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RE/ POST-HATCH NANO-ZINC SUPPLEMENTATIONS EFFECTS ON HATCHABILITY, GROWTH PERFORMANCE, CARCASS TRAITS, BONE CHARACTERISTICS AND PHYSIOLOGICAL STATUS OF INSHAS CHICKS

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ABSTRACT: Two trials were conducted to evaluate the efficiency of nano-zinc (ZnO-NPs) supplementations on hatchability, growth performance, carcass traits, bone characteristics and physiological status of Inshas chick.In the initial experiment, on day 18 of the incubation 1020 eggs in four groups (n=85, r=3) were injected with ZnO-NPs at levels of 0, 60, 80, and 100µg /egg.Results showed that *in ovo* injection with ZnO-NPs at different levels has no significant effect on the hatched chick weight. In addition, nano-zinc at the level up to 60µg/ egg has no adverse effect on hatchability percentage.In the second experiment 480 one-day-old unsexed chicks from the initial experiment with an initial weight of 33.64 ±0.98gwere fed on a diet containing nano-zinc at the level of 0 or 30 mg / kg diet. The interaction of ZnO-NPs pre/post-hatch supplementations showed a significant effect on final body weight, weight gain, feed intake, and feed conversion ratio and the non-treated chicks in the control group showed the lowest values. The highest spleen weightwas recorded at the level of 100 µg ZnO-NPs/egg. The highest content of zinc in the breast meat was observed in chicks supplemented with 100µg/egg and fed 30 mg/ kg diet. Tibia bone ash content increased with increasing nano-zinc in the diet, meanwhile tibia bone Zn and P contents were increased with increasing nano-zinc *in ovo* supplementation. Chicks fed 30 mg ZnO-NPs/ kg diet showed highest values for tibia length, thickness when pre-hatch eggs supplemented with 100 µg ZnO-NPs/egg and the highest strength when pre-hatch supplemented with 60 µg ZnO-NPs/egg. ZnO-NPs *in ovo* supplementation had no significant effect on serum biochemical parameters. Total protein, albumin, ALT, AST, HDL, LDL, and serum Zn content values were increased with increasing nano-zinc in the diet except LDL.The highest phagocytic activities were observed in chicks fed 30 mg/ kg diet and pre-hatched with 60, 80, and 100 µg ZnO-NPs/egg.

Key words: Nano-zinc – Poultry -*in ovo*-Hatchability - Bone quality

INTRODUCTION

Many of the modern technologies that are currently widely used have contributed to the development of poultry production in all stages in terms of quantity and quality, such as genetic improvement, the use of nanoparticles, and *In-ovo* nutrition technology (Fathi *et al*., 2016; Kucharska-Gaca *et al.*, 2017). *In-ovo* nutrition technology can be defined as an appropriate injection of eggs with external substances, such as immune-stimulants, hormones, minerals, vitamins, amino acids, and carbohydrates, which support better fetal development, hatching and prepare chicks for intensive growth (Liu *et al*., 2011; Ebrahimi *et al*., 2012;Selim *et al*., 2012; Roto *et al.*, 2016).

Normal growth of chicken requires a balanced diet containing all the necessary nutrients, including minerals, which vary in the diet according to a number of interrelated factors including their forms (Das *et al.*, 2014; Sheoran, 2017; Uniyal *etal.,* 2017). Nanoparticles are nowadays exceedingly used in several sectors; including nutrition mainly in the preparation of nano-minerals mostly trace minerals, which bioavailability is low in addition to reduce intestinal mineral antagonism (Ankamwar *et al*., 2005; El Basuini *et al*., 2017; Gopi *et al*., 2017). Studies have reported that nanoparticles supplementations boosted the nutrients utilization and bioavailability, immunity, and performance in livestock and poultry (Sahoo *et al.*, 2014[a]; Vijayakumar and Balakrishnan, 2014; Ognik *et al.*, 2016; Al-Beitawi *et al.*, 2017).

Zinc is an essential trace element for the physiological function of livestock animals, and it plays an important role in many biological processes Sloup *et al*., 2017; Zhang *et al.*, 2018). Since materials at the nano-scale dimension exhibit novel properties (Thulasi et al., 2013), Nano-Zn (ZnO-NPs) has attracted attention (Ognik et al., 2016).

Previous studies have indicated that*in ovo* supplementation of nano-zinc has no detrimental effect on the hatchability and can boost the post-hatch performance of broiler chicken (Oliveira *et al.*, 2015[b]; Joshua *et al.*, 2016). Knowledge about the combination effects of nano-zinc*in ovo* and dietary supplementations on Inshas chicks is restricted. Thus, this study aims to investigate the effect of *in ovo* and post-hatch feeding by ZnO-NPs at different levels on hatchability, growth performance, carcass traits, bone characteristics and physiological status of Inshas chicks.

MATERIALS AND METHODS

1. Preparation of zinc nanoparticles (ZnO-NPs)

Zinc in nanoparticles form was prepared usingprimary materials namely zinc sulfate and sodium hydroxide according to Kumar *et al*.(2013). Briefly, zinc sulfate was dissolved by slowly adding sodium hydroxide drops in a molar ratio of 1:2 under continuous vigorous stirring for 12 hrs. The precipitate was filtered, washed thoroughly with deionized water and dried at 100℃ and then ground to a fine powder using an agate mortar. The size of the prepared ZnO-NPs was determined by scanning electron microscopy and ranged between 20-85 nm (Fig.1).

2.2. Experimental design and animals

This study was carried out in the animal research station, Sakha agricultural research center, Egypt.Two trials were conducted to evaluate the efficiency of ZnO-NPs supplementations for Inshas strain hatchability, growth performance, carcass traits, bone characteristics and

physiological status. Inshas strain is developed from a cross between Sina and Plymoth Rock breeds, which characterized by its ability to withstand unfavorable conditions imposed in the Egyptian farm (Abou El-Ghar and Abd El-Karim, 2016).

Experiment 1:1020 fertilehatching eggs(have the same weight) from Inshas strain were chosenaftercandling at 14 day of incubation period to get rid of unfertilized, contaminated, or/and dead embryos eggs. Eggs were randomly divided into 4 experimental groups in triplicates (n=85 eggs, r = 3) and injected on day 18 of the incubation into embryonic amnion sac by gauge needle at 25 mm depth with 0.5 ml of *in-ovo*-feeding solutions consisting of a saline solution (0.9 % NaCl) containing nano-zinc (ZnO-NPs) at levels of 0 (control = C0), 60 (C60), 80 (C80), and 100 (C100) µg /egg. After injection, special care was taken to cover holes using glue. Hatchability was calculated and expressed as a percentage of fertilized eggs. Also, hatch weight of chicks was recorded.

Experiment 2: A total of 480 one-day-old unsexed Inshas strain chicks obtained from the first experiment were allocated into 4 main groups (n=60) with 33.64 ±0.98 g initial body weight. Each one of the main group was divided into two sub-groups according to the feeding diet, where ZnO-NPs supplemented at the level of 0 and 30 mg/kg diet resulted in 4×2 factorial designas follows:

D0C0=0 mg/kg ZnO-NPs dietary supplementation+0 µg ZnO-NPs/egg (*in ovo* supplementation) (control group).

D0C60 =0 mg/kg ZnO-NPs dietary supplementation + 60 µg ZnO-NPs /egg *(in ovo* supplementation).

D0C80 =0 mg/kg ZnO-NPs dietary supplementation + 80 µg ZnO-NPs /egg *(in ovo* supplementation).

D0C100 =0 mg/kg ZnO-NPs dietary supplementation + 100 µg ZnO-NPs /egg (*in ovo* supplementation).

D30C0 =30 mg/kg ZnO-NPs dietary supplementation + 0 µg ZnO-NPs /egg *(in ovo* supplementation).

D30C60 =30 mg/kg ZnO-NPs dietary supplementation + 60 µg ZnO-NPs /egg (*in ovo* supplementation).

D30C80 =30 mg/kg ZnO-NPs dietary supplementation + 80 µg ZnO-NPs /egg (*in ovo* supplementation).

D30C100=30 mg/kg ZnO-NPs dietary supplementation + 100 µg ZnO-NPs /egg (*in ovo* supplementation).

Chicks were raised for 10 weeks in a controlled and disinfected system and feed was offered ad-libitum along with clean drinking water. The formulation and proximate composition of the basal diet are shown in Table 1 which contains about 19.7% crude protein, 3.71% lipid. Prior to the final sampling, feeding was discontinued and the parameters of growth and nutrient utilization were calculated using the following formulas:

Weight Gain (WG, g) = Final weight – Initial weight.

Feed Intake (FI, g/ bird/ 10 weeks) = (dry diet given – dry remaining diet recovered)/no. of birds.

Feed Conversion Ratio (FCR) = dry feed intake, g/ live weight gain, g.

3. Samples collection and analytical procedure

Chemical analysis of experimental diets were performed using standard methods (AOAC, 2000) for dry matter by dehumidification through drying to constant weight at 110 °C, crude protein content by applying the method of Kjeldahl, crude lipid by Soxhlet solvent

extraction method and ash by burning for 4h at 550 °C in Muffle furnace. At the end of the feeding trial, three birds per replicate were randomly collected, slaughtered after fasting for 8 hrs and the internal organs (liver, spleen, heart, gizzard, bursa, and thymus) were carefully dissected out, weighed individually and expressed as a percentage of body weight.Tibia bones were separated; their cartilages were removed;besides weight and length were determined. Tibia bone breaking strength was determined according to the method of Crenshaw *et al*.(1981). Minerals contents (Ca, Zn, and P) were measuredcalorimetrically using standard methods of(AOAC, 2000).

Serum was collected using non-heparinized disposable tubes after whole blood was clotted and centrifuged at 3000 rpm for 20 min and serum samples were kept at -20°C until the analysis. Serum total protein, albumin were determined using calorimetric method according to Doumas *et al*. (1971); Henry (1964).Globulin was calculated by subtracting the albumin value from the total protein value of the same sample (Coles, 1986). Also, serum zinc concentration, triglyceride, AST, and ALT were detected spectrophotometrically according to the improved calorimetric methods (Gottfried and Rosenberg, 1973; Johnson et al., 1977; Reitman and Frankel, 1957). Determination of cholesterol, HDL, and LDL were performed according to Herrmann *et al.*(1983); Okada and Ishida, (2001).

A Phagocytic activity (PA) was measured according to Rashid et al.(1994). Briefly, 100 µl of serum samples were mixed with 100 µl of heat killed *Candida albicans* yeast suspension (5×10^6/ml), incubated at 37° C for 30 minutes and then centrifuged at 1000 rpm for 5 minutes. The resulted supernatants were removed and the pellets were re-suspended to be used in preparing smears. Smears were air dried, fixed with methyl alcohol and stained with Giemsa stain. The numbers of heterophils ingesting Candida were counted from one hundred heterophils.

Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells.

Phagocytic index (PI) = Number of yeast cells phagocytized/ Number of phagocytic cells.

4. Statistical analysis

Statistical analysis was performed with general linear model procedure using statistical analysis system (SAS version 9.1.3, 2007) for Windows. Means were tested for significant differences using Duncan's multiple range test and differences between treatments were considered significant ($P<0.05$). First experiment data were analyzed by one-way ANOVA according to the following model:

$Y_{ij}= \mu + C_i + E_{ij}$

Where, µ is the overall mean; C_i is the fixed effect of i^{th}*in ovo* nano-zinc supplementation; and E_{ij} is the random error.

Second experiment data were analyzed using two-way ANOVA according to the following model:

$Y_{ijk}= \mu + C_i + D_j + CD_{ij} + E_{ijk}$

Where, µ is the overall mean; C_i is the fixed effect of i^{th}*in ovo* nano-zinc supplementation; D_j is the fixed effect of j^{th} nano-zinc dietary supplementation; CD_{ij} is the interaction effect of i^{th} nano-zinc *in ovo* and dietary supplementations; and E_{ijk} the random error.

RESULTS

1. Hatching performance:

Hatchability of fertile eggs percentage and hatched chick weight of Inshas eggs injected by graded levels of ZnO-NPs are shown in Table 2. Egggroups supplemented with experimental concentrations of ZnO-NPs represented numerical increase of hatched chick weight compared with control, while groups of C60 and C80showed numerical increase by 7.74 and 4.97% respectively over control group.The hatchability percentage was significantlyinfluenced ($P<0.05$) by high levels of *in ovo*nano-zinc supplementation. High levels of nano-zinc in the C80 and C100 groups resulted in a low hatching rate, while there was no significant difference between the control group and the C60 group.

3.2. Growth parameters and nutrient utilization

Effects of ZnO-NPs *in ovo* and post hatch supplementation on Inshas chick'sgrowth performance and nutrient utilizationare shown in Table 3. The effect of ZnO-NPs pre/post-hatch supplementations interactions on (FnWt), (WG), (FI), and (FCR) are significant($P<0.05$). The control group showed the lowest values for FnWt, WG and FI meanwhile the best improvement of FCR was observed in group D30C0 compare with other interactions.

3. Carcass characteristics:

Table 4 represents carcass characteristics of *in ovo* and post hatch ZnO-NPs supplemented Inshas chicks. No significant differences were detected in the relative weight of heart, bursa, thymus, abdominal fat, and breast meatamong treatments. Only ZnO-NPs *in ovo* supplementations had a significant factor on spleen weight percentageand the highest values were recorded at the level of 100 μg ZnO-NPs/egg ($P < 0.05$). Meanwhile, effect of ZnO-NPs *in ovo* and post hatch supplementations interactions on the content of zinc in the breast meat were significant and the highest zinc content was observed in D30C100 while D0C0 showed the lowest value.

4. Tibia bone characteristics:

Tibia bone characteristics as affected by ZnO-NPs are shown in Table 5. ZnO-NPs pre/post-hatchsupplementations interactionshad a significant effect on all tibia bone characteristics in terms of length, weight, strength and minerals content. While, the effect of nano-zinc dietary supplementation was only significant on tibia bone ash contentas it increased with increasing nano-zinc in the diet. In addition, the effect of nano-zinc *in ovo* supplementation was significant on tibia bone Zn and P contentsas it increased with increasing nano-zinc. The highest values for tibia length and thickness were observed for birds in D30C80 and highest strength was in D30C60 while the highest weight was found in D30C0.

5. Blood biochemical Parameters:

Table 6 represents the blood parameters of Inshas chicks due to egg injection and dietary supplementation with ZnO-NPs. The effect of nano-zinc dietary supplementation was significant on Total protein, albumin, ALT, AST, HDL, LDL, and serum Zn content astheir values were increased with increasing nano-zinc in the dietexcept that of LDL. In contrast, ZnO-NPs *in ovo* supplementations had no significant effect on serum biochemical parameters. The interaction effect was significant for ALT, AST, cholesterol, HDL, and LDL and the highest values were recorded in the chick of D30C100except LDL in D0C0.

6. Phagocytic activity:

Phagocytic activity of Inshas chicks fed tested diets for 10 weeks and pretreated by nano-zinc *in ovo* supplementation is shown in Table 7. *In ovo* feeding of ZnO-NPs had no significant effect while dietary supplementation and the interaction showed a significant on phagocytic activity ($P<0.05$). The highest phagocytic activities were observed in D30C60, D30C80, and D30C100 groups.

DISCUSSIONS

Zinc at adequate level plays serious roles in the overall performance of animals including poultry, as it involves in an assortment of biological systems and is the main component of a large number of enzymes(Akbari *et al*., 2016, 2018; Torres and Korver, 2018; Zhang *et al*., 2018). Poultryrequirements of zinc range from 30 to 40 µg / kg depending on a number of factors, including species or breeds, age,environmental conditions, diet composition, supplementation levels, and forms (Burrell *et al*., 2004; Rossi*et al*., 2007; Roy *et al*., 2013). Nanoparticles have been reported to be more efficient for animals than larger particles at low doses due to the easy absorption and biological availability as well as appear to interact better with other materials due to the significance of the active surface(Khalil *et al*., 2013; Wang *et al.*, 2015; El Basuini *et al.*, 2016, 2017).

In the present study, hatchability percentage of Inshas strain was affected by *in ovo* zinc supplementations, which is in line with the previous studiesreported by Joshua *et al*.(2016);Jose *et al*. (2018); Sun *et al*. (2018). Results indicate that*in ovo* ZnO-NPs by up to 60 µg/ egg has no adverse effect onhatchability percentagebut decreases with increasingZnO-NPs above 60 µg per egg. It has been reported that, *in ovo* zinc injection at a proper level has no adverse effect on developing chicken embryo or hatchability(Tako *et al*., 2005; Yair *et al*., 2013). Meanwhile, high levels of zinc showed a reduction in the hatchability and this may be attributed to theimbalance of amnion minerals content that interfered with embryogenesis during the late incubation or the toxicity of zinc nano-form due to its high availability(Star *et al*., 2012; Swain *et al.*, 2016Jose *et al.*, 2018;). The weight of the hatched chicks evolved insignificantly with ZnO-NPs *in ovo* supplementation which consist with the previous results (Oliveira *et al*., 2015[a];Joshua *et al*., 2016).

Injection of ZnO-NPs *in ovo* or dietary supplementations showed no significant effect on (FnWt), (WG), (FI) and (FCR) of Inshas chicks while their interactionsexhibited a significant effect ($P<0.05$). In accordance with our results, post-hatch growth performance did not affected by ZnO-NPs *in ovo* administration (Yair *et al*., 2013; Oliveira *et al*., 2015[a]) or dietary zinc supplementation (Rossi *et al*., 2007; Sunder *et al.*, 2013). Contrary, Joshua *et al*.(2016) reported a significant effect of zinc nano-form *in ovo* feeding at the level of 40 µg/ egg on weight gain and feed conversion ratio. In addition, nano-zinc dietary supplementation promote growth performance and feed utilization at the level of 20 mg/kg (Fathi *et al*., 2016) and 20 – 60 mg /kg (Zhao *et al*., 2014). The positive effect of ZnO-NPs pre/post-hatch supplementations interaction on growth performance and feed utilization may be linked to the improvement in the intestine development as reportedTako *et al*. (2005) or/ and up-regulation of growth-related genes (Goel *et al*., 2012).

The characteristics of the carcass did not change in general with the addition of

zinc whether *in ovo* or dietary sources except that for abdominal fat% with dietary zinc, spleen with *in ovo* zinc, and breast meat zinc content with zinc *in ovo* and dietary supplementations interaction. The results of the present study are in agreement with the previous works which reported no significant effect of zinc on carcass traits (Yogesh *et al*., 2013; Karthikeyan *et al*., 2017). On the other hand, a significant effect of zinc supplementation on carcass traits and internal organs weight were reported byMehran *et al*. (2015; Olukosi *et al*.(2018). In addition, Zn supplementation was found to boost the intramuscular fat content in broilers breast muscle (Liu *et al*., 2015) and weight of digestive and lymphoid organs (Mohammadi *et al*., 2015).

The skeleton of poultry is characterized by rapid growth during its short productive life cycle, playing a key role in production not only by providing structural support but also as a source of minerals for metabolic process (Bao *et al*., 2007;Sahraei *et al*., 2012). Zinc has a key role in bone formation and maintenance(Park et al., 2004). Results of the present study showed that tibia bone characteristics were improved by pre/post-hatch zinc supplementations. These results are in consistence with those obtained byYair and Uni (2011); Yair *et al*.(2013);Tomaszewska *et al*.(2017). In addition, ZnO-NPs *in ovo* and dietary supplementation at high levels did not antagonize calcium or phosphorusbut on the contrary increased their bone content and this can be attributed to improved zinc properties when used in nano form(Sahoo *et al*., 2014[a]; Swain *et al*., 2016).

Blood biochemistry parameters are important signals of the physiological response as well as the general health condition of animals (Abou-Zeid *et al*.,2015 ; El Basuini *et al*., 2017). The blood parameters of treated Inshas chicks were varied significantly in terms of total protein, albumin, ALT, AST, HDL, LDL, and serum Zn content as their values increased with increasing zinc level except LDL decreased.Total serum protein is a good signal for animal enhanced immunity (Coeurdacier *et al*., 2011). The increased values of total protein and albumin with zinc supplements refer to the basic role of zinc in protein synthesis(MacDonald, 2000). While ALT and AST values reflect the functional liver and kidney condition, as their increased value may indicate toxicity with high levels of zinc as previously reported (Sahoo *et al*., 2014[b]; Sharma *et al*., 2012; Wang *et al*., 2008). Alteration of serum lipid content (HDL, LDL) with zinc addition indicates the role of zinc inlipid digestion and absorption(Al-Daraji and Amen, 2011; Fathi *et al*., 2016). Zinc supplementation also significantly increasedserum/ plasma Zn contents (Sunder *et al*., 2013; Olukosi *et al*., 2018).

Phagocytic activity is a vital component of the host to withstand pathogens(Sornplang *et al*., 2015). In this study, phagocytic activities were developed due to the interaction between *in ovo* and dietary zinc supplementations at high levels.Zinc is very substantialfor a number of immune system functions(Park *et al*., 2004). As a result of the active surface of the nanoparticles, there is a possibility that when these particles contact with the immune cells, an interaction will occur with a high probability to increase or inhibit immune functions(Dobrovolskaia and McNeil,

2007; Zolnik *et al*., 2010 Smith *et al.*, 2014).

To sum up, the present study is preliminary work for evaluating the supplementation of nano-zinc starting from embryogenesis using *in ovo* technique along with dietary supplementations after hatching.

In conclusion,*in ovo*feeding of ZnO-NPs at different levels has no significant effect on the hatched chick weight. In addition, nano-zinc at the level up to 60 µg/ egg has no adverse effect on the hatchability. Interactionsof ZnO-NPs *in ovo*supplementation along with dietary supplementation showed better results than the effect of each of them separately. It is likely that 60µgZnO-NPs/ egg combined with 30 mg/ kg in the diet is sufficient to improve the growth performance of Inshas chicks.

Table (1): Formulation and chemical analysis of the basal diet (Starter diet 0-12 weeks).

Ingredients	%
Yellow corn	62.3
Soybean meal (44%)	32.1
Dicalcium phosphate	1.9
Limestone	1.8
Vitamin and mineral premix*	0.3
Salt	0.3
Methionine	0.1
Sand	1.2
Total	**100**
Proximate composition	
Crude protein	19.7±0.08
Crude lipids	3.71±0.12
ME (KJ/g) **	11.74±0.20
Calcium	1.20±0.03
Available phosphorus	0.74±0.03
Methionine	0.44±0.01
Lysine	1.09±0.07

* Vitamin and mineral premix (kg): Vitamin A (12000 IU), Vitamin D (2200 IU), Vitamin E (10 mg), Vitamin k3 (2mg), Vitamin B1 (1mg), Vitamin B2 (5mg), Vitamin B6 (1.5 mg), Vitamin B12 (10 Mcg), Nicotinic acid (30 mg), Folic acid (1 mg), Pantothenic acid (10 mg), Biotin (50 Mcg), Choline chloride (500 mg), Copper (10 mg), Iron (30 mg), Manganese (60 mg), Zinc (0.05 mg), Iodine (1 mg), Selenium (0.1 mg) and Cobalt (0.1 mg).

** Calculated using combustion values for protein, lipid and carbohydrate of 23.6, 39.5 and 17.2 kJ/ g, respectively.

Table (2): Effect of Nano-zinc in ovo feeding on Inshas hatching performance

Parameters	C0	C60	C80	C100
Hatched chick weight (g)	32.41±0.75	34.92±1.18	34.02±0.54	33.25±0.72
Hatchability of fertile eggs%	80.23±1.31^{a}	79.82±2.60^{a}	55.72±2.98^{b}	49.53±1.91^{b}

Means with different letters in the same row are significantly different (P≤ 0.05).

Table (3): Growth and nutrient utilization of Inshas chicks (average initial body weight: 33.64 ±0.98 g) fed tested diets for 10 weeks.

Item	InWt	FnWt	WG	FI	FCR
D0C0	32.40	1266.22^{c}	1233.82^{c}	2243.84^{f}	1.82^{ab}
D0C60	34.91	1304.44^{b}	1269.53^{b}	2324.39^{e}	1.83^{ab}
D0C80	34.00	1305.65^{b}	1271.65^{b}	2498.93^{b}	1.97^{a}
D0C100	33.27	1344.65^{a}	1311.38^{a}	2632.75^{a}	2.01^{a}
D30C0	32.41	1314.17^{b}	1281.76^{b}	2282.56^{d}	1.78^{b}
D30C60	34.89	1313.48^{b}	1278.59^{b}	2466.74^{c}	1.93^{a}
D30C80	34.02	1319.59^{ab}	1285.57^{b}	2682.34^{a}	2.09^{a}
D30C100	33.25	1312.61^{b}	1279.36^{b}	2348.88^{d}	1.84^{ab}
MSE	0.83	28.47	19.84	21.52	0.23
Two way ANOVA (*P*-Value)					
Dietary Nano-Zn	0.980	0.568	0.564	0.876	0.976
In Ovo Nano-Zn	0.444	0.458	0.221	0.193	0.185
Interaction	0.782	0.042	0.043	0.039	0.037

Means with different letters in the same column are significantly different (P≤ 0.05).
InWt= initial body weight, FnWt= final body weight, WG= weight gain, FI=feed intake, FCR= feed conversion ratio.

Table (4): Effect of Nano-Zn supplementation levels on carcass characteristics of Inshas chicks.

Item	**Heart %**	**Liver %**	**Spleen %**	**Bursa %**	**Thymus %**	**Abd. fat%**	**Breast meat%**	**Zn in Breast Meat%**
D0C0	0.54	1.96	0.13^{ab}	0.24	0.38	0.36^{b}	15.91	0.029^{b}
D0C60	0.51	1.94	0.10^{b}	0.22	0.41	0.27^{c}	15.67	0.030^{ab}
D0C80	0.57	2.03	0.10^{b}	0.26	0.38	0.28^{c}	15.56	0.032^{a}
D0C100	0.48	1.89	0.16^{a}	0.18	0.32	0.25^{c}	13.94	0.032^{a}
D30C0	0.48	1.83	0.11^{b}	0.22	0.31	0.34^{b}	13.07	0.030^{ab}
D30C60	0.47	1.87	0.09^{b}	0.16	0.27	0.40^{a}	15.84	0.032^{a}
D30C80	0.57	2.07	0.12^{ab}	0.18	0.33	0.41^{a}	15.80	0.033^{a}
D30C100	0.53	1.69	0.19^{a}	0.27	0.42	0.34^{b}	13.11	0.036^{a}
MSE	0.03	0.22	0.02	0.08	0.14	0.03	2.84	0.02
Two way ANOVA (*P*-Value)								
Dietary Nano-Zn	0.694	0.323	0.854	0.577	0.32	0.036	0.404	0.219
In Ovo Nano-Zn	0.191	0.167	0.022	0.839	0.97	0.868	0.250	0.178
Interaction	0.074	0.093	0.057	0.127	0.62	0.081	0.088	0.032

Means with different letters in the same column are significantly different (P≤ 0.05)

Table 5: Effectof *in ovo* and Post-hatch Nano-Zn supplementation on tibia bone characteristics of Inshas chicks.

Traits / Treatments	Length (cm)	Weight (g)	Thickness (cm)	Weight/Length Index (mg/mm)	Strength (N)	Ash%	Zn%	Ca%	P%
D0C0	11.15^{c}	5.22^{e}	0.85^{b}	46.81^{d}	277.00^{c}	38.43^{d}	0.029^{b}	34.78^{d}	15.49^{c}
D0C60	10.21^{f}	5.25^{e}	0.80^{c}	51.42^{ab}	235.11^{f}	31.64^{g}	0.030^{ab}	36.12^{b}	16.32^{b}
D0C80	10.31^{e}	5.14^{f}	0.88^{b}	49.84^{b}	206.10^{g}	32.11^{g}	0.033^{a}	36.81^{a}	16.30^{b}
D0C100	11.31^{b}	5.54^{c}	0.84^{b}	48.98^{d}	196.80^{h}	36.07^{f}	0.035^{a}	35.63^{c}	16.89^{ab}
D30C0	11.25^{b}	5.86^{a}	0.87^{b}	52.09^{a}	281.10^{b}	39.47^{c}	0.029^{b}	35.84^{c}	15.50^{c}
D30C60	11.27^{b}	5.63^{b}	0.87^{b}	49.96^{b}	326.90^{a}	41.31^{b}	0.030^{ab}	36.33^{b}	16.35^{b}
D30C80	11.45^{a}	5.48^{c}	0.93^{a}	47.86^{e}	282.30^{b}	45.43^{a}	0.034^{a}	36.94^{a}	16.49^{b}
D30C100	10.85^{d}	5.32^{d}	0.88^{b}	49.03^{c}	243.90^{e}	38.52^{d}	0.036^{a}	35.71^{c}	17.03^{a}
MSE	0.06	0.06	0.04	1.14	2.33	0.69	0.05	0.25	0.37
Two way ANOVA (*P*-Value)									
Dietary Nano-Zn	0.188	0.096	0.086	0.731	0.069	0.025	0.824	0.495	0.836
In Ovo Nano-Zn	0.855	0.893	0.355	0.807	0.542	0.967	0.001	0.054	0.047
Interaction	0.047	0.044	0.039	0.041	0.030	0.029	0.027	0.033	0.042

Means with different letters in the same column are significantly different ($P \leq 0.05$).

Table (6): Effect of in ovo and Post-hatch Nano-Zn supplementation on serum biochemical parameters of Inshas chicks

Item	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALT/GPT (U/L)	AST/GOT (U/L)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Serum Zn (µmol/L)
D0C0	3.12^{b}	1.92^{d}	1.20	7.80^{d}	105.03^{c}	69.67	97.13^{c}	32.71^{d}	60.88^{a}	25.00^{c}
D0C60	3.18^{b}	1.92^{d}	1.26	7.94^{d}	105.52^{c}	69.33	96.82^{c}	32.69^{d}	58.12^{c}	25.54^{c}
D0C80	3.20^{b}	1.94^{d}	1.26	7.96^{d}	105.77^{c}	68.87	97.32^{c}	32.87^{d}	59.59^{b}	25.55^{c}
D0C100	3.25^{ab}	1.95^{c}	1.30	8.09^{d}	106.04^{c}	68.41	98.08^{b}	33.08^{d}	59.36^{b}	25.64^{c}
D30C0	3.31^{a}	1.98^{c}	1.33	9.33^{c}	107.12^{b}	66.11	97.89^{c}	38.58^{c}	54.07^{d}	27.67^{b}
D30C60	3.30^{a}	1.97^{c}	1.33	10.57^{b}	108.74^{b}	66.84	98.71^{b}	40.22^{b}	53.91^{d}	28.11^{a}
D30C80	3.32^{a}	2.06^{b}	1.26	11.92^{ab}	109.36^{a}	67.52	99.18^{b}	41.12^{a}	53.49^{d}	28.09^{ab}
D30C100	3.38^{a}	2.14^{a}	1.24	12.39^{a}	110.85^{a}	69.88	102.66^{a}	42.95^{a}	54.17^{d}	28.55^{a}
MSE	0.07	0.03	0.13	0.94	1.53	1.12	1.25	1.15	0.37	0.52
Two way ANOVA (*P*-Value)										
Dietary Nano-Zn	0.005	0.041	0.305	0.004	0.005	0.136	0.081	0.0001	0.0001	0.0001
In Ovo Nano-Zn	0.790	0.635	0.923	0.888	0.821	0.879	0.498	0.977	0.985	0.98
Interaction	0.061	0.057	0.221	0.037	0.040	0.119	0.045	0.033	0.049	0.212

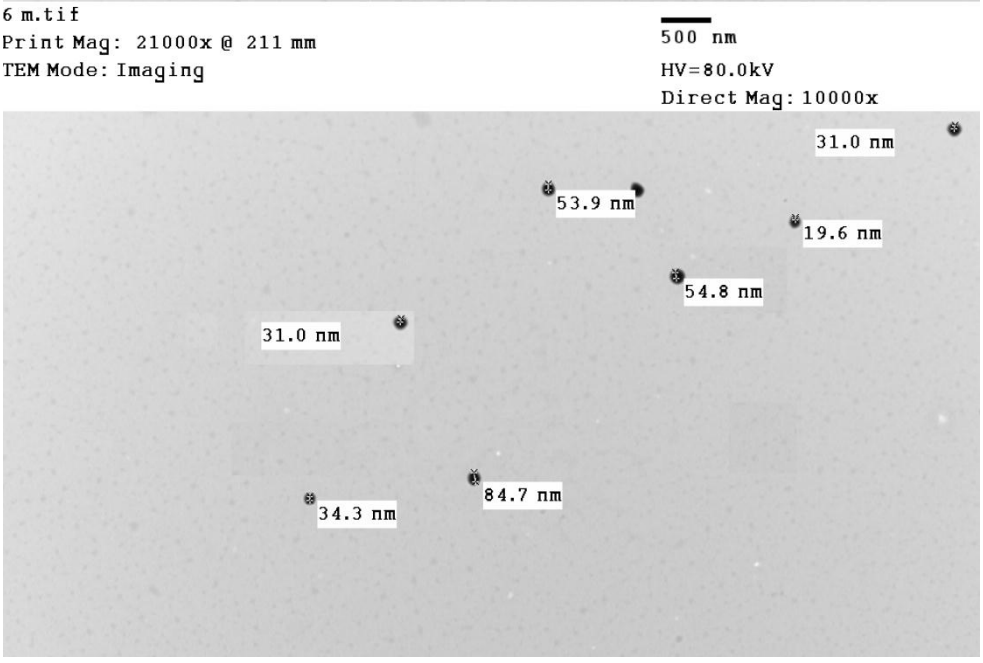
Means with different letters in the same column are significantly different ($P \leq 0.05$).

Table (7): Effect of in ovo and Post-hatch Nano-Zn supplementation on phagocytic activity of Inshas chicks

Item	Phagocytes activity	Phagocytes index
D0C0	54.00^{c}	3.53^{b}
D0C60	54.66^{b}	3.63^{b}
D0C80	56.66^{b}	3.76^{b}
D0C100	56.00^{b}	3.73^{b}
D30C0	54.66^{c}	3.60^{b}
D30C60	69.66^{a}	4.43^{a}
D30C80	69.00^{a}	4.43^{a}
D30C100	69.66^{a}	4.63^{a}
MSE	1.33	0.23
Two way ANOVA (*P*-Value)		
Dietary Nano-Zn	0.035	0.041
In Ovo Nano-Zn	0.682	0.630
Interaction	0.040	0.039

Means with different letters in the same column are significantly different (P≤ 0.05). Means having same subscript letters were not significantly different. Absence of letters indicates no significant difference between treatments.
PA= Phagocytic activity, PI= Phagocytic index.

Figure (1): Scanning Electron Microscopy for Nano-Zn



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الملخص العربي

تأثيرات إضافة النانو زنك قبل وبعد الفقس علي أداء الفقس ، الاداء الانتاجي ، خصائص الذبيحة ، خصائص العظام والحالة الفسيولوجية لدجاج أنشاص.

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أجريت تجربتان لتقييم كفاءة إضافة النانو زنك قبل وبعد الفقس علي أداء الفقس ، الاداء الانتاجي ، خصائص الذبيحة ، خصائص العظام والحالة الفسيولوجية لدجاج أنشاص. في التجربة الاولي وعند عمر 18 يوم من بداية التفريخ تم اختيار 1020 بيضه مخصبه من سلالة انشاص وقسمت عشوائيا الي 4 مجموعات تجريبية ، قسمت داخليا الي 3 مكررات بكل منها 85 بيضه وحقنت جميها بالنانو زنك بتركيزات (صفر ، 60 ، 80 ، 100 ميكروجرام/بيضه). أظهرت النتائج أن تغذية الأجنة داخل البيض بالمستويات المختلفة من نانو أكسيد الزنك لم يكن لها تأثيرات معنوية علي وزن الكتكوت عند الفقس. علاوة علي ذلك فان حقن الاجنة بمستوي 60 ميكروجرام /بيضه لم يكن له تأثير سلبي علي نسبة الفقس.

في التجربة الثانية ، تم تغذية 480 كتكوتاً عمر يوم واحد غير مجنسه ناتجة من التجربة الأولي ذات وزن اولي قدره 33.64 ± 0.98 جرام علي عليقة تحتوي على مستويين من نانو أكسيد الزنك (0 أو 30 مجم / كجم). بين التداخل بين اضافة النانو زنك قبل وبعد الفقس تأثيرا معنويا في كلا من وزن الجسم النهائي ، معدل الزيادة في وزن الجسم ، العلف المستهلك ، الكفاءة التحويلية علي عمر 10 أسابيع من بداية التجربة الثانية. و حققت الطيور التي لم تعامل بالنانو زنك قبل وبعد الفقس (الكنترول) أقل المعدلات.

حققت الطيور المعاملة بمعدل 100 ميكروجرام/ بيضه من نانو اكسيد الزنك اعلي وزن نسبي للطحال ، كما لوحظ أن الطيور التي تغذت علي عليقة مزودة بالنانو زنك بمعدل 30 مجم /كجم عليقة وحقنت أجنتها داخل البيض بمعدل 100 ميكروجرام/ بيضه أعلي محتوي من الزنك في عضلات الصدر.

زاد محتوى الرماد في عظم الساق مع زيادة مستوي النانو زنك في العلائق وفي الوقت نفسه زاد تركيز الزنك والفسفور في عظمة الساق مع زيادة محتوي العليقة من النانو زنك وزيادة معدل الزنك المحقون في الاجنة. وأظهرت الكتاكيت التي تغذت على النانو زنك بمعدل 30 مجم/كجم عليقه أعلى القيم لطول عظمة الساق ، وسمكها عندما غذيت أجنتها علي 100 ميكروجرام/بيضه، بينما حققت الطيور التي غذيت أجنتها علي 60 ميكروجرام/ بيضه أعلى قوة لعظمة الساق.

لم يكن لحقن الأجنة داخل البيض بالنانو زنك اي أثر معنوي علي القياسات البيوكيميائية لسيرم الدم. بينما حدث زيادة معنوية في تركيز كلا من البروتين الكلي ، الالبيومين ،انزيمات الكبد والكولسترول عالي الكثافة ومحتوي السيرم من الزنك بزيادة مستوي الزنك في العليقة وكان ذلك متبوعا بانخفاض تركيز الكوليسترول منخفض الكثافة. كما لوحظ أن أعلى نشاط للخلايا البلعمية في الكتاكيت التي تغذت على 30 مجم / كجم والتي غذيت اجنتها داخل البيضه علي المستويات المختلفة من النانو زنك.