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Anandhi M
Assistant Professor, Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

Jayanaik
Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

V Malathi
Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

H Indresh
Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

TM Prabhu
Department of Animal Nutrition, Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

AV Elangovan
Principal Scientist, National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka, India

Corresponding Author
Anandhi M
Assistant Professor, Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University, Karnataka, India

Effect of in ovo supplementation of nano trace minerals on hatchability and post hatch performance of broiler chicks

Anandhi M, Jayanaik, V Malathi, H Indresh, TM Prabhu and AV Elangovan

Abstract
The current study was aimed to ascertain the effect of in ovo injection of nanoparticles of zinc, copper and chromium on the hatchability and post hatch performance of broiler chicks. Four hundred fertile broiler eggs from Cobb 430 flock were randomly divided into five treatments (80 eggs each) 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 each and all birds were fed with broiler starter diet (1-21 days) and finisher diet (22-42 days) as per NRC (1994) in ovo feeding of nano forms of zinc, copper and chromium at 18th day of incubation through amniotic route does not harm the developing embryo and does not affect hatchability. No significant variation (P>0.05) existed in the egg weight and hatchability per cent between the treatments. Significant variation (P>0.05) found in chick weight and ratio of chicks weight to egg weight% between treatment and control group. In ovo supplementation of zinc, copper and chromium increased the weight (% of live weight) of small intestine on seventh day of hatch.

Keywords: In ovo injection, nano trace minerals, broilers, performance

Introduction

The in ovo feeding technology has established a new science of perinatal nutrition that will open greater opportunities for improving production efficiency and animal welfare issues (fasting) of broilers. 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 ovo and neonatal feeding enhances digestive capacity, growth rate, muscle development, breast meat yield, feed conversion efficiency, body weight and decreases the incidence of skeletal disorders, post hatch mortality and morbidity (Joshua *et al*., 2016) [7]. In in ovo feeding technology, among different sites of injection, amniotic route is commonly used. Embryo consumes the amniotic fluid and its contents are exposed to the intestines and the enteric cells. Therefore, substances administered to this region will be consumed along with the amniotic fluid and presented to enteric tissues (Lokesha *et al*., 2017) [9]. In ovo administration of nano particles, acting as bioactive agents and as carries of nutrients may be seen as a new method of nano nutrition (Sawosz *et al*., 2012) [13].

Nano technology deals with the conversion of larger molecules to nanometer size. The process of conversion of these larger molecules to tiny one cause changes in the innate physical and chemical nature of the base material. The mineral antagonism in the intestine or cellular level leads to mineral imbalance at absorption, transportation and excretion. 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 also reduce their requirements and excretion (Gopi *et al*., 2017) [6].

Microminerals that are important to bone formation and strength include Cu, Zn, and Mn, which are greatly reduced in concentration in the egg by the 17th d of incubation (Yair and Uni, 2011) [20]. Zinc is important in immune system of the broiler embryo (Kidd *et al.*, 2003) [8]. Cardoso *et al*., (2007) [4] reported that additional zinc in diet of broiler has improved antibody. Zinc enhances cells mediating non-specific immunity such as neutrophils and natural killer cells (Shankar and Prasad, 1998) [14].

Copper is part of the linkage between elastin and collagen, which gives the bone its tensile strength (Carlton and Henderson, 1964) [5]. Supplemental Nano chromium picolinate improved the retention of Zn, Fe, Ca and also increased the number of lymphocytes in broiler chickens (Nattapon *et al.,* 2012) [10].

Hence, the present study was planned to examine the effect of in ovo injection of nano forms of Zinc, Copper and Chromium on the hatchability and post hatch performance of broiler chicks.

2. Materials and methods

The present nutritional study was carried out at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka. The eggs were collected from a broiler breeder (Cobb 400) farm located at Bangalore. The eggs were fumigated and cleaned with egg shell sanitizer and incubated with broad end up in forced draft automatic chicken incubator. Throughout 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 day 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 were 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, with handling temperature not lower than 35°C (Bhanja *et al*., 2004) [3]. A validation test using a water soluble dye was carried out to confirm the site (Amnion) of deposition of mineral solution. Prior to each injection (between eggs) the needle was immersed in 99.90% ethanol and replaced between treatments. 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 hatchability percent was calculated using the following formulae: Hatchability percent (%) = (Number of hatched chicks / number of eggs that were fed in ovo) × 100. Chick weight to egg weight ratio = (Chick weight (g)/ Egg weight (g)) ×100.

Table 1: Design of biological experiment

Treatments	Nano forms of minerals	In ovo feeding of nano trace minerals		No. of 18th day incubated eggs required for In ovo injection	Growth performance study after In ovo injection (No. of birds required)
		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 and all birds were fed with broiler starter diet (1-21 days) and finisher diet (22-42 days) as per NRC (1994) (Table 2).

The experimental treatments were as follows.

T1: Chicks produced from un-injected treatment as control

T2: Chicks produced from the injection with normal saline (NS).

T3: Chicks produced from the injection of nano zinc (ZnNPs).

T4: Chicks produced from the injection of nano copper (CuNPs).

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

At the end of first week of the experiment, two birds from each replicate were sacrificed and gut development was measured by recording the weights and length of duodenum, jejunum and ileum.

Table 2: Per cent ingredient and nutrient composition of experimental basal diet

Ingredients	Starter (0-21 days)	Finisher (22-42 days)
Yellow maize	47.5	57
Soya bean meal	44.3	35.5
Vegetable oil	6	5
Dicalcium phosphate	1.2	1.5
Common salt	0.30	0.35
Mineral mixture*	0.5	0.5
Vitamin premix **	0.10	0.10
DL-Methionine	0.10	0.05
Total	100.00	100.00
Metabolizable energy (Kcal/Kg)	3197	3205
Crude Protein (%)	22.94	20.00
Calcium (%)	1.00	0.90
Phosphorus (%)	0.45	0.35
Lysine (%)	1.12	1.00
Methionine (%)	0.50	0.38

* Mineral mixture: Each 100 g contains Calcium- 30%, Phosphorus- 9%, Manganese- 0.4%, Zinc-0.4%, and Iron- 2000 ppm, Copper- 500 ppm, Iodine- 100 ppm and Selenium- 23 ppm.
** Vitamin premix: Each gram contains Vitamin A-82,500 IU, Vitamin B_2-50 mg Vitamin D_3-12,000 IU and Vitamin K-10 mg, Vitamin B_1- 4 mg, Vitamin B_6- 8 mg, Vitamin B_{12}- 40 mcg, Vitamin E- 40 mg, and Calcium D pantothenate- 40 mg and Niacin-60 mg.

The data were subjected to one way analysis of variance (ANOVA) using SPSS statistical software (Version 20 for windows, SPSS). Values were expressed as mean ± SE. Means were compared by Duncan multiple range comparison test (Steel and Torrie, 1981) [10] with level of significance (P<0.05).
All the experimental procedures were assessed and approved by the Institutional Animal Ethics Committee from Karnataka veterinary, Animal and Fisheries Sciences University, Bidar and all the institutional guidelines were followed.

3. Result and Discussion

Effects of in ovo nutrition with nano forms of zinc, copper and chromium on hatchability and first week intestinal parameters are summarized in Table 3. No significant variation (P>0.05) existed in the egg weight and hatchability per cent between treatments. Chick weight (g) and ratio of chicks weight to egg weight% were significantly different (P<0.05) between treatment groups in ovo feeding of nano copper produced significantly (P< 0.05) heavier chicks (49.58 g) than other treatment groups. Nano chromium, nano copper and normal saline injected groups produced significantly (P< 0.05) heavier chicks than control group. Nano copper injected treatment group recorded significantly (P< 0.05) higher ratio of chicks weight to egg weight% (81.65) than other treatment groups. Nano chromium, nano zinc and normal saline injected groups recorded 79.73, 77.91 and 77.85 per cent chicks weight to egg weight% respectively which were significantly (P< 0.05) higher than the control group (77.76%).
Amal M. Hassan (2018) [1] who noticed that the nano selenium recorded the highest value by 4.73, 4.31and 4.73%, for egg weight(g) and ratio of chicks weight to egg weight% and hatchability%, while, nano zinc recorded 2.28, 2.17 and 3.30% for hatching egg weight (g) and Ratio of chicks weight to egg weight% and hatchability% than control, respectively. Patric Joshua *et al*., (2016) [7] recorded that in ovo feeding of nano minerals (at 5 ppm level of zinc, copper and selenium) did not significantly influence (P>0.05) the hatching egg weight, ratio of chicks weight to egg weight% and hatchability%. Oliveira *et al*. (2015) observed that injection of 0.5 mg of zinc along with manganese and copper did not influence the hatch weight of chicks compared to control. The degree of response to in ovo feeding may depend on genetics, breeder hen age, egg size, and incubation conditions (Uni *et al*., 2000) [18]. The hatchability percentage obtained in this study for nano forms of zinc, copper and chromium were higher than previous study reported by Bakyaraj *et al*., (2012) [2], who reported hatchability of 81.3% on in ovo feeding of selenium 0.3 µg, Zinc 80 µg, Copper 16 µg and manganese 120mg/egg. They also reported a hatchability of 61.3% on in ovo feeding of selenium 0.3 µg, zinc 80 µg, iron 160 µg and iodine 0.7 µg/egg. Salmanzadeh *et al*., (2012) [12] reported a reduced hatchability on in ovo injection of glucose and attributed it to the development of allergic reactions under air sac that stopped the respiration of the embryo and caused its death. Such type of change occurred with regard to in ovo feeding of coarse form of minerals when particle size was larger. However, nano form of minerals owing to its particle size, have an ability to remain in colloidal state and might have not caused harm to the embryo. Oliveira *et al*. (2015) [11] observed that injection of 0.5 mg of zinc along with manganese and copper did not influence the hatch weight of chicks compared to control. Uni *et al*. (2003) [19] indicated that during the last 3 days of incubation, the weight of the intestine as a proportion of embryo weight increased from approximately 1% at 17 days of embryonic age to 3.5% at hatch.

Table 3: Effect of in ovo injection in broiler eggs with nano form of trace minerals (Mean ±SE) on egg weight, hatch weight of chicks, their ratio, hatchability percent and small intestine weight and length of chicks at 7 day of age

Treatment	Hatching egg weight (g)	Hatch of chicks weight (g)	Ratio of chicks weight to egg weight%	Hatchability%	SIW (%)	SIL (cm)
T1	60.71±0.77	47.21^{c}±0.80	77.76^{c} ±0.78	88.01± 4.11	6.45±3.01	47.00±2.40
T2	60.78±0.82	47.32^{bc}±0.77	77.85^{bc} ±0.83	89.21±3.57	7.38±2.71	47.50±2.52
T3	60.86±0.73	47.42^{bc}±0.94	77.91^{bc} ±1.02	87.06±5.08	7.40±2.90	48.00±2.32
T4	60.72±0.83	49.58^{a}±0.94	81.65^{a} ±1.02	89.36±3.08	7.49±2.88	49.57±2.50
T5	60.88±0.91	48.54^{b}± 0.75	79.73^{b} ±0.90	88.25±2.15	7.45±2.41	49.10±2.87
Sig.	ns	*	*	ns	ns	ns

a,b,c: Means within a column with different superscripts are significantly different (P< 0.05).
Sig. = Significant, * (P< 0.05), ns = not significant. SIW - Small intestine weight, SIL - Small intestine length.

At first week of age, small intestine weight and length did not differ significantly between treatment groups. Amal M. Hassan (2018) [1] also reported the same result while injecting the broiler eggs with 15 ppm/egg nano selenium and nano zinc. Highest value of small intestine weight and length were recorded in nano copper injected gruop (7.49% and 49.57% respectively) and lowest values were recorded in control group (6.45% and 47% respectively). Nano zinc injected group recorded highest value of small intestine length (49.57 cm) and the lowest value was recorded in control group (47.00 cm).
In chicks, at 3–7 days of age, the digestive organs will grow at a faster rate as compared to other organs and the small intestine increases in weight more quickly than the body mass during the first week of age (Sklan, 2001) [15]. 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) [18]. Tako *et al*., (2005) [17] observed that in ovo injection of zinc and methionine in amniotic fluid on 18th day of incubation increased the villus surface area and enhanced the expression of genes and biochemical activity of intestinal transporters and enzymes and thus accelerated intestinal development.

4. Conclusion
In ovo feeding of nano forms of zinc, copper and chromium at 18th day of incubation through amniotic route does not harm the developing embryo and does not affect hatchability.

No significant variation (*P*>0.05) existed in the egg weight and hatchability per cent between the treatments groups. Significant variation (*P*>0.05) found in chick weight and ratio of chicks weight to egg weight% between treatment and control group. In ovo supplementation of zinc, copper and chromium increased the weight of small intestine during the first week of its age.

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