International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; SP-9(6): 01-05

www.veterinarypaper.com
Received: 02-08-2024
Accepted: 06-09-2024

Dr. M Anandhi
Assistant Professor, Department of Poultry Technology, College of Poultry Production and Management, Hosur, Tamil Nadu, India

Corresponding Author:
Dr. M Anandhi
Assistant Professor, Department of Poultry Technology, College of Poultry Production and Management, Hosur, Tamil Nadu, India

In-ovo supplementation of nano forms of trace minerals in the development of intestine and lymphoid organs of broiler chicken

Dr. M Anandhi

Abstract
The current study was aimed to ascertain the effect of *in-ovo* injection of nano particles of zinc, copper and chromium on intestine and lymphoid organs development of broiler chicken. Four hundred fertile broiler eggs from Cobb 430 were randomly divided into five treatment (each 80 eggs) groups. First treatment was without injection (control), second was injected with 0.5 ml normal saline, third, fourth and fifth treatment eggs were injected with 40, 12 and 0.5 µg/egg of nano zinc, nano copper and nano chromium, respectively. After hatching, 240 day old straight run broiler chicks were allocated to five treatment groups each consisting of four replicates with twelve chicks per replicate and all birds were fed with broiler starter diet (1-21 days) and finisher diet (22-42 days) as per NRC (1994)[11]. *In-ovo* feeding of nano forms of zinc, copper and chromium at 18th day of incubation through amniotic route significantly ($p \leq 0.05$) increased the histomorphometry of small intestine at early age and increased the lymphoid organs weight of broilers.

Keywords: *In-ovo* injection, Nano zinc, nano copper, nano chromium, broilers, histomorphometry, lymphoid organs

Introduction
The perinatal period is a most crucial time in the development of a young chick as, this is a transitional period in which the chicks undergo metabolic and physiological shifts from the utilization of egg nutrients to exogenous feed (Ferket and Uni, 2012) [6]. The *in-ovo* feeding allows the delivery of various supplements directly to chicken embryos, facilitate early establishment of a healthy GIT microbiome before it is exposed to any pathogenic bacteria. In the poultry diet, trace minerals in the form of nano particles have recently been examined. Nano technology deals with the conversion of larger molecules to nanometer size. The process of conversion of these larger molecules to tiny one causes changes in the innate physical and chemical nature of the base material. As the technology engineers to nano level, their properties differ fundamentally and unpredictably compared to a larger scale. The mineral nano particles not only increase the bioavailability of minerals but also reduce their requirements and excretion (Gopi *et al*., 2017) [8]. Due to their extreme small size and unique physical properties, the nanoparticles are likely to be different when compared to their conventional forms. Mineral feed additives with particle sizes less than 100 nanometer increase their absorption, resulting in improved tissue delivery and performance. Therefore, the aim of this study was to assess the effect of *In-ovo* supplementation of nano forms of trace minerals on development of small intestine and lymphoid organs of broiler chicken

Materials and Methods
The eggs were collected from a broiler breeder (Cobb 400) farm and were fumigated and cleaned with egg shell sanitizer and incubated with broad end up in forced draft automatic chicken incubator. During incubation, a dry-bulb temperature ranging from 99-100°F and wet-bulb temperature of 85-87°F were maintained from day 1 to 18 days of incubation. The hatching eggs in the setter were turned by 45° angle on either side at hourly interval until they were transferred to the hatcher. *In-ovo* injection was carried out on 18th day of incubation with various trace mineral solutions. The trace mineral nano particles were procured from M/s.

Matrix Nano Pvt. Ltd., Noida, India. Mineral nano particles were characterized by Scanning Electron Microscopy (SEM) method. The average particle size was found to be 50-80 nm and the purity was 99 per cent. Required amount of nano trace minerals were weighed and dissolved in the normal saline in such that a concentration of 0.5 ml contained the required amount of trace mineral to be injected in one egg.

On 18th day of embryonic age, the eggs showing viable embryo were injected with nano particles of minerals at the broad end of the egg into amnion using a 24-gauge hypodermic needle (25 mm long) under laminar flow system. The injection area was disinfected with 99.90% ethyl alcohol and the hole was sealed with melted paraffin wax and transferred to hatching trays. After completion of *In-ovo* injection, all the eggs were transferred and incubated in hatching trays at the dry bulb temperature of 97.34°F and the wet bulb temperature of 86.36°F without turning from 19- 21 days the design of experiment is presented in Table 1.

Table 1: Design of biological experiment

Treatments	Nano forms of minerals	*In-ovo* feeding of nano trace minerals		No. of 18th day incubated eggs for *in-ovo* injection	Growth performance study after *in-ovo* injection (No. of birds)
		Basal solvent (ml/egg)	Levels (µg/egg)		
T_1	Control (Non-injected)	0	0	80	48
T_2	Injected control	0.5	0	80	48
T_3	Nano zinc	0.5	40	80	48
T_4	Nano copper	0.5	12	80	48
T_5	Nano Chromium	0.5	0.5	80	48

A total of 400 fertile eggs with uniform weight were randomly divided into 5 treatment groups with four replicates of 20 eggs each. After hatching, 240 day old straight run broiler chicks were allocated into five treatment groups each consisting of four replicates with twelve chicks each. All birds were fed broiler starter diet (1-21 days) and finisher diet (22 - 42 days) as per NRC (1994) [11].

The experimental treatments were as follows.

T_1: Chicks produced from un-injected treatment as control

T_2: Chicks produced from the injection with normal saline (NS).

T_3: Chicks produced from the injection of nano zinc (ZnNPs).

T_4: Chicks produced from the injection of nano copper (CuNPs).

T5: Chicks produced from the injection of nano chromium (CrNPs).

At the end first week of the experiment, one male and one female from each replicate (six birds from each treatment) were randomly selected and slaughtered as per the method of Arumugam and Panda (1970) [1]. The gut samples (2 cm) were taken from duodenum, jejunum and ileum for histomorphometric study. The duodenum was collected from duodenal loop, jejunum (between the duodenal loop and Meckel's diverticulum) and ileum between Meckel's diverticulum and ileo-cecal junction as per the method described by Miller (2007) [10]. After fixing the tissues in10 per cent neutral buffered formalin, the tissues were embedded in paraffin. Serial tissue sections of 5 µm thick were cut by a microtome and were fixed on slides. The tissue sections were stained with haematoxylin and eosin stain. The sections were examined for histomorphological studies *viz.*, villus height, width and crypt depth.

At the end of sixth weeks, one male and one female from each replicate (Six birds from each treatment) were randomly selected and slaughtered as per the method of Arumugam and Panda (1970) [1]. Data on pre-slaughter weight, eviscerated carcass weight, giblets weight, ready-to-cook carcass weight and the weights of lymphoid organs *viz*., bursa of Fabricius, thymus and spleen were recorded. Per cent slaughter weights to live body weight were calculated.

The data obtained in this study were statistically analyzed with general linear model procedure of SPSS statistical software (Version 20 for windows, SPSS). Significant differences among treatment means were tested by Duncan multiple range test. A level of $P \leq 0.05$ was used as the criterion for statistical significance. All the experimental procedures were assessed and approved by the Institutional Animal Ethics Committee from Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar.

Results and Discussion

Histomorphometry of small intestine

The result of influence of *in-ovo* feeding of nano trace minerals on 18th day of incubation on histomorphometry of small intestine at the end of first of age is presented in Table 2.

Table 2: Mean (±SE) duodenal, jejuna and ileal histomorphometry of broiler chicken at first week of age as influenced by *in-ovo* feeding of nano trace minerals on 18thday of incubation

Experimental group	Description of the treatment	Duodenal histomorphometry			Jejunal histomorphometry			Ileal histomorphometry		
		Villi height (µm)	Villi width (µm)	Crypt depth (µm)	Villi height (µm)	Villi width (µm)	Crypt depth (µm)	Villi height (µm)	Villi width (µm)	Crypt depth (µm)
T_1	Negative control	1092.63±9.99^{c}	67.44±7.39	59.88±9.99^{b}	473.13±10.48^{d}	59.63±5.54	68.94±0.89	301.13±43.43^{d}	64.38±6.39	62.5±3.38
T_2	Positive Control	1160.07±11.42^{b}	67.69±10.05	57.63±7.39^{b}	507.38±10.48^{c}	74.69±7.92	73.50±1.67	457.66±23.94^{c}	63.97±2.84	69.19±5.05
T_3	Nano Zn	1298.59±11.28^{a}	77.88±10.48	97.30±8.28^{a}	634.59±11.28^{a}	93.88±10.48	86.63±9.99	755.88±10.48^{a}	68.04±3.23	78.13±10.48
T_4	Nano Cu	1278.63±10.48^{a}	74.25±2.03	96.81±11.28^{a}	584.13±9.99^{b}	84.38±9.99	80.38±10.48	532.88±9.99^{b}	69.13±4.86	71.88±9.99
T_5	Nano Cr	1152.38±10.48^{b}	73.66±3.27	89.38±10.48^{a}	576.38±10.48^{b}	78.63±10.48	84.49±10.48	470±15.57^{bc}	67.29±3.21	73.25±7.59

Means with in a column bearing different superscripts differ significantly ($p \leq 0.05$)

At first week of age, duodenal villi height was significantly ($p \leq 0.05$) more in *in-ovo* injected chicks than chicks hatched without *in-ovo* injection group. The duodenal villi height was significantly ($p \leq 0.05$) highest in nano zinc injected group (1298.59 µm) followed by nano copper injected group (1278.63 µm). The chicks with out *in-ovo* injection recorded the shortest villi (1092.63 µm) and there was no significant difference noticed among nano chromium and normal saline

injected chicks. There was no significant difference recorded in duodenal villi width between treatment groups. Duodenal crypt depth was significantly ($p \leq 0.05$) higher in nano trace mineral injected groups (from 89.38 to 97.30 µm) than positive and negative control chicks (57.63 and 59.88µm, respectively) which were not significantly differed from each other.

Significantly ($p \leq 0.05$) tallest jejunal villi was recorded in nano zinc injected chicks (634.59 µm) followed by other nano trace mineral injected groups (584.13 and 576.38 µm). There was significant ($p \leq 0.05$) difference noticed in jejunal villi height among positive and negative control groups. No significant difference recorded in jejunal villi width and crypt depth among treatment groups at first week of age.

The ileal villi height at first week of age differed significantly ($p \leq 0.05$) between treatment groups. Nano zinc injected chicks produced tallest ileal villi (755.88 µm) and non-injected chicks had shortest ileal villi (301.13 µm). No significant difference recorded among nano copper and nano chromium injected groups.

However, no significant difference recorded in ileal villi width and ileal crypt depth among treatment groups at first week of age.

Increased duodenal and jejunal villi height were recorded by Ferket and Uni (2012) [6], Chous *et al.* (2009) [5] as has been observed in this study. Smirnov *et al.* (2006) [15] observed that *in-ovo* feeding of carbohydrates to the amniotic fluid of Cobb embryos at 17.5 days of incubation increased jejunal villus surface area at hatch and 3 days post hatch about 27% and 21%, respectively than non-injected control. The present study results also supported by Chous *et al.* (2009) [5] who reported that *in-ovo* supplementation of 25-hydroxy cholecalciferol increased duodenum and jejunum villi length. Sogunle *et al*., (2018) [16] studied the effect of *in-ovo* injection of inorganic salts of Zn (80 µg. egg-1), Se (0.3 µg. egg-1) and Cu (16 µg. egg-1) and found significantly ($p<0.05$) higher gut morphometry values than control.

In-ovo feeding results in gastrointestinal tract (GIT) of hatchlings to be functionally similar to that of conventional 2 day old chicks offered feed immediately after hatch (Uni *et al.*, 2003)[18]. They also indicated that during the last 3 days of incubation, the weight of the intestine with a proportion of embryo weight increased from approximately 1% at 17 days of embryonic age to 3.5% at hatch. Rapid intestinal growth is due to increase in cell number and size, accelerated enterocyte proliferation and differentiation and intestinal crypt formation (Uni *et al*., 2000; Geyra *et al*., 2001) [17, 7].

Digestive organs length

The result of influence of *in-ovo* feeding of nano trace minerals on 18th day of incubation on digestive organs length at six weeks of age is presented in Table 3.

Table 3: Mean (±SE) digestive organs length (cm/100g live weight) of broiler chicken as influenced by *in-ovo* feeding of nano trace minerals on18thday of incubation

Experimental group	Description of the treatment	Duodenum	Jejunum	Ileum	Small Intestine
T_1	Negative control	1.75±0.04	3.45±0.15	3.01±0.27	8.21±0.58
T_2	Positive control	1.66±0.05	3.44±0.13	3.18±0.08	8.28±0.13
T_3	Nano Zn	1.69±0.05	3.47±0.09	3.23±0.07	8.26±0.05
T_4	Nano Cu	1.69±0.04	3.49±0.11	3.07±0.07	8.25±0.18
T_5	Nano Cr	1.68±0.04	3.49±0.11	3.15±0.06	8.32±0.05

At the end of six weeks, there was no significant difference noticed in length of duodenum, jejunum, ileum and small intestine between treatment groups. The length of duodenum, jejunum, ileum and small intestine ranged from 1.66 to 1.75 cm/100 g live weight, 3.44 to 3.49 cm/100 g live weight, 3.01 to 3.23 cm/100 g live weight and 8.21 to 8.32 cm/100 gram live weight, respectively.

This result is in accordance with the following research findings. Bhanja and Mandal (2005) [4] who studied the effect of *in-ovo* injection of limiting amino acid at 14 days of incubation and concluded that the *in-ovo* injection of amino acid did not have any influence on the length and weight of the digestive organs. Pedroso *et al.* (2006) [12] carried out three experiments to evaluate the effects of *in-ovo* inoculation of linoleic acid and glucose at 0, 100, 200 or 300 mg/µl levels and glutamine at 0, 10, 20 or 30 mg per chicken egg at 16th day of incubation and concluded that the development of digestive organs was not affected by the *in-ovo* injection. The relative weight of the liver and small intestine was higher in glucose injected chicken embryos than in un-injected controls on the day of hatch (Bhanja *et al*., 2008) [3]. Salahi *et al.* (2011) [14] concluded that *in-ovo* injection of butyric acid in broiler eggs significantly affect the weight of the duodenum and liver than control at hatch. Razani *et al.* (2017) [13] found at 1 and 7 days of post hatch, the weight of small intestine in the nano zinc treatments were higher than control.

Lymphoid organs weight

The result of influence of *in-ovo* feeding of nano trace minerals on 18th day of incubation on lymphoid organs weight at six weeks of age is presented in Table 4.

The weight of the spleen was not significantly differed ($p \leq 0.05$) between treatment groups at the end of six weeks. The weight of bursa of Fabricius and thymus were significantly ($p \leq 0.05$) differed between treatment groups. Nano chromium injected group recorded significantly higher ($p \leq 0.05$) bursa of Fabricius weight (0.23 g/100g live body weight) than all other groups. No significant difference noticed between nano zinc and normal saline injected groups and also between nano copper and non-injected groups.

Table 4: Mean (±SE) weight of lymphoid organs (g/100g live body weight) of broiler chicken as influenced by *in-ovo* feeding of nano trace minerals on18thday of incubation

Experimental group	Description of the treatment	Lymphoid organs weight (g/100g live bodyweight)		
		Spleen	Bursa of Fabricius	Thymus
T_1	Negative control	0.11±0.01	0.15±0.01^{c}	0.37±0.04^{c}
T_2	Positive control	0.10±0.01	0.16±0.01^{bc}	0.40±0.06^{b}
T_3	Nano Zn	0.14±0.03	0.19±0.02^{b}	0.48±0.03^{a}
T_4	Nano Cu	0.12±0.02	0.16±0.02b^{c}	0.46±0.02^{ab}
T_5	Nano Cr	0.13±0.01	0.23±0.14^{a}	0.48±0.03^{a}

Means with in a column bearing different superscripts differ significantly ($p \leq 0.05$)

Nano chromium and nano zinc injected groups recorded significantly higher thymus weight (0.48 g/100g live body weight) which was not differed from nano copper injected group (0.46 g/100g live body weight). The lowest thymus weight recorded in non-injected group (0.37 g/100g live body weight).

In contrary to this result, Bakhshayesh *et al*. (2018) [2] found that *in-ovo* injection of different levels of nano zinc oxide had no effect on bursa of Fabricius weight and spleen weight in broilers. Hassan *et al*. (2021) [9] found that there was no significant difference in relative weights of spleen and bursa and immune response when broiler eggs injected with 0, 8 or 16 µg/egg of Cu sulphate, Cu acetate and nano Cu, respectively.

Conclusion

The results revealed that *in-ovo* feeding of nano trace minerals significantly ($p \leq 0.05$) improved intestinal growth and lymphoid organs weight resulting in increased the digestion and absorption of nutrients and immunity in broiler chicken.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Arumugam MP, Panda B. Processing and inspection of poultry. Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. 1970.
2. Bakhshayesh SJ, Seifdavati SF, Mirzaei A, Agheshlagh H, Abenmar Vahedi. The effect of *in-ovo* injection of nanoparticles of zinc oxide on hatching, growth performance and carcass yield of broiler chicks. Animal Science. 2018;9(21):86-92.
3. Bhanja SK, Mandal AB, Agarwal SK, Majumdar S. Effect of *in-ovo* glucose injection on post-hatch growth, digestive organ development and blood biochemical profiles in broiler chickens. Indian Journal of Animal Sciences. 2008;78:869-872.
4. Bhanja SK, Mandal AB. Effect of *in-ovo* injection of critical amino acids on pre- and post-hatch growth, immune competence, and development of digestive organs in broiler chickens. Asian-Australasian Journal of Animal Sciences. 2005;18:524-531.
5. Chous H, Chung K, Yu B. Effects of supplemental 25-hydroxy cholecalciferol on growth performance, small intestinal morphology, and immune response of broiler chickens. Poultry Science. 2009;88:2333-2341.
6. Ferket PR, Uni Z. Early feeding *in-ovo* feeding enhances early gut development and digestive capacity of poultry. XII European Poultry Conference, Verona, Italy. 2012, 10-14.
7. Geyra A, Uni Z, Sklan D. Enterocyte dynamics and mucosal development in the post-hatch chick. Poultry Science. 2001;80:776-782.
8. Gopi M, Pearlin B, Kumar RD, Shanmathy M, Prabakar G. Role of nanoparticles in animal and poultry nutrition: modes of action and applications in formulating feed additives and food processing. International Journal of Pharmacology. 2017;13:724-731.
9. Hassan HA, Arafat AR, Farroh KY, Bahnasi MS, El-Wardany I, Elnesr SS. Effect of *in-ovo* copper injection on body weight, immune response, blood biochemistry, and carcass traits of broiler chicks at 35 days of age. Animal Biotechnology. 2021;29:1-8.
10. Miller CR. Developmental gene expression of nutrient transporters in the small intestine of chickens from lines divergently selected for high or low juvenile body weight. M.Sc. thesis, Virginia Polytechnic Institute University; c2007.
11. National Research Council. Nutrient requirements of poultry. 8th rev. ed. National Academy Press, Washington, DC; c1994.
12. Pedroso AA, Chaves LS, Lopes KLA, Leandro NSM, Cafe MB, Stringhini JH. Nutrient inoculation in eggs from heavy breeders. Revista Brasileira de Zootecnia. 2006;5:2018-2026.
13. Razani K, Mottaghitalab M, Moghaddam SHH. The effect of *in-ovo* injection of zinc-methionine and nano-zinc methionine on Zn-T1 gene expression, alkaline phosphatase, and maltase activity in broiler small intestine. Animal Production Research. 2017;6:73-87.
14. Salahi A, Mousavi SN, Foroudi F, Khabisi MM, Morozi M. Effects of *in-ovo* injection of butyric acid in broiler breeder eggs on hatching parameters, chick quality, and performance. Global Veterinaria. 2011;7:468-477.
15. Smirnov A, Tako E, Ferket PR, Uni Z. Mucin gene expression and mucin content in chicken intestinal goblet cells are affected by *in-ovo* feeding of carbohydrates. Poultry Science. 2006;85:669-673.
16. Sogunle OM, Elangovan AV, David CG, Ghosh J, Awachat VB. Response of broiler chickens to *in-ovo* administration of inorganic salts of zinc, selenium, and copper or their combination. Journal of Animal Science. 2018;51(1):8-19.
17. Uni Z, Geyra A, Ben-Hur H, Sklan D. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. British Poultry Science. 2000;41:544-551.
18. Uni Z, Smirnov A, Sklan D. Pre- and post-hatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. Poultry Science. 2003;82(2):320-327.

19. Uni Z, Tako E, Gal GO, Sklan D. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poultry Science. 2003;82:1747-1754.

How to Cite This Article
Anandhi M. *In-ovo* supplementation of nano forms of trace minerals in the development of intestine and lymphoid organs of broiler chicken International Journal of Veterinary Sciences and Animal Husbandry. 2024;SP-9(6):01-05.