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

Effect of *In ovo* supplementation of nano forms of zinc, copper and chromium on production performance and serum biochemistry of broiler chicken

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 nano particles of zinc, copper and chromium on production performance and serum biochemistry of broiler chicken. Four hundred fertile broiler eggs from Cobb 430 flock 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). *In ovo* feeding of nano forms of zinc, copper and chromium at 18th day of incubation through amniotic route did not influence the growth performance of broiler chicken. However, better serum glucose, total protein and globulin levels were observed in nano trace mineral supplemented groups.

Keywords: *In ovo* injection, Nano zinc, nano copper, nano chromium, broilers, performanc, serum biochemistry

Introduction
The *In ovo* feeding allows the delivery of various supplements directly in to chicken embryos, facilitates 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) [11]. Nano technology deals with the conversion of larger molecules to nanometer size. The process of conversion of these larger molecules in 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.
The mineral nano particles not only increase the bioavailability of minerals also reduce their requirements and excretion (Gopi *et al*., 2017) [8]. Cardoso *et al*., (2007) [3] 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) [19]. Copper is part of the linkage between elastin and collagen, which gives the bone its tensile strength (Carlton and Henderson, 1964) [4]. Supplemental Nano chromium picolinate improved the retention of Zn, Fe, Ca and increased the number of lymphocytes in broiler chickens (Nattapon *et al*., 2012) [16]. Hence, the present study was planned to examine the effect of *In ovo* injection of nano forms of zinc, copper and chromium on production performance and serum biochemistry of broiler chicken.

Materials and methods
The present nutritional study was carried out at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru, Karnataka. The eggs were fumigated and cleaned with eggshell 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 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, with handling temperature not lower than 35°C (Bhanja *et al.*, 2004) [2]. 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 (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 and all birds were fed with broiler starter diet (1-21 days) and finisher diet (22-42 days) as per NRC (1994).

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).

Data on hatch weight, body weight, weight gain, feed consumption, FCR and serum glucose, total protein, albumin, globulin were recorded. The body weights of the broiler chicken were recorded every week by using an electronic balance with 0.2 g accuracy. The broiler chickens were fed *ad libitum* feed during the experimental period. Feed consumption up to 6th week was recorded. Feed conversion ratio was calculated by dividing average feed consumption by average body weight gain. The serum glucose values were estimated by using commercial kit from AGAPPE based on Glucoseoxidase/Peroxidase (GPO-PAP) method. The serum total protein was estimated by using AGAPPE kit based on Direct Biuret modified method (Gornall *et al.*, 1949) [9] and serum albumin based on Bromocresol Green method (Doumas *et al*., 1971) [5]. The serum globulin level was calculated by subtracting serum albumin from serum total protein level and expressed as g/dl. 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) [20] 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 production performance and serum biochemistry are summarized in table 2 and table 3, respectively.

Table 2: Effect of *In ovo* injection of nano trce minerals on overall weight gain, feed consumption and feed coversion ratio of broilers (1-6weeks)

Experimental group	Description of the treatment	Body weight (g)	Feed consumption (g/bird)	Feed conversion ratio
T_1	Negative control	2104.73± 42.41	3787.51 ± 31.38	1.83 ± 0.03
T_2	Positive Control	2143.06 ± 50.47	3779.74 ± 29.33	1.79 ± 0.03
T_3	Nano Zn	2163.63 ± 41.34	3780.76 ± 41.27	1.78 ± 0.04
T_4	Nano Cu	2158.54 ± 43.47	3722.26 ± 42.42	1.75 ± 0.03
T_5	Nano Cr	2136.49 ± 44.12	3757.51 ± 30.34	1.79 ± 0.05

Means within a column bearing different superscripts differ significantly ($P\leq0.05$)

Table 3: Effect of *In ovo* injection of nano trce minerals on serum glucose, total protein, albumen and globulin at 6 weeks of age

Experimental group	Description of the treatment	Glucose (mg/dl)	Total protein (g/dl)	Albumen (g/dl)	Globulin (g/dl)
T_1	Negative control	162.89 ± 0.87^c	4.46 ± 0.21^c	2.22± 0.05	2.24 ± 0.22^c
T_2	Positive Control	160.66 ± 3.19^c	4.75± 0.02b^c	2.23 ± 0.01	2.52 ± 0.03^c
T_3	Nano Zn	149.47 ± 2.58^b	4.99 ± 0.17^{ab}	2.24 ± 0.03	2.75 ± 0.17^{ab}
T_4	Nano Cu	145.38 ± 1.54^b	5.20 ± 0.06^{ab}	2.23 ± 0.04	2.97 ± 0.07^a
T_5	Nano Cr	138.40 ± 1.39^a	5.35 ± 0.15^a	2.26 ± 0.03	3.09 ± 0.14^a

Means within a column bearing different superscripts differ significantly ($P \leq 0.05$)

In ovo feeding of nano trace minerals was not significantly differed (P≤0.05) on overall body weight, feed consumption and feed conversion ratio between treatment groups. These findings were in agreement with the report of Jose *et al.* (2017) [10] who found that *In ovo* injection in broiler eggs with nano zinc at 0.04 and 0.08 mg per egg had no difference (P≤0.05) in body weight gain, while Wang *et al.* (2011) [21] observed improved body weight and weight gain in broilers subjected to *In ovo* feeding with copper-loaded chitosan nanoparticles. Bello *et al.* (2014) [1] observed no significant difference in body weight gain of broilers injected with different levels of 25- hydroxy cholecalciferol.

In the present study, the cumulative feed consumption was not significantly differed between the treatment groups. This is in agreement with Bhanja *et al.* (2008) [2] who reported that there was no difference in feed intake of broilers injected with different amino acids on the 18th day of incubation into amniotic fluid. Whereas, increased feed consumption was recorded by Kadam *et al*. (2013) [13] in broilers injected with betaine through intra amnion on 18th day of incubation. Pedroso *et al*. (2006) [17] observed no difference in feed intake and feed conversion ratio of chicks inoculated with glucose at 16 days of incubation.

At the end of 42nd day, the serum glucose level was significantly lower (P≤0.05) in nano chromium injected group (138.40 mg/dl) than all other treatment groups. The highest serum glucose level was recorded in non-injected control group (162.89 mg/dl) and this level was significantly higher (P≤0.05) than other injected groups. This result was in agreement with Natalia *et al*. (2013) [14] who observed that *In ovo* injection of colloidal nano copper at 60 ppm in broiler eggs at 18th day of incubation contributed to reduced concentrations of blood serum glucose. However, non-significant changes in the serum glucose were observed by Lotfi *et al.* (2013) [13] by the *In ovo* injection of gherline in broilers. But Ebrahimnezhad *et al*. (2011) [6] recorded higher levels of serum glucose in *In ovo* nutrients fed broiler than control during the entire study period.

In the present study, no significant difference was noticed in serum albumen level between treatment groups. But significant increase (P≤0.05) in serum total protein (5.35 g/dl) and globulin (3.09 g/dl) levels were recorded in nano chromium fed group than all other treatment groups. There was no significant difference (P≤0.05) noticed in serum total protein and globulin levels among other nano trace mineral (nano zinc and nano copper) injected groups and were significantly higher than other control groups. Shokraneh *et al*. (2020) [19] also found that *In ovo* injection of 40 µg Nano-Se and 500 µg Nano-ZnO significantly increased serum total protein in broilers. This finding was concurred with Foye *et al.* (2006) [7] who reported that *In ovo* injection of egg white proteins and glucose were significantly improved serum protein profile.

4. Conclusion

In ovo feeding of nano forms of zinc, copper and chromium at 18th day of incubation through amniotic route did not influence the growth performance of broiler chicken. However, better serum glucose, total protein and globulin levels were observed in nano trace mineral supplemented groups.

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