



Effect of different sources of zinc in *ovo* injection on hatching traits, growth and some physiological parameters of broiler chicks

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ABSTRACT:

A total of 546 fertile eggs were divided randomly to 7 treatments in three replicates, 26 eggs in each, as follows: The 1st treatment served as a control (without injection), the 2nd, 3rd and 4th treatments were injected with 0.1 ml deionized water containing 25, 50 and 75 µg/egg zinc sulfate, respectively and treatments 5th, 6th and 7th injected with 0.1 ml deionized water containing 25, 50 and 75 µg/egg ZnO-NPs, respectively. Eggs injection was performed at the 9th day of incubation.

The results could be summarized as follow:

The treatments did not cause any significant differences in the hatchability %, embryonic mortality, chick weight, chick weigh %, the relative weight of residual yolk sac, heart, liver, gizzard and proventriculus to body weight (BW) or yolk free body weight of chicks at one day of age, slaughter parameters (percent of carcass, breast, thighs, abdominal fat, liver, heart and gizzard) at 35 days of age or hematological parameters and plasma constituents at one and 35 days of age compared to the control.

Significant differences were found in body weight (BW) at 21 and 35 days of age, and the highest value was recorded of the group 75 µg/egg zinc sulfate followed by 25 µg/egg ZnO-NPs *in ovo* injection. It is concluded that using of 75 µg/egg zinc sulfate or 25 µg/egg ZnO-NPs in *ovo* injection at 9 days of incubation, could be recommended to , enhance the growth performance traits without harmful effects on the physiological responses.

Key words: In *ovo* injection, zinc oxid nanoparticles, hatching traits, growth, blood parameters.

INTRODUCTION:

In modern chicken strains, the stored nutrients in the egg might not be sufficient for embryonic development and postnatal growth (Pineda et al., 2013), this is attributed to having higher metabolic rate of embryos with higher nutritional requirements therefore, deficiency to meet these nutritional requirements has negative

effects on some traits such as embryonic development and hatchability (Karagecili and Karadaş 2017). Also, Yair and Uni (2011) reported that, the consumption of mineral is higher during the days 11–17 of egg incubation, therefore, embryo development in this period can be affected by mineral deficiency.

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The method of *in ovo* administration is considered effective tool for feeding, in which the exogenous nutrients are safely transferred into fertilized eggs and providing embryos with a supplementary amount of nutrients. These nutrients are transferred into the amniotic fluid, then swallowed, and absorbed by the embryo at the final steps of embryonic development (Uni and Ferket, 2004; Uni et al., 2005). Also, Yair and Uni (2011) found higher consumption of zinc (Zn) between day 20 of incubation and day of hatch of trace mineral *in ovo* injected group than un injected group. And that reflected on improving growth performance, immunity of chicks through improving digestive capacity, and reducing post-hatch mortality (Ferket, 2011; Yair and Uni, 2011).

The nanotechnology considerably increases surface area of the compounds which allow the opportunity for biological reactions, chemical stability and physical activity in nanoparticles (Hartemann et al., 2015). The nanoparticles can penetrate deeply into the tissues, optimizing nutrient supply across tissues, keeping nutrients safe against damage before reaching target cells (Joshua et al., 2016).

Zinc is essential trace mineral that important to all living organisms, it involved in many digestive, physiological, and biosynthetic processes within the body. They act primarily as catalysts in enzyme systems inside the cells or as coenzyme agents. Also they are components of hundreds of proteins

MATERIALS AND METHODS:

The current study included two phases, the 1st phase at El-Azab hatching plant, and the 2nd phase at laboratory of the Poultry Production Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt,

involved in intermediary metabolism, hormone secretion pathways, and immune defense systems (Milanović et al., 2008 ; Sunder et al., 2008).

Zinc oxide nanoparticles (ZnO-NPs) has strong chemical activity and participates in oxidative stress reactions. Furthermore, the permeability of ZnO-NPs can help prevent harmful intestinal reactions and improve absorption as reported by Zhao et al. (2014). Moreover, using ZnO-NPs had better effects in bioavailability when compared to other sources of zinc as well as being less toxic to broilers chicks (Sahoo et al., 2014).

Sogunle et al. (2018) showed increasing in hatchability % in group of Zn-injected eggs with level 80 µg / egg compared to the other groups, and the weight of chicks were significantly highest at 7 days of age but not at the final weight (5 weeks of age) compared to the other groups. But, Joshua et al. (2016) reported that, *in ovo* feeding of graded levels of Zn nanoparticles (20, 40, 60 and 80 µg / egg) at 18 days of incubation did not affect significantly the hatchability %.

Much research are still needed to confirm the effectiveness and safety of nanotechnology, and avoid harming birds. Therefore, this study was carried out to determine the effect of *in ovo* injection of broilers eggs with different sources of zinc [zinc sulfate and zinc oxide nanoparticles (ZnO-NPs)] on hatching traits and growth of broiler chicks.

Preparation and characterization of zinc oxide nanoparticles (ZnO-NPs).

All the preparation and characterization processes of ZnO-NPs were carried out in the Central Laboratory for Nanotechnology and Advanced Materials, Agricultural Research Center,

Egypt. The ZnO-NPs were prepared by chemical precipitation method using zinc sulfate heptahydrate as precursor salt according to **Kumar et al. (2013)**. X-Ray powder diffraction patterns of the

synthesized ZnO-NPs is shown in Figure (1). The size of ZnO-NPs ranged from 9.75-15.42 nm as determined by high resolution transmission electron microscopic

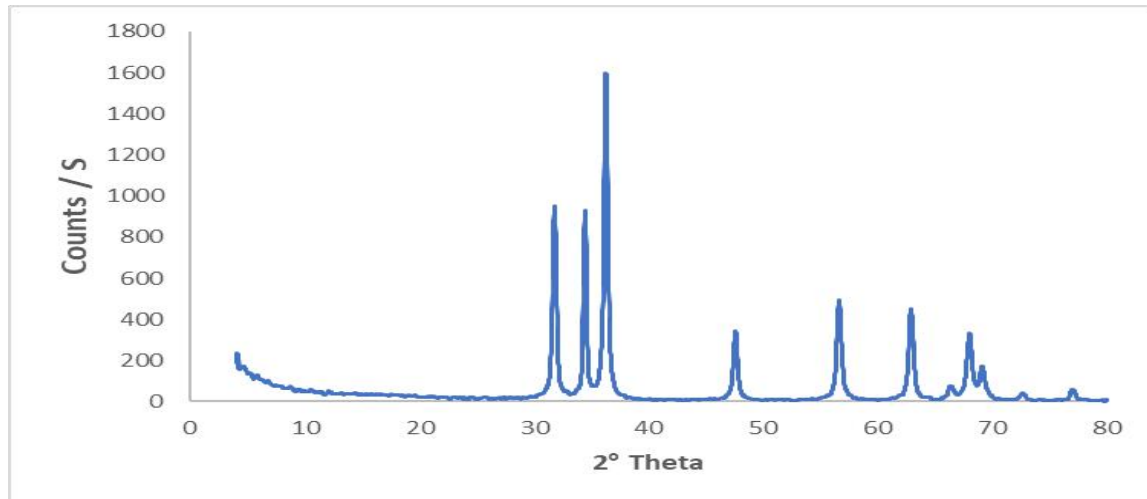


Fig.1. Characterization of zinc oxide nanoparticles (ZnO-NPs) by X-Ray diffraction patterns

Experimental design

A total number of 672 eggs were obtained from a commercial broiler breeder flock (Arbor Acres, 42 weeks of age), stored 2 days at 63 °F and 70-80 % relative humidity. All eggs were marked, weighed individually and initially divided into similar 7 groups, 96 eggs each. The eggs were fumigated and incubated under standard conditions (99-100 °F, 60-70 % relative humidity and turned each one-hour for the first 18 days of incubation in automatic electrical incubator. After that the eggs were transferred to the hatchery with a temperature of 97-98 °F and 80-90 % relative humidity for three days). After candling the fertile eggs (546 with average weight of 63.97 ± 0.16 g) were divided randomly into 7 treatments in three replicates, 26 eggs in each. The distribution of treatments as follows: The 1st treatment as control (without injection), the 2nd, 3rd and 4th treatments injected with 0.1 ml deionized water containing 25, 50 and

75 µg/egg zinc sulfate, respectively on the 9th days of incubation and treatments 5th, 6th and 7th injected with 0.1 ml deionized water containing 25, 50 and 75 µg/ ZnO-NPs, respectively

Procedure of *in ovo* injection

The solution of experiments were injected on the 9th day of incubation into amnion, before injection the broad end of each egg was sterilized with ethyl alcohol and amniotic route was marked and a small pinpoint hole was made in the broad end to injection. According to **Bhanja et al. (2004)**, the solutions were injected in the eggs using automatic syringe by a 24G hypodermic needle (25 mm long) and the pinpoint hole was sealed by sterile paraffin wax.

Hatching traits:

The hatched chicks from all treatments were counted after hatching and weighed individually. Also, Number of no hatched eggs were recorded and checked to calculate hatching %, chicks weight % and

embryonic mortality by the following formulas.

Hatchability %= (Number of hatched chicks / Number of fertile eggs) * 100

Chick weight %= (chick weight / egg weight) * 100

The embryonic mortality was calculated as a percent of fertile eggs

Traits studied on chicks at one day post hatch

After hatching, the chicks were weighed individually of each treatment and selection were made for healthy chicks to determine some parameters at one day of age and further rearing experiment

A number of forty two healthy unsexed one-day broiler chicks (six chicks around the mean of each treatment) were taken to slaughter and the blood samples were collected in two tubes the first tube (EDETA as anticoagulant) to determine the hematological parameters as red blood cells (RBCs), hematocrit (Ht), and hemoglobin (Hb) and calculate the hematological parameters indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) as reported by Perkins (2009) and the second tube contained heparin to separate the plasma, and store it in sterile tubes at -20 °C until the time of plasma constituents determination. Plasma lipid profiles concentrations (cholesterol, triglyceride, , high density lipoprotein (HDL),), calcium, phosphorus, aspartate aminotransferase (AST), total protein, albumin, total antioxidant and superoxide dismutase (SOD) were determined calorimetrically using commercial kits purchased from BioVision Company, and low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated. The different organs (heart, liver, gizzard, proventriculus) and the residual yolk sac

were weighed to calculate their relative weights.

Rearing stage conditions

Healthy unsexed 399 one-day broiler chicks around the mean of each treatment, randomly allocated to 7 treatments in three replicates, that were wing banded, weighed individually and housed in raised wire floor battery supplied with light and heaters. The birds were fed on basal diet throughout a three-stage feeding program: starter (23% CP and 3050 kcal ME / kg diet), grower (21% CP and 3160 kcal ME / kg diet) and finisher (19 CP and 3250 kcal ME / kg diet) to meet the nutrient requirement of strain, pellet diet and fresh water were provided ad libitum. The birds reared under similar managerial and hygienic conditions.

Live body weight (LBW)

Live body weight (LBW) was recorded at 1, 21 and 35 days of age as indication for the growth performance.

Slaughtering, blood analysis and carcass measurements

At 35 days of age (end of the experiment), 6 birds (3♀+3♂) were chosen around the average of each treatment the feed withdrawal was done overnight to that birds, weighed individually, and slaughtered by severing the jugular vein, and the blood sample of each bird was collected in two tubes as previously mentioned. Carcass, liver, gizzard, heart, and abdominal fat were weighed and expressed as a percentage of live body weight.

Statistical analysis

The data obtained in this study was statistically analyzed general linear models procedure (GLM) of SPSS for windows, 23 (SPSS, 2016). Significant differences among treatment means were tested by Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION:**Hatching traits**

The results in Figure (2) show that numerical differences were found in hatchability % among the eggs of control group and the groups of eggs that injected with 25, 50 or 75 µg/egg ZnSO₄ or ZnO-NPs. In this respect, **Joshua et al. (2016)** found that, *in ovo* feeding of zinc nanoparticles (20, 40, 60 and 80 µg / egg) did not affect significantly the hatchability %. And, **Biria et al. (2020)** found similar results by using nano-ZnO with levels of 50, 75 and 100 ppm/ egg. While, **Jose et al. (2018)** found in one of their experiments, that *in ovo* administration of 500 µg/egg ZnSO₄ or zinc methionine and two levels of nano zinc (250 or 500 µg/egg) resulted in complete failure of hatchability %, and found in other experiment the groups of nano Zn (40 or 80 µg/egg) *in ovo* administration had around 81% hatchability in compared to the administered group (92%). However, **El-Damrawy et al. (2019)** indicated that *in ovo* injection by 60 µg/ egg ZnO-NPs has no adverse effect on hatchability% while, it decreased with increasing the levels of ZnO-NPs to 80 or 100 µg / egg. Also, **Shahbandi et al. (2019)** reported that, injected of 50 µL deionized water containing 3.64 or 5.23 M zinc acetate caused decreasing the hatchability %. Similarly, **Palouj et al. (2021)** noted that *in ovo* administration with 20 µL nano-ZnO (0.5 mg and 0.6 mg of nano-ZnO in 50 mL saline respectively), reduced hatchability % . On the contrary, **Sogunle et al. (2018)** observed increasing in hatchability % in group of Zn-injected eggs with level 80 µg / egg compared to the other groups.

Results presented in Figure (3) show that numerical differences were existed in embryonic mortality % among the groups

of eggs that injected with 25, 50 or 75 µg/egg zinc sulfate or Zn-ONPs and the eggs of control group Also, **Shahbandi et al. (2019)** noted no effect on middle and late mortality by feeding the fertile eggs with 50 µl deionize water containing 3.64 or 5.23 M zinc acetate compared to the control. Also, **Biria et al. (2020)** found no significant differences in embryonic mortality at days 18-21 of the incubation period when injected the eggs by 50, 75 or 100 ppm nano-ZnO at 18 days of incubation. However, **Palouj et al. (2021)** observed that *in ovo* administration with 20 µL nano-ZnO (0.5 mg and 0.6 mg of nano-ZnO in 50 mL saline respectively), caused higher early and late embryonic mortality compared to the negative control.

Results presented in Table (1) indicated that there were insignificant effects for *in ovo* ZnSO₄ or ZnO-NPs injection with three levels (25, 50 or 75 µg/egg) on each of chick weight and chick weight% compared to the control. In agreement with our results. **Joshua et al. (2016)** reported that no significant differences in the chick weight or chick weigh% when the fertile eggs injected with graded levels of nano zinc (20, 40, 60 and 80 µg/egg) compared with the control. Similarly, **Jose et al. (2018)** reported doses of nano zinc (40 and 80 µg / egg) *in ovo* administration did not show any significant effect on chick weight. Also, **El-Damrawy et al. (2019)** found that *in ovo* injection with ZnO-NPs at different levels (60, 80, and 100 µg /egg) has no significant effect on the chick weight compared with the control. As well as **Shahbandi et al. (2019)** observed no significant effect on body weight at one day of hatch by injecting the eggs by of 50 µL deionized water containing 3.64 or 5.23 M zinc acetate at 3 days of incubation

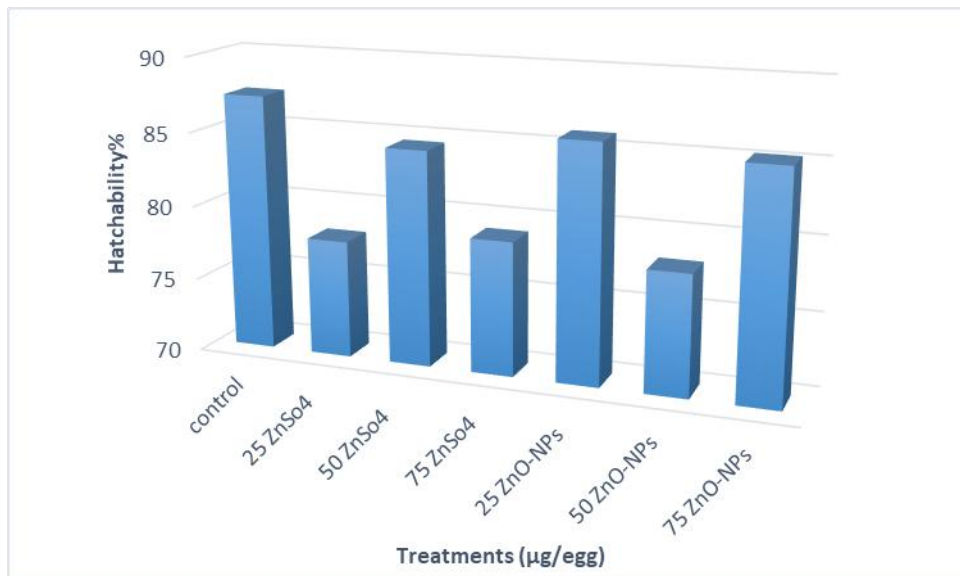


Fig. 2. Effects of in ovo injection by different zinc sources on hatchability%

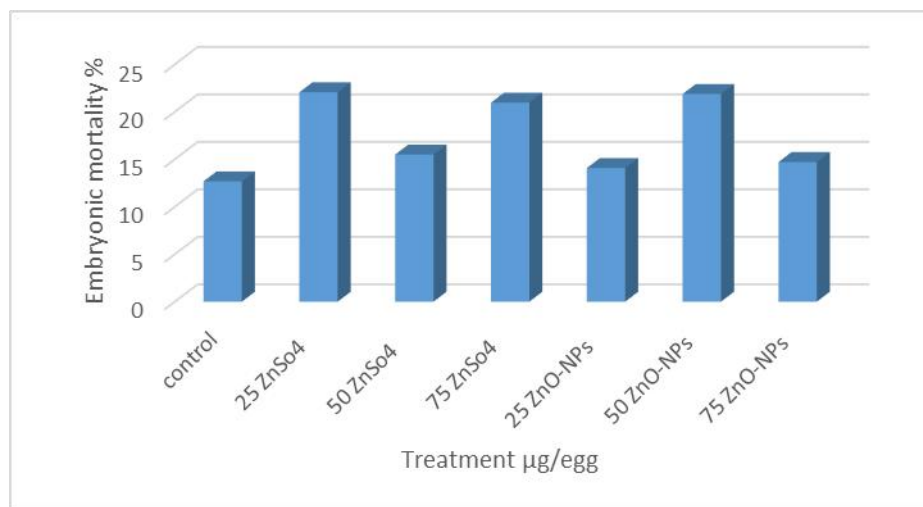


Fig.3. Effects of in ovo injection by different zinc sources on mid and late embryonic mortality

Table 1. Effects of in ovo injection by different zinc sources on broiler chick weight and chick weight %

Treatments		Egg weight (g)	Chicks weight (g)	Chicks Weight %
control		63.99	41.49	64.84
25 µg/egg	Zinc sulfate	64.24	42.72	66.51
50 µg/egg		63.86	41.60	65.14
75 µg/egg		63.86	41.77	65.40
25 µg/egg	ZnO-NPs	64.04	41.31	64.50
50 µg/egg		63.91	42.30	66.19
75 µg/egg		64.13	42.23	65.85
SEM		0.43	0.44	0.69
Sig		NS	NS	NS

NS: Not significant

One day post hatch traits

The relative weight of yolk sac and organs (heart, liver, gizzard and proventriculus) of broiler chicks at one day of age

No significant differences were found in the relative weight of yolk sac and organs % (heart, liver, gizzard and proventriculus) of chicks at one day of age by injecting the eggs with different levels of ZnSO₄ or ZnO-NPs at 9 days of incubation period as shown in Table (2). Also, **Sogunle et al. (2018)** found no significant differences in percentage of liver, gizzard, proventriculus

of broiler chicks resulted from administrating the eggs by 80 µg inorganic zinc but found increasing in heart % compared with the control group at 7 days of age. However, **Shahbandi et al. (2019)** observed increasing in liver % at 7 days of age by administering the eggs with 50 µL deionized water containing 3.64 or 5.23 M zinc acetate. But **Lukasiewicz et al. (2020)** observed increasing the heart weight and decreasing the liver weight of embryos at 19 days of incubation by the effect of in ovo injection of nano-ZnO (50, 100, 500 mg/L).

Table 2. Effects of in ovo injection by different zinc sources on the relative weight of yolk sac and organs (heart, liver, gizzard and Proventriculus) of broiler chicks at one day of age.

Treatments		Yolk sac %	Heart %	Heart* %	Liver %	Liver* %	Gizzard %	Gizzard* %	Proventriculus %	Proventriculus* %
Control		4.10	0.91	0.95	4.14	4.31	5.80	6.06	1.21	1.25
25 µg/egg	Zinc sulfate	5.19	0.81	0.85	4.32	4.57	5.82	6.14	1.05	1.10
50 µg/egg		3.64	0.81	0.84	4.07	4.23	6.22	6.46	1.13	1.18
75 µg/egg		5.71	0.82	0.87	3.64	3.87	7.32	7.75	1.02	1.10
25 µg/egg	ZnO-NPs	6.86	0.69	0.74	3.83	4.12	5.90	6.32	1.14	1.22
50 µg/egg		5.46	0.74	0.78	3.68	3.90	6.13	6.48	1.15	1.23
75 µg/egg		3.84	0.73	0.76	3.68	3.83	5.99	6.23	1.17	1.21
SEM		1.27	0.54	0.06	0.20	0.22	0.50	0.50	0.10	0.11
Sig		NS	NS	NS	NS	NS	NS	NS	NS	NS

Body weight (BW) of broiler chicks

60 µg/egg inorganic zinc compared to the control. The improvement in BW may be because zinc supplementation improved morphological structures of the intestinal as reported by **Tako et al. (2005)**; **Shah et al. (2019)**. While, **Lukasiewicz et al. (2020)** reported that the injection of eggs by different levels of nano-ZnO (50, 100, 500 mg/L) at one day of incubation did not effect on BW of chicks.

And **Palouj et al. (2021)** noted insignificant differences were found in BW of chicks at 42 days of age by injecting the eggs with 20 µL of nano-ZnO (0.5 mg of nano-ZnO in 50 mL saline), and 20 µL of nano-ZnO (0.6 mg of nano-ZnO in 50 mL saline) compared to the control.

Results presented in Table (3) indicated that there were significant differences in BW at 21 and 35 days of age and the highest value in BW was of the group 75 µg/egg ZnSO₄ in ovo injection followed by group of 25 µg/egg ZnO-NPs. Also, **Sogunle et al. (2018)** showed the weight of chicks hatched from 80 µg Zn-injected eggs were significantly highest at 7 days of age compared to the other groups. Similarly, **Shahbandi et al. (2019)** reported injecting the eggs with zinc acetate improved significantly the BW during the first week of age. And **kim and kang (2022)** found significant increase in BWG (from 1-35 days of age) of broiler chicks that resulted from injected eggs with

Table 3. Effects of in ovo injection by different zinc sources on body weight of broiler chicks

Treatments		Body weight (g)		
		1 Day	21 days	35 days
Control		41.71	760.18 ^c	1732.93 ^b
25 µg/egg	zinc sulfate	43.06	816.03 ^{bc}	1857.57 ^{ab}
50 µg/egg		41.71	762.06 ^c	1863.62 ^{ab}
75 µg/egg		42.19	858.86 ^a	1958.81 ^a
25 µg/egg	ZnO-NPs	41.05	843.33 ^{ab}	1894.22 ^a
50 µg/egg		42.41	789.10 ^{bc}	1827.59 ^{ab}
75 µg/egg		42.51	771.58 ^c	1811.23 ^{ab}
SEM		0.52	19.18	48.51
Sig		NS	***	*

Means having different superscripts within each effect in the same column are significantly different at accompanied probability. NS: Not significant
*: significant at P ≤ 0.05 , ***: significant at P ≤ 0.001

Carcass characteristics of broiler chicks:

No significant differences were existed in slaughter parameters (carcass, breast, thighs, abdominal fat, liver, heart and gizzard) of broiler chicks resulted from broiler fertile eggs administered with ZnSO₄ (25, 50 and 75 µg/egg) or ZnO-NPs (25, 50 and 75 µg/egg) related to control group as shown in Table (4). Also, **Sogunle et al. (2018)** found no significant

differences in percentage of heart, liver and gizzard of broiler chicks at 35 days of age resulted from injecting eggs by 80 µg/egg inorganic zinc compared to the control group. And **El-Damrawy et al. (2019)** found similar result of heart % and liver% by injecting the eggs with 60, 80 and 100 µg/egg ZnO-NPs. In addition to, **Palouj et al. (2021)** detected no differences in the percentages of carcass characteristics

(breast, thigh, abdominal fat, liver, heart and gizzard) of broiler treated groups resulted from injecting egg with 20 μL of nano-ZnO (0.5 mg of nano-ZnO in 50 mL

saline), and 20 μL of nano-ZnO (0.6 mg of nano-ZnO in 50 mL saline) compared to the control.

Table 4. Effects of in ovo injection by different zinc sources on carcass characteristics of broiler chicks at 5 weeks of age

Treatments	RBC ($10^6/\text{mm}^3$)	HT (%)	Hb (%)	MCV (μm)	MCH (pg)	MCHC (%)	
Control	2.37	31.02	9.97	130.87	42.10	32.18	
25 $\mu\text{g}/\text{egg}$	zinc sulfate	2.35	32.28	10.70	137.44	45.54	33.34
50 $\mu\text{g}/\text{egg}$		2.12	27.98	9.25	131.72	43.58	33.10
75 $\mu\text{g}/\text{egg}$		2.15	26.97	8.60	125.77	40.07	31.87
25 $\mu\text{g}/\text{egg}$	ZnO-NPs	2.20	28.30	8.93	129.27	40.97	31.68
50 $\mu\text{g}/\text{egg}$		2.43	30.64	9.60	126.09	39.51	31.33
75 $\mu\text{g}/\text{egg}$		2.01	25.22	8.53	125.79	42.51	33.90
SEM	0.18	2.29	0.63	4.41	0.92	1.05	
Sig	NS	NS	NS	NS	NS	NS	

NS: Not significant

Hematological parameters of broiler chicks at one and 35 days of age

In significant alteration were observed in the hematological parameters (Ht, Hb, RBCs, MCV, MCH and MCHC) of broiler chicks at one and 35 days of age by treating the eggs with 25, 50 and 75 $\mu\text{g}/\text{egg}$ zinc sulfate or ZnO-NPs as illustrated in Tables (5 and 6). In agreement with the our result **Biria et al.(2020)** found no differences in hematocrit % of broiler chicks at 35 days of

age by the effect of in ovo injection with levels 50, 75 and 100 ppm/egg nano-ZnO. Also, **William, et al. (2020)** found similar results of Ht, Hb, RBCs, MCV, MCH and MCHC at 7 and 42 days of age by the effect of injecting 80 $\mu\text{g}/\text{egg}$ inorganic zinc. In addition to, **kim and kang (2022)** found in significant variation in RBCs, Hb, HT and MCHC of broiler chicks at 35 days of age that resulted from injected eggs with 60 $\mu\text{g}/\text{egg}$ inorganic zinc

Table 5. Effects of in ovo injection by different zinc sources on hematological parameters of broiler chicks at one day of age

Items %	Control	Zinc sulfate ($\mu\text{g}/\text{egg}$)			Zno-NPs ($\mu\text{g}/\text{egg}$)			SEM	Sig.
		25	50	75	25	50	75		
Carcass	68.66	67.93	71.43	70.90	71.50	68.48	72.43	2.06	NS
Breast	39.41	39.04	41.06	39.72	40.64	38.59	42.74	2.65	NS
Thighs	29.85	28.89	30.37	31.19	30.86	29.89	29.69	0.77	NS
Abdominal fat	1.22	1.60	1.47	1.47	1.48	2.38	0.97	0.27	NS
Liver	2.63	3.14	2.37	2.31	2.48	3.22	2.1	0.28	NS
Heart	0.62	0.77	0.56	0.76	0.60	0.58	1.17	0.30	NS
Gizzard	1.19	1.16	0.87	1.21	0.74	0.93	1.07	0.13	NS

NS: Not significant

Table 6. Effects of in ovo injection by different zinc sources on hematological parameters of broiler chicks at 5 weeks of age

Treatments	Lipid profiles					Calcium (mg/dl)	Phosphorus (mg/dl)	AST (U/L)
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)			
Control	308.29	195.13	34.22	235.04	39.03	10.52	4.18	29.64
25 µg/egg	zinc sulfate	258.20	228.71	31.19	181.27	45.74	10.08	31.85
50 µg/egg		224.34	307.30	28.24	134.64	61.46	9.59	26.84
75 µg/egg		255.03	237.23	31.22	176.37	47.45	11.27	42.90
25 µg/egg	ZnO-NPs	236.33	284.67	26.52	152.88	56.93	13.88	26.25
50 µg/egg		173.02	224.82	34.20	93.86	44.96	9.81	22.76
75 µg/egg		269.84	237.96	29.30	192.95	47.59	9.34	32.36
SEM		46.52	37.69	2.48	45.19	7.54	1.68	0.37
Sig		NS	NS	NS	NS	NS	NS	NS

NS: Not significant

Plasma constituents of broiler chicks

No differences were observed in plasma lipid profiles concentrations (cholesterol, triglyceride, HDL LDL and VLDL), calcium, phosphorus, aspartate aminotransferase (AST), total protein, Albumin, globulin A/G ratio, total antioxidant and superoxide dismutase (SOD) at one day or 35 days of broiler chicks by feeding the eggs with 25, 50 and 75 µg/egg ZnSO₄ or ZnO-NPs as found in Tables (7, 8, 9 and 10). Similarly, **El-Damrawy et al.(2019)** reported that, ZnO-NPs in ovo supplementations had no significant effect on serum biochemical parameters. Also, **kim and kang (2022)** noted that in ovo Zn injection did not significantly affect cholesterol, triglyceride, AST and total

protein at 35 days of age. On the contrary, **Biria et al. (2020)** observed that the groups of nano zinc oxide in ovo injection (50, 75 and 100 ppm/egg) had lower serum triglyceride compared to the control, however, the groups of 75 and 100 ppm/egg had higher concentrations of serum cholesterol, LDL and HDL compared to groups of 50 ppm/egg and control. Also, **Łukasiewicz et al.(2020)** found decrease in level of serum triglyceride of chicks that resulted from eggs injecting by 100 and 500 mg/L nano-ZnO groups. While, **Shokraneh et al. (2020)** found that the group of nano zinc in ovo injection had higher concentration of triglyceride and total protein than the control group.

Table 7. Effects of in ovo injection by different zinc sources on plasma constituents of newly hatched broiler chicks

Treatments	RBC (10 ⁶ /mm ³)	HT (%)	Hb (%)	MCV (µm)	MCH (pg)	CHC (%)
Control	2.84	36.58	18.75	129.13	66.58	51.57
25 µg/egg	zinc sulfate	2.97	36.16	18.03	122.20	49.91
50 µg/egg		2.74	35.06	18.30	128.21	66.86
75 µg/egg		3.04	38.21	19.68	126.02	65.02
25 µg/egg	ZnO-NPs	2.64	32.69	17.04	124.30	64.86
50 µg/egg		2.78	34.65	18.22	124.61	65.57
75 µg/egg		2.68	33.44	17.33	125.07	64.82
SEM		0.16	1.75	0.79	2.45	1.63
Sig		NS	NS	NS	NS	NS

NS: Not significant

Table 8. Effects of in ovo injection by different zinc sources on plasma proteins, superoxide dismutase (SOD) and total antioxidant of newly hatched broiler chicks

Treatments	Lipid profiles					Calcium (mg/dl)	Phosphorus (mg/dl)	AST (U/L)	
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)				
Control	134.92	136.01	36.11	71.61	27.20	9.18	4.25	38.31	
25 µg/egg	zinc sulfate	157.41	181.39	38.26	82.87	36.28	7.40	4.09	27.77
50 µg/egg		133.07	215.33	35.76	54.24	43.07	10.08	3.72	32.19
75 µg/egg		144.18	156.57	38.78	74.09	31.31	7.19	4.20	14.52
25 µg/egg	ZnO-NPs	170.37	172.26	37.35	98.57	34.45	8.55	3.38	37.46
50 µg/egg		173.54	178.83	38.93	98.85	35.77	6.72	4.21	20.72
75 µg/egg		154.23	170.80	35.13	84.95	34.16	7.40	4.29	26.71
SEM		14.40	18.69	3.62	14.33	3.74	0.94	0.28	9.29
Sig		NS	NS	NS	NS	NS	NS	NS	NS

NS: Not significant

Table 9. Effects of in ovo injection by different zinc sources on plasma lipid profiles ,calcium, phosphorus and aspartate aminotransferase (AST) of broiler chicks at 5 weeks of age

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/Glo ratio	SOD U/g protein	Total antioxidant (mM/L)	
Control	5.45	1.93	3.52	0.58	0.050	0.21	
25 µg/egg	zinc sulfate	5.52	2.57	2.95	0.90	0.047	0.13
50 µg/egg		5.03	2.08	2.95	0.71	0.020	0.14
75 µg/egg		5.27	2.51	2.76	0.91	0.070	0.16
25 µg/egg	ZnO-NPs	5.16	2.48	2.68	1.02	0.020	0.14
50 µg/egg		5.13	2.32	2.81	0.94	0.010	0.09
75 µg/egg		4.65	2.47	2.18	1.16	0.025	0.18
SEM	0.52	0.20	0.51	0.18	0.01	0.06	
Sig	NS	NS	NS	NS	NS	NS	

NS: Not significant

Table 10. Effects of in ovo injection by different zinc sources on proteins, superoxide dismutase (SOD) and total antioxidant of broiler chicks at 5 weeks of age

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/Glo ratio	SOD U/g protein	Total antioxidant (mM/L)
Control	4.23	2.15	2.08	1.62	0.07	0.29
25 µg/egg	zinc sulfate	2.88	1.89	0.98	2.58	0.02
50 µg/egg		3.58	2.14	1.44	1.68	0.07
75 µg/egg		3.13	2.04	1.09	1.94	0.07
25 µg/egg	ZnO-NP	4.03	2.21	1.82	1.29	0.04
50 µg/egg		4.38	2.70	1.67	1.67	0.07
75 µg/egg		3.34	2.19	1.15	2.59	0.06
SEM	0.51	0.26	0.40	0.61	0.03	0.03
Sig	NS	NS	NS	NS	NS	NS

NS: Not significant

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الملخص العربي

تأثير حقن البيض بمصادر مختلفة من الزنك على صفات الفقس والنمو وبعض القياسات الفسيولوجية لكتاكيت التسمين

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تم استخدام 546 بيضة مخصبة وقسمت عشوائيا الى 7 معاملات، ثلاث مقررات فى كلا منها 26 بيضة. توزيع المعاملات على النحو التالي : المجموعة الاولى خدمت ككنترول (بدون حقن) و المعاملات 2 ، 3 و 4 حقنت ب 0.1 مل ماء منزوع الأيونات يحتوي على 25 ، 50 و 75 ميكروجرام / بيضة من كبريتات الزنك على الترتيب والمعاملات 5 ، 6 و 7 حقنت ب 0.1 مل ماء منزوع الأيونات يحتوي على 25 ، 50 و 75 ميكروجرام / بيضة من جسيمات اكسيد الزنك النانوية على الترتيب. تم اجراء الحقن فى اليوم التاسع من التحضين. ويمكن تلخيص النتائج كما يلى- لم تتسبب المعاملات فى اى اختلافات معنوية فى نسبة الفقس ، النفوق الجنينى ، وزن الكتكوت ، نسبة وزن الكتكوت ، الوزن النسبى لكيس المح و القلب ، الكبد، القانصة والمعدة الغدية نسبة لوزن الجسم او لوزن الجسم الخالى من كيس المح للكتاكيت عند عمر يوم ، قياسات الذبح (النسبة المئوية للذبيحة، الصدر ، الفخذ، ودهن البطن، الكبد، والقلب، والقانصة) عند 35 يوم من العمر و القياسات الهيماتولوجية ومكونات البلازما عند يوم و 35 يوم من العمر مقارنة بالكنترول . -وجدت اختلافات معنوية فى وزن الجسم عن 21 و 35 يوم من العمر وكان لمجموعة 75 ميكروجرام / بيضة كبريتات الزنك القيم الاعلى تليها المجموعة ب 25 ميكروجرام / بيضة جسيمات اكسيد الزنك النانوية. **ونستنتج** ان استخدام 75 ميكروجرام / بيضة من كبريتات الزنك او 25 ميكروجرام / بيضة من جسيمات اكسيد الزنك النانوية لحقن البيض عند 9 ايام من التحضين يمكن التوصية بها وذلك لتعزيز اداء النمو دون اثار ضارة على الاستجابات الفسيولوجية.