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Effects of Different Zinc Sources on Performance, Bio Distribution of Minerals and Expression of Genes Related to Metabolism of Broiler Chickens

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Abstract

Different sources of Zinc (Zn) were compared to assess their possible effects on performance, nutrients retention, mineral distribution and some serum parameters of broiler chickens. A total of 200 one-day old Ross 308 chicks were divided into in equal four dietary treatments groups with five replicates each of ten chicks. The experimental groups were given the basal diet (inorganic ZnO), basal diet supplemented with organic Zn (Zn methionine), nano-ZnO and Zn-mix (organic Zn and nano-ZnO) at a concentration of 50 mg/kg of diet. After 42 days of feeding trial, the group supplemented with nano-ZnO exhibited the best final body weight and feed conversion ratio (2380 g/bird and 1.69, respectively). Nano-ZnO and Zn-mix supplementation significantly increased crude fat retention (86.70 and 86.75%, respectively). All sources of supplemented Zn other than inorganic ZnO significantly increased ($P<0.05$) Zn retention especially in the group supplemented with nano-ZnO (41.8%). Organic Zn and/or nano-ZnO sources supplemented to broiler diets significantly increased ($P<0.05$) iron and copper contents in the hepatic tissue and Zn content in the tibia. The mean of serum total cholesterol, triglycerides and very low density lipoprotien were significantly reduced ($P<0.05$) by dietary supplementation of organic Zn and/or nano-ZnO. The activity of malondialdehyde was significantly decreased ($P<0.05$), while Cu/Zn superoxide dismutase activity was significantly increased ($P<0.05$) by addition nano-ZnO or Zn-mix. Dietary Zn-mix and nano-ZnO positively affected mRNA expression of insulin like growth factor-1 and growth hormone genes in broilers when compared to the inorganic ZnO source. The present findings prospected that replacing traditional inorganic ZnO source with nano-ZnO or combining nano-ZnO and Zn methionine at applied concentration, promoted the growth of broilers, enhanced Zn up take and antioxidant status without negative effect on selected minerals distribution in tissues.

Keywords: Zinc Sources, Performance, Nutrients Retention, Minerals Bio Distribution, Gene Expression, Broilers.

Introduction

Appropriate trace mineral supplementation is fundamental for several physiological capacities, growth and health of poultry. Zinc (Zn) is an essential trace element required for development and growth of broiler chickens. This essential element has a key part in the structure and maintenance of the skeleton and acts as cofactor in many metabolic processes that necessary for hormone secretion i.e. growth and insulin hormones [1], DNA synthesis, gene expression and cellular division [2], vision photochemistry [3], enhanced the immune status [4] via energy creation, protein synthesis and cell membranes protection from endotoxins producing bacteria [5] gene transcription and RNA synthesis. Also, zinc is vital free radical's scavenger of the in-antioxidant defense system [6]. Traditionally Zn is supplemented in inorganic forms in poultry diets such as sulfates, oxides and carbonates as they are of low cost. However, in monogastric animals the bioavailability of inoragnic zinc is much low [7]. In the gastrointestinal tract, the inorganic Zn combined with phytic acid found in most of the broilers grains based diets thus impair zinc and calcium absorption [8], consequently affected the tissue uptake of Zn. Nevertheless,

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the degree of mineral absorption based on the synergism (Zn and Mn) or antagonism (Zn and Cu) of different minerals [9]. In contrast, organic minerals which combined with amino-acids did not interact with phytic acid as they are lacking the free divalent cations needed for chelation in the intestine [10] and thus, they are metabolized in diverse way to facilitate their absorption [11]. Many authors documented that the organic form of dietary zinc for poultry was more bioavailable than its inorganic form, which improved the immunity [12]. On the other hand, with continued genetic modification of commercial broiler strains, the recommended NRC [13] level of Zn 40 mg/kg of diet, is no longer provided sufficient growth, health and reproduction for modern broiler strains. Additionally, increased supplementation of Zn may influence other trace elements balance and reduced other nutrient stability and long-term exposure that can cause Zn deposit inside the animal body [6]. The inorganic trace minerals may be replaced by organic sources to reduce the over supplementation and decrease their excretion [14]. The supplementation of organic Zn in broiler diets is restricted until now due to its relatively high cost. Thus, to get better Zn bio-availability, other approaches should be considered.

The application of nanotechnology gives rise to nano-sized particles from Zn called nano Zn which can replace other zinc forms. As their large surface area, their absorption, utilization and chemical constancy have increased in animal body [15]. The nanoparticles from zinc oxide are most widely used due to its unique physical and chemical properties, easy preparation and environmental-friendless [16]. Supplementation of nano-Zn to broilers exhibited better results on bioavailability when compared with other Zn sources and also be less toxic for broilers [17]. In addition, Zinc also has a prominent role on many physiological processes that influence signal transduction, gene transcription and RNA synthesis [18]. Yu et al [19] cleared that zinc availability can affect IGF-I gene transcription. Accordingly, this study was designed to evaluate the effects of different dietary Zn sources or combining nano-ZnO and organic Zn on growth performance, nutrient retention, mineral biodistribution (Zn, Fe, Cu, Mn) in different tissues (liver, kidney, muscle and bone) and metabolism of broiler chickens.

Material and Methods

Experimental birds and management

All the experimental procedures were approved by the local experimental animal care committee and in accordance with the institutional ethics committee.

A total of 200 one-day old unsexed broiler chicks (Ross 308) obtained from a local hatchery were used. The chicks were weighed on arrival and randomly distributed in equal four groups with five replicates each of ten chicks. Birds were reared under hygienic condition at a density of 10 birds/m^2. The temperature was maintained at 33±1°C then decreased gradually 2°C each week until reach 21±1°C at the 6th week. The relative humidity during the experimental rearing ranged from 67 to 77%. All birds were vaccinated with Hitchner B_1 (1st day/eye drop), Coccivac-B (2nd day/eye drops) against *Eimeria acervulina, E.mivati, E. maxima, E. tenella* live oocysts, Avian influenza (H5N1, 7 days/S/C injection), Gumboro 78 (11 days/eye drop), Lasota (17 days/drinking water) and Gumboro 78 (22 days /drinking water).

Experimental diets and design

The experimental diet was formulated to Ross 308 broiler nutrition specification (Table 1). The birds were reared for 42 days and the feeding period was divided to starter and finisher period. The diet was not supplemented with phytase. The proximate analysis of the feed ingredients was carried out according to the standard procedures of the AOAC [20]. Four dietary treatment groups were designed as the following: control (inorganic zinc oxide, ZnO, sigma Aldrich), organic-Zn (Zn methionine, Zinpro products), nano-ZnO and Zn-mix groups. The level of Zinc was 50 mg/kg diet in control with inorganic Zn, organic-Zn and nano-ZnO groups, while the Zn-mix group was contained 25 mg/kg diet from organic Zn and 25 mg/kg from nano-ZnO.

Zinc oxide nanoparticles (ZnO NPs)

The ZnO NPs were purchased from Faculty of Science of Beni Suef University which was

a white powder with a measured ZnO NPs content of purity ≥ 99.99% and size of nanoparticles was 27 nm. The phase characterization of nanoparticles was performed by means of X-ray diffraction (XRD) (Figure 1) using EMPYREAN diffractometer. The morphologies and particle sizes of the Zinc Oxide (ZnO) samples were characterized by JEM- 200CX transmission electron microscopy (TEM) working at 30 kV and Scanning electron microscopy (SEM) images were obtained with a ZIESS EM 902A scanning electron microscope, the characterization of zinc oxide nanoparticles includes**:** Ref. Code 01-080-7099, Score: 100, Displacement [°2Th.]: 0.047, Scale Factor: 1.028

Broiler growth performance and Digestibility trails for estimation of nutrient retention

Body weight, body weight gain (BWG), feed intake (FI), feed conversion ratio was calculated through the rearing period. At the end of rearing period the allover performance parameters were recorded. Four digestibility trials were conducted. At the end of the growth trial ten birds from each group were randomly selected to carry out the digestibility trail and kept in separate pens and plastic dishes were used for collecting total excreta. Chromic oxide (Cr_2O_3) was incorporated into the diets of birds in different groups at a rate of 0.3% as analytical marker [21] for 12 days (seven-days of adaptation period plus five-days for collection of excreta). The collected excreta were dried in hot air oven at 55°C, ground and stored at 4°C until analysis. Digestibility of dry matter, crude protein and crude fat were determined according to AOAC procedures [20].

Zinc analysis in feed and feces

One gram of excreta was heated for 5 h in a furnace at 550°C for ashing. Ten mL 3 N HCl was added to the ash sample and heated until the solution became clear. After cooling, filtered and diluted to 50 mL with 0.1 N HCl. For zinc analysis, lanthanum 185.4 L 50000 mg/kg was added to 6 mL of the sample solution. Then, analysis was performed using spectrometer with the wavelength set at 400 nm [20].

Samples collection and serum biochemical parameters

At the end of the feeding trial, two birds were randomly selected from each replicate and slaughtered and then the blood samples were collected without anticoagulant. The selected tissues samples including, liver, kidney, thigh muscle and bone (tibia) were stored at -20°C. The separated serum was used for determining the glucose (GAGO-20, kits from Sigma-Aldrich), total cholesterol (MAK043, kits from Sigma-Aldrich), triglyceride (TAG) (TR0100, kits from Sigma-Aldrich), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) (MAK045, kits from Sigma-Aldrich), Malonaldehyde (MDA) (MAK085, kits from Sigma-Aldrich), aspartate aminotransferase (AST) (MAK055, kits from Sigma-Aldrich) and Alanine Amino transferase (ALT) (MAK052, kits from Sigma-Aldrich) concentration calorimetrically. The activity of Cu/Zn superoxide dismutase (Cu/Zn-SOD) was determined by OxiSelect™ ABIN2344995 kits.

Determination of mineral biodistribution

The mineral concentrations of Zn, Fe and Cu in tissues, including liver, kidney, thigh muscle and bones (tibia) were determined [22]. Briefly, tissues (2 g) were digested in digested tube using an acid mixture (15 mL of nitric acid (HNO_3), 10 mL of per-chloric acid ($HClO_4$) and 5 mL of hydrochloric acid (HCl) (HNO_3: $HClO_4$: HCl = 3:2:1). The digest (25 mL) was with demineralized water. Blanks and standard solutions were prepared. After that, mineral concentrations were determined via an atomic absorption spectrophotometer.

Table 1: Nutrients composition of starter and grower-finisher basal diet

Ingredients (%)	Starter diet (day 1-21)	Grower-finisher diet (day 22-42)
Corn, ground	56.6	59.6
Soybean meal (48%)	32.2	26.5
Corn gluten, 60%	3.2	5.9
Soybean oil	4	4.3
Calcium carbonate	1.3	1.3
Calcium dibasic phosphate	1.50	1.25
Common salt	0.3	0.3
L-Lysine (78%)	0.2	0.2
DL-Methionine (98%)	0.2	0.15
Vitamin premix*	0.25	0.25
Mineral premix**	0.25	0.25
Total	**100**	**100**
Calculated nutrients**		
ME (Kcal/Kg)	3100	3200
Protein (%)	22.68	20.08
EE (%)	6.42	6.82
Calcium (%)	1.06	1.05
Avail. Phos (%)	0.47	0.44
Zn (mg/kg)	27.31	25.46
Lysine (%)	1.28	1.14
Methionine (%)	0.55	0.51

*Vitamin premix supplied the following per kilogram of diet: vit A, 12,000 IU; vit. D3, 2,300 IU; vit E, 50 IU; vit K, 0.5 mg; vitamin B12, 5 mg; thiamine, 1.5 mg; riboflavin, 5mg; folic acid, 0.8 mg; biotin, 0.15 mg; niacin, 60 mg; pyridoxine, 5 mg; pantothenic acid, 0.02 mg.

**Mineral premix supplied the following per kilogram of diet: iron, 80 mg; manganese, 21.8 mg; selenium, 0.1 mg; iodine, 0.35 mg.

Real-time quantitative RT-PCR

At the end of the experiment, immediately after slaughter liver was dissected and frozen in trizole for assay of growth hormone, insulin- like growth factor-1 genes. Samples were stored at -20°C in a plastic tube for RNA isolation. Total RNA was extracted from tissue samples using Qiagen RNA extraction kits, (Cat, No. 74104). Total RNA purity was determined using NanoDrop_ND-1000 Spectrophotometer. The total RNA was reverse transcribed into cDNA via QIAGEN Long Range 2 Step RT-PCR Kit. One µL of total cDNA was mixed with 12.5 µL of 2x SYBR_Green PCR mix with ROX from Bio-Rad, 5.5 µL of RNAse-free water and 0.5 µL of each forward and reverse primers for the measured genes. The up-and downstream primer sequences of growth hormone, insulin-like growth factor-1 genes were: GH primers F: 5'- AAC ACA GAT ACC CAA CAG CC-3', R: 5'- AGA AGT CAG TGT TTG TCA GGG-3'; IGF-1 primers (F: 5'- CAC CTA AAT CTG CAC GCT-3' and R: CTT GTG GAT GGC ATG ATC T-3'); β-actin primers (F: 5'- ACC CCA AAG CCA ACA GA-3' and R: 5'- CCA GAG TCC ATC ACA ATA CC-3'). The β-actin gene was used as a regulator for normalization [23].

Statistical analyses

Analysis of variance revealed that the difference between replicates were not significant. The data were analyzed using the General Linear Model procedure, (SPSS, 18 Inc., USA). Difference among treatment means were compared using Duncan's multiple range test. Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

The statstical model was as the following:

$$Y_{ijk} = \mu + T_i + R_j + e_{ijk}$$

Where: µ is the overall mean for each trait, T_i is the fixed effect of diet supplementation (i=1, 2,.,4), R_j is the fixed effect of replicates (j=1, 2,…,5) and e_{ijk} is the random residual effect.

Table 2: Effects of dietary different forms of Zn on performance (1-42 days) and nutrient retention of broiler chickens on mineral concentration of selected tissues at 42 days (means ± standard error)

	Dietary groups			
Items	**Control[1]**	**Organic-Zn[2]**	**Nano-ZnO[3]**	**Zn-mix[4]**
Performance parameters				
Body weight (g)	2018±19.20^{d}	2106± 9.54^{c}	2380±12.66^{a}	2302± 4.16^{b}
Body weight gain, g/bird	1972 ± 32.35^{d}	2061±16.86^{c}	2335 ±22.91^{a}	2256 ± 7.57^{b}
Feed intake (g)	4043±86.02	3938± 58.59	3954±83.64	3948±12.90
Feed conversion ratio	2.05± 0.02^{a}	1.91± 0.02^{b}	1.69±0.03^{d}	1.75± 0.01^{c}
Protein efficiency ratio	2.33± 0.02^{c}	2.51± 0.02^{b}	2.83± 0.05^{a}	2.73± 0.02^{ab}
Nutrient retention (%)				
Dry matter	88.10±0.05	89.45±0.02	89.37±0.03	89.8±0.05
Crude protein	86.92±0.02	87.20±0.01	87.65±0.01	87.32±0.03
Crude fat	85.39±0.06^{b}	85.90±0.02^{b}	86.70±0.01^{a}	86.75±0.03^{a}
Zinc	30.25±0.2 ^{c}	38.47±0.08^{b}	41.8±0.09^{a}	40.59±0.1^{a}
Tissues bio-bistribution				
Liver				
Zn (mg/kg)	70.68±0.06	71.64±0.04	71.95±0.02	68.48±0.02
Fe (mg/10g)	12.25±0.09^{b}	13.9±0.04^{a}	15.64±0.05^{a}	14.66±0.04^{a}
Cu (mg/kg)	13.21±0.08^{b}	14.9±0.05^{a}	15.3±0.05^{a}	15.54±0.04^{a}
kidney				
Zinc (mg/kg)	15.24±0.04	16.31±0.07	15.98±0.07	15.9±0.03
Fe (mg/kg)	257.36±0.09	255.87±0.09	259.9±0.06	261.58±0.07
Cu (mg/kg)	16.64±0.03	16.2±0.03	16.80±0.07	15.97±0.05
Thigh muscle				
Zn (mg/kg)	25.31±0.04	25.00±0.09	25.6±0.1	25.2±0.03
Fe (mg/kg)	160.25±0.08	160.2±0.06	163.2±0.06	162.3±0.04
Cu (mg/kg)	5.5±0.04	5.92±0.05	6.1±0.03	6.1±0.02
Tibia bone				
Zn (mg/kg)	250.32±0.04^{b}	265.3±0.04^{a}	265.36±0.06^{a}	260.32±0.04^{a}
Fe (mg/kg)	12.50±0.06	13.34±0.01	13.61±0.03	14.10±0.02
Cu (mg/kg)	11.10±0.09	11.50±0.1	12.54±0.04	12.35±0.08

[1]Control: group supplemented with inorganic zinc oxide; [2]Organic-Zn: group supplemented with Zn methionine; [3]Nano-ZnO: group supplemented with nano Zn-oxide and [4]Zn-mix: group supplemented with both Zn methionine and nano Zn-oxide.

[a-b-c-d]Means in a row with different superscripts were significantly different (P<0.05).

Results and Discussion

Performance parameters

Zinc is practically added to all formulated broiler diets to support their development and growth. As the inorganic source of Zn has low bioavailability especially when incorporated with grain based diet, it is an urgent need to increase their dietary level than recommended by the NRC. However, this can lead to higher Zn excretion and environmental pollution. From this point, our dietary strategy has been directed for applying other sources of supplemental Zn with higher bioavailability in broiler diets as organic Zn and the most recently available nano-Zn. The effects of different dietary sources of Zn on performance parameters of broiler chickens are shown in Table (2). The data of broiler performance (1-42 days) were significantly affected by different supplemental Zn sources except for feed consumption. The broilers group received nano-ZnO had significantly (P<0.05) higher body weight gain, better feed conversion and protein utilization followed by the group supplemented with Zn-mix (oraganic and nano-ZnO) and finally organic Zn (Zn methionine) supplemented group when compared with the traditionally inorganic ZnO

supplemented diet. Replacing inorganic ZnO with nano-ZnO source, Zn-mix and Zn methionine caused an increase in weight gain at the end of the rearing period (approximately 15.5, 12.5 and 4.3%, respectively). These Results showed that using of supplemental organic and/or nano-Zn promoted growth performance of broilers. The nanoparticles can efficiently satisfy the mineral requirement by animals, enhanced their production performance and feed efficiency [24]. Its application in animal feeding with low level, can be substituted to antibiotic growth promoters and diminish the environmental pollution [25]. In accordance with these findings Zhao *et al.*, [6] demonstrated that feeding of broilers on nano-ZnO (20 or 60 mg/kg) were improved the feed conversion and growth rate than feeding organic Zn. In addition, dietary supplementation of nano-Zn in combination with an organic Zn source (80 mg/kg) enhanced the growth performance of broiler chickens than when dietary nano-Zn supplemented with inorganic Zn source with the same level [26]. Also, the growth performance of broilers was improved due to higher bioavailability of supplemented organic Zn sources [27] and the positive properties of Zn methionine on nutrients digestion and absorption. Also, if Zn methionine did not modify during absorption and transportation, its supply for tissues will increase and thus improves animal productivity [28]. These positive effects were more prominent with nano -Zn than organic Zn due to the higher bioavailability of nanoparticles.

The development of indigestible complexes between minerals and other dietary ingredients may be prevented by addition of organic trace minerals as well as it can also decrease antagonism between minerals [29], thus will affect the broilers growth rate. The current results of feed consumption agreed with Puchala *et al.* [30] who reported that adding of graded levels of organic Zn (15, 30, 45 and 60 ppm/ kg diet) to broiler chickens did not significantly affect their feed intake when compared with the inorganic Zn.

Table 3: Effects of dietary different forms of zinc on serum parameters of broiler chickens (means ± standard error)

	Dietary groups			
Parameters	**Control[1]**	**Organic-Zn[2]**	**Nano-ZnO[3]**	**Zn-mix[4]**
Glucose (mg/dL)	242.30±0.07	240.24±0.05	245.26±0.02	247.50±0.06
Total cholesterol (mg/dL)	165.25±0.08^{a}	158.36±0.2^{b}	155.34±0.09^{b}	147.32±0.1^{c}
TAG[5] (mg/dL)	276.23±0.05^{a}	198.32±0.08^{b}	180.5±0.08^{c}	188.25±0.05^{bc}
HDL-C[6](mg/dL)	46.94±0.01^{b}	47.55±0.03^{a}	47.65±0.04^{a}	47.21±0.04^{a}
VLDL-C[7] (mg/dL)	55.25±0.08^{c}	39.66±0.06^{b}	36.1±0.09^{a}	37.55±0.09^{ab}
AST[8] (mg/dL)	176.99±0.02	175.61±0.07	179.65±0.01	178.32±0.06
ALT[9] (mg/dL)	53.32±0.04	53.20±0.08	56.3±0.06	54.32±0.06
MDA[10](nmoL/mL)	2.63±0.08^{a}	2.46b±0.04^{b}	2.30±0. 1^{b}	2.42±0.3^{c}
CuZn-SOD[11](U/mgp)	238.22±0.09^{c}	247.25±0.1^{b}	255.36±0.09^{a}	247.40±0.2^{ab}

[1]Control: group supplemented with inorganic zinc oxide; [2]Organic-Zn: group supplemented with Zn methionine; [3]Nano-ZnO: group supplemented with nano Zn-oxide; [4]Zn-mix: group supplemented with both Zn methionine and nano Zn-oxide; [5]TAG: Triglyceride; [6]HDL-C: High-density lipoprotein; [7]VLDL-C: Very low-density lipoprotein; [8]AST: Aspartate amino transferase; [9]ALT: Alanine amino transferase; [10]MDA: Malondialdehyde; [11]CuZn-SOD: Cu/Zn superoxide dismutase. $^{a\text{-}b\text{-}c}$ Means in a row with different superscripts were significantly different (P<0.05).

Nutrient retention

Dietary supplementation of different zinc sources on nutrient retention of broilers are shown in table 2. Dry matter, crude protein retention were not affected by different zinc supplementation (P > 0.05), while the retention of crude fat and zinc were affected. Fat retention was higher in nano-Zn and mix- Zn groups than that in organic Zn and the control group (P < 0.05). The addition of organic Zn and/or nano-Zn to broiler diets significantly increased (P < 0.05) Zn retention in the body when compared with the control group. The

same results were obtained by Tsai *et al.* [1] who stated that feeding of laying hens on dietary inorganic, organic and nano-Zn had no significant affect in nutrient retention, although Zn retention was significantly increased in groups supplemented with organic and nano-Zn when compred with inorganic Zn group. As the inorganic trace minerals dissociate in the upper gastrointestinal tract due to the low pH, thus these separated minerals can antagonize other minerals or combined with other dietary components in the digesta and hinder their absorption in the small intestine [31]. In addition, Zn-methionine supplementation in broiler chickens had higher bioavailability than inorganic Zn which absorbed through amino acid transport systems, resulting in higher bioavailability [32]. High concentrations of Zn in poultry excreta due to their lower bioavailability cause environmental pollution and soil phytotoxicity when these excreta used as fertilizers and the high bioavailability of organic and nano-Zn will decrease Zn excretion [33]. Similarly, several studies reported that supplementation of organic minerals for broiler diets can improve the mineral uptake, enhance gain and reduce the excretion of minerals [34,35]. Nanoparticles revealed unique physical characteristics of transport and uptake that display higher absorption efficiencies when compared with the inorganic and organic Zn sources [36]. The current results concluded that the supplementation of required level of organic and/or nano-Zn minerals lead to higher mineral retention with higher bioavailability. Additionally, the results related to fat retention are in harmony with Tronina *et al.* [37] who reported that dietary Zn-Gly (organic source) could increase body fat content of broilers. The intramuscular fat of the broiler breast meat fed diets supplemented with Zn was higher than the control [38] which may be attributed to that the dietary Zn can influence fat metabolism in liver and enhance the lipid synthesis for broilers by controlling the activities and gene expressions of lipogenic enzymes [39].

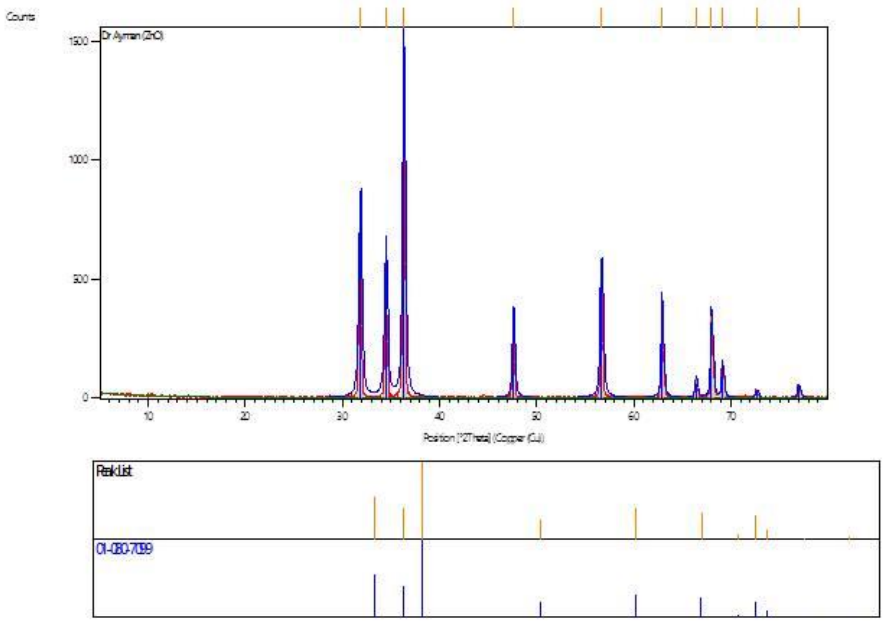

Figure 1. X-ray Diffraction of zinc oxide nano particles

Mineral contents of selected tissues

The influence of different Zn sources in tissues mineral contents is shown in table 2. There was no significant difference in Zn contents of the selected tissues except for tibia, where the highest Zn concentration was estimated in groups supplemented with organic Zn and /or nano-ZnO compared to the control group. Supplementation of organic Zn and/or nano-ZnO had no significant effect on Fe and Cu contents in the kidney, thigh muscle and tibia, while hepatic Fe and Cu contents were significantly increased ($P < 0.05$) in the groups fed diets supplemented with organic Zn and/or nano-ZnO. Thus, introducing the organic or nano-Zn trace elements into animal nutrition could alter mineral deposition in tissues due to their proper bioavailability when compared with the inorganic sources. The ability of nano minerals to pass through the small intestine and distribute in the body are much greater than inorganic and organic minerals [40]. Tissue mineral biodistribution can be used as an index of mineral storages in the body [41]. In the same line, Sunder *et al.* [42] reported that the addition of organic Zn to broilers (80 ppm/kg diet) lead to higher minerals precipitation (Zn and Mn) in the bone. Also, Mwangi *et al.* [43] specified that increasing dietary Zn and Mn supplementation were desired for a higher mineral storage in the liver. Ivanišinová, *et al.* [44] reported that higher bioavailability of organic Zn associated with its higher concentration in the hepatic tissues. It was described that zinc influenced the absorption and biodistribution of Cu, Fe and Mn trace minerals [45]. It appears from our results that the organic and/or nano-Zn content in broilers diets did not affect the mineral distribution in tissues except for the liver. Ao *et al.* [46] reported that there is an antagonism between Zn and Cu when the inorganic sources used, while this antagonism was not existed when organic sources of minerals were incorporated in broiler diet. Nevertheless, feeding of mice on dietary nano-Zn oxide by the level of (50, 500 and 5000 mg/kg) had no significant effect on the Cu, Fe, and Mn content in the kidney, thigh and testis and significantly affect on the hepatic Fe content and pancreas Mn level [47]. Tibia zinc concentration in nanoparticle zinc samples from broiler chickens was significantly higher relative to the control and zinc-sulphate groups [48].

Serum parameters

The serum biochemical parameters in relation to different dietary sources of Zn are shown in Table (3). There was no significant difference in glucose levels among different treated groups. The results indicated that the serum total cholesterol, triglyceride and very low-density lipoprotein were significantly decreased ($P < 0.05$) in groups supplemented with organic Zn and/or nano Zn, while the lowest values were observed in broilers group fed on nano Zn. The high density lipoprotein was not affected by organic Zn and/or nano Zn supplementation. Serum malondialdehyde (MDA) level was significantly decreased ($P < 0.05$) while the Cu/Zn superoxide dismutase (Cu/Zn-SOD) level was significantly increased ($P < 0.05$) with dietary organic Zn and/or nano ZnO, which indicated that the addition of organic Zn and/or nano Zn enhanced the antioxidant activity of broilers. The hepatic activities of aspartate amino transferase and alanine amino transferase were not significantly affected by the different dietary sources of Zn. Zinc has an important role in scavenging of free radicals and reactive oxygen species and considered as an essential element in the formation and function of Cu-Zn superoxide dismutase [6]. One of the most important antioxidant functions of Zn is related to elevation of Cu/Zn SOD activity in chicken liver [48]. The results of our study indicate that organic zinc and/or nano Zn sources had a similar effect on the activity of Cu/Zn SOD in tissues. In the same line, Fathi *et al.* [49] revealed that serum concentrations of MDA in broiler chickens was significantly ($P<0.05$) decreased at 20 mg/kg nano-ZnO. ZONPs associated with reduction of serum low density lipoproteins, triglyceride and cholesterol, besides increased high density lipoproteins compared to the control treatment [50]. This can be attributed to reduction of the absorption of dietary lipids and decline in caloric intake [51]. Also, Berg and Shi [52] reported that there was a contrary connection between zinc oxide nanoparticles and serum MDA concentration. Supplementation of nano-Zn to broiler diets (0.06 mg/kg)

accompanied by decreasing the blood cholesterol level and improving the bird's immunity when compared with organic and inorganic Zn sources [17]. The alteration of blood cholesterol levels may be linked to the zinc's role in enzymes systems (metalloenzymes) which plays a vital role in fat metaboilsm [53]. Ahmadi *et al.* [50] reported that the dietary nano-ZnO had no significant role in aspartate amino transferase and alanine amino transferase activities in serum of broilers.

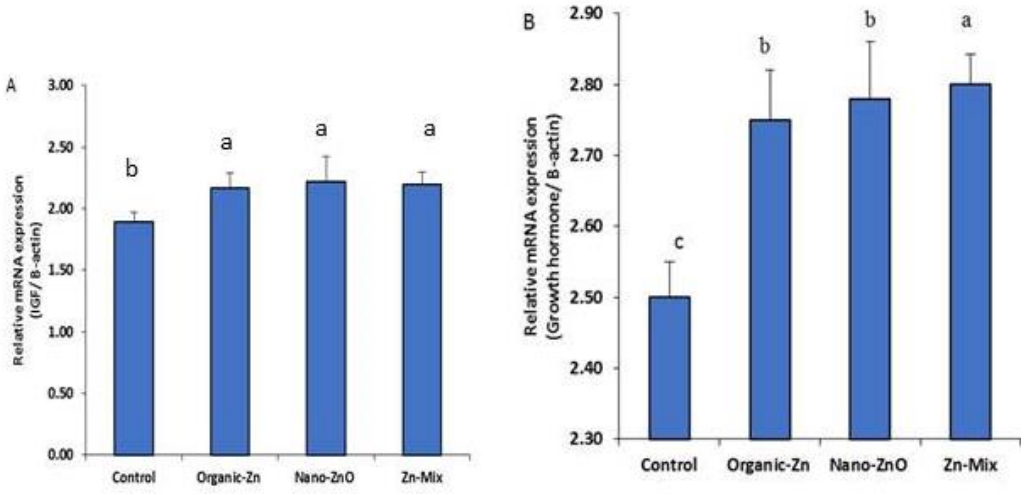

Figure 2: Effect of different dietary forms of zinc on the relative mRNA expression of A) insulin like growth factor-1 and B) growth hormone genes of broilers chicken. Control = group supplemented with inorganic zinc oxide, Organic-Zn = group supplemented with Zn methionine, Nano-ZnO= group supplemented with nano Zn-oxide and Zn-mix = group supplemented with both Zn methionine and nano Zn-oxide.

Insulin like growth factor-1 and growth hormone genes expression

Data regarding the gene expression are presented in Figure (2). The present results showed that supplementation of broiler diets with organic Zn and/or nano ZnO significantly increased ($p < 0.05$) mRNA expression of insulin like growth factor gene (IGF-I). Moreover, the mRNA expression of IGF-I gene tended to be increased in nano-ZnO supplemented group than in Zn-mix and organic groups. Compared to inorganic ZnO, addition of nano-ZnO and Zn-mix significantly increased ($p < 0.05$) mRNA expression of growth hormone followed by the group supplemented with Zn methionine. The high bioavailability of organic Zn and nano-ZnO indicates that more Zn was taken through the body and not only deposited in bone compared with inorganic ZnO, thus affected cellular function responsible for hormones and growth factor secretions. Similarly, Zn augmented the growth factor synthesis, as IGF-1 and influenced the action of calcium-regulating hormones [54]. Also, Zn plays an important role in the formation of insulin through its enzyme systems [7]. Compared with inorganic Zn supplemented group, the serum level of IGF-1 was increased in the organic-Zn group [55]. Other results in mice, showed that dietary Zn- methionine increased the body weight gain and up-regulated mRNA expression of IGF-I more than dietary inorganic Zn [56].

Conclusion

The present study suggested that supplementation of nano-ZnO or even combing organic Zn and nano-ZnO with a dose of 50 mg/kg diet have positive effects on broiler performance, Zn retention and enhanced their antioxidant activity. In addition, the trace mineral contents of selected tissues in relation to dietary treatment not affected except for Fe and Cu, which increased in hepatic tissues. Also, supplemental nano-ZnO or combination organic Zn and nano-ZnO improved the broiler's metabolism by increased the activities of insulin like growth factor and growth hormone genes.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

تأثير إضافة مصادر الزنك المختلفة على الأداء، وتوزيع المعادن داخل الجسم والجينات المتعلقة بالتمثيل الغذائي في بداري التسمين

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الهدف من هذه التجربة هو دراسة تأثير إضافة المصادر المختلفة من الزنك علي الأداء و إحتجاز المواد الغذائية وتوزيع المعادن داخل الجسم والتنظيم الجيني المسئول عن الأيض الغذائي في بداري التسمين. تم تقسيم 200 طائر عشوائيا إلي أربعة مجموعات متساوية بكل مجموعة خمس تكرارات وبكل تكرار 10 طيور وتم تغذية المجاميع كالتالي: عليقة ضابطة تحتوي علي الزنك الغيرعضوي,العليقة الثانية تحتوي علي الزنك العضوي والعليقة الثالثة تحتوي علي جزئيات النانو من الزنك ,والعليقة الرابعة تحتوي علي خليط من الزنك العضوي وجزئيات النانو من الزنك بتركيز 50 مجم/كجم عليقة ويمكن إيجاز أهم النتائج في نهاية مرحلة التربية (42 يوم) كما يلي: تم تسجيل أعلي نمو في الطيور التي تم تغذيتها علي عليقة تحتوي علي جزئيات النانو من الزنك يليها المجموعة التي غذيت علي الزنك العضوي وجزئيات النانو من الزنك ثم المجموعة التي غذيت علي الزنك العضوي وذلك بالمقارنة بالمجموعة التي تحتوي علي الزنك الغيرعضوي. وأبرزت النتائج أيضا أن التغذية علي عليقة تحتوي علي جزئيات النانو من الزنك أو التي تحتوي علي الزنك العضوي وجزئيات النانو من الزنك قد أدت إلي إحتجاز الدهن بالإضافة إلي أن إستخدام الزنك العضوي أو جزئيات النانو من الزنك أدي إلي زيادة إحتجاز الزنك في جسم الطائر وذلك بالمقارنة بالزنك الغيرعضوي. وقد أدي إضافة الزنك العضوي و/أو جزئيات النانو من الزنك إلي زيادة محتوي الكبد من الحديد والنحاس وزيادة كمية الزنك في عظمة الساق. وقد أظهرت النتائج أن متوسطات كلا من الكوليسترول والدهون الثلاثية والبروتين الدهني ذات الكثافة المنخفضة جدا قد أنخفضت بإضافة الزنك العضوي و/أو جزئيات النانو من الزنك إلي علائق بداري التسمين. وإنخفض بشكل ملحوظ نشاط المالوندیالدهيد وزاد نشاط سوبر أكسيد الديسموتازمن الزنك والنحاس وذلك بإضافة جزئيات النانو من الزنك أو الزنك العضوي وجزئيات النانو من الزنك. وتم تحسين في الأداء الجيني للجينات المرتبطة بالتمثيل الغذائي في المجموعات التي غذيت علي عليقة تحتوي علي جزئيات النانو من الزنك أو الزنك العضوي وجزئيات النانو من الزنك. وقد أوضحت الدراسة الحالية إلى أن الإستعاضة عن مصدر أكسيد الزنك غير العضوي التقليدي بمكمل جزئيات النانو من أكسيد الزنك أو الجمع بين جزئيات النانو من أكسيد الزنك والزنك العضوي عند التركيز التطبيقي، قد أدي إلي تعزيز نمو الدجاج اللاحم وزيادة إستفادة الجسم من الزنك وتحسين إستجابة الجسم ضد مضادات الأكسدة دون التأثير الجانبي السلبي على توزيع المعادن المختارة في الأنسجة في بداري التسمين.