 GNR benchtop X-ray diffractometer.

2.3. Experimental procedures

2.3.1. Fumigation and incubation

Fumigation was done using potassium permanganate (KMnO4) and formalin.The concentrations can be achieved by the reaction of 45 ml (40% formalin solution) with 30 g potassium permanganate crystals per m3. The eggs were incubated at 37.7°C and relative humidity of 52-54% from days 0 to 18, for days 19 to 21, the incubator was re-adjusted to 37.2°C and 70-75% relative humidity, and the eggs were set in a horizontal position. Eggs were candled on 7th and 18th days to remove infertile eggs.

2.3.2. In ovo feeding procedure

On the 7th day of incubation, candling of eggs was carried out, and a small pinpoint hole was made in the broad end of the egg in the middle of the air sac using a 24G hypodermic needle (25 mm long), and the pinpoint hole was sealed using paraffin wax. The eggs were placed back in the incubator with hatcher temperature at 37.7 °C and relative humidity of 52-54 %.

2.3.3. Hatch characteristics and embryonic mortality

On hatching days, the number of chicks was counted in each replicate to calculate the hatchability percentage. Then, the embryonic mortality and fertile and infertile eggs were examined by breaking the unhatched eggs. All infertile eggs were opened and examined macroscopically for evidence of embryonic mortality. All unhatched eggs were analyzed for the developmental stage of dead embryos. The time of embryonic death was assigned to one of four categories: early dead (≤ 7 days), mid-dead (8-16 days), late dead (17-21 days), and pips. The percentage of embryonic mortality and hatching percentage were expressed based on the eggs placed into the incubator. The percentage of fertile eggs and efficiency of converting eggs to chick were presented as described by Palouj et al [21].

NVVJ., Vol. 5, Issue (1), 2025

2.4. Body weight

Hatched chicks from different experimental groups are weighted and subjected to statistical analysis for calculating the mean body weights for each group.

2.5. Blood analysis

Five blood samples per treatment on a random basis were collected into heparinized tubes to determine the blood picture parameters (white blood cells (WBCs), lymphocytes, MID-range absolute count (MID), granulocytes, red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and thrombocytes) according to the method described by Nengsih and Mustika [22].

2.6. Intestinal histomorphology

At one day old, the birds (5 from each group) were euthanized by neck severing and necropsied. Samples of duodenum, jejunum, ileum and cecum (2-cm segments from each) were flushed with 0.9% saline, then immersed in a formalin solution (10%) for 72 h and processed according to the method described by [23] and embedded in paraffin wax. Processed sections were cut (5 µm) using a microtome, placed on a glass slide, and then stained with hematoxylin-eosin. The samples were analyzed under a light microscope (Leika DM 500) to determine morphometric indices. The morphometric variables, including villus length (VL), crypt depth (CD), VL/CD ratio, and villus width (VW), were recorded and the villus surface area (VS) was calculated using the following formula: $(2\pi) \times (VW/2) \times (VH)$ (Sakamoto et al., 2000). There were 3 cross sections/one sample/bird, and the mean from five villi per cross section was used as the average value for analysis. The following parameters in H&E-stained sections were measured on one cross section per bird and intestinal segment:

- **Villus length:** five villi were measured from their base at the level of the crypt's entrance through to their distal tips. Only full finger-shaped and well-oriented villi were used.
- **Villus width (VW):** it is the distance between the two mid borders
- **Crypt depth:** five crypts were measured from the crypt's base to the closest villus base. The ratio of villus length to crypt depth was calculated by dividing villus height by crypt depth.
- **Thickness of the tunica muscularis:** this parameter was defined as the distance between the lamina muscularis mucosae internally and the tunica serosa externally. Five measurements were performed per intestinal segment.

2.7. Economic efficiency

The economic efficiency was calculated according to the price of injected materials, the price of eggs, the hatchability rate, and finally the total price of a one-day chick at the time of the experiment(Table 4).

2.8. Statistical analysis

The statistical analyses were implemented using R Software [24]; the data on body weight, internal organ weights, blood picture, and intestinal parameters were analyzed with a one-way analysis of variance ($P \leq 0.05$) followed by Tukey's test; the data were tabulated and expressed as mean ± S.E.

2.9. Ethical approval

The present research was carried out in accordance with the Animal Care and Use Committee guidelines of the Assuit University, Egypt, ethical number 04-2023-100067. The hatching eggs and chickens in this study were given proper care and management without causing them any unnecessary distress.

3. Results

3.1. Hatched chicks body weight

Results obtained of hatched chicks body weights (Fig. 1) have shown that there is no significant ($p \leq 0.05$) change in the average body weights among tested groups.

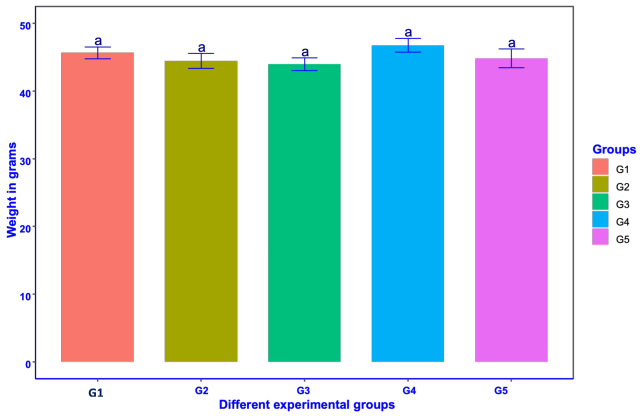

Figure 1: Effects of CurNps and/or ZnNps IO on broilers hatched chicks body weights (g) at one day old (mean ± SE, n = 5).

3.2. Erythrogram

Hematological findings of experimental groups (Table 1) showed a comparable non-significant change ($p \leq 0.05$) in the RBCs count and MCHC means. While the Hb and PCV showed a significant ($p \leq 0.05$) increase in G4 compared to the other tested ones. MCV of G1 expressively increased ($p \leq 0.05$) relative to G5.

Table 1: Effect of CurNps and/or ZnNps on broilers erythrogram at one day old chicks (mean ± SE,n=5)

Item	RBCs (10^{12}/L)	HB (mmol/L)	PCV (%)	MCV (fl)	MCH (fmol)	MCHC (mmol Hb/L)
G1	1.74 ± 0.08^{a}	7.53±0.07^{ab}	26.30 ± 0.55^{ab}	152.8 ± 3.41^{a}	4.40 ± 2.38^{a}	28.81 ± 0.72^{a}
G2	1.61 ± 0.09^{a}	6.64 ± 0.38^{c}	23.03 ± 0.69^{c}	145.53 ± 3.95^{ab}	4.18 ± 1.21^{abc}	28.90 ± 0.43^{a}
G3	1.60 ± 0.41^{a}	6.81 ± 0.41^{c}	23.63 ± 0.88^{c}	149.17 ± 3.08^{ab}	4.29 ± 0.96^{ab}	28.84 ± 0.33^{a}
G4	2.01 ± 0.07^{a}	7.84 ± 0.30^{a}	28.23 ± 0.49^{a}	140.9 ± 3.75^{ab}	3.89 ± 1.28^{c}	27.82 ± 1.19^{a}
G5	1.80 ± 0.06^{a}	6.95 ± 0.16^{bc}	24.63 ± 0.5^{bc}	137.97 ± 2.57^{b}	3.92 ± 1.87^{bc}	28.38 ± 0.53^{a}

Groups mean labeled different superscript letters (a, b, c, . .etc.) in the same column are significantly ($p \leq 0.05$) different. Control negative without IO (G1). The IO groups (G2: G5) were administered by: distilled water (G2), CurNps (G3), ZnNps (G4), and CurNps + ZnNps (G5).

3.3. Leukogram

WBCs of G2 and G3 (Table 2) showed a major ($p \leq 0.05$) decrease compared to the G1, G4, and G5. Study outcome results of heterophils count (Table 2) showed a significant ($p \leq 0.05$) increase in G4 and G5 compared to other experimental groups. While the lymphocyte count has shown a significant reduction in the Zn-treated group (G4) compared to the G1, G2, and G3 inoculated groups. The H/L ratio of G4 and G5 chicks showed a significant ($p \leq 0.05$) increase relative to the G1 and G2 ones. The monocyte counts of G4 showed a substantial ($p \leq 0.05$) increase against the other experimental groups.

Table 2: Effects of CurNps and/or ZnNps IO on broilers leukogram at one day old (mean ± SE, n = 5).

Item	Monocytes (%)	H/L ratio	Lymphocytes (%)	Heterophils (%)	WBCS (10^{9}/L)
G1	1.67± 0.13^{cd}	0.16± 0.01^{c}	85.00± 0.45^{ab}	13.33± 0.47^{c}	13.81±0.35^{ab}
G2	1.33± 0.13^{d}	0.13 ± 0.02^{c}	87.67 ± 1.52^{a}	11.00 ± 1.40^{c}	13.06±2.80^{c}
G3	2.00 ± 0.00^{bc}	0.23 ± .01^{bc}	79.67 ± 0.34^{bc}	18.33 ± 0.34^{b}	13.00±2.13^{c}
G4	3.00 ± 0.22^{a}	0.45 ± 0.08^{a}	69.67 ± 3.62^{d}	27.33 ± 1.19^{a}	14.25±0.77^{a}
G5	2.33 ± 0.13^{b}	0.36 ±0.03^{ab}	72.33 ± 1.49^{cd}	25.33 ± 1.44^{a}	13.57±0.67^{bc}

Groups mean labeled different superscript letters (a, b, c, . .etc.) in the same column are significantly ($p \leq 0.05$) different. Control negative without IO (G1). The IO groups (G2: G5) were administered by: distilled water (G2), CurNps (G3), ZnNps (G4), and CurNps + ZnNps (G5).

3.4. Weights of the internal organs

The outcomes of the internal organ weights (Table 3) showed a significant ($p \leq 0.05$) increase in G1 in comparison with the other groups. Heart of the G3 showed a marked ($p \leq 0.05$) increase in weights in contrast to other treated groups. The intestine weights of G5 showed a noticeable ($p \leq 0.05$) rise compared to other groups. Liver weights of G3 and G5 presented a major ($p \leq 0.05$) increase vs. the other groups. Spleen and yolk sac weights showed no significant ($p \leq 0.05$) changes among different treatments.

3.5. Intestinal histomorphometric changes

Histomorphometric changes (Table 5 and Fig. 2) among groups revealed that:

- **Duodenum:** The villus width and villus length showed marked ($p \leq 05$) increase at both Zn and Cur-Zn treatments compared to other experimental groups. While the crypt depth and villus length/crypt depth ratio showed a significant decrease ($p \leq 0.05$) at all treated groups compared to the control one. The muscular thickness did not show any significant ($p \leq 0.05$) variation among treatments.
- **Jejunum:** The villus width of G5 showed a significant ($p \leq 05$) increase relative to other experimental treatments. The villus length of G2 recorded a marked

NVVJ., Vol. 5, Issue (1), 2025

Table 3: Effects of CurNps and/or ZnNps IO on broilers internal organ weights (g) at one day old (mean ± SE, n = 5).

Item	Yolk sac	Volume of yolk sac	Thigh	Spleen	Liver	Intestine	Heart	Bursa Fabricius
G1	5.87±0.15^{ab}	8±0.49^{b}	3.07±0.03^{a}	0.02±0.00^{ab}	1.21±0.06^{b}	2.68±0.07^{a}	0.36±0.01^{b}	0.16±0.04^{a}
G2	6.73±0.44^{ab}	9.67±0.13^{a}	3.32±0.15^{a}	0.01±0.0^{b}	1.11±0.00^{b}	1.77±0.08^{c}	0.37±0.01^{b}	0.04±0.00^{b}
G3	5.51±0.52^{b}	8.17±0.06^{b}	3.13±0.15^{a}	0.01±0.00^{b}	1.49±0.08^{a}	2.69±0.17^{a}	0.53±0.07^{a}	0.05±0.00^{b}
G4	5.65±0.37^{b}	6.17±0.50^{c}	3.18±0.23^{a}	0.07±0.02^{a}	1.19±0.01^{b}	2.27±0.07^{b}	0.38±0.01^{b}	0.06±0.00^{b}
G5	7.23±0.06^{a}	10±0.22^{a}	2.94±0.06^{a}	0.06±0.02^{ab}	1.19±0.01^{b}	1.56±0.06^{c}	0.38±0.01^{b}	0.05±0.01^{b}

Groups mean labeled different superscript letters (a, b, c, . .etc.) in the same column are significantly ($p \leq 0.05$) different. Control negative without IO (G1). The IO groups (G2: G5) were administered by: distilled water (G2), CurNps (G3), ZnNps (G4), and CurNps + ZnNps (G5).

Table 4: Economic study of one-day chicks after in-ovo CurNps and/or ZnNps injection.

	G1	G2	G3	G4	G5
1-Price of one egg (L.E.)	12	12	12	12	12
2-No. of eggs	120	120	120	120	120
3-Price of eggs/group (L.E.)	1440	1440	1440	1440	1440
4-Price of injected materials/ml or gm					
a- Sterile distilled water (L)	—-	20	—-	—-	—-
b- Nano-zinc oxide (5 g)	—-	—-	—-	1750	—-
c- Nano-curcumin (5 g)	—-	—-	900	—-	—-
d- Nano- Zn + Cur	—-	—-	—-	—-	2650
5-Volume of injected materials/egg (ml)	—-	0.05	0.05	0.06	0.03+0.025
6-Price of injected materials/egg	—-	0.001	0.721	0.00141	0.72121
7- Price of injected materials/group	—-	0.12	86.52	0.1452	86.55
8-Hatchability rate %	85%	78%	77%	91%	63%
9- No. of chicks/group	102	93.6 (94)	92.4 (92)	109.2	75.6 (76)
10-*Total price of one-day chick = "Steps [3+7] ÷ 9"	1440÷102 **14.12**	1440+0.12 ÷ 94 = **15.32**	1440+86.52 ÷ 92 = **16.59**	1440+0.15 ÷109= **13.21**	1440+86.55÷76 = **20.08**

The IO groups (G2: G5) were administered by: distilled water (G2), CurNps (G3), ZnNps (G4), and CurNps + ZnNps (G5). *Total price of one-day chick (L.E., without incubation process price).

($p \leq 0.05$) decrease compared to all groups. In contrast, the crypt depth illustrated a non-significant ($p \leq 0.05$)change. Villus length/crypt depth ratio presented an important ($p \leq 0.05$) increase at both G4 and G5 in matching with other experimental groups, while the muscle thickness showed a non-significant ($p \leq 0.05$) change.

- **Ileum:** Villus width revealed a significant ($p \leq 0.05$) increase at G4 compared to all experimental groups. The villus length of both G4 and G5 registered an expressive increase, contrary to other tested groups. The crypt depth and muscular thickness showed a non-remarkable variation among groups. The villus length/crypt depth ratio of G2 showed a significant ($p \leq 0.05$) reduction.
- **Cecum:** Villus width, villus length, crypt depth, and villus length/crypt depth ratio showed a non-significant ($p \leq 0.05$) change among experimental groups.

3.6. Economic efficiency

The highest price was observed in G5, while the lowest price in G4 (ZnNps) of the one-day-old chick in the current study is due to the parallel to hatchability percentages that were reflected on the price.

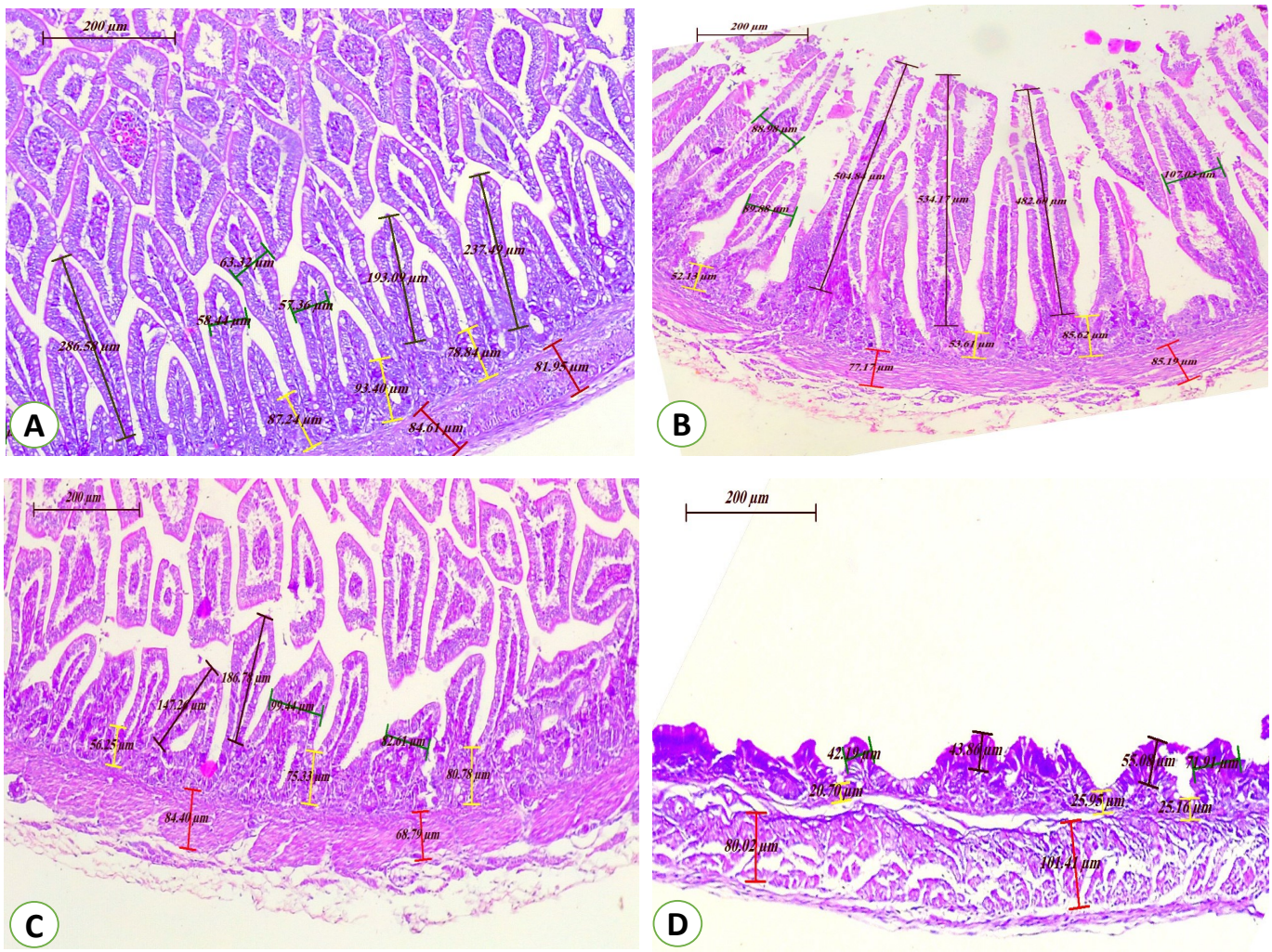

Figure 2: Photomicrograph from A) duodenum, B) Jugenum, C) Ilium and D) Ceacum of one-day-old chick (IO by ZnNps in fertilized eggs) showing villus length (VL, dark line), villus width (VW, green line), crypt depth (CD, yellow line), and muscle thickness (MT, red line). H&E stain scale bar 200 um).

4. Discussion

The existent literature appeared to suggest that zinc and curcumin nanoparticles in ovo inoculation of fertilized chicken eggs could improve some physiological parameters and gut health of the day-old chick. Study outcomes showed that concomitant zinc-curcumin nanoparticles in ovo inoculation caused significant ($p \leq 0.05$) improvement in gut health and increased intestinal weights. There is some evidence to suggest that combining zinc and curcumin may have synergistic effects. This occurs throughout several aspects; for example, zinc can enhance the absorption of curcumin in the body [25]. Curcumin can inhibit the excretion of zinc from the body, leading to increased zinc levels in various tissues. This helps in maintaining higher zinc concentrations in the body, contributing to its overall bioavailability [26]. Furthermore, curcumin has chelating properties; this may increase the bioavailability stability of metal ions, including zinc [27].

Average body weight of hatched chicks is considered a good indicator about general health condition and expected future growth [28]. The effect of in ovo feeding on day-old chicks body weights has been studied extensively; some in ovo feeding has been found to have a positive impact on the body weights of chicks at hatch and during certain stages of their early life. Several bioactive compounds (GABA and carbohydrates), when supplemented

NVVJ., Vol. 5, Issue (1), 2025

Table 5: Effects of CurNps and/or ZnNps IO on broilers intestinal histomorphometric changes at one day old (mean ± SE, n = 5).

Item	Villus width (µm)	Villus length (µm)	Crypt depth (µm)	VL/CD ratio	Muscle thickness (µm)
Duodenum					
G1	46.77± 0.76^{c}	194.01± 1.43^{c}	59.37± 1.05^{a}	3.29± 0.06^{a}	80.6± 0.70^{a}
G2	47.24± 1.07^{c}	194.28± 1.21^{c}	101.5± 0.90^{a}	1.92± 0.02^{d}	81.13± 0.87^{a}
G3	52.57± 0.82^{b}	214.21± 2.84^{b}	96.63±0.77^{b}	2.22± 0.03^{c}	81.67± 0.96^{a}
G4	60.91± 1.44^{a}	241.03± 1.44^{a}	86.52± 1.64^{c}	2.81± 0.06^{b}	82.26± 1.42^{a}
G5	59.37± 1.05^{a}	243.36± 0.85^{a}	87.39± 1.20^{c}	2.79± 0.04^{b}	80.79± 1.15^{a}
Jejunum					
G1	96.83 ± 1.6^{ab}	500.68±10.74^{ab}	65.44 ± 2.52^{a}	7.82 ± 0.28^{b}	80.93 ± 0.62^{a}
G2	97.9 ± 1.39^{b}	496.08±10.31^{b}	66.17 ± 2.13^{a}	7.61 ± 0.24^{b}	82.47 ± 0.82^{a}
G3	52.57 ± 1.37^{c}	498.81 ± 8.29^{ab}	64.97 ± 1.70^{a}	7.77 ± 0.24^{b}	82.6 ± 1.27^{a}
G4	100.9 ± 1.44^{b}	538.81 ± 13.72^{a}	59.64 ± 1.37^{a}	9.11 ± 0.29^{a}	83.13 ± 1.00^{a}
G5	110.10±2.40^{a}	539.01 ± 10.51^{a}	60.31 ± 1.14^{a}	8.98 ± 0.2^{a}	83.53 ± 0.98^{a}
Ileum					
G1	90.10 ± 1.41^{c}	140.61 ± 1.52^{b}	70.04 ± 1.64^{a}	2.04 ±0.07^{bc}	76.00 ± 1.09^{a}
G2	90.5 ± 1.32^{bc}	142.15 ± 1.62^{b}	71.57 ± 1.26^{a}	2.00 ± 0.05^{c}	77.4 ± 1.20^{a}
G3	94.44±1.74^{abc}	146.15 ± 2.22^{b}	70.44 ± 1.67^{a}	2.10 ±0.06^{abc}	75.6 ± 1.07^{a}
G4	98.64 ± 2.01^{a}	155.68 ± 20^{a}	69.51 ± 1.65^{a}	2.26 ± 0.06^{a}	77.87 ± 1.47^{a}
G5	97.24±2.31^{ab}	156.41 ± 1.60^{a}	70.91 ± 1.31^{a}	2.22 ±0.04^{ab}	78.8 ± 1.60^{a}
Cecum					
G1	61.57 ± 1.37^{a}	48.51 ± 0.66^{a}	24.71 ± 0.66^{b}	48.51 ±0.66^{a}	87.47 ± 1.27^{a}
G2	62.44 ± 1.45^{a}	49.17 ± 0.57^{a}	25.71 ± 0.72^{ab}	49.17 ±0.57^{a}	88.53 ± 0.95^{a}
G3	62.44 ± 1.45^{a}	48.71 ± 0.76^{a}	26.04 ± 0.7 ^{ab}	48.71 ±0.76^{a}	88.86 ± 1.07^{a}
G4	63.71 ± 0.99^{a}	50.51 ± 0.58^{a}	26.71 ± 0.63^{ab}	50.51 ±0.58^{a}	90.33 ± 0.93^{a}
G5	61.04 ± 1.68^{a}	49.84 ± 1.49^{a}	27.51 ± 0.67^{a}	49.84 ±1.49^{a}	91.26 ± 0.97^{a}

Groups mean labeled different superscript letters (a, b, c, . etc.) in the same column are significantly ($p \leq 0.05$) different. Control negative without IO (G1). The IO groups (G2: G5) were administered by: distilled water (G2), CurNps (G3), ZnNps (G4), and CurNps + ZnNps (G5). VL/CD = villus length / crypt depth ratio.

in ovo, have shown beneficial effects on body weight gain at hatch and throughout the post-hatch period [29]. In contrast, the current work outcomes showed that in ovo zinc and curcumin nanoparticles has a non-significant influence on the hatched chick's body weights. This finding matches the ones reported by Iqbal et al. [30], who stated that the main determinant factor of the hatched chick body weights is the egg size and weights. Notably, some authors [31]. declared the specific effects on the chicks weights vary depending on the type of bioactive compound. Results outcomes presented no significant changes in most blood parameters in day-old chicks due to zinc and curcumin nanoparticle inoculation. Rath et al.[32], who studied the effect of selenium nanoparticles in ovo inoculation, reported similar findings. These findings showed no marked changes in most of the blood parameters in Japanese quail eggs. Equally, some authors [33] declared parallel outcomes when using copper nanoparticles in ovo feed in broiler chicks. The existing work outcomes have shown that there are no significant changes in the WBCS counts among different experimental groups. Although the cur-zn (G5)-treated group

showed increased heterophil counts, lowered lymphocyte counts, increased H/L ratio, as well as increased monocyte numbers compared to the control one. These findings are usually accompanied by stress, inflammation, and infectious conditions. Previous studies showed that Average body weight of hatched chicks is considered a good indicator about general health condition and expected future growth [28]. The effect of in ovo feeding on day-old chicks body weights has been studied extensively; some in ovo feeding has been found to have a positive impact on the body weights of chicks at hatch and during certain stages of their early life. Several bioactive compounds (GABA and carbohydrates), when supplemented in ovo, have shown beneficial effects on body weight gain at hatch and throughout the post-hatch period [29]. In contrast, the current work outcomes showed that in ovo zinc and curcumin nanoparticles has a non-significant influence on the hatched chick's body weights. This finding matches the ones reported by Iqbal et al.[30], who stated that the main determinant factor of the hatched chick body weights is the egg size and weights. Notably, some authors [31] declared the specific effects on the chicks weights vary depending on the type of bioactive compound. Results outcomes presented no significant changes in most blood parameters in day-old chicks due to zinc and curcumin nanoparticle inoculation. Rath et al.[32], who studied the effect of selenium nanoparticles in ovo inoculation, reported similar findings. These findings showed no marked changes in most of the blood parameters in Japanese quail eggs. Equally, some authors [33] declared parallel outcomes when using copper nanoparticles in ovo feed in broiler chicks. The existing work outcomes have shown that there are no significant changes in the WBCS counts among different experimental groups. Although the curzn (G5)-treated group showed increased heterophil counts, lowered lymphocyte counts, increased H/L ratio, as well as increased monocyte numbers compared to the control one. These findings are usually accompanied by stress, inflammation, and infectious conditions. Previous studies showed that development and integrity of the intestinal mucosa [34]. Zinc is required for cell proliferation and differentiation, which are essential for the maintenance of a healthy intestinal epithelium [35]. Inflammation negatively affects the intestinal development. Curcumin is well known for its anti-inflammatory properties and contributes to keeping a healthy intestinal environment [36]. Notably, curcumin possesses an antioxidant activity that may protect intestinal cells from oxidative stress that indirectly helps in the development and integrity of the small intestine [37] . Zinc and curcumin (G5) possess immunomodulatory properties and could influence immune responses in the gut; this may strongly influence the intestinal micro- and macro-morphology [38].

Conclusion

The current study findings indicate that in ovo ZnNps feeding in the air sac does not harm the developing embryo and gave the best percentage of hatchability. The research clearly shows IO of zinc nanoparticles has better results than curcumin nanoparticles. It increases significantly HB, PCV, WBCs, monocyte, and heterophil count. As well as improving the gut health and integrity. This technique can be used to improve the post-hatch performance of broiler chickens. From the economic point of view, the ZnNps-treated group recorded the lowest price of hatchlings relative to other experimental groups. However, further advanced research is required to explore further beneficial effects and safety of ZnNps for maximum efficacy to improve economic outcomes and profits.

Conflict of interest

The authors declare that they have no competing interests.

References

[1] I. Abbasi, A. Shamim, M. Shad, H. Ashari and I. Yusuf, *Journal of Cleaner Production*, 2024, 140–368.

[2] S. Roto, Y. Kwon and S. Ricke, *Frontiers in veterinary science*, 2016, **3**, year.

NVVJ., Vol. 5, Issue (1), 2025

[3] K. El-Sabrout, S. Ahmad and A. El-Deek, *J. Anim. Health Prod*, 2019, **7**, 126–130.

[4] S. Abdelnour, M. Alagawany, N. Hashem, M. Farag, E. Alghamdi, F. Hassan, R. Bilal, S. Elnesr, M. Dawood and S. Nagadi, *Animals*, 2021, **11**, year.

[5] D. Kamel, A. Abdel-Khalek and S. Gabr, *Journal of Animal and Poultry Production*, 2020, **11**, 565–571.

[6] H. Al-Zahrani, K. Nahar, H. Alharby, H. Alsamadany, K. Hakeem and M. Hasanuzzaman, *Agronomy*, 2022, **12**, year.

[7] S. Gomez, C. Anjali, B. Kuruvila, P. Maneesha and M. Joseph, *Food Production, Processing and Nutrition*, 2023, **5**, 1–13.

[8] R. Partovi, S. Seifi, M. Pabast, A. Mohajer and P. Sadighara, *Effect of dietary supplementation of nanocurcumin on oxidant stability of broiler chicken breast meat infected with eimeria species*, Veterinary Research Forum. Faculty of Veterinary Medicine, Urmia University, Urmia, 2020.

[9] I. Walbi, M. Ahmad, J. Ahmad, M. Algahtani, A. Alali, S. Alsudir, A. Aodah and H. Albarqi, *Polymers*, 2022, **14**, year.

[10] H. Jahangirian, E. Lemraski, T. Webster, R. Rafiee-Moghaddam and Y. Abdollahi, *A review of drug delivery systems based on nanotechnology and green chemistry: green nanomedicine*, 2017.

[11] H. Ayalew, J. Wang, S. Wu, K. Qiu, A. Tekeste, C. Xu, D. Lamesgen, S. Cao, G. Qi and H. Zhang, *Poultry Science*, 2023, 103–130.

[12] J. Deshpande, M. Joshi and P. Giri, *Int J Med Sci Public Health*, 2013, **2**, 1–6.

[13] B. Razavi, M. Ghasemzadeh Rahbardar and H. Hosseinzadeh, *Phytotherapy Research*, 2021, **35**, 6489–6513.

[14] R. Mala, R. Keerthana and P. Mohan, *Sustainable Agriculture Reviews*, 2021, **55**, 263–315.

[15] D. Samak, Y. El-Sayed, H. Shaheen, A. El-Far, M. Abd El-Hack, A. Noreldin, K. El-Naggar, S. Abdelnour, E. Saied and H. El-Seedi, *Environmental Science and Pollution Research*, 2020, **27**, 19058–19072.

[16] S. Oladokun, J. Macisaac, B. Rathgeber and D. Adewole, *Animals*, 2021, **11**, year.

[17] N. Heidary and N. Kornienko, *Chemical science*, 2020, **11**, 1798–1806.

[18] A. Biria, B. Navidshad, F. M. Aghjehgheshlag and S. Nikbin, 2020.

[19] R. Mahdavi and S. Talesh, *Ultrasonics Sonochemistry*, 2017, **39**, 504–510.

[20] A. Pandit and D. Zeugolis, *Twenty-five years of nano-biomaterials: have we revolutionized healthcare?*, 2016.

[21] J. Palouj, M. Kazemi-Fard, M. Rezaei and Z. Ansari-Piresaraei, *Iranian Journal of Applied Animal Science*, 2021, **11**, 595–603.

[22] R. Nengsih and A. Mustika, *Acta Veterinaria Indonesiana*, 2020, **8**, 9–15.

[23] N. Elbarbary, N. Abdelmotilib, R. Gomaa, F. Elnoamany, A. Fotouh, E. Noseer and R. Zaki, *The Egyptian Journal of Aquatic Research*, 2023, **49**, 530–536.

[24] RSoftware, *R: A language and environment for statistical computing*, 2020.

[25] S. Prasad and R. Lall, *Journal of Trace Elements in Medicine and Biology*, 2022, **73**, year.

[26] C. Singh, G. Chhabra, A. Patel, H. Chang and N. Ahmad, *Nutrients*, 2021, **13**, year.

[27] E. Smirnova, M. Moniruzzaman, S. Chin, A. Sureshbabu, A. Karthikeyan, K. Do and T. Min, *Antioxidants*, 2023, **12**, year.

[28] M. Michalczuk, M. Stepinska and M. O.-S. S. Lukasiewicz, *Effect of the initial body weight of Ross 308 chicken broilers on the rate of growth*, 2011.

[29] R. Jha, A. Singh, S. Yadav, J. Berrocoso and B. Mishra, *Frontiers in Veterinary Science*, 2019, **6**, year.

[30] J. Iqbal, S. Khan, N. Mukhtar, T. Ahmed and R. Pasha, *Effects of egg size*, 2016.

[31] R. Das, P. Mishra and R. Jha, *Frontiers in Veterinary Science*, 2021, **8**, year.

[32] P. Rath, S. Bhanja, M. Mehra, A. Goel and P. Aswathi, *Indian journal of poultry science*, 2018, **53**, 188–191.

[33] A. Scott, K. Vadalasetty, E. Sawosz, M. Łukasiewicz, R. Vadalasetty, S. Jaworski and A. Chwalibog, *Animal Feed Science and Technology*, 2016, **220**, 151–158.

[34] A. Hassan, K. Sada, S. Ketheeswaran, A. Dubey and M. Bhat, *Cureus*, 2020, **12**, year.

[35] W. Ohashi, T. Hara, T. Takagishi, K. Hase and T. Fukada, *Digestive diseases and sciences*, 2019, **64**, 2404–2415.

[36] N. Wang, G. Wang, J. Hao, J. Ma, Y. Wang, X. Jiang and H. Jiang, *Digestive diseases and sciences*, 2012, **57**, 1792–1801.

[37] C. Wang, Z. Han, Y. Wu, X. Lu, X. Tang, J. Xiao and N. Li, *Food and Chemical Toxicology*, 2021, **151**, year.

[38] M. Abdel-Tawwab, E.-S. Eissa, W. Tawfik, H. Abd Elnabi, S. Saadony, W. Bazina, R. J. Ahmed and Biochemistry, *Dietary curcumin nanoparticles promoted the performance, antioxidant activity, and humoral immunity, and modulated the hepatic and intestinal histology of Nile tilapia fingerlings*, 2022.