
EFFECT OF *IN OVO* AND ORAL SUPPLEMENTATION OF COPPER AND ZINC NANOPARTICLES ON HATCHABILITY OF BROILER HATCHING EGGS AND CHICK BODY WEIGHT

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ABSTRACT

In ovo supplementation of nutrients is emerging as an innovative technique and can be used to enhance the productivity of broiler chicken. Mineral supplementation *in ovo* can benefit the embryonic development in the later stage. *In ovo* inoculation with nanoparticles of minerals will help in exploring the potential of nanotechnology in embryonic nutrition. It may improve the overall health and performance of broiler chicks post-hatch. This study has been proposed to investigate the impact of *in ovo* and post hatch oral supplementation of nano copper (CuNP) and nano zinc (ZnNP) on the hatchability of broiler hatching eggs and the body weight of chicks at hatch and up to two weeks of age. Six hundred eggs were procured, candled, sorted and randomly allotted into seven treatment groups with 80 eggs each. At the 18th day of incubation the control

group was *in ovo* inoculated with normal saline. Two treatment groups were *in ovo* inoculated with 0.5 ml of nano copper solution (12µg/egg), one without and other with post hatch oral supplementation (T2 and T4, respectively). T3 group was not *in ovo* inoculated but supplemented orally. Similarly, two treatment groups were *in ovo* inoculated with nano zinc solution (80 µg/egg), one without and another with oral supplementation post hatch (T5 and T7, respectively). T6 group was orally supplemented nano zinc solution. Results of the study indicate that hatchability rates were not adversely influenced by *in ovo* supplementation. The day-old body weight of chicks was also not affected showing no significant difference between treatment groups subjected to *in ovo* supplementation

Keywords: Poultry, Broiler, Hatchability, *In Ovo*, Copper nanoparticles, Zinc nanoparticles

INTRODUCTION

The increased demand for meat and eggs has led to phenomenal expansion in the poultry business worldwide. The Indian poultry operation has evolved significantly in the past 20 years, going from being an inexpensive backyard activity to a significant enterprise. This industry has experienced remarkable development, with growth rates for egg and broiler production of 8.51 and 7.52 per cent, respectively (BAHS, 2019). Hatchability is a critical parameter in the poultry industry, directly impacting the productivity and profitability of hatcheries and poultry farms. Various factors, including nutrition, genetics, environment, and management practices, influence hatchability rates. Among these factors, micronutrient supplementation has gained significant attention due to its potential to improve the health and performance of chicks.

In ovo supplementation of nano minerals represents an innovative approach to optimize nutrient delivery and support embryonic development and post-hatch growth in poultry. Various nutrients have been *in ovo* inoculated with favourable response. The beneficial effects of *in ovo* supplementation of carbohydrates on growth (Smirnov *et al.*, 2006), vitamins on hatchability (Yair *et al.*, 2015) and minerals

on growth (Hassan *et al.*, 2021) have been documented. Recent advancements in nanotechnology have opened new avenues for utilisation of nanoparticles of minerals in poultry nutrition. Nanoparticles of minerals, such as copper (Cu) and zinc (Zn), offer increased bioavailability and efficacy compared to conventional mineral forms. Hence, this study was designed to evaluate the effect of *in ovo* inoculation of copper and zinc nanoparticles on hatchability of broiler hatching eggs and day-old body weight of hatched out chicks.

MATERIALS AND METHODS

Six hundred hatching eggs of broiler chicken (Vencobb 400Y), were purchased from a breeding farm (FIC, Pollachi) for the study. After removing the eggs from the packaging, it was fumigated using potassium permanganate and formalin (3X concentration). The experimental design comprised of seven treatment groups, each consisting of 80 hatching eggs. The hatching eggs were selected by candling and set for incubation in the setter compartment of the incubator. The incubator was run ensuring standard temperature (37.2 – 37.5°C) and relative humidity (55-60%) conditions. After candling, the eggs were moved to the hatcher chamber on the eighteenth day of incubation. For the experiment, only fertile eggs containing viable embryos were used.

A cotton swab dipped in 70 per cent isopropyl alcohol was used to sanitize the surface of the eggs. The *in ovo* inoculation procedure followed was as per the method standardised by Bhanja *et al.* (2004). A pinhole was punched using a sterile 20 gauge needle on the broad end of the egg. The needle was sterilized by dipping in 70 per cent isopropyl alcohol after punching the hole to prevent contamination. A sterile 24 gauge hypodermic needle of 25.0 mm length was used for the *in ovo* inoculation procedure. The punch hole was sealed immediately using molten paraffin wax. After the sealing process, the eggs were transferred to the hatcher compartment and incubated under standard temperature (36.8 – 37.2°C) and relative humidity (60-65 per cent) conditions till the 21st day of incubation.

The control group (T1) was *in ovo* inoculated with 0.5 ml of normal saline. Two treatment groups were *in ovo* inoculated with copper nanoparticles (12 µg/egg), one without and the other with post hatch oral supplementation of copper nanoparticles (12 mg/L) (T2 and T4, respectively). Similarly, two treatment groups were *in ovo* inoculated with nano zinc solution (80 µg/egg), T5 without post hatch oral supplementation of zinc nanoparticles and T7 with post hatch oral supplementation of zinc nanoparticles (80 mg/L). T3 group

was not *in ovo* inoculated but orally supplemented copper nanoparticles (12 µg/mL). Similarly, T6 group was not *in ovo* inoculated but supplemented orally zinc nanoparticles (80 mg/L). Hatchability was determined as the per cent chicks hatched from the total number of eggs set. The body weight of chicks at day of hatch was recorded.

$$\text{Hatchability} = \frac{\text{Number of chicks hatched out} \times 100}{\text{Number of eggs set}}$$

Statistical analysis of the data collected was done by one way ANOVA using SPSS version 24.0.

RESULTS AND DISCUSSION

The mean values of hatchability of broiler hatching eggs and day-old body weight of the chicks hatched out of treatment groups subjected to *in ovo* supplementation of Cu and Zn nanoparticles have been presented in Table 1. Analysis of the hatchability across the seven treatment groups revealed no significant ($p > 0.05$) differences. The supplementation of normal saline, copper nanoparticles, and zinc nanoparticles resulted in mean hatchability values ranging from 94.67 per cent to 97.33 per cent across groups, indicating no adverse effect of *in ovo* supplementation of CuNPs and ZnNPs on hatchability of broiler chicks

under standard incubation conditions. The day-old body weight of the chicks is presented in Table 1. The mean values of body weight in day-old chicks showed no significant difference between treatment groups supplemented *in ovo* of copper and zinc nanoparticles, the normal saline inoculated control group and the treatment groups which were not supplemented *in ovo*. The chicks in treatment groups *in ovo* inoculated copper and zinc nanoparticles showed no significant difference with the groups supplemented copper and zinc nanoparticles orally post hatch and the control group in the first and second weeks of age.

The findings of this study agree with the observation of Anandhi *et al.* (2021) in broiler hatching eggs subjected to *in ovo* supplementation with zinc, copper and chromium nanoparticles at 18th day of incubation. Similar results on hatchability were reported by Bakyaraj *et al.* (2012) with *in ovo* supplementation of a combination of zinc, copper, selenium and manganese, Goel *et al.* (2013) with *in ovo* supplementation of copper and iron on the 14th day of incubation and Sogunle *et al.* (2018) with *in ovo* administration of inorganic salts of selenium, copper and their combination in broiler fertile eggs on the 18th day of incubation. One possible

Table 1. Mean (\pm SE) hatchability of hatching eggs subjected to *in ovo* inoculation with copper and zinc nanoparticles, per cent

Parameter	Treatments							P value
	T1	T2	T3	T4	T5	T6	T7	
Hatchability (%)	96.00 \pm 0.33	96.00 \pm 2.31	94.67 \pm 1.33	97.33 \pm 1.33	96.00 \pm 2.31	97.33 \pm 1.33	97.33 \pm 1.33	0.87 ^{ns}

ns- non significant

Table 2. Mean (\pm SE) weekly body weight of broiler chicks subjected to *in ovo* inoculation and post hatch oral supplementation with CuNPs and ZnNPs, g

Age in weeks	Treatments							P value
	T1	T2	T3	T4	T5	T6	T7	
Day-old	44.57 \pm 1.30	44.69 \pm 0.41	44.60 \pm 0.28	44.68 \pm 0.59	43.91 \pm 0.69	43.74 \pm 0.99	43.80 \pm 0.51	0.25 ^{ns}
1	152.33 \pm 5.58	155.79 \pm 4.96	158.25 \pm 3.01	159.93 \pm 1.38	148.97 \pm 3.00	155.55 \pm 6.07	159.61 \pm 2.20	0.50 ^{ns}
2	384.78 \pm 3.40	394.97 \pm 10.62	399.86 \pm 3.98	409.20 \pm 8.47	385.73 \pm 11.23	387.16 \pm 10.14	397.84 \pm 9.89	0.49 ^{ns}

ns- non significant

explanation could be the size and surface characteristics of the nanoparticles used, which might influence their aggregation behaviour and bioavailability (Kim and Kang, 2022).

The *in ovo* inoculation of the minerals did not significantly influence day-old body weight of the chicks as the copper and zinc content of the egg were optimum as stated by Awachat *et al.* (2020). Similar findings with the present study were observed in day-old body weight of broiler chicks by Scott *et al.* (2018) on *in ovo* supplementation of copper nanoparticles and Bakyaraj *et al.* (2012) with *in ovo* supplementation of a combination of zinc, copper, selenium and manganese. The *in ovo* inoculation and post-hatch oral supplementation of copper and zinc nanoparticles showed no significant difference between treatment groups in the mean weekly body weight up to two weeks of age. The findings of this study were in agreement with the observations of Mroczek-Sosnowska *et al.* (2015) on *in ovo* supplementation of CuNPs, Scott *et al.* (2018) in broilers subjected to *in ovo* supplementation with copper sulphate and Sogunle *et al.* (2018) on *in ovo* copper sulphate supplementation.

The findings of this study suggest that the concentrations and methods of administration of copper and zinc

nanoparticles used in this experiment did not influence hatchability in broiler chicks. Despite the presence of bioavailable forms of copper and zinc, no significant effects were observed in body weight at hatch and up to two weeks of age. This suggests that the metabolic pathways typically modulated by these minerals might not be significantly influenced at the nanoparticle scale.

CONCLUSION

The *in ovo* supplementation of copper nanoparticles (12 µg/egg) and zinc nanoparticles (80 µg/egg) showed no significant effect on hatchability rates which signifies the fact there were no adverse reactions. The body weight of chicks at day-old and upto two weeks of age also showed no significant difference between treatment groups implying that *in ovo* supplementation of copper and zinc nanoparticles on the 18th day of incubation does not significantly influence the body weight of chicks at the time of hatch or during the initial growth period. Further research in the dosages and biochemical effects of copper and zinc nanoparticles are necessary.

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