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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 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.

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,

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.

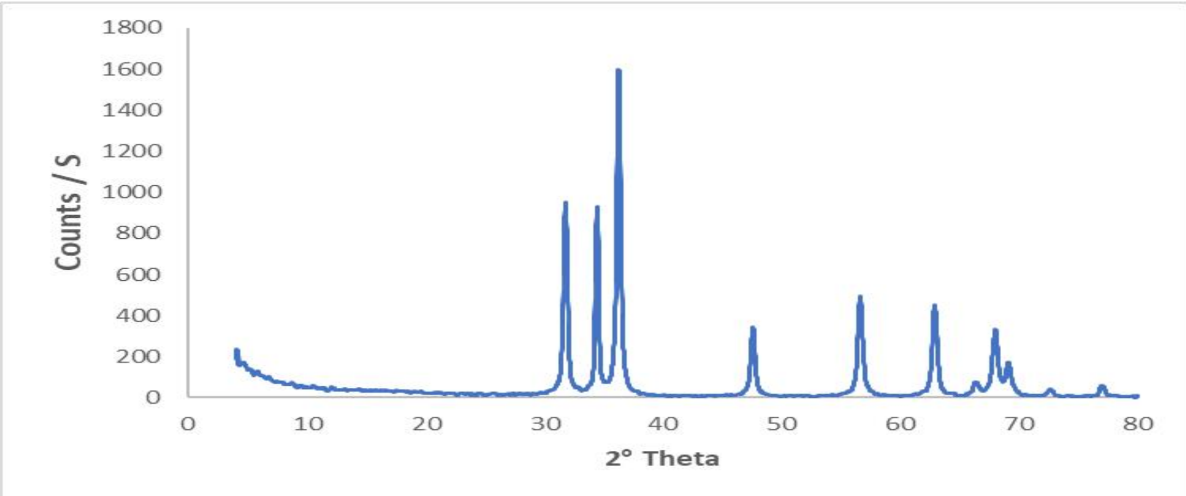

Figure (1): Characterization of zinc oxide nanoparticles (ZnO-NPs) by X-Ray diffraction patterns

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 ºF 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 ºF , 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 ºF 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 market 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, triglycereid, , 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 $ZnSO_4$ 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 $ZnSO_4$ 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* $ZnSO_4$ 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

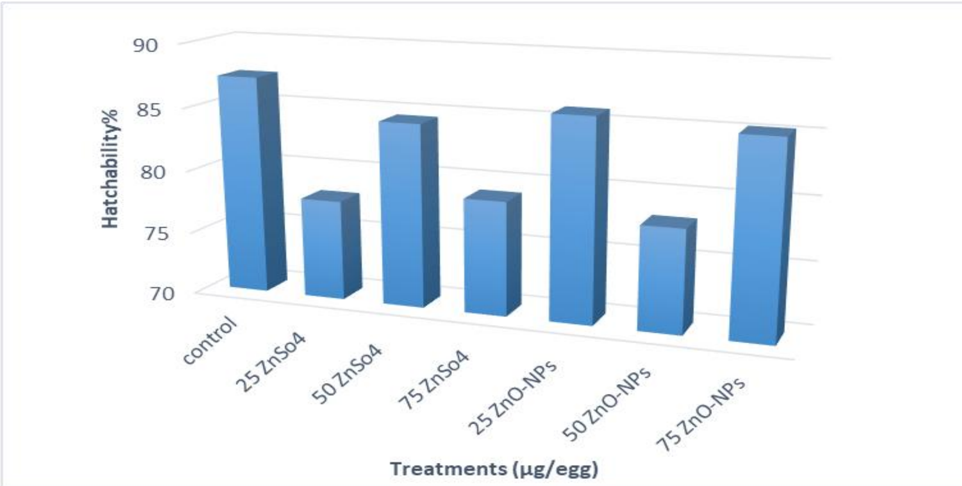

Figure 2: Effects of *in ovo* injection by different zinc sources on hatchability%

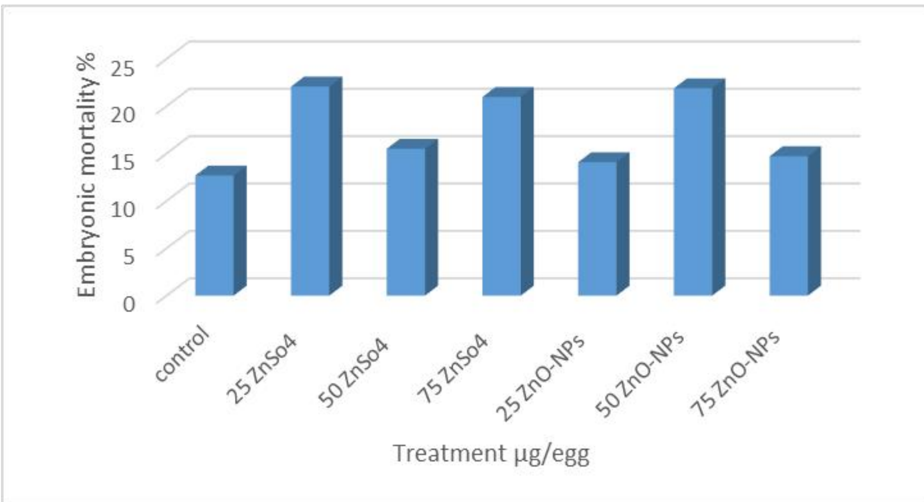

Figure 3: Effects of *in ovo* injection by different zinc sources on embryonic mortality %

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	Zinc sulfate	63.86	41.60	65.14
75 µg/egg	Zinc sulfate	63.86	41.77	65.40
25 µg/egg	ZnO-NPs	64.04	41.31	64.50
50 µg/egg	ZnO-NPs	63.91	42.30	66.19
75 µg/egg	ZnO-NPs	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 proventriculs) 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 $ZnSO_4$ 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 Proventriculs) 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	**Zinc sulfate**	3.64	0.81	0.84	4.07	4.23	6.22	6.46	1.13	1.18
75 µg/egg	**Zinc sulfate**	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	**ZnO-NPs**	5.46	0.74	0.78	3.68	3.90	6.13	6.48	1.15	1.23
75 µg/egg	**ZnO-NPs**	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

NS: Not significant *** relative to yolk-free body weight**

Body weight (BW) of broiler chicks

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 $ZnSO_4$ *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 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, **Łukasiewicz *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.

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	zinc sulfate	41.71	762.06^{c}	1863.62^{ab}
75 µg/egg	zinc sulfate	42.19	858.86^{a}	1958.81^{a}
25 µg/egg	ZnO-NPs	41.05	843.33^{ab}	1894.22^{a}
50 µg/egg	ZnO-NPs	42.41	789.10^{bc}	1827.59^{ab}
75 µg/egg	ZnO-NPs	42.51	771.58^{c}	1811.23^{ab}
SEM		0.52	19.18	48.51
Sig		NS	***	*

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

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 $ZnSO_4$ (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

Items %	Control	Zinc sulfate (µg/egg)			Zno-NPs (µg/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

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

In significant alteration were observed in the heamatological 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 µg/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 µg/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 µg/egg inorganic zinc.

Treatments		RBC (10^6/mm^3)	HT (%)	Hb (%)	MCV (µm)	MCH (pg)	MCHC (%)
Control		2.37	31.02	9.97	130.87	42.10	32.18
25 µg/egg	zinc sulfate	2.35	32.28	10.70	137.44	45.54	33.34
50 µg/egg	zinc sulfate	2.12	27.98	9.25	131.72	43.58	33.10
75 µg/egg	zinc sulfate	2.15	26.97	8.60	125.77	40.07	31.87
25 µg/egg	ZnO-NPs	2.20	28.30	8.93	129.27	40.97	31.68
50 µg/egg	ZnO-NPs	2.43	30.64	9.60	126.09	39.51	31.33
75 µg/egg	ZnO-NPs	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

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

NS: Not significant

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	61.10	49.91
50 µg/egg	zinc sulfate	2.74	35.06	18.30	128.21	66.86	52.16
75 µg/egg	zinc sulfate	3.04	38.21	19.68	126.02	65.02	51.60
25 µg/egg	ZnO-NPs	2.64	32.69	17.04	124.30	64.86	52.18
50 µg/egg	ZnO-NPs	2.78	34.65	18.22	124.61	65.57	52.61
75 µg/egg	ZnO-NPs	2.68	33.44	17.33	125.07	64.82	51.85
SEM		0.16	1.75	0.79	2.45	1.63	0.77
Sig		NS	NS	NS	NS	NS	NS

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

NS: Not significant

Plasma constituents of broiler chicks

No differences were observed in plasma lipid profiles concentrations (cholesterol, triglycereid, 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 $ZnSO_4$ 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 lipid profiles ,calcium, phosphorus and aspartate aminotransferase 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		308.29	195.13	34.22	235.04	39.03	10.52	4.18	29.64
25 µg/egg	zinc sufate	258.20	228.71	31.19	181.27	45.74	10.08	3.94	31.85
50 µg/egg	zinc sufate	224.34	307.30	28.24	134.64	61.46	9.59	4.10	26.84
75 µg/egg	zinc sufate	255.03	237.23	31.22	176.37	47.45	11.27	4.00	42.90
25 µg/egg	ZnO-NPs	236.33	284.67	26.52	152.88	56.93	13.88	4.39	26.25
50 µg/egg	ZnO-NPs	173.02	224.82	34.20	93.86	44.96	9.81	4.86	22.76
75 µg/egg	ZnO-NPs	269.84	237.96	29.30	192.95	47.59	9.34	4.08	32.36
SEM		46.52	37.69	2.48	45.19	7.54	1.68	0.37	6.17
Sig		NS	NS	NS	NS	NS	NS	NS	NS

NS: Not significant

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	zinc sulfate	5.03	2.08	2.95	0.71	0.020	0.14
75 µg/egg	zinc sulfate	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	ZnO-NPs	5.13	2.32	2.81	0.94	0.010	0.09
75 µg/egg	ZnO-NPs	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

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

NS: Not significant

Table 9: Effects of *in ovo* injection by different zinc sources on plasma lipid

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	zinc sulfate	133.07	215.33	35.76	54.24	43.07	10.08	3.72	32.19
75 µg/egg	zinc sulfate	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	ZnO-NPs	173.54	178.83	38.93	98.85	35.77	6.72	4.21	20.72
75 µg/egg	ZnO-NPs	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

profiles ,calcium, phosphorus and aspartate aminotransferase (AST) of broiler chicks at 5 weeks of age

NS: Not significant

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	0.22
50 µg/egg	zinc sulfate	3.58	2.14	1.44	1.68	0.07	0.23
75 µg/egg	zinc sulfate	3.13	2.04	1.09	1.94	0.07	0.18
25 µg/egg	ZnO-NPs	4.03	2.21	1.82	1.29	0.04	0.23
50 µg/egg	ZnO-NPs	4.38	2.70	1.67	1.67	0.07	0.24
75 µg/egg	ZnO-NPs	3.34	2.19	1.15	2.59	0.06	0.25
SEM		0.51	0.26	0.40	0.61	0.03	0.03
Sig		NS	NS	NS	NS	NS	NS

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

NS: Not significant

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الملخص العربى

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 بـ 0.1 مل ماء منزوع الأيونات يحتوي على25 ، 50 و 75 ميكروجرام / بيضة من كبريتات الزنك على الترتيب والمعاملات 5 ، 6 و7 حقنت ب 0.1 مل ماء منزوع الأيونات يحتوي على25 ، 50 و 75 ميكروجرام / بيضة من جسيمات اكسيد الزنك النانونية على الترتيب. تم اجراء الحقن فى اليوم التاسع من التحضين.

ويمكن تلخيص النتائج كما يلى- لم تتسبب المعاملات فى اى اختلافات معنوية في نسبة الفقس ،النفوق الجنينى ، وزن الكتكوت ، نسبة وزن الكتكوت ، الوزن النسبى لكيس المح و القلب ،الكبد، القانصة والمعدة الغدية نسبه لوزن الجسم او لوزن الجسم الخالى من كيس المح للكتاكيت عند عمر يوم ، قياسات الذبح (النسبة المئوية للذبيحة، والصدر ، الفخذ، ودهن البطن،الكبد ،والقلب، والقانصه)عند 35 يوم من العمر و القياسات الهيماتولوجية ومكونات البلازما عند يوم و 35 يوم من العمر مقارنه بالكنترول .

-وجدت اختلافات معنوية فى وزن الجسم عن 21و 35 يوم من العمر وكان لمجموعة 75 ميكروجرام / بيضة كبريتات الزنك القيم الاعلى تليها المجموعة ب 25 ميكروجرام / بيضة جسيمات اكسيد االزنك النانونية.

ونستنتج ان استخدام 75 ميكروجرام / بيضة من كبريتات الزنك او 25 ميكروجرام / بيضة من جسيمات اكسيد الزنك النانونية لحقن البيض عند 9 ايام من التحضين يمكن التوصية بها وذلك لتعزيز اداء النمو دون اثار ضارة على الاستجابات الفسيولوجية.