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IMPACT OF DIETARY ZINC OXIDE NANO-PARTICLES ON ANTIOXIDANT STATUS, LIVER AND KIDNEY FUNCTIONS IN ALEXANDRIA CHICKENS

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ABSTRACT: This study aimed to evaluate the effect of different forms of Zinc oxide as bulk and nano particles supplemented diet on hematological profile, antioxidant status, liver and kidney functions of Alexandria chickens. A total of 150 females and 25 males of Alexandria chickens at an age of 32 weeks were randomly divided into five groups in each group 30 female and 5 male for 12 weeks. The first group served as control group. Birds of the 2nd and 3rd groups were fed basal diet containing 40 and 80 mg zinc oxide (Bulk shape, ZnO) per kg diet and the 4th and 5th groups were fed basal diet containing 40 and 80 mg zinc oxide (Nano shape, ZnO-NPs) per kg diet, respectively. The results indicated that Hb and MCHC were significantly increased with the dose of 40 mg/kg ZnO. Data obtained revealed that there is a significant effect of zinc oxide forms on antioxidant status. While, 80 mg/kg ZnO-NPs caused a significant increase on catalase (CAT), superoxide dismutase (SOD) and total antioxidant capacity (TAC), but malondialdehyde (MDA) concentration was the lowest with the dose of 80 mg/kg ZnO-NPs. Total protein and albumin were affected by zinc oxide forms, sex and the interaction between them, but globulin was not significantly affected. Also, zinc oxide forms supplementation had no significant effect on liver and kidney functions. Generally, it can be considered that zinc oxide nanoparticles (ZnO-NPs) at a dose of 80 mg/kg addition to Alexandria chickens' diet can enhance physiological and antioxidant statuses.

Keywords: Alexandria chickens, Zinc oxide ZnO, nano shape, antioxidant status.

INTRODUCTION

The poultry industry needs advanced modern technologies to revolutionize the poultry industry with different and new tools, including the use of nanotechnology to enhance the birds' ability to absorb nutrients and thus improve the response of the productive and physiological performance of poultry. Essential trace elements play important roles as metabolism of nutrients and antioxidants and a component of many mineral enzymes and proteins (Yatoo *et al.*, 2013). Adequate supply of minerals and vitamins in diet are the key for good poultry production. The feeding of vitamins and minerals deficient diet can produce numerous health problems for chicks including death in some cases.

The poultry farmer should keep a watch on the health of chicks every day. Hence, it is emphasized to develop the practice for feeding a balanced diet with required minerals and vitamins so that deficiency diseases can be reduced in birds (Pal, 2017). Minerals are inorganic nutrients that are required in small quantities but participate in orchestration of different biological processes that drive normal growth, development, and function. Minerals are also essential for the formation of bones; as essential constituents of body fluids and tissues; as components of enzyme systems and for normal nerve function (Upadhaya and Kim, 2020). Mineral nanoparticles are now-a-days widely used in various sectors, nutrition, therapy, targeted drug delivery, preparations of vaccines and various purifications processes in textile industries (Marappan *et al.*, 2017). These nanoparticles have the ability to transport various components under various environmental conditions (Hong *et al.*, 2021). One of these nanoparticles are zinc oxide nanoparticles (ZnO-NPs), Zinc (Zn) is an important nutrient in poultry and its deficiency has been linked with various disorders, in addition to depressed growth and performance. It is now recognized that Zn has an essential role in antioxidation status, growth and development, production, immune system and stress related issues (prasad, 2009). Supplementation of Zn can enhance growth, augment immunity, improve antioxidant capacity, increase endocrine secretion and interact with other minerals in bird gut (Naz *et al.*, 2016). Zinc is a vital trace element for poultry, acts as a co-factor in more than 300 metal enzymes and plays a major role in many metabolic pathways, such as protein synthesis. Also, zinc readily available stored pools in birds' body are limited, then there is a need to daily Zn supply via diet (Asheer *et al.*, 2018). Inadequate zinc consumption negatively affects feed intake which related to body weight, growth rate and feed conversion ratio, defects protein and carbohydrate metabolism and cause abnormalities in immune responses, reproductive performance, skeletal and skin disorders (Navidshad *et al.*, 2016).The present study was designed to evaluate the effect of zinc oxide bulk (ZnO) or nanoparticles (ZnO-NPs) on hematological profile, antioxidant status, liver and kidney functions of Alexandria chickens.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Center and Laboratories of the Poultry Production Department, Faculty of Agriculture (El-Shatby), Alexandria University from April to June, 2021 (12 weeks). All treatments and birds care procedures were approved by the Institutional Animal

Care and Use Committee in AU- IACUC, Alexandria University, Egypt with the review report number AU08191217253. Authors declare that the procedures imposed on the birds were carried out to meet the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals and birds used for scientific purpose.

Zinc oxide

Zinc oxide bulk and zinc oxide nanoparticles (ZnO-NPs) used in this study were purchased from City of Scientific Research and Technological Applications (SRTA). The purity of the ZnO-NPs content was measured to be ≥99%. The ZnO-NPs varied in size between 20 and 40 nm with an average of 30 nm. Zinc oxide nanoparticles were manufactured by high energy laboratory planetary ball miller (Retsch PM VERDER SCIENTIFIC, Haan, Germany). Miller process was lasted for 40 minutes.

Birds, housing and experimental design

The experiment was carried out on a total of one hundred and seventy-five (150 female and 25 male) of local chicken strain (Alexandria) at 8 months of age for a period of 90 days. Chickens were assigned randomly into five treatment groups of 35 birds in each treatment (30 female and 5 male). Females were distributed with 3 replicates in divided breeding houses with an area of (250 cm long x 250 cm wide x 300 cm height). Birds were reared on floor in an open sided poultry house, laying hens were subjected to a photoperiod of 16-hr light and 8-hr dark/day. Males were distributed in individual gregarious battery cages (30 cm long x 30 cm wide x 40 cm height), set up on open- sided laying house. All birds in the experimental design were kept under the same managerial and environmental conditions. An identical and adequate feeding and watering space was provided to all the birds throughout the experimental period. Birds were given free access to fresh, clean and wholesome drinking water throughout the experimental period. All birds were fed on a corn-soybean basal diet supplemented with two forms of zinc. The first group was served as control group (T1) that fed basal diet without Zinc oxide supplementation. While, the first form is zinc oxide (Bulk) with two levels (40 and 80 mg/kg diet) which were (T2 & T3) respectively. The second form is zinc oxide (Nanoparticles) with two levels also (40 and 80 mg/kg diet) which were (T4 & T5) respectively. The basal diet was formulated to meet or exceed the nutrient requirements of laying hens as recommended by the NRC (1994), except for Zn. The basal diet contained 50 g/ton of Zn, which was determined by atomic absorption spectroscopy. The composition and calculated analysis of the basal diet are shown in Table 1.

Data collected

Hematological parameters

At the end of the experiment, six samples of females and males (three from each) from each treatment were randomly taken at the early morning between 08:00-09:00 am and about 3 ml blood was collected from the wing vein into sterile tubes with or without containing K3-EDTA (1 mg/ ml). The first part was used to test shortly after collection for estimating erythrocyte parameters like red blood cells (RBCs, 10^6 /ml), hemoglobin (Hb, g/dl), hematocrit (HCT, %), whereas, mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dl) counts were calculated according to (Feldman *et al.*,

2000).

Biochemical blood parameters

All blood biochemical variables were centrifuged at 4000 rpm for 15 min and the clear plasma and serum was separated and stored in a deep freezer at–20ºC until biochemical analysis. Serum glutathione per oxidase (GPx), catalase (CAT), superoxide dismutase (SOD) activity, malondialdehyde (MDA) and total antioxidant capacity (TAC, mM/L) were also measured using commercial kits obtained from Bio-diagnostic, Giza, Egypt as described by (Koracevic *et al*., 2001). Plasma total protein (TP) and albumin (Alb) were determined by a colorimetric method using a commercial kit. However, serum globulin level was calculated by subtraction of Alb from TP. Serum alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), were determined using a commercial kits of sentinel CH Company, Milano, Italy according to Senanayake *et al.,* 2015. Uric acid and creatinine were determined using enzymatic colorimetric or kinetic methods, according to the manufacturer's instructions (Marono *et al.,* 2017) Statistical analysis The experiment was set in a completely randomized design. Data were analyzed by analysis of variance using the general liner model procedure (Proc GLM; SAS Institute, 2001). Differences among means were determined using Duncan's test (Duncan, 1955).The following model was used:

$Y_{ijkl} = \mu + T_i + S_j + (T \times S)_{ij} + R_k + e_{ijkl}$

Where;

Y_{ijkl} the dependent variable under study,

μ overall mean,

T_i effect of Zn treatment,

S_j effect of sex,

$(S \times D)_{ij}$ effect of Zn treatment X sex interaction,

R_k effect of replicate,

E_{ijkl} the random residue error

RESULTS AND DISSCUSION

Hematological parameters

It is clear from the data analysis of variance that different forms of Zinc oxide (ZnO), sex and the interaction between them had various effects on hematological profile (Table 2).

The effect of each of the different forms of zinc oxide and sex on RBCs count, HCT and MCV, the highest value of RBCs in males' blood was with the dose of 80 mg/kg ZnO bulk which reached 116.98% compared with the control group. While the values of HCT and MCV showed the highest values among males with 80 mg/kg ZnO- NPs which were (16.61 and 9.11%) for HCT and MCV, respectively, but between females the highest values were with 80 mg/kg ZnO bulk (6.85 and 15.91%) for HCT and MCV, respectively.

There is a significant effect of zinc oxide forms on Hb, HCT and MCH, regardless of sex effect. Where the dose of 40 mg/kg ZnO-NPs increased Hb and MCH concentrations to reach (113.34 and 115.92 %) compared to control respectively. But HCT concentration was the highest value with dose of 80 mg/kg ZnO bulk compared to control (9.10%). Also, results refer to that sex affects Hb, HCT, MCV and MCHC. Whereas Hb, HCT and MCHC concentrations in male blood are higher than them in female by (20.91, 8.89 and 10.99 %) respectively, except for MCV which was higher in female blood than male by 10.56%. It is clear that there is a relative improvement in the hematological blood traits with the effect of zinc oxide nanoparticles. In general, RBCs, Hb, and HCT levels of Alexandria chickens either in the control or increasing levels of ZnO are in the

normal range of levels reported for chickens. This finding is in agreement with the results of Dönmez and Keskin, (1999) and Nelson *et al.,* (1984).

Along the same line, the increment in hemoglobin among zinc supplemented broiler chicken could be attributed to its essentiality in erythropoiesis. Zinc also plays a catalystic role in the activity of alfa-aminolevunilic acid dehydrogenase which is responsible for hem synthesis (Aksu *et al.,* 2010). The increase in Hb in treatment groups supplemented with zinc could be attributed to their antioxidant characteristics and their ability to improve the synthesis, stability and activity of enzymes in the body (Ali *et al.,* 2018). Zinc has been believed to be associated with RBCs and Hb production, and male goats living at high altitudes with increased erythrocytosis and blood hemoglobin levels have been confirmed to have higher serum zinc levels (Gonzoles *et al.,* 2011).

Similarly, in the studies reported by Sobhanirad and Naserian (2012) they found that supplementation of zinc in goats and cattle increase the RBCs and Hb concentrations and a significant increase was found in the RBCs and Hb values estimated from the samples obtained on the 30^{th} day in the zinc supplemented groups compared to control group.

Antioxidant status

Significant differences were observed in glutathione per oxidase (GPx), catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC) and level of malondialdehyde (MDA) in serum as presented in (Table 3). Regardless of sex factor, there was a significant effect of different dietary Zinc oxide forms on GPx, CAT, SOD, MDA and TAC levels. The dose of 80 mg/kg ZnO-NPs significantly ($P<0.05$) reduced MDA by 23.31% compared to the control group, increased CAT, SOD activity and TAC to reach (140.36, 138.18 and 135.45%) of control. While the dose of 40 and 80 mg/kg ZnO-NPs significantly ($P<0.05$) increased GPx activity by 62.70 and 75.13% compared to the control treatment respectively. Along the same line, Zhang *et al.* (2020) illustrated that SOD activity was significantly ($P\leq0.001$) increased as a result of dietary zinc supplementation. In the same study, it is noticed that dietary zinc levels caused a quadratic decrease ($P\leq0.01$) in MDA content of Longyan layer duck. MDA is an important index of lipid peroxidation and oxidative damage caused by reactive oxygen species (ROS) (Nielsen *et al.,* 1997). Zn is considered a cofactor and it is a component of more than 240 enzymes and can influence oxidative processes.

Generally, the chronic effect of antioxidation results in increased sensitivity to certain oxidative stressors (Powell, 2000). Cunningham-Rundles *et al.* (1990) showed that Zn acts as an antioxidant to reduce cell membrane damage due to free radicals, although the mechanism was not specified in their study. The total antioxidant capacity (TAC) in the body contributes mainly to the dynamic balance of active oxygen, where TAC is an integrative parameter reflecting the status of all the antioxidants in serum and body fluids. Hepatic injury may lead to a reduction in TAC in broilers (Zhao *et al.,* 2014). Moreover, it has been found that Zn is an essential component in SOD, and dietary Zn levels positively correlate with SOD activity. Prasad (2008) and Ozturk and Gumuslu, (2004) found that SOD is involved in the cellular scavenging of free radicals and ROS. Zhao *et al.* (2014) reported that 20 mg/kg ZnO-NPs had a significant effect

on SOD activity in poultry serum, while higher concentrations of ZnO-NPs (40 mg/kg) were not associated with a significant growth in SOD activity in serum suggesting that excess nano-ZnO does not contribute to biological function. These findings are consistent with those of previous reports (Duzguner and Kaya 2007; Fathi *et al.* 2016) who reported that appropriate concentrations of nano-ZnO may stimulate SOD activity, and that enhanced SOD will suppress the generation of ROS and thus decrease MDA. Also, it is obvious that sex affects GPx, CAT, MDA and TAC, whereas GPx, CAT and TAC concentrations in female blood are higher than them in male by (21.89, 10.40 and 24.35 %) respectively. MDA concentration was low in male blood, the decrease ratio was 13.30%. Taking into consideration the effect of each, the different forms of zinc oxide and sex on CAT, SOD and MDA, the highest values of CAT and SOD in both female and male blood were with the dose of 80 mg/kg ZnO-NPs which reached (136.60, 145.09 and 137.33, 139.11 %) for CAT and SOD of control group, respectively. But for MDA level the lowest value was with the dose of 80 mg/kg ZnO-NPs which were reduced by (19.42 and 27.54%) for blood females and males, respectively.

Hassan *et al.* (2016) found that the addition of zinc oxide nanoparticles at a dose of (25 ug/kg) to bird feed possesses hepato-protective effect through scavenging of free radicals, or by enhancing the activity of antioxidant, which then detoxify the free radicals. Also, Bannister *et al.* (1971) and McCord *et al.* (1971) showed that superoxide dismutase plays an important role in protecting cells and tissue from damage by superoxide radical. It has been well discussed that Zn is able to exert antioxidant effects by stimulating the expression of metallothioneins, as potent electrophilic scavengers and cell protective agents and activation of antioxidant proteins and enzymes, such as GPx and CAT (Jarosz *et al.*, 2017). Also, Kazemi *et al.* (2018) noticed that Zn supplementation partially improved TAC and SOD activity in the broiler chickens during summer conditions.

Protein profile

Results of plasma protein profile as affected by different dietary forms of ZnO, sex and the interaction between them were summarized in Table (4). Regardless of sex factor, results showed a significant ($P \leq 0.05$) increase in plasma total protein and albumin concentrations in the groups treated with 80 mg/kg ZnO-NPs (22.93 and 41.89%) compared to the control group, respectively. But globulin levels were not affected by different forms of Zinc oxide supplementation or sex and by the interaction between them as well. Also, results showed that there is a significant influence of sex on plasma TP and Alb levels whereas TP and Alb concentrations in female blood are higher than them in male, the increment ratios were 25.81 and 36.42 % for total protein and albumin, respectively. Taking into consideration the effect of each, different forms of zinc oxide and sex on protein profile (total protein and albumin), the highest values of total protein and albumin in female and male blood also were with the dose of 80 mg/kg ZnO-NPs which reached (131.24, 150.31 and 113.88, 132.09%), respectively compared with the control group. Supplemental zinc improves amino acid uptake by tissues and muscle cells and increase protein retention (Yuan

et al., 2020). It is well known that zinc is involved in protein metabolism. In addition, zinc has a role in nucleic acid metabolism as an increase in stimulation of amino acid incorporation into liver protein in vitro (Marchesini *et al.,* 1996). Higher plasma total protein values may be explained by increased digestion time with slower passage time of feed into the digestive system due to the effect of inorganic Zn since it plays a key role in the body enzyme system, physiology, metabolism and growth and it is essential for promoting protein synthesis (Berger, 2006). Feng *et al.* (2010) noticed that increased blood total protein level with zinc-glycine chelate supplementation to Ross one day-old broilers diet.

Liver and Kidney functions

Results illustrate that serum ALP, ALT, AST, uric acid and creatinine were not significantly affected by different dietary Zinc oxide forms (Table 5). Our findings reveal that ZnO-NPs were safe with respect to liver function, as reflected by unchanged values of the activities of ALT, AST and concentrations of uric acid, creatinine in the serum of treated birds, this finding is in agreement with the results of (El-Bahr *et al.,* 2020) who reached the same result when using with Japanese quail diets. Results showed that only sex affects ALP, whereas ALP concentration was higher in male blood than female by 24.36%. Regardless of the ZnO forms effect, there is a signific ant effect of sex on ALT, AST and creatinine. While, the values of them are low in male blood compared to female blood where the decrease ratios were 33.64, 19.57 and 42.31% for ALT, AST and creatinine respectively. But uric acid was not significantly affected by sex. (Zhang *et al.* 2020) reported that the ALP activity in plasma was quadratically increased ($P \leq 0.01$) by dietary zinc supplemented to Longyan layer duck.

Taking into consideration the effect of each, the different forms of zinc oxide and sex on ALT, the lowest concentration of ALT in females' blood was with the dose of 40 mg/kg ZnO-NPs which was reduced by 12.42% compared with the female control. But the lowest ratio of ALT in males' blood was the control treatment (105.33, U/L). Fawzy *et al.* (2016) noticed that there were no effects of the test supplements on liver and kidney function tests, which may be due to their antioxidative function. Similar results were obtained by Yalçinkaya *et al.,* (2012) and Okunlola *et al*., (2015).

CONCLUSION

Data obtained from this study indicated that the utilization of 80 mg/kg diet zinc oxide (ZnO) as nano particles may have beneficial effects on hematological profile, antioxidant status, liver and kidney functions of Alexandria chickens.

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Table (1): Composition and calculated analysis of the basal diet un-supplemented with (ZnO) in Alexandria chickens.

Ingredients	%
Yellow corn	60.80
Soybean meal 48%	23.65
Corn gluten meal 60%	3.45
Vegetable oil	1.00
Mono-Calcium phosphate	1.70
Limestone	8.50
Salts (NaCl)	0.35
L-lysine	0.10
Premix *	0.20
Choline-chloride	0.10
D-L-Methionine	0.05
Sodium bicarbonate	0.10
Total	100
Calculated analysis:	
Crude protein (CP %)	18.00
Metabolizable energy (Kcal/kg)	2800
Ether extract (EE %)	4.02
Crude fiber (CF %)	2.20
Calcium (%)	3.60
Available phosphorus (%)	0.50
L-Lysine (%)	1.05
Methionine (%)	0.40
Sodium (%)	0.17
Methionine + Cysteine (%)	0.68

*Each 2 kg of vitamin and mineral mixture contains: Vit. A, 12.00 MIU; Vit. D_3, 4.00 MIU; Vit. E,
15.00 MIU; Vit. K_3 2.00 g; Vit. B_1, 1.00 g; Vit. B_2, 8.00 g; Pantothenic acid, 10.87 g; Nicotinic acid,
30.00 g; Vit. B_6, 2.00 g; Vit. B_{12}, 10.00 mg; Folic acid, 1.00 g; Biotin, 150.0 mg; Cupper, 5.00 g;
Manganese, 70.00 g; Iodine, 0.50 g; Selenium, 0.15 g; Zinc, 60.00 g and Antioxidant, 10.00 g.
**ME calculated according to (Singh and Panda, 1988).

Table (2): Effect of (Mean ± SE) different forms of zinc oxide, sex and the interaction between them on hematological profile of Alexandria chickens.

Traits		RBCs (10^6/mm^3)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Treatments	Control	4.16±0.06	12.97±0.46^{b}	38.15±0.80^{bc}	91.7±1.6	31.16±1.05^{b}	34.11±1.59
	40 ZnO Bulk	4.22±0.26	14.10±0.89^{a}	37.68±1.38^{c}	90.0±3.0	33.44±0.98^{ab}	37.37±1.76
	80 ZnO Bulk	4.36±0.27	13.77±0.41^{ab}	41.62±0.65^{a}	97.0±5.4	31.89±1.15^{b}	33.07±0.79
	40 ZnO Nano	4.07±0.21	14.70±0.92^{a}	39.55±1.16^{abc}	98.2±4.6	36.12±1.18^{a}	37.16±2.04
	80 ZnO Nano	4.27±0.17	14.43±0.62^{a}	40.37±1.79^{ab}	94.7±2.6	33.91±1.24^{ab}	35.83±0.96
***p*- value**		0.1313	0.0204	0.0097	0.1336	0.0298	0.1225
Sex	Female, F	3.83±0.07^{b}	12.67±0.18^{b}	37.79±0.71^{b}	99.0±2.4^{a}	33.22±0.77	33.66±0.72^{b}
	Male, M	4.60±0.07^{a}	15.32±0.33^{a}	41.15±0.68^{a}	89.6±1.5^{b}	33.39±0.86	37.36±1.01^{a}
***p*- value**		<0.0001	<0.0001	<0.0001	0.0004	0.8547	0.0052
Interaction	Control * F	4.09±0.11^{cd}	12.07±0.48	38.37±1.39^{cd}	93.9±2.3^{cd}	29.60±1.74	31.62±2.32
	40 ZnO bulk * F	3.69±0.16^{e}	12.40±0.46	35.00±1.11^{d}	95.1±2.4^{bcd}	33.70±1.29	35.42±0.50
	80 ZnO bulk * F	3.77±0.11^{e}	12.90±0.17	41.00±0.78^{abc}	108.8±1.3^{a}	34.27±0.93	31.48±0.62
	40 ZnO nano * F	3.62±0.07^{e}	12.80±0.46	38.10±1.91^{cd}	105.6±7.2^{ab}	35.43±1.55	33.72±1.65
	80 ZnO nano * F	4.00±0.24^{d}	13.17±0.23	36.50±0.30^{d}	91.8±4.9^{cd}	33.09±1.76	36.08±0.74
	Control * M	4.24±0.04^{c}	13.87±0.14	37.93±1.10^{cd}	89.4±1.7^{cd}	32.71±0.36	36.61±0.99
	40 ZnO bulk * M	4.76±0.12^{ab}	15.80±0.93	40.37±1.07^{bc}	84.9±3.6^{d}	33.17±1.75	39.32±3.38
	80 ZnO bulk * M	4.96±0.03^{a}	14.63±0.24	42.23±1.06^{ab}	85.2±2.2^{d}	29.52±0.41	34.67±0.47
	40 ZnO nano * M	4.52±0.08^{bc}	16.60±0.62	41.00±1.00^{abc}	90.8±0.6^{cd}	36.82±2.01	40.61±2.48
	80 ZnO nano * M	4.53±0.11^{bc}	15.70±0.51	44.23±1.03^{a}	97.6±0.2^{bc}	34.73±1.97	35.58±2.00
***p*- value**		0.0010	0.1678	0.0089	0.0061	0.1201	0.3850

$^{a,b,c...}$Means in the same column followed by different letters are significantly different at P≤0.05.
RBCs: red blood cells, Hb: hemoglobin, HCT: hematocrit, MCV: blood mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

Table (3): Effect of (Mean ± SE) different forms of zinc oxide, sex and the interaction between them on antioxidant status of Alexandria chickens.

Traits		GPx (U/L)	CAT (U/mL)	SOD (U/mL)	MDA (µMol/L)	TAC (mMol/L)
Treatments	Control	1.85±0.22^{d}	6.02±0.74^{c}	114.2±6.2^{c}	14.37±1.32^{a}	1.10±0.09^{c}
	40 ZnO Bulk	2.15±0.13^{c}	6.77±0.44^{bc}	127.3±9.5^{c}	13.03±0.78^{ab}	1.28±0.11^{b}
	80 ZnO Bulk	2.68±0.11^{b}	7.53±0.35^{b}	138.6±6.7^{b}	13.17±0.58^{ab}	1.33±0.06^{ab}
	40 ZnO Nano	3.01±0.13^{a}	8.18±0.60^{ab}	152.4±8.4^{ab}	11.93±0.36^{b}	1.26±0.08^{bc}
	80 ZnO Nano	3.24±0.15^{a}	8.45±0.59^{a}	157.8±7.5^{a}	11.02±0.83^{c}	1.49±0.07^{a}
***p*- value**		<0.0001	<0.0001	<0.0001	0.0091	0.0020
Sex	Female, F	2.84±0.14^{a}	7.75±0.46^{a}	141.6±11.4	13.61±0.42^{a}	1.43±0.04^{a}
	Male, M	2.33±0.16^{b}	7.02±0.41^{b}	134.5±9.8	11.80±0.52^{b}	1.15±0.05^{b}
***p*- value**		<0.0001	<0.0001	0.2201	<0.0001	<0.0001
Interaction	Control * F	2.33±0.11	6.53±0.27^{d}	117.6±6.6^{d}	14.93±0.42^{a}	1.28±0.08
	40 ZnO bulk * F	2.32±0.23	7.00±0.29^{cd}	133.0±7.3^{c}	14.53±1.12^{a}	1.51±0.04
	80 ZnO bulk * F	2.80±0.13	7.63±0.26^{bc}	139.9±5.6^{c}	13.43±0.88^{b}	1.41±0.07
	40 ZnO nano * F	3.22±0.07	8.67±0.27^{a}	156.0±7.0^{a}	13.10±0.67^{bc}	1.37±0.11
	80 ZnO nano * F	3.54±0.06	8.92±0.41^{a}	161.5±4.9^{a}	12.03±0.86^{c}	1.61±0.08
	Control * M	1.37±0.05	5.50±0.46^{e}	110.7±5.9^{d}	13.80±1.10^{b}	0.92±0.08
	40 ZnO bulk * M	1.97±0.04	6.53±0.31^{d}	121.7±6.5^{d}	11.53±0.43^{d}	1.05±0.09
	80 ZnO bulk * M	2.55±0.16	7.42±0.34^{c}	137.3±6.5^{c}	12.90±1.39^{c}	1.25±0.09
	40 ZnO nano * M	2.80±0.15	7.68±0.25^{bc}	148.7±5.2^{bc}	10.77±0.73^{d}	1.15±0.10
	80 ZnO nano * M	2.95±0.13	7.98±0.55^{b}	154.0±3.8^{ab}	10.00±0.97^{d}	1.37±0.07
***p*- value**		0.1144	0.0095	<0.0001	0.0401	0.3607

$^{a,b,c...}$Means in the same column followed by different letters are significantly different at P≤0.05.

GPx: glutathione per oxidase, CAT: catalase, SOD: superoxide dismutase, MDA: malondialdehyde, TAC: total antioxidant capacity.

Table (4): Effect of (Mean ± SE) different forms of zinc oxide, sex and the interaction between them on protein profile of Alexandria chickens.

Traits		Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Treatments	Control	5.19±0.14^{c}	2.96±0.14^{d}	2.23±0.13
	40 ZnO Bulk	5.90±0.35^{b}	3.48±0.25^{c}	2.42±0.16
	80 ZnO Bulk	5.93±0.36^{b}	3.84±0.31^{b}	2.09±0.11
	40 ZnO Nano	6.12±0.38^{b}	4.01±0.32^{ab}	2.11±0.09
	80 ZnO Nano	6.38±0.34^{a}	4.20±0.30^{a}	2.17±0.12
***p*- value**		<0.0001	<0.0001	0.3333
Sex	Female, F	6.58±0.17^{a}	4.27±0.17^{a}	2.31±0.08
	Male, M	5.23±0.07^{b}	3.13±0.09^{b}	2.10±0.07
***p*- value**		<0.0001	<0.0001	0.0602
Interaction	Control * F	5.41±0.23^{cd}	3.24±0.05^{de}	2.17±0.25
	40 ZnO bulk * F	6.67±0.07^{b}	3.98±0.22^{c}	2.68±0.17
	80 ZnO bulk * F	6.74±0.09^{b}	4.51±0.15^{b}	2.23±0.15
	40 ZnO nano * F	6.97±0.07^{ab}	4.73±0.07^{ab}	2.25±0.13
	80 ZnO nano * F	7.10±0.08^{a}	4.87±0.02^{a}	2.23±0.06
	Control * M	4.97±0.03^{e}	2.68±0.12^{f}	2.29±0.16
	40 ZnO bulk * M	5.13±0.10^{de}	2.97±0.10^{ef}	2.16±0.20
	80 ZnO bulk * M	5.12±0.07^{de}	3.17±0.14^{e}	1.95±0.15
	40 ZnO nano * M	5.27±0.08^{de}	3.30±0.10^{de}	1.97±0.09
	80 ZnO nano * M	5.66±0.18^{c}	3.54±0.07^{d}	2.12±0.25
***p*- value**		0.0002	0.0060	0.4590

$^{a,b,c..}$.Means in the same column followed by different letters are significantly different at P≤0.05.

Table (5): Effect of (Mean ± SE) different forms of zinc oxide, sex and the interaction between them on liver and kidney functions of Alexandria chickens.

Traits		ALP (U/mL)	ALT (U/L)	AST (U/L)	Uric acid (mg/dL)	Creatinine (mg/dL)
Treatments	Control	326.6±22.0	139.8±15.8	41.17±3.88	2.06±0.12	0.79±0.11
	40 ZnO Bulk	358.4±17.8	146.2±16.5	49.00±5.62	1.83±0.14	0.78±0.09
	80 ZnO Bulk	370.3±24.3	140.2±14.2	51.67±4.36	1.89±0.14	0.86±0.14
	40 ZnO Nano	355.6±26.3	138.7±8.1	39.50±2.25	2.00±0.12	0.88±0.12
	80 ZnO Nano	355.2±24.0	155.5±15.0	47.10±2.78	1.87±0.08	0.79±0.10
***p*- value**		0.3815	0.1533	0.0669	0.6031	0.5159
Sex	Female, F	314.9±12.5^{b}	173.2±4.2^{a}	50.64±2.43^{a}	1.89±0.08	1.04±0.04^{a}
	Male, M	391.6±6.6^{a}	114.9±3.4^{b}	40.73±2.20^{b}	1.97±0.08	0.60±0.03^{b}
***p*- value**		<0.0001	<0.0001	0.0027	0.4091	<0.0001
Interaction	Control * F	298.2±39.9	174.3±5.7^{ab}	47.33±4.05	2.21±0.13	1.03±0.05
	40 ZnO bulk * F	328.5±25.8	182.0±7.1^{a}	58.67±2.33	1.60±0.12	0.93±0.10
	80 ZnO bulk * F	333.6±35.5	169.0±7.0^{ab}	59.67±2.73	2.03±0.23	1.15±0.11
	40 ZnO nano * F	307.6±32.0	152.7±8.4^{b}	37.67±3.73	1.82±0.05	1.14±0.06
	80 ZnO nano * F	306.5±20.6	188.0±6.7^{a}	49.87±0.94	1.78±0.11	0.98±0.06
	Control * M	355.0±30.2	105.3±4.9^{c}	35.00±4.58	1.91±0.19	0.56±0.07
	40 ZnO bulk * M	388.3±24.4	110.3±4.3^{c}	39.33±7.69	2.06±0.19	0.64±0.07
	80 ZnO bulk * M	407.0±18.4	111.3±11.3^{c}	43.67±4.84	1.74±0.15	0.57±0.04
	40 ZnO nano * M	403.7±11.2	124.7±7.7^{c}	41.33±2.85	2.19±0.19	0.63±0.05
	80 ZnO nano * M	404.0±18.1	123.0±4.7^{c}	44.33±5.49	1.97±0.11	0.60±0.09
***p*- value**		0.8038	0.0449	0.1273	0.0834	0.3442

$^{a,b,c,...}$Means in the same column followed by different letters are significantly different at P≤0.05.
ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate aminotransferase.

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الملخص العربى

تأثير إضافة جزيئات أكسيد الزنك النانويه على حالة مضادات الأكسده ، وظائف الكبد والكلي لعلائق دجاج الإسكندريه

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الهدف من هذه الدراسه هو تقييم تأثير الصور المختلفه لأكسيد الزنك كأسيد زنك عادي وفي صورة جزيئات نانو كإضافه علفيه على خصائص الدم وحالة مضادات الأكسده ووظائف الكبد والكلى في دجاج الإسكندريه. تم استخدام عدد إجمالي 175 طائر (١٥٠ أنثى و ٢٥ ذكر) من دجاج الإسكندريه بعمر ٣٢ أسبوعاً وتم تقسيمهم بشكل عشوائي إلى خمس مجموعات في كل مجموعة ٣٠ أنثى و ٥ ذكور لمدة ١٢ أسبوعاً. إستُخدمت المجموعة الأولى كمجموعة كنترول . تم تغذية طيور المجموعتين الثانيه والثالثه على عليقه أساسيه تحتوي على ٤٠ و ٨٠ مجم من أكسيد الزنك (الصوره العاديه) لكل كيلوجرام من العلف ، بينما تم تغذية المجموعه الرابعه والخامسه على عليقه أساسيه تحتوي على ٤٠ و ٨٠ مجم من أكسيد الزنك (صوره جزيئات النانو) على التوالي. أشارت النتائج إلى أن نسبة الهيموجلوبين و متوسط تركيز الهيموجلوبين في كرية الدم الحمراء زادت بشكل ملحوظ مع استخدام الجرعة ٤٠ مجم / كجم من أكسيد الزنك (الصوره العاديه). كما لوحظ أن هناك تأثير معنوي لصور أكسيد الزنك على حالة مضادات الأكسدة ، في حين أن ٨٠ مجم / كجم من أكسيد الزنك (صوره جزيئات النانو) أدت إلى زيادة CAT و SOD و TAC ، ولكن شوهد أن تركيز MDA إنخفض مع المجموعه المعامله بالجرعه ٨٠ مجم / كجم أكسيد الزنك (صوره جزيئات النانو) .كما وجد أن البروتين الكلي والألبيومين تأثر بكل من صور أكسيد الزنك والجنس والتفاعل بينهما ، لكن الجلوبيولين لم يتأثر بشكل معنوي. كما أن استخدام صور مختلفه لأكسيد الزنك ليس له تأثير ضار على وظائف الكبد والكلى .عموماً ، نستطيع من هذه الدراسه اعتبار أن استخدام جزيئات أكسيد الزنك النانويه بجرعة ٨٠ ملجم / كجم كإضافه علفيه لعلائق دجاج الإسكندريه عززت الحاله الفسيولوجيه ونشاط مضادات الأكسده دون إحداث تأثير ضار على وظائف الكبد والكلى.

الكلمات الداله: دجاج الإسكندريه، أكسيد الزنك، صورة جزيئات النانو، حالة مضادات الأكسده، وظائف الكبد والكلى.