Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.4.704-711

Effect of *in ovo* and post hatch oral supplementation of copper and zinc nanoparticles on growth and immune response in broilers#

A.M. Vimal[1*], P. Anitha[2], C. Binoj[2], C.J. Beena[2] and R. Ambily[3]

[1]College of Avian Sciences and Management, Thiruvazhamkunnu, Palakkad – 678 601, [2]Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, [3]Assistant Professor, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

Citation: Vimal, A.M., Anitha, P., Binoj, C., Beena, C.J. and Ambily, R. 2024. Effect of in ovo and post hatch oral supplementation of copper and zinc nanoparticles on growth and immune response in broilers.
J. Vet. Anim. Sci. **55** (4):704-711

Received:12.06.2024 Accepted:26.09.2024 Published: 31.12.2024

Abstract

In ovo supplementation of nutrients is emerging as an innovative technique used to enhance the productivity of broiler chicken. In ovo inoculation with mineral nanoparticles help in exploring the potentials of nanotechnology in embryonic nutrition. This study has been conducted to investigate the effect of in ovo supplementation of copper nanoparticles (CuNP) and zinc nanoparticles (ZnNP) on the growth and immune response of broiler chicken. Six hundred eggs were incubated. At 18 days of incubation the eggs were candled and the fertile eggs allotted into seven treatment groups with 80 eggs each. The control group (T1) was in ovo inoculated with normal saline. The treatment groups T2, T3 and T4 were supplemented CuNP in ovo (12µg/egg), orally post hatch and in combination (in ovo + oral) respectively. The treatment groups T5, T6 and T7 were supplemented with ZnNP (80 µg/egg) in ovo, orally post hatch and in combination. The results of weekly body weight and bodyweight gain up to six weeks of age showed that in ovo supplementation of copper and zinc nanoparticles did not influence growth significantly. The feed consumption and FCR did not differ significantly between treatment groups. The humoral and cell mediated immunity immunity were non-significant in the copper and zinc nanoparticles supplemented groups. Thus, the in ovo and dietary supplementation of copper and zinc nanoparticles has similar effects on growth and immune response of broiler chicken.

Keywords*: Broiler, growth, immune response, in ovo nutrition, copper, zinc, nanoparticles*

The incubation period and early chick period covers about 50 per cent of the lifespan of a broiler chicken, which increases the importance of the period of embryonic development. Any factor that promotes growth during this period will have a remarkable effect on the performance and health of broilers (Noy and Uni, 2010). Day-old chicks may not have feed access for about 48–72 hours during the hatch day operations and transportation from the hatchery to farms. This delay may have an impact on the early chick nutrition, development of intestinal villi and the initiation of stimulation of the immune mechanism of chicks (Lingens *et al*., 2021).

In ovo inoculation of nutrients was proposed as a measure to overcome these shortcomings affecting chick growth. It is the method of inoculation of nutrients directly into the amniotic sac of the developing embryo at a later stage

#Part of PhD thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala
**Corresponding author: vimal@kvasu.ac.in Ph. 9447770207*

of embryonic development. The *in ovo* supplementation of eggs can provide early nutrients and additives to embryos, stimulate gut microflora, and mitigate the adverse effects of starvation during post-hatch periods (Uni *et al.*, 2005). *In ovo* nutrient supplementation is an emerging technique, which is gaining importance due to its beneficial effects on embryonic development and post hatch growth and immune status in chicken. Different compounds are delivered to the embryo at day 17 to 18 of incubation by amniotic sac route. Energy sources like glucose when supplemented *in ovo* showed improved hatchability and body weight of chicks at hatch (Naeem *et al.,* 2022). *In ovo* supplementation with vitamins improved immune response in broiler chicken (Bhanja *et al.,* 2012). Minerals like zinc, iron, copper and manganese when *in ovo* inoculated showed favourable responses with better hatchability (Bakyaraj *et al*., 2012), growth and immune response (Anandhi *et al*., 2022).

Inorganic copper and zinc are commonly used for the dietary supplementation in chicken. The application of nanotechnology can bring about drastic changes in the properties of copper and zinc (Scott *et al*., 2018). Hence this experiment was planned to study the effects of *in ovo* supplementation of copper and zinc nanoparticles along with its post hatch oral supplementation on growth and immune status of chicken.

Materials and methods

Experimental design

Six hundred hatching eggs of commercial broiler (Vencobb-430Y) were procured from the broiler breeder farm, M/S Farms India Chicken, Udumalpet. The eggs were randomly allotted into seven treatments which were incubated under standard conditions. At eighteen days of incubation, the eggs were candled and sixty fertile eggs from each treatment were transferred to the hatcher. The seven treatments are detailed below in Table 1.

Nanoparticles

The nanoparticles of copper and zinc for *in ovo* inoculation and post-hatch oral supplementation in broilers for this study were procured from Sigma Aldrich (Darmstadt, Germany). The copper nanoparticles (CuNPs) with average particle size of 25 nanometer and zinc oxide nanoparticles (ZnNPs) with average particle size of 50 nanometer were stored in amber-coloured bottles within airtight containers to ensure stability.

Nanoparticle solutions

The nano copper and nano zinc solutions were prepared in normal saline for *in ovo* inoculation (Anandhi *et al*., 2022). Nano copper solution was prepared by dissolving 2.4 mg of CuNPs in 2 mL ethanol using a magnetic stirrer, which was further made up to 100 mL with normal saline.

Nano zinc oxide solution was prepared by mixing 16 mg of ZnNPs in 100 mL normal saline using a magnetic stirrer followed by ultrasonic mixing for 20 minutes using an ultrasonic mixer. The prepared solutions of Cu and Zn nanoparticles were autoclaved and cooled to 37°C. The *in ovo* inoculation was carried out using these solutions.

Incubation and in ovo inoculation

The eggs were fumigated using potassium permanganate and formalin (3X concentration). All the eggs were inspected for any shape or shell abnormalities, weighed, and subsequently assigned randomly to seven treatment groups. The eggs were marked and then placed into setter trays, which were subsequently loaded into the incubator compartment.

The incubator was run ensuring standard temperature (37.2 – 37.5^{0}C) and relative humidity (55-60%) conditions. The eggs were transferred to the hatcher compartment on the 18th day of incubation. The eggs were candled at the time of transfer and the fertile eggs with live embryo were taken for the experiment. The eggs were divided into seven treatments of sixty eggs each.

The surface of the eggs at the broad end was sanitised by swabbing using a cotton swab dipped in an alcoholic sanitiser (70 per cent isopropyl alcohol). The *in ovo* inoculation was done as per the method prescribed by Bhanja *et al*. (2004). At the centre of the air cell on the broad end, a pinhole was punched using a sterile 20 gauge

Table 1. Experimental design of different treatment groups for *in ovo* and post-hatch oral supplementation of CuNPs and ZnNPs in broilers (n=12 in each group)

Treatment	*In ovo* inoculation at 18th day of incubation	Oral supplementation in drinking water to chicks
T1 (Control)	Normal saline	Nil
T2	CuNPs 12 µg/egg	Nil
T3	Nil	CuNPs 12 mg/L
T4	CuNPs 12 µg/egg	CuNPs 12 mg/L
T5	ZnNPs 80 µg/egg	Nil
T6	Nil	ZnNPs 80 mg/L
T7	ZnNPs 80 µg/egg	ZnNPs 80 mg/L

needle. This hole was used for the inoculation of the nano copper and nano zinc solutions into the amniotic cavity of the embryo. After each hole is punched the needle was sterilized by dipping in 70 per cent isopropyl alcohol to prevent contamination. A sterile 24 gauge hypodermic needle of 25 mm length was used for the *in ovo* inoculation procedure. This was immediately followed by sealing of the punch hole using molten paraffin wax. The eggs were then transferred to the hatcher compartment and incubated under standard conditions till the 21st day of incubation.

The day-old chicks were weighed, wing banded and 12 chicks allotted to each respective labelled replicate pen. From day-old onwards the chicks in the treatment groups allotted oral supplementation were given the nano mineral solutions through drinking water till the end of the experimental period.

The birds were fed with pre-starter diet up to seven days of age, starter diet from eight to 21 days of age and then finisher diet up to 42 days of age. The feed and water were given *ad libitum*. The feed samples were subjected to proximate analysis as per AOAC (2016). Standard management practices were followed throughout the experiment.

The body weight (g) of individual birds were recorded at weekly intervals from day-old to six weeks of age and weekly body weight gain was calculated. Feed consumed (g) by birds in each replicate was recorded at weekly intervals up to six weeks of age and from this data cumulative feed consumption for the entire period was calculated. Feed conversion ratio (kg of feed consumed per kg weight gain) was calculated replicate wise, based on the data on body weight gain and feed consumption.

Humoral and cell mediated immune response

Blood from three birds in each treatment was collected at 0, 14, 28 and 42 days of age and the serum separated. The antibody titre in the serum against Newcastle Disease was tested using the standard Haemagglutination Inhibition test (HI) as per OIE, (2021).

Three chicks from each treatment were inoculated with 0.25 mL of 2, 4-dinitrochlorobenzene (DNCB) at 28 days of age. The inoculation was done in the interdigital space between the third and fourth toes of the right foot by intradermal injection. In the same interdigital space of the left foot of the same bird, 0.25 mL acetone was injected as control. Prior to inoculation, 24, 48 and 72 hours post challenge, the cell reaction was evaluated by measuring skin thickness with vernier callipers. Based on the above measurements, the following calculations were made.

1) DNCB Response = thickness of the right foot after DNCB inoculation – thickness of the right foot before DNCB inoculation (mm)

2) Acetone control response = thickness of the left foot after acetone inoculation – thickness of the left foot before DNCB inoculation (mm)

The cutaneous hypersensitivity at each evaluation time was calculated as the difference between DNCB and acetone response.

Results and discussion

Body weight

The mean day-old body weight and weekly body weight recorded up to six weeks of age in broiler chicks are presented in Table 2. The day-old body weight of chicks showed no significant difference between treatment groups supplemented with copper and zinc nanoparticles *in ovo*, orally and in combination and the control group *in ovo* inoculated with normal saline. Similar results were observed in day-old body weight of broiler chicks by Scott *et al*. (2018) with *in ovo* supplementation of copper sulphate and copper nanoparticles on the 18th day of incubation and Olatunbosun *et al*. (2022) with *in ovo* supplementation of organic zinc, organic copper and their combination. This indicates that the *in ovo* inoculation of copper or zinc have not adversely affected the growth of the embryo.

The mean weekly body weight of broiler chicks from the first to sixth week of age was also not significantly influenced by *in ovo* inoculation, oral supplementation and their combination with copper and zinc nanoparticles in this study. The post-hatch growth of broiler chicks was not significantly influenced by *in ovo* and post hatch oral supplementation of copper and zinc nanoparticles as the feed provided during the growth period contained optimum amount of these minerals.

Similar findings of non-significant effect of these mineral nanoparticles on body weight were reported in broiler chicks by Scott *et al*. (2018) with *in ovo* supplementation of copper sulphate and copper nanoparticles along with oral supplementation in drinking water. Similar results were reported by Jose *et al*. (2018) in broiler chicks hatched from the eggs *in ovo* supplemented with zinc oxide nanoparticles. Liu *et al*. (2023) also observed similar results in chicks supplemented copper nanoparticles orally.

Feed consumption

The data on mean feed consumption of broilers under different treatment groups up to six weeks of age (Table 3) did not differ significantly between the groups supplemented with CuNPs and ZnNPs *in ovo*, orally post-hatch and in combination.

Reports of similar trends in feed consumption were reported by Jose *et al.* (2018) in broiler chicken hatched from the eggs *in ovo* supplemented zinc oxide

Table 2. Mean (± 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							F value	P value
	T1 Control	T2 CuNPs *In ovo*	T3 CuNPs Oral	T4 CuNPs *In ovo*+ oral	T5 ZnNPs *In ovo*	T6 ZnNPs Oral	T7 ZnNPs *In ovo*+ oral		
Day old	44.57 ± 1.30	44.69 ± 0.41	44.60 ± 0.28	44.68 ± 0.59	43.91 ± 0.69	43.74 ± 0.99	43.80 ± 0.51	2.93	0.25^{ns}
1	152.33 ± 5.58	155.79 ± 4.96	158.25 ± 3.01	159.93 ± 1.38	148.97 ± 3.00	155.55 ± 6.07	159.61 ± 2.20	1.03	0.45^{ns}
2	384.78 ± 3.40	394.97 ± 10.62	399.86 ± 3.98	409.20 ± 8.47	385.73 ± 11.23	387.16 ± 10.14	397.84 ± 9.89	0.92	0.5^{ns}
3	813.86 ± 13.49	823.09 ± 22.23	821.56 ± 4.52	838.03 ± 11.81	791.80 ± 22.49	798.46 ± 17.82	824.66 ± 13.51	0.97	0.49^{ns}
4	1304.20 ± 48.70	1317.28 ± 25.14	1285.49 ± 32.81	1326.44 ± 20.39	1289.39 ± 31.33	1250.33 ± 35.21	1275.44 ± 11.15	0.82	0.57^{ns}
5	1825.22 ± 89.96	1855.03 ± 15.48	1790.41 ± 28.21	1883.33 ± 13.69	1840.12 ± 3.82	1755.84 ± 60.68	1796.83 ± 11.80	1.56	0.23^{ns}
6	2382.83 ± 93.71	2396.15 ± 26.20	2382.59 ± 29.27	2473.86 ± 48.67	2457.51 ± 29.21	2306.29 ± 53.18	2350.91 ± 27.18	1.94	0.15^{ns}

ns-non significant

Table 3. Mean (± SE) weekly feed consumption and cumulative six-week feed consumption of broiler chicks subjected to *in ovo* inoculation and post hatch oral supplementation with CuNPs and ZnNPs, g

Age in weeks	Treatments							F value	P value
	T1 Control	T2 CuNPs *In ovo*	T3 CuNPs Oral	T4 CuNPs *In ovo*+ oral	T5 ZnNPs *In ovo*	T6 ZnNPs Oral	T7 ZnNPs *In ovo*+ oral		
1	119.50 ± 0.50	118.33 ± 4.37	110.67 ± 9.87	119.33 ± 3.76	113.00 ± 3.06	118.67 ± 5.55	109.67 ± 2.33	0.80	0.58^{ns}
2	289.36 ± 3.22	294.91 ± 6.13	277.18 ± 18.20	307.48 ± 8.92	295.15 ± 15.41	278.49 ± 10.75	265.97 ± 9.45	1.51	0.25^{ns}
3	561.48 ± 13.4	568.58 ± 19.48	524.67 ± 36.15	563.70 ± 10.16	538.39 ± 21.05	552.27 ± 2.86	586.91 ± 5.75	1.19	0.36^{ns}
4	839.42 ± 39.98	846.33 ± 28.61	744.66 ± 64.37	835.79 ± 5.56	851.79 ± 14.64	813.18 ± 32.31	745.58 ± 31.22	1.72	0.18^{ns}
5	906.12 ± 61.68	939.06 ± 9.53	843.27 ± 84.25	946.67 ± 14.99	963.91 ± 35.15	782.30 ± 95.33	773.85 ± 29.46	1.96	0.13^{ns}
6	1123.97 ± 16.81	1098.30 ± 19.85	1157.00 ± 168.66	1199.15 ± 107.79	1241.39 ± 64.18	1122.21 ± 10.14	1022.94 ± 36.63	0.75	0.61^{ns}
Cumulative feed consumption upto 6 weeks	3628.84 ± 157.20	3543.14 ± 32.07	3352.61 ± 271.73	3640.94 ± 93.01	3669.75 ± 61.07	3361.19 ± 125.65	3212.67 ± 36.81	1.71	0.19^{ns}

ns-non significant

nanoparticles on the 18th day of incubation, and Awachat *et al*. (2020) by *in ovo* and post-hatch oral supplementation of zinc and copper. The lack of significant differences in feed consumption between treatment groups suggests that nanoparticles do not affect feed intake or nutrient absorption markedly.

Feed conversion ratio

The mean weekly and cumulative feed conversion ratio (FCR) values up to six weeks of age in the treatment groups *in ovo* and post hatch supplemented with CuNPs and ZnNPs presented in Table 4 showed no significant difference between treatment groups and the control group. This finding agrees with the earlier report by Goel *et al.* (2013) who observed no significant effect on FCR in broiler chicks *in ovo* supplemented copper and iron on 14th day of incubation. Supporting the present findings, Sahr *et al.* (2020) reported no significant effect on cumulative FCR with oral supplementation of CuNPs. Palouj *et al.* (2021), Anandhi *et al.* (2022) and Hassan *et al.* (2023) also reported similar results in their experiments.

Table 4. Mean (± SE) weekly and cumulative six weeks feed conversion ratio of broiler chicks subjected to *in ovo* inoculation and post hatch oral supplementation with CuNPs and ZnNPs

Age in Weeks	Treatments							F value	P value
	T1 Control	T2 CuNPs *In ovo*	T3 CuNPs Oral	T4 CuNPs *In ovo*+ oral	T5 ZnNPs *In ovo*	T6 ZnNPs Oral	T7 ZnNPs *In ovo*+ oral		
1	1.11 ± 0.04	1.05 ± 0.02	1.02 ± 0.02	1.02 ± 0.03	1.06 ± 0.03	1.01 ± 0.01	1.02 ± 0.04	1.49	0.25^{ns}
2	1.25 ± 0.02	1.23 ± 0.01	1.24 ± 0.03	1.27 ± 0.01	1.22 ± 0.04	1.23 ± 0.02	1.21 ± 0.07	0.32	0.92^{ns}
3	1.35 ± 0.00	1.32 ± 0.01	1.33 ± 0.01	1.31 ± 0.01	1.34 ± 0.02	1.37 ± 0.04	1.41 ± 0.01	3.45	0.06^{ns}
4	1.68 ± 0.00	1.70 ± 0.01	1.70 ± 0.01	1.67 ± 0.00	1.69 ± 0.02	1.71 ± 0.01	1.68 ± 0.01	0.74	0.63^{ns}
5	1.76 ± 0.01	1.76 ± 0.01	1.77 ± 0.01	1.75 ± 0.00	1.77 ± 0.01	1.76 ± 0.02	1.78 ± 0.01	0.69	0.66^{ns}
6	2.03 ± 0.02	2.03 ± 0.02	2.04 ± 0.02	2.05 ± 0.04	2.07 ± 0.04	2.04 ± 0.02	2.04 ± 0.07	0.11	0.99^{ns}
Cumulative FCR upto 6 weeks	1.65 ± 0.01	1.64 ± 0.01	1.65 ± 0.01	1.64 ± 0.01	1.69 ± 0.02	1.70 ± 0.01	1.73 ± 0.01	10.51	0.06^{ns}

ns-non significant

Table 5. Mean (± SE) antibody titre (log_2) against Newcastle disease vaccine by HI in broiler chicks subjected to *in ovo* inoculation and post hatch oral supplementation with CuNPs and ZnNPs

Treatments	Days of age			
	Hatch day	14	28	42
T1 (control)	3.33 ± 0.33	6.33 ± 0.33	6.67 ± 0.67	6.33± 0.33
T2	3.67 ± 0.33	6.67 ± 0.33	7.33 ± 0.33	7.00 ± 0.58
T3	3.33 ± 0.33	6.67 ± 0.67	7.33 ± 0.33	6.67 ± 0.33
T4	3.33 ± 0.33	7.00 ± 0.58	7.00 ± 0.58	6.67 ± 0.67
T5	3.67 ± 0.33	7.33 ± 0.33	7.33 ± 0.33	7.33 ± 0.33
T6	3.33 ± 0.33	7.00 ± 0.58	8.00 ± 1.00	7.67± 0.33
T7	3.67 ± 0.33	7.67 ± 0.33	8.67± 0.58	7.33 ± 0.58
P value	0.93^{ns}	0.5^{ns}	0.08^{ns}	0.97^{ns}

ns-non significant

Table 6. Mean values of cutaneous hypersensitivity reaction to interdigital inoculation of DNCB in broiler chicks subjected to *in ovo* inoculation and post hatch oral supplementation with CuNPs and ZnNPs

	Hours after inoculation			
	0	24	48	72
T1	0.07 ± 0.01	0.71 ± 0.04	0.52 ± 0.06	0.33 ± 0.03
T2	0.06 ± 0.01	0.64 ± 0.06	0.54 ± 0.06	0.30 ± 0.04
T3	0.09 ± 0.02	0.68 ± 0.10	0.51 ± 0.07	0.39 ± 0.03
T4	0.05 ± 0.03	0.69 ± 0.01	0.55 ± 0.01	0.27 ± 0.01
T5	0.09 ± 0.01	0.70 ± 0.07	0.50 ± 0.02	0.36 ± 0.03
T6	0.08 ± 0.03	0.74 ± 0.07	0.56 ± 0.08	0.29 ± 0.01
T7	0.09 ± 0.01	0.71 ± 0.04	0.56 ± 0.05	0.31 ± 0.01
P value	0.38^{ns}	0.62^{ns}	0.53^{ns}	0.42^{ns}

ns-non significant

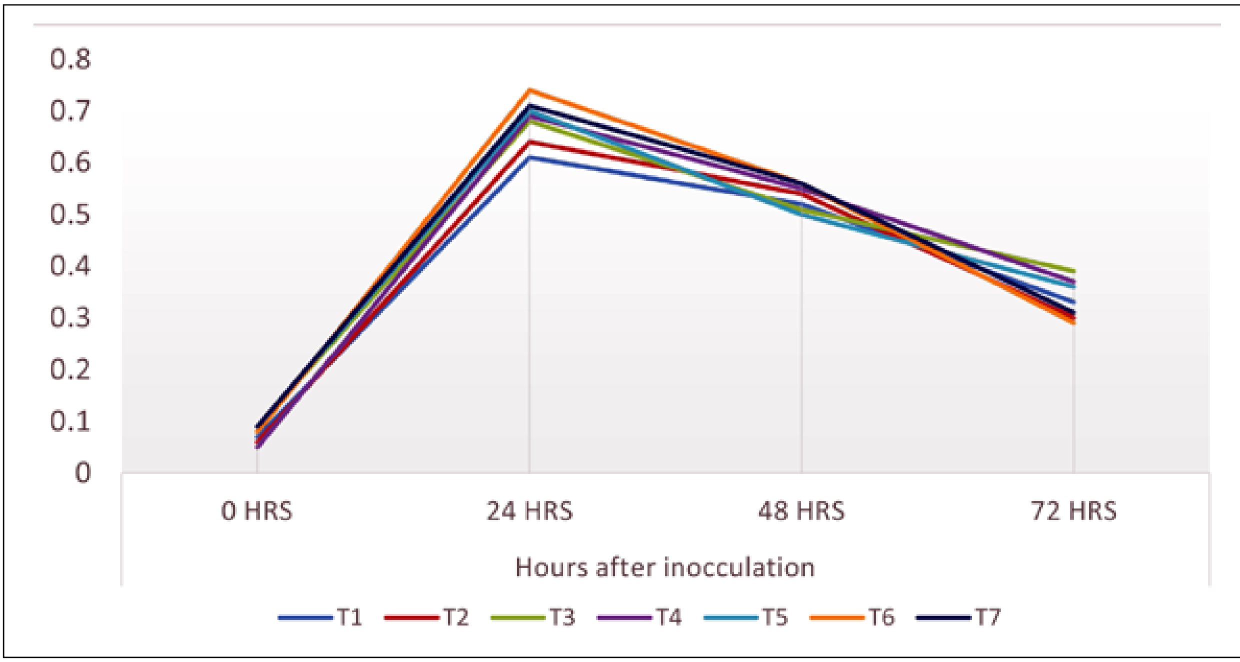

Fig. 1. Cutaneous hypersensitivity reaction to interdigital inoculation of DNCB in broiler chicks subjected to *in ovo* inoculation and post hatch oral supplementation with CuNPs and ZnNPs

The results of this study indicated that CuNPs and ZnNPs do not significantly influence the feed conversion ratio in broiler chicks.

Haemagglutination Inhibition

The haemagglutination inhibition (HI) test done to detect antibody level in serum samples of the broiler chicks at different ages, before and after Newcastle disease vaccination is shown in Table 5.

The mean HI titre value of the control group on the day of hatch did not differ significantly between treatment groups and were negative in all groups. The mean HI titre at 14 days of age also showed no significant difference between treatment groups.

The HI titre on 28^{th} day of age of the control group T1 after Newcastle disease booster dose vaccination was 6.67. The treatments groups supplemented with CuNPs *in ovo*, orally post hatch and in combination (T2, T3 and T4) showed mean values of 7.33, 7.33 and 7.00, respectively. The treatments groups supplemented with ZnNPs *in ovo*, orally post hatch and in combination (T5, T6 and T7) showed mean values of 7.33, 8.00 and 8.67, respectively. The mean values did not differ significantly between treatment groups.

On the 42^{nd} day of age the mean HI titre values of various treatment groups showed no significant difference between treatment groups. The humoral immune response observed in this study was in agreement with the observations of Goel *et al.* (2013) on *in ovo* supplementation of copper, Palouj *et al.* (2021) on zinc oxide nanoparticle supplementation and Anandhi *et al.* (2022) on *in ovo* supplementation of zinc, copper and chromium nanoparticles.

Cutaneous hypersensitivity

The cutaneous hypersensitivity reaction to interdigital injection with 2,4 Dinitro chlorobenzene (DNCB) in the footpad of broilers in different treatment groups was calculated and expressed in Table 8 and graphically represented in Fig. 1.

The mean values of cutaneous hypersensitivity evaluated 24, 48 and 72 hours after the DNCB injection did not show any significant difference between treatment groups. The results of the current study are in agreement with the observations of Palouj *et al.* (2021) in broiler chicken *in ovo* supplemented zinc oxide nanoparticles at the 10^{th}day of incubation. Sogunle *et al.* (2018) also reported no significant effect of *in ovo* supplementation of zinc sulphate, sodium selenite and copper sulphate on cell mediated immunity response in broilers.

Conclusion

The present study on *in ovo* inoculation of copper and zinc nanoparticles in broiler hatching eggs and their post-hatch drinking water supplementation showed no significant improvement in growth in broilers supplemented with copper and zinc nanoparticles. The humoral immune response was insignificant even though the values were numerically better in groups supplemented with zinc nanoparticles *in ovo*, orally and in combination. Thus, the supplementation of nanoparticles of copper and zinc *in ovo* and orally post-hatch, have shown no beneficial effect in improving the productivity and health of broiler chicken.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

Anandhi, M., Jayanaik, M., Malathi, V., Indresh, H., Prabhu, T.M. and Elangovan, A.V. 2022. Effect of *in ovo* supplementation of nano trace minerals on hatchability and post hatch performance of broiler chicks. *Pharma Innov. J.* **10**: 1300-1303.

AOAC [Association of Official Analytical Chemists]. 2016. Official Methods of Analysis. (20th Ed.). Association of Official Analytical Chemists International, Rockville, Maryland, 1885p.

Awachat, V.B., Elangovan, A.V., Sogunle, O.M., David, C.G., Ghosh, J., Gowda, S.N.K., Bhanja, S.K. and Majumdar, S. 2020. Influence of *in ovo* and pre-starter zinc and copper supplementation on growth performance and gastrointestinal tract development of broiler chickens. *Acta Agriculturae Slovenica*. **115**: 237–245.

Bakyaraj, S., Bhanja, S.K., Majumdar, S. and Dash, B. 2012. Modulation of post-hatch growth and immunity through *in ovo* supplemented nutrients in broiler chickens. *J. Sci. Food Agric.* **92**: 313-320.

Bhanja, S.K., Mandal, A.B. and Goswami, T.K. 2004. Effect of in ovo injection of amino acids on growth, immune response, development of digestive organs and carcass yields of broiler. *Indian J. Poult. Sci.* **39:** 212-218.

Bhanja, S.K., Mandal, A.B., Majumdar, S., Mehra, M. and Goel, A. 2012. Effect of in ovo injection of vitamins on the chick weight and post-hatch growth performance in broiler chickens. *Indian J. Poult. Sci.* **47**: 306-310.

Goel, A., Bhanja, S., Mehra, M., Majumdar, S., Pande, V. and Pandey. 2013. Effect of *in ovo* copper and iron feeding on post-hatch growth and differential expression of growth or immunity related genes in broiler chickens. *Indian J. Poult. Sci.* **48**: 279-285.

Hassan, H.A., Arafat, A.R., Farroh, K.Y., Bahnas, M.S., El-Wardany, I. and Elnesr, S.S. 2023. Effect of *in ovo* copper injection on body weight, immune response, blood biochemistry and carcass traits of broiler chicks at 35 days of age. *Anim. Biotech*. **33**: 1134-1141.

Jose, N., Elangovan, A.V., Awachat, V.B., Shet, D., Ghosh, J. and David, C.G. 2018. Response of *in ovo* administration of zinc on egg hatchability and immune response of commercial broiler chicken. *J. Anim. Physiol. Anim. Nutri.* **102:** 591-595.

Lingens, J.B., Abd El-Wahab, A., Ahmed, M.F.E., Schubert, D.C., Surie, C. and Visscher, C. 2021. Effects of early nutrition of hatched chicks on welfare and growth performance: a pilot study. *Anim.* [online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles /PMC8532627/.

Liu, J., Lin, S., Wu, S., Lin, Q., Fan, Z., Wang, C., Ye, D. and Guo, P. 2023. Dietary supplementation with nano-composite of copper and carbon on growth performance, immunity, and antioxidant ability of yellow-feathered broilers. *J. Anim. Sci.* [online]. **101**. Available: 10.1093/jas/skad362

Naeem, M.A., Chamani, M., Mousavi, S.N. and Asgh, A. 2022. The effect of the *in ovo* injection of some carbohydrates and antioxidants on incubating parameters, blood and immunological parameters, intestinal morphometry and post1hatching production performance in broiler chickens. *Italian J. Anim. Sci*. **21**: 749-763.

Noy, Y. and Uni, Z. 2010. Early nutritional strategies. *World Poult. Sci. J.* **66***:* 639-646.

OIE [Office International des Epizooties]. 2021. OIE Terrestrial Manual. Office International des Epizooties, 22p.

Olatunbosun, O. B., Sogunle, O. M., Adetola, O.O., Odutayo, O.J., Ayodeji, T.M., Ayoola, A.A. and Abiona, J.A. 2022. *In ovo* feeding of organic salts of zinc and copper: effects on growth performance and health status of two strains of broiler chickens. *Tropical Agric.* **99**: 146-165.

Palouj, J., Kazemi-Fard, M., Rezaei, M. and Ansari-Piresaraei, Z. 2021. Effects of *in ovo* injection of nano zinc oxide on the hatchability, immunity and antioxidant responses, and relative gene expressions of interleukin 2 and 12 in broiler chickens. *Iran. J. Appl. Anim.* **11**:595-603.

Sahr, W.B., Odutayo, O.J., Sogunle, O.M., Ayo-Ajasa, O.Y., Fafiolu, A.O. and Fatunmbi, F.A. 2020. Effects of *in ovo* injection of inorganic salts of Zn, Cu and Mn on hatching traits and post-hatch performance of broiler chickens in the tropics. *Nigerian J. Anim. Sci.* **22**: 113-125.

Scott, A., Vadalasetty, K.P., Chwalibog, A. and Sawosz, E. 2018. Copper nanoparticles as an alternative feed additive in poultry diet: a review. *Nanotech. Reviews*. **7**: 69-93.

Scott, A., Vadalasetty, K.P., Łukasiewicz, M., Jaworski, S., Wierzbicki, M., Chwalibog, A. and Sawosz, E. 2018. Effect of different levels of copper nanoparticles and copper sulphate on performance, metabolism and blood biochemical profiles in broiler chicken. *J. Anim. Physiol. Anim. Nutri*. **102**: 364-373.

Sogunle, O.M., Elangovan, A.V., David, C.G., Ghosh, J. and Awachat, V.B. 2018. Response of broiler chicken to *in ovo* administration of inorganic salts of zinc, selenium and copper or their combination. *Slovak J. Anim. Sci.***51**: 8-19.

Uni, Z., Ferket, P.R., Tako, E. and Kedar, O. 2005. *In ovo* feeding improves energy status of late-term chicken embryos. *Poult. Sci.* **84**: 764-770. ■