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EFFICACY OF DIETARY ZINC OXIDE NANOPARTICLES SUPPLEMENTATION ON SERUM BIOCHEMICAL, NUTRIENTS RETENTION AND CHEMICAL COMPOSITION OF MEAT AND TIBIA IN BROILER CHICKENS

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ABSTRACT: A total of 192 unsexed day-old "Cobb" broiler chicks were used to evaluate the effects of different levels of dietary nano zinc oxide (N-ZnO) on nitrogen and minerals retention, blood serum measurements, meat chemical composition and tibia minerals of broiler chickens. Chicks were randomly distributed into six treatments of four replicates eight chicks each. A control contained 100 mg inorganic ZnO/kg diet (I-ZnO) and 100, 80, 60, 40 and 20 mg N-ZnO/kg diet were fed. Diets supplemented with different levels of N-ZnO had significant effect on blood serum parameters except for total protein and cholesterol. The highest contents of (albumin, high density lipoprotein, low-density lipoprotein, lactate dehydrogenase, superoxide dismutase, calcium, phosphorous and zinc) and the lowest (triglycerides, creatinine, uric acid, aspartate transferase, alanine transferase, alkaline phosphatase and malondialdehyde) were obtained with 20 mg N-ZnO/kg diet. A significant reduction in moisture and fat and significant increase in crude protein, ash and minerals (Ca, P and Zn) of breast and thigh meats were observed with N-ZnO treatments; the lowest and the highest percentages, respectively, of the former criteria were recorded with 20 mg N-ZnO. Birds fed N-ZnO (40 and 20), (60, 40 and 20) and 20 mg/kg diet had the highest significant tibia Ca, Zn and P percentages, respectively. Nano-ZnO treatments significantly increased the retention percentages except for Zn. Birds fed 40 mg N-ZnO/kg diet exhibited the highest nitrogen, Ca, P and Zn retention. It could be concluded that supplementation of N-ZnO to broiler diet improved birds' physiological status, meat carcass quality, bone mineralization and nutrients retention. The lower levels of N-ZnO (20 and 40 mg/kg diet) revealed promising results with no harmful effect on birds' health status.

Key words: broiler, zinc oxide nano particle, nutrients retention, meat quality, serum parameters.

INTRODUCTION

Zinc (Zn) as a vital trace mineral plays three important roles in the body to facilitate the biological functions: as a catalyst, regulator and structural constituent (Stefanidou *et al.,* 2006). It is a cofactor of over 300 enzymes (metalloenzymes) from all six enzyme classes. Thus it has been known to be essential for immune function, wound healing, growth, metabolism, fertility, and oxygen-free radical scavenging in animals (Kietzmann and Braun, 2006; Chand *et al.,* 2014 and Vinus and Sheoran, 2017). Although, the recommended level of Zn in broiler diet is 40 mg/kg diet (NRC, 1994); the optimal level was estimated to be 80.50 to 84.09 mg/kg of diet (Huang *et al.,* 2007). However, Štenclová *et al.* (2016) reported that supplementation of 120, 40 or 20 mg Zn oxide (ZnO) to corn-wheat-soybean meal basal diet, containing 25.84 mg Zn/kg, had no significant effect on the growth performance of broiler chickens. Moreover, diets supplemented with high levels of Zn may increase Zn excretion in the excreta resulting in environmental pollution, influence the other trace elements balance and decrease vitamins and nutrients stability. Also, the long-term implementation might increase Zn residual in the body (Case and Carlson, 2002; Broom *et al.,* 2003 and Sundaresan *et al.,* 2008)

The bio-available Zn to poultry vary depending on the form added to diets; it is higher in organic form compared with inorganic but organic Zn application in poultry feed is limited due to its higher cost (Zhao *et al.,* 2014). Nanoparticles as feed additives have been recently used in animal feed. Elements processed in nanoparticles gain unique physical characteristics such as a large surface area with high activity, many surface-active centers and, high catalytic efficiency which resulted in better absorption and utilization (Rai and Ingle, 2012). Nano trace elements enter the animal body through direct cell membrane penetration; therefore, its utilization rate is higher than the ordinary inorganic trace elements. It was found that the utilization coefficient of the inorganic trace elements about 30 % while it was close to 100 % with nano trace elements. Consequently, positive effects were resulted such as improving appetite, increasing daily gain, decreasing feed to gain ratio, improving immunity and diminish use of antibiotics thus increasing food safety for the consumer. Also, it can reduce the odor of poultry manure, which is conducive to bring environment improvement (Huang *et al.,* 2015). In addition, some improvements were detected in meat chemical composition, muscle Zn concentration, blood serum parameters, antioxidant defense and cellular immunity in broilers with the supplementation of nano ZnO particles (Esfahani *et al.,* 2015; Hafez *et al.,* 2019 and Ramiah *et al.,* 2019).

The lack of information with a satisfactory degree on usage nanotechnology in poultry nutrition needs more investigation. Therefore, the aim of the current study was to investigate the effect of dietary ZnO nanoparticles (N-ZnO) on nitrogen and minerals retention, blood serum measurements, meat chemical composition and tibia minerals of broiler chickens.

MATERIALS and METHODS

Birds and management:

An experiment was carried out at the Poultry Research Farm, Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. One hundred and ninety-two, one-day-old unsexed, "Cobb" broiler chicks obtained from a commercial hatchery were used. Chicks were individually weighed, wing-banded and randomly distributed into 24 groups (similar in average body weight) of eight chicks each. Chicks were raised in brooder batteries with wire mesh floors. Feed and water were provided *ad-libitum* during the 6 weeks experimental period. Artificial light was provided for 23 hours per day and the ambiance temperature was 34-35^{O}C during the first week of age and gradually decreased 2-3^{O}C per week till the third week of age. Electric fans were used to provide ventilation. All chicks were kept under the same management, hygienic and environmental conditions.

Treatments and the experimental diets:

A completely randomized design of six treatments of four replicate each was used to evaluate the effects of different levels of dietary N-ZnO particles on nitrogen and minerals retention, blood serum measurements, meat chemical composition and tibia minerals. The first treatment (control) contained 100 mg inorganic ZnO/kg diet (I-ZnO, as recommended by the manual of Cobb, 2015). Diets were supplemented with 100, 80, 60, 40 and 20 mg N-ZnO/kg diet to form treatments two to six, respectively. Chicks were fed three types of diets in mash form and were formulated to be iso-caloric and iso-nitrogenous. Diets were starter (0 to 3 weeks of age), grower (4 to 5 weeks of age) and finisher (6th week of age). The experimental diets composition and chemical analysis (A.O.A.C, 1990) are shown in Table 1.

Synthesis of nano zinc oxide particles:

Zinc oxide nanoparticles were prepared by the precipitation method (Kumar *et al.*, 2013). Sodium hydroxide solution (122.40 g dissolved in 1500 ml of deionized water) was added drop wise under magnetic stirring to zinc sulfate heptahydrate solution (431.31 g dissolved in 1500 ml of deionized water) and the stirring was continued for 12 hr after the completed addition. The precipitates were filtered, washed by pure water several times, dried at 100◦C for 30 min and calcined at 500◦C for 2 hr. The crystalline and phase structure of the synthesized ZnO was studied by an X-ray diffract meter (XRD, X′ Pert PRO). The morphology and size were determined by the transmission electron microscopy (TEM, JEOL JEM-2100). All chemicals used were used without further purification (Research-Lab Fine Chem Industries-Mumbai 400-002, India). All the preparation and characterization processes were conducted at Nanotechnology and Advanced Materials Central Lab, Agricultural Research Center, Egypt.

Nutrients balance trial:

At five weeks of age, digestion trial was performed. The total apparent retention of nitrogen (N), Calcium (Ca), phosphorus (P) and Zn was determined using one bird per replicate (four birds per treatment). The finisher diet was fed. The trial was extended for six days, three days of adaptation followed by three days of feces collection. Birds were housed in individual

digestion cages. Feed intake and excreta voided were accurately determined. The excreta from each bird was separately collected, cleaned from feathers, weighed and dried in a forced oven at 70° C for 24 hr. The dried excreta were weighed, grind and placed in glass jars until chemical analyses. Calcium, P and Zn in the experimental diets and excreta were determined according to Fodor *et al.* (1973), while nitrogen was determined according to the A.O.A.C (1990).

Blood serum parameters:

Close to the average body weight, two birds were chosen from each replicate at the end of the experimental period (6 weeks of age). Birds were fasted overnight with free access to water. Selected birds were individually weighed and slaughtered. During bleeding, blood samples were collected from each bird in a tube. Serum was separated by centrifugation and stored at -20°C until analysis. Serum constituent measurements of total protein, albumin, triglyceride, cholesterol, high density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine, uric acid, Ca, P, Zn, superoxide dismutase (SOD), alkaline phosphatase (ALP), aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH) and malondialdehyde (MDA) were colorimetrically determined using commercial kits (Egyptian Company for Biotechnology, S.A.E, and Diamond, D-P, International) as described by **Young and Friedman (2001).**

Chemical analysis of meat:

The eviscerated carcass from each bird was deboned; meat of the breast and thigh was minced and dried. The chemical composition of the dry meat samples were done according to **A.O**.A.C. (1990). The dry matter was evaluated by drying in an oven at 135°C and moisture percentage was calculated. The crude protein (CP) was determined by Kjeldahl method and crude fat by Soxhlet method using petroleum ether. The crude ash was determined by ashing meat sample in a muffle furnace oven at a temperature of 550°C for 2 hr. Nitrogen-free extract (NFE) was calculated by the difference. Calcium, P and Zn were evaluated using the atomic absorption spectrometry (Fodor *et al.*, 1973).

Tibia minerals:

The right tibia of each carcass was cleaned of adhering tissues, including cartilage caps, and washed with distilled water. Tibia bones were dried in a hot air oven at 105°C for 24 hours. The dried tibias were ashed at 600° C for 24 hours in a muffle furnace and the ash was weighed. Percentages of Ca and P and Zn (ppm) were determined in the ash (Fodor *et al.*, 1973).

Statistical analysis:

Data were subjected to analysis for significance by a one-way ANOVA model (as a completely randomized design) using the General Linear Models (GLM) procedures of SPSS (IBM SPSS statistics, version 22, USA). Treatments differences were considered significant at $P \leq 0.05$ and $P \leq 0.01$ for all measurements. Means comparisons were performed using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

1. Nano zinc oxide particles

The size of N-ZnO powder was investigated by the XRD and TEM analyses and has shown clear results.

1.1. XRD analysis results:

The XRD pattern of the synthesized ZnO nano-particles is shown in figure (1). The peaks at 2θ = 31.77°, 34.4°, 36.26°, 47.54°, 56.60°, 62.86°, 66.38°, 67.95°, 69.09°, 72.57°, and 76.97° were assigned to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) of ZnO nano-particles, indicating that the crystalline structure of synthesized ZnO nano-particles presented a hexagonal phase structure of the wurtzite (Zincite, JCPDS 5-0664).

1.2. Transmission Electron Microscopic analysis results:

High Resolution Transmission Electron Microscopic (HR-TEM) studies were carried out to find out the exact particle size of synthesized ZnO. Transmission Electron Microscopic images as illustrated in Figure (2), ZnO nano-particles which having particle size in the range of 19–39 nm with nearly spherical shaped particles.

2. Blood serum parameters:

2.1. Serum constituents:

Diets supplemented with N-ZnO increased serum total protein compared with the control with no significant difference (Table 2). This increase might be attributed to the role of Zn in protein synthesis **(**Ibs and Rink, 2003). An increase (P≤0.01) in serum albumin and a reduction (P≤0.01) in creatinine and uric acid were observed with the supplementation of N-ZnO particles; the highest and the lowest values, respectively, of the former serum parameters were obtained with level of 20 mg N-ZnO/kg diet and the vice versa for the control.

These results were partially consistent with Hassan *et al.* (2017) who showed that serum total protein was increased with the supplementation of 60 and 30 mg N-ZnO/kg of rabbit diets in comparison with Zn-free premix diet or supplemented with 60 mg I-ZnO/kg of diet. However, the present data divergent with those of El-Katcha *et al.* (2017) who observed that the replacement of I-ZnO by N-Zn in broiler diets had no significant effect on blood serum total protein, albumin and uric acid concentrations.

A significant reduction in triglycerides and a significant increase in HDL and LDL contents were recorded with N-ZnO levels (Table 2). The lowest (P≤0.01) triglycerides and the highest (P≤0.01) HDL values were observed with 40 and 20 mg N-ZnO diets while 60, 40 and 20 mg N-ZnO diets recorded the highest (P≤0.05) LDL values in comparison with the control. Likewise, the lowest cholesterol content was observed with 20 mg N-ZnO diet compared with the control but no significant difference.

In similar direction, the replacement of I-Zn with different levels of N-Zn particles reduced (P≥0.05) blood serum triglycerides while increased total cholesterol and HDL. However, no effect on serum LDL and very low density lipoprotein (VLDL) concentrations of broilers was found (El-Katcha *et al.,* 2017). In addition, N-Zn decreased (P≤0.05) serum total cholesterol, VLDL and triglyceride, in broiler chicks with the lowest values were observed with N-Zn diets. In spite of that, N-Zn supplementation had no effect on serum lipid and HDL (Ibrahim *et al.,* 2017). Similar results have been obtained by Ahmadi *et al.*(2013) who reported that supplementation of 60 or 90 mg N-ZnO/kg of broiler diet decreased serum

triglycerides, total cholesterol and LDL-cholesterol with no significant differences, however, HDL-cholesterol increased (P <0.05) in comparison with the control diet (zero N-ZnO). Besides, the highest serum HDL was observed with 90 mg N-ZnO /kg of diet while 120 mg N-ZnO recorded the highest LDL. Although, serum total cholesterol and HDL were significantly increased at 20 mg N-ZnO/kg; no significant effect was detected by N-ZnO (10, 20 and 40 mg) on serum triglyceride of broiler chickens (Fathi *et al.*, 2016). A change of cholesterol levels in blood plasma may be due to the role of Zn in enzyme action as Zn forms an integral part of several enzymes (metalloenzymes) that are severed in lipid digestion and absorption Al-Daraji and Amen (2011).

2. 2. Serum enzymes and minerals:

Supplementation of different levels of N-ZnO decreased (P ≤0.01) liver enzymes (AST and ALT), ALP and MDA contents, the lowest contents were obtained by level of 20 mg N-ZnO/kg diet (Table, 3). Conversely, a significant increase in serum LDH (P ≤0.05) and SOD (P≤0.01) enzyme contents were obtained by N-ZnO treatments, the lowest values recoded by the control diet in comparison with the other treatments.

These data are in harmony with those obtained by Berg and Shi (1996) and Walsh *et al.* (1994) who stated inverse relationship between N-ZnO and MDA values and serum ALT and AST enzyme concentrations. Besides, Ibrahim *et al.* (2017) reported that serum MDA level was decreased (P≤0.05), while, the Cu/Zn-SOD level was increased (P≤0.05) with dietary N-ZnO, which indicated that adding of N-Zn increased the antioxidant activity in broilers. However, the hepatic activity of AST and ALT were not significantly influenced by different dietary sources of Zn. Also, the activity of serum AST, ALT, ALP and LDH enzymes were significantly decreased with the supplementation of different levels of N-ZnO in broiler diets. On the other side, SOD was significantly increased and MDA was decreased at 60 or 90 mg N-ZnO/kg of diet in comparison with the other levels (zero, 30, 120 mg N-ZnO/kg). They concluded that 60 or 90 mg N-ZnO/kg of diet improved serum enzymes activity and antioxidant condition of broiler chicks (Ahmadi *et al.*, 2014).

However, Fathi *et al.* (2016) demonstrated that serum HDL, ALP and SOD were significantly increased and MDA reduced at 20 mg N-ZnO/kg. In addition, N-ZnO (10, 20 and 40 mg) had no significant effect on serum AST and ALT of broiler chickens. Moreover, Zhao *et al.* (2014) affirmed that 20 mg N-ZnO/kg of broiler diet had a similar effect on the action of Cu-Zn-SOD1 in serum and liver tissue as that of the control (60 mg ZnO). They attributed this due to the role of zinc as a cofactor of antioxidant enzyme (Cu-Zn-SOD1) and it is also required to activate these enzymes that scavenge reactive oxygen species, reducing oxidative stress.

Data in Table 3 showed that serum Ca, P and Zn were significantly increased (P≤0.01) with the supplementation of N-ZnO in the diets. Birds fed 40 and 20 mg N-ZnO diets had the highest Zn in serum while 20 mg N-ZnO was enough to record the highest contents of Ca and P. The present data are in agreement with those obtained by Pei *et al.* (2018) who reported

that serum Zn was increased when pigs fed basal diet supplemented with 150 and 300 mg N-ZnO or 450 and 3000 mg I-ZnO for 21 days. Moreover, El-Katcha *et al.* (2017) observed that replacing the I-ZnO with lower levels of N-ZnO in broiler diets increased blood serum Ca or P compared with the basal or I-ZnO diets. Furthermore, Li *et al.* (2016) revealed that serum Zn concentration of weaning piglet was increased by 120 mg N-ZnO compared with 80 and 120 mg I-ZnO.

3. Meat nutrients composition:

The effect of N-ZnO on the chemical composition and minerals content of breast and thigh meats was similar and significant ($P \leq 0.05$ and 0.01) except for NFE of breast meat (Tables 4 and 5). Diets supplemented with different levels of N-ZnO decreased moisture and crude fat and increased crude protein and ash and minerals content in breast and thigh meats. The lowest significant moisture and crude fat percentages were recorded by N-ZnO levels (40 and 20 mg) and 20 mg, respectively, in comparison with the control. In contrast, a significant increase ($P \leq 0.01$) in meat crude protein, ash, Ca and P percentages and Zn (ppm) were observed and 20 mg N-ZnO/kg of diet recorded the highest values compared with the control.

In agreement with the present results, Ramiah *et al.* (2019) established that thigh muscle Zn content was increased with reducing dietary N-ZnO level from 100 mg to 60 and 40 mg/kg of broiler diet. Also, Esfahani *et al.* (2015) found that a mixture of 50 N-ZnO + 2 *Curcuma longa*/kg of broiler diet decreased humidity and crude fat and increased the crude protein of breast and thigh meat, resulted in improved carcass quality. Similar results were observed by Selim *et al.* (2014) who noticed that supplementation of N-ZnO (40 and 80 mg/kg) to broiler diet significantly increased muscles Zn content by 41.4 and 10.6% relatively with $ZnSO_4$ and Zn methionine as well as reduced total lipids contents especially with the high Zn level (80 mg). On the other hand, muscles Zn content was the highest with the supplementation of 15 ppm I-ZnO to broiler diet and this was significantly different with zero and 0.03 ppm N-Zn/kg (Sahoo *et al.*, 2014).

4. Tibia minerals:

Birds fed 40 and 20 mg N-ZnO diets were significantly higher ($P \leq 0.01$) in tibia Ca percentages compared with 100 mg of I-ZnO or N-ZnO (Table 6). Also, tibia P percentages were significantly increased ($P \leq 0.01$) with chicks fed 20 mg N-ZnO while the highest ($P \leq 0.01$) Zn was recorded by birds fed 60, 40 and 20 mg N-ZnO diets compared with the control. The increase in the crude protein and ash of meat, as well as the minerals (Ca, P and Zn) in meat and tibia, is due to increasing the same nutrients in blood serum and to improving their digestibility. The results of the current study agree with those reported by Ibrahim *et al.* (2017) who showed that the highest tibia Zn content was recorded with diets supplemented with N-ZnO compared with that supplemented with I-ZnO of broiler chicks. Similarly, tibia Zn content was significantly higher with the addition of 80 mg N-Zn/kg to broiler diet in comparison with 80 mg Zn-sulphate or the unsupplemented control (Mohammadi *et al.*, 2015). Furthermore, Sahoo *et al.* (2014) stated that N-ZnO (0.06 ppm)

significantly increased Zn content of tibia bone compared with I-$ZnSO_4$ and Zn-Methionine. Similar results were reported in laying hens where Abedini *et al.* (2017) found that 80 mg of N-ZnO increased tibia Zn deposition.

5. Nitrogen and minerals retention:

Diets supplemented with N-ZnO increased the retention percentages of N, Ca, P and Zn compared with the control. This retention increment was significant ($P \leq 0.05$) with N, Ca and P (Table 6). Birds fed 40 mg N-ZnO exhibited the highest N, Ca, P and Zn retention percentages compared with those fed the control which recorded the lowest percentages. In harmony with the current data, Ibrahim *et al.* (2017) on broilers and Tsai *et al.* (2016) on laying hens concluded that Zn retention was significantly increased by N-ZnO supplementation compared with I-ZnO because of its higher bioavailability. Also, Yan and Waldroup (2006) reported that Zn excretion could be reduced in the excreta by the supplementation of N-Zn to poultry diets. Similar results were reported with pigs, supplementation of 150 and 300 mg N-ZnO (Pei *et al*., 2018) or 800 mg N-ZnO /kg of diet (Wang *et al.,* 2018) decreased fecal Zn excretion compared with I-ZnO.

CONCLUSION

It could be concluded that the improvements in studied criteria may be related to more bio-available Zn resulted from the new physicochemical properties of N-ZnO particles. Low levels supplementation of N-ZnO to broiler diets was more efficient than high levels. Diets supplemented with 40 or 20 mg N-ZnO/kg improved breast and thigh meats quality, enhanced immune enzymes and, increased blood serum and tibia mineral contents. Also, N-ZnO increased the retention of nitrogen, Ca, P and Zn, decreasing their excretion, which reduced environmental pollution. Further studies are suggested to explore the absorption mechanism, metabolic pathways, toxicological levels and residues in tissues of N-Zn.

Table (1): Formula and chemical analysis (determined and calculated) of the experimental basal diets.

Ingredients (%)	**Starter (0-3wk)**	**Grower (3-5wk)**	**Finisher (6th wk)**
Yellow corn	57.00	60.60	64.90
Soybean meal (44%CP)	30.00	27.00	24.31
Corn gluten meal (60%CP)	6.70	5.00	3.00
Veget.oil (Soybean +sun flower)	1.82	3.01	3.92
Limestone	1.24	1.07	1.00
Di-calcium phosphate	1.68	1.57	1.40
Mineral premix (Zn free)[1]	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.30	0.25
Sodium chloride	0.40	0.50	0.37
DL- Methionine	0.23	0.21	0.28
L-Lysine	0.33	0.29	0.22
Choline chloride	0.10	0.20	0.10
Total	100.00	100.00	100.00
Chemical calculated values (according to NRC, 1994)			
Metabolizable energy (Kcal/kg)	**3000.64**	**3100.80**	**3200.26**
Crude protein, %	22.07	20.02	18.02
Lysine, %	1.321	1.196	1.052
Methionine, %	0.610	0.553	0.582
Methionine + Cystine, %	0.984	0.896	0.822
Calcium, %	0.939	0.842	0.771
A. Phosphorous, %	0.450	0.422	0.384
Zinc (ppm)	26.15	24.91	23.79
Chemical determined analysis %			
Moisture	8.66	8.97	8.95
Crude protein	21.65	19.87	17.58
Crude fiber	3.65	3.44	3.15
Ether extract	4.97	5.71	6.76
Crude ash	5.60	5.61	4.91
Nitrogen free extract	55.47	56.40	58.65

[1)] Each 1 kg of vitamin mixture contained: 10.000.000 IU vit. A, 5.000.000 IU vit. D3, 80.000 mg vit. E, 3.000 mg vit. K3, 3.000 mg vit. B1, 9.000 mg vit. B2, 4.000 mg vit. B6, 20 mg vit. B12, 15.000 mg pantothenic acid, 60.000 mg Nicotinic acid, 2.000 mg Folic acid and 150 mg Biotin.
[2)] Each 2 kg of minerals mixture contained: 500.000 mg choline chloride, 150.000 mg Cu, 1.000 mg I; 40.000 mg Fe, 100.000 mg Mn. and 350 mg Se.

Table (2): Effect of dietary nano zinc oxide (N-ZnO) on blood serum constituents of broiler chicks at 42 days of age (M±SE).

Treatments	Total protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	3.62±0.10	1.43^{d}±0.09	3.02^{a}±0.05	8.64^{a}±0.28	67.49^{a}±1.80	155.76±1.53	50.11^{d}±1.84	40.57^{b}±1.30
100 I-ZnO*	3.84±0.07	2.01^{c}±0.13	2.79^{ab}±0.09	7.87^{b}±0.28	62.70^{ab}±2.04	154.60±1.36	56.45^{c}±1.73	43.26^{ab}±1.12
100 N-ZnO**	3.66±0.06	1.96^{c}±0.17	2.61^{bc}±0.11	7.40^{bc}±0.26	58.97^{bc}±2.36	150.67±1.73	59.17^{bc}±1.35	44.08^{ab}±1.79
80 N-ZnO	3.80±0.09	2.21^{bc}±0.17	2.38^{c}±0.09	6.83^{c}±0.19	56.25^{c}±1.56	151.19±2.11	62.67^{b}±1.96	46.32^{a}±1.48
60 N-ZnO	3.84±0.06	2.51^{ab}±0.14	1.87^{d}±0.10	5.12^{d}±0.24	49.71^{d}±2.35	152.79±1.26	68.29^{a}±1.58	47.62^{a}±2.32
40 N-ZnO	3.74±0.06	2.65^{a}±0.09	1.63^{d}±0.10	5.00^{d}±0.33	48.56^{d}±2.17	150.20±2.94	69.50^{a}±2.33	48.07^{a}±1.48
20 N-ZnO	NS	P≤0.01	P≤0.01	P≤0.01	P≤0.01	NS	P≤0.01	P≤0.05
Sig.								

$^{a-d}$ means within each column followed by different letters differ significantly , NS =Non significant , * I-ZnO = inorganic zinc oxide and ** N-ZnO = nano zinc oxide .

Table (3): Effect of dietary nano zinc oxide (N-ZnO) on blood serum enzymes and minerals of broiler chicks at 42 days of age (M±SE).

Treatments	Enzymes						Minerals		
	AST (U/l)	ALT (U/l)	ALP (U/L)	LDH (U/L)	SOD (U/ml)	MDA (nmol/ml)	Calcium (mg/dL)	Phosphorus (mg/dL)	Zinc (µg/dL)
Control	177.48^{a} ±2.21	7.85^{a} ±0.13	331.33^{a} ±3.75	2992.00^{b} ±63.15	160.26^{d} ±2.34	3.01^{a} ±0.06	9.10^{e} ±0.23	4.72^{e} ±0.16	98.53^{e} ±3.05
100 I-ZnO*									
100 N-ZnO**	173.57^{ab} ±2.41	6.93^{b} ±0.20	324.22^{ab} ±2.80	3078.38^{ab} ±61.69	165.16^{cd} ±2.51	2.86^{ab} ±0.02	9.51^{de} ±0.25	5.30^{de} ±0.27	106.93^{de} ±3.42
80 N-ZnO	168.97^{bc} ±2.06	6.43^{bc} ±0.29	318.81^{bc} ±4.10	3117.50^{ab} ±58.53	167.44^{bc} ±2.49	2.71^{bc} ±0.06	9.90^{cd} ±0.26	5.81^{cd} ±0.24	113.94^{bc} ±3.09
60 N-ZnO	165.32^{cd} ±2.45	5.90^{cd} ±0.37	313.66^{bcd} ±3.16	3186.69^{a} ±36.71	172.98^{b} ±2.18	2.56^{c} ±0.10	10.51^{bc} ±0.25	6.29^{bc} ±0.21	122.18^{b} ±3.20
40 N-ZnO	161.05^{d} ±3.29	5.37^{de} ±0.28	307.99^{cd} ±3.91	3206.50^{a} ±39.59	179.50^{a} ±2.13	2.36^{d} ±0.07	11.07^{ab} ±0.28	6.69^{ab} ±0.27	135.49^{a} ±2.94
20 N-ZnO	160.29^{d} ±3.16	5.12^{e} ±0.26	305.57^{d} ±4.20	3212.44^{a} ±50.17	180.10^{a} ±2.25	2.26^{d} ±0.05	11.29^{a} ±0.19	6.97^{a} ±0.20	137.95^{a} ±2.77
Sig.	P≤0.01	P≤0.01	P≤0.01	P≤0.05	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01

$^{a-e}$ means within each column followed by different letters differ significantly,* I-ZnO = inorganic zinc oxide and ** N-ZnO = nano zinc oxide .

Table (4): Effect of dietary nano zinc oxide (N-ZnO) on breast meat chemical composition (%) of broiler chicks at 42 days of age (M±SE).

Treatments	Percentage (%)							Zinc (ppm)
	Moisture	Crude protein	Crude fat	Crude ash	NFE	Calcium	Phosphorus	
Control	6.99^{a}±0.13	54.88^{e}±0.13	33.74^{a}±0.25	3.51^{c}±0.13	0.68±0.06	0.469^{f}±0.005	0.668^{f}±0.006	211.88^{f}±2.13
100 I-ZnO*	6.96^{ab}±0.14	56.22^{d}±0.26	32.20^{b}±0.16	3.96^{b}±0.11	0.66±0.06	0.516^{e}±0.006	0.726^{e}±0.009	219.75^{e}±1.74
100 N-ZnO**	6.64^{abc}±0.13	57.12^{c}±0.20	31.41^{c}±0.23	4.10^{ab}±0.14	0.73±0.03	0.558^{d}±0.005	0.764^{d}±0.007	230.38^{d}±1.19
80 N-ZnO	6.63^{abc}±0.16	57.52^{c}±0.19	31.05^{c}±0.25	4.15^{ab}±0.15	0.65±0.03	0.604^{c}±0.006	0.804^{c}±0.008	236.25^{c}±1.06
60 N-ZnO	6.50^{bc}±0.22	58.86^{b}±0.16	29.67^{d}±0.18	4.26^{ab}±0.09	0.71±0.04	0.656^{b}±0.005	0.825^{b}±0.006	245.13^{b}±1.16
40 N-ZnO	6.25^{c}±0.11	59.90^{a}±0.12	28.67^{e}±0.18	4.46^{a}±0.14	0.71±0.04	0.706^{a}±0.007	0.858^{a}±0.008	258.50^{a}±1.20
20 N-ZnO	P≤0.05	P≤0.01	P≤0.01	P≤0.01	NS	P≤0.01	P≤0.01	P≤0.01
Sig.								

$^{a-f}$ means within each column followed by different letters differ significantly, NS =Non significant, * I-ZnO = inorganic zinc oxide and ** N-ZnO = nano zinc oxide .

Table (5): Effect of dietary nano zinc oxide (N-ZnO) particles on thigh meat chemical composition (%) of broiler chicks at 42 days of age (M±SE).

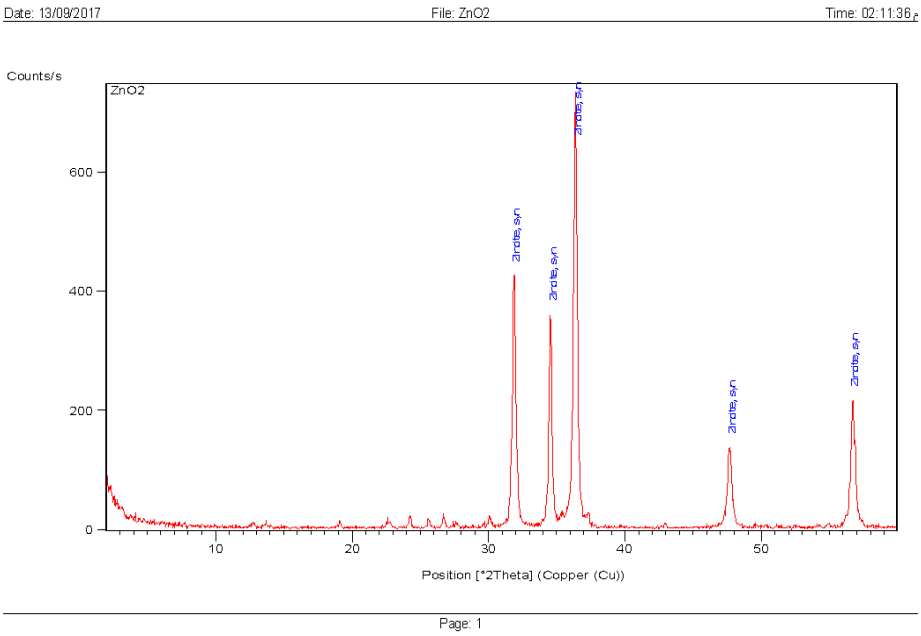
Treatments	Percentage (%)							Zinc (ppm)
	Moisture	Crude protein	Crude fat	Crude ash	NFE	Calcium	Phosphorus	
Control	6.00^{a}±0.12	50.75^{f}±0.16	39.01^{a}±0.19	2.63^{c}±0.18	1.48^{a}±0.10	0.423^{e}±0.005	0.605^{e}±0.006	417.25^{e}±3.94
100 I-ZnO*	5.96^{a}±0.12	51.75^{e}±0.16	38.15^{b}±0.21	2.88^{bc}±0.13	1.39^{ab}±0.09	0.431^{e}±0.006	0.621^{e}±0.008	436.50^{d}±1.55
100 N-ZnO**	5.73^{ab}±0.14	53.00^{d}±0.19	36.85^{c}±0.21	3.00^{bc}±0.00	1.28^{ab}±0.06	0.463^{d}±0.006	0.649^{d}±0.004	444.75^{c}±1.67
80 N-ZnO	5.56^{abc}±0.14	54.08^{c}±0.30	35.50^{d}±0.19	3.25^{ab}±0.16	1.40^{ab}±0.06	0.518^{c}±0.013	0.668^{c}±0.005	457.75^{b}±0.90
60 N-ZnO	5.49^{bc}±0.19	54.90^{b}±0.40	34.75^{e}±0.16	3.52^{a}±0.20	1.23^{b}±0.04	0.571^{b}±0.007	0.688^{b}±0.008	462.88^{b}±1.62
40 N-ZnO	5.22^{c}±0.14	55.99^{a}±0.19	33.63^{f}±0.18	3.61^{a}±0.18	$1.39a^{b}$±0.07	0.618^{a}±0.011	0.729^{a}±0.005	493.25^{a}±2.30
20 N-ZnO	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.05	P≤0.01	P≤0.01	P≤0.01
Sig.								

$^{a-f}$ means within each column followed by different letters differ significantly, *I-ZnO = inorganic Znic oxide and ** N-ZnO = nano zinc oxide .

Table (6): Effect of dietary nano zinc oxide (N-ZnO) particles on tibia minerals and retention percentages of nitrogen, calcium, phosphorus and zinc of broiler chicks at 42 days of age (M±SE).

Treatments mg/kg diet	Retention percentage (%)				Tibia minerals		
	Nitrogen	Calcium	Phosphorus	Zinc	Calcium (%)	Phosphorus (%)	Zinc (ppm)
Control	93.84^{b}±0.29	63.28^{c}±2.78	69.36^{b}±1.94	45.44±2.77	31.50^{c}±0.28	12.78^{e}±0.15	162.69^{d}±0.51
100 I-ZnO*	94.91^{ab}±0.28	69.60^{bc}±2.19	72.97^{ab}±2.48	45.59±5.04	32.43^{bc}±0.55	13.25^{d}±0.15	165.02^{c}±0.31
100 N-ZnO**	95.38^{ab}±0.45	73.38^{ab}±2.49	75.78^{ab}±2.30	45.92±2.04	33.08^{ab}±0.31	13.53^{cd}±0.15	168.18^{b}±0.32
80 N-ZnO	94.88^{a}±0.61	74.20^{ab}±2.82	73.95^{ab}±2.88	46.68±3.66	33.22^{ab}±0.41	13.87^{bc}±0.16	170.74^{a}±0.39
60 N-ZnO	96.05^{a}±0.24	79.38^{a}±0.70	81.87^{a}±0.85	48.41±4.71	33.75^{a}±0.52	14.23^{ab}±0.16	172.07^{a}±0.71
40 N-ZnO	95.22^{ab}±0.76	76.50^{ab}±4.60	76.90^{ab}±5.05	47.17±6.11	33.80^{a}±0.34	14.64^{a}±0.20	172.20^{a}±0.91
20 N-ZnO	P≤0.05	P≤0.05	P≤0.05	NS	P≤0.01	P≤0.01	P≤0.01
Sig.							

[a-e] means within each column followed by different letters differ significantly, NS =Non significant ,* I-ZnO = inorganic zinc oxide and ** N-ZnO = nano zinc oxide .



Figure(1): XRD pattern of the synthesized ZnO nanoparticles

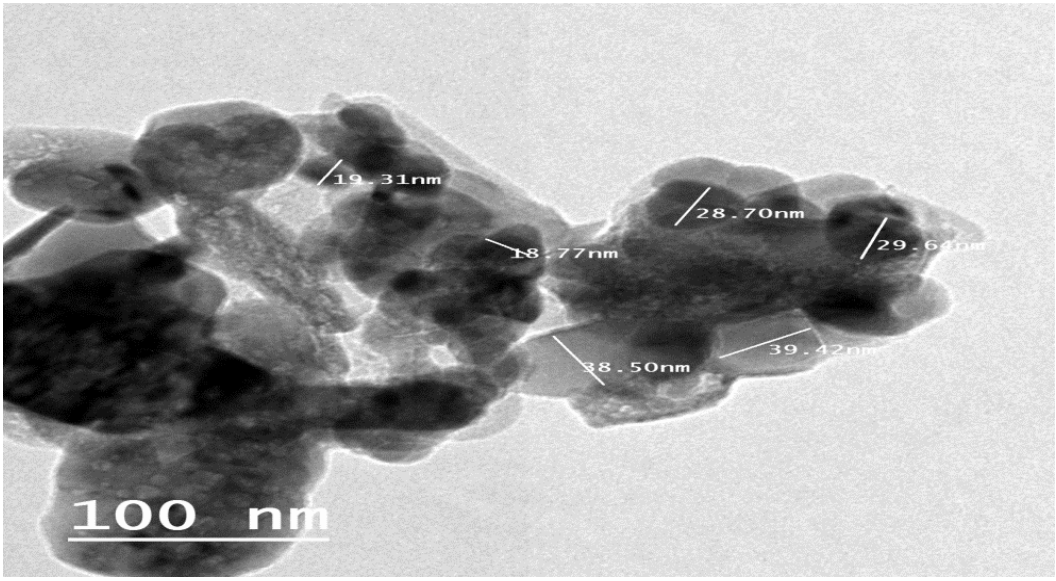


Figure (2): TEM images of the synthesized ZnO nanoparticles

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الملخص العربى

تأثير إضافة أكسيد الزنك في الصورة النانونية علي الكيمياء الحيوية لسيرم الدم والمركبات المحتجزة، والتركيب الكيميائي للحم وعظمة الساق في دجاج التسمين.

هيام سيد عبد الحليم[1] و فاتن عبد العزيز محمود عطية[1] و حماده صلاح صابر محمد[1] و إسماعيل حافظ هرمس[1]و خالد يحي فروح[2]

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تم استخدام ما مجموعه 192 كتكوت من سلالة دجاج التسمين "Cobb" غير المجنس في عمر يوم لتقييم تأثير إستخدام مستويات مختلفة من أكسيد الزنك في الصورة النانونية (N-ZnO) على كل من قدرة إحتجاز النيتروجين والمعادن في الجسم ، مقاييس سيرم الدم ، والتركيب الكيميائي للحم ، ومعادن الساق لدجاج التسمين. تم توزيع الكتاكيت عشوائياً على ستة معاملات بكل منها أربعة مكررات كل مكررة تضم ثمانية كتاكيت. إحتوت عليقة المقارنة على 100 ملجم من أكسيد الزنك الغير عضوي أما باقي المعاملات من الثانية إلي السادسة فاحتوت علي 100 و 80 و 60 و 40 و 20 ملجم /كجم عليقة من أكسيد الزنك في الصورة النانونية. أثرت العلائق المحتوية علي نسب مختلفة من أكسيد الزنك في صورة النانو تأثيرا معنويا علي مقاييس سيرم الدم بإستثناء البروتين الكلي والكوليسترول. تم الحصول على أعلي معدلات لكل من (الألبيومين ، البروتين الدهني عالي الكثافة ، البروتين الدهني منخفض الكثافة ، لاكتات الهيدروجينيز ، فوق أكسيد الديسموتيز ، الكالسيوم ، الفوسفور والزنك) وأقل معدلات لكل من (الدهون الثلاثية ، الكرياتينين ، حمض اليوريك ، الأسبارتيز ترانسفيريز ، ألانين البروتينيز ، الفوسفاتيز القلوي و المالون داي الديهايد) تم الحصول عليها مع مستوي 20 ملجم منN-ZnO /كجم. لوحظ انخفاض كبير في الرطوبة والدهون وزيادة ملحوظة في البروتين الخام والرماد والمعادن (Ca و P و Zn) في لحم الصدر والفخذ باستخدام المعاملات المحتوية علي N-ZnO ؛ تم تسجيل أدنى وأعلى النسب المئوية ، على التوالي ، من المقاييس السابقة مع مستوي20 ملغ N-ZnO. أعطت الطيور التي غذيت علي المعاملات المحتوية علي (40 و 20) و (60 و 40 و 20) و 20 ملجم من N-ZnO أعلى نسب مئوية من الكالسيوم والفوسفور والزنك في الساق على الترتيب. أظهرت الطيور التي غذيت على 40 ملجم من N-ZnO أعلى نسبة إحتجاز النيتروجين والكالسيوم والفوسفور والزنك.

يمكن أن نخلص إلى أن إضافة الزنك في الصورة النانونية (N-ZnO) في علائق التسمين أدي إلي تحسن الحالة الفسيولوجية للطيور وجودة ذبائح اللحم وزيادة ترسيب المعادن بالعظام وزيادة المركبات الغذائية المحتجزة بالجسم.

أعطت المستويات المنخفضة من N-ZnO (20 و 40 ملجم/كجم) نتائج واعدة مع عدم وجود تأثير ضار على الحالة الصحية للطيور.