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Effect of Nano Zinc Oxide and Traditional Zinc (Oxide and Sulphate) Sources on Performance, Bone Characteristics and Physiological Parameters of Broiler Chicks

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ABSTRACT

Zinc is one of the most importance trace minerals in poultry nutrition. Therefore, the objective of this study is to investigate the influence of nano zinc oxide (green and chemical) and traditional zinc (Oxide and Sulphate) on broiler performance, bone characteristics and some biochemical parameters from 1-35 day of age. A total of 240 a-day-old male chicks (Arbor Acres) were assigned at random into 4 treatments. Treatments 1 and 2 contained zinc in traditional form as zinc oxide (ZnO) and zinc sulphate (Zn SO_4) and treatments 3 and 4 contain zinc in nano form; green synthesized zinc oxide nanoparticles (GZnO NPs) and chemically synthesized zinc oxide nanoparticles (CZnO NPs); respectively. The results showed that the birds fed diet treated with nano-Zn sources (GZnO NPs and CZnO NPs) showed the best results ($P<0.01$) in weight gain and feed conversion ratio. There were no statistical differences between the two sources of zinc in the traditional form as well as between the two sources of zinc in the nano form. In addition, chicks fed nano zinc sources gave the best values in bone mineral density and bone mineral concentration compared to those fed traditional sources of zinc. These results reflect the values obtained for tibia length, width, breaking strength and ash since the dietary GZnO NPs and CZnO NPs were recorded for the highest values compared with the other traditional form. Non-significant differences were detected in the metabolic hormones (T3, T4) levels and their ratio T3/T4 among different dietary treatments. The results of the liver function (ALT, AST and albumin), the kidney function (creatinine) and alkaline phosphatase were within the normal range in all treatments. From the current results it can be suggested that using zinc in nano form instead of traditional form enhances performance and bone parameters of broiler chicks.

Key words: Nanomaterials, Broilers, Performance, Bone characteristics, Blood parameters, Zinc sources.

INTRODUCTION

Zinc is an essential trace mineral in poultry diets for ideal performance and production. Zinc is vital for the proliferation of cells and their differentiation and serves as a metal cofactor for many enzymes (Bonaventura et al. 2015; Sharif et al. 2021). Zinc catalyzes enzyme reactions and controls many cellular processes including proteins and regular growth, hormone production, membrane stability and improves bones and feathers (Jahanian and Rasouli 2015; Kwiecien et al. 2017; Gammoh and Rink 2017). Also, zinc improves the effectiveness of antioxidant and thus participates in the production of glutathione peroxidase (Saleh et al. 2018). Shortage of zinc in broiler diets might impair the development of performance and health status. Therefore, Aviagen (2019) suggested that more amount of zinc is needed for chicks' requirements to get higher performance. Higher Zn supplementation may be increase feed cost and nutrients excretion thus might lead to more environmental contamination, and imbalance of minerals. So, improving the bioavailability of zinc may support to resolve this issue. Usually, traditional sources of zinc are used in poultry diets such as inorganic zinc oxide and zinc sulfate. However, the bioavailability of inorganic zinc sources is low because inorganic Zn combined with phytic acid found in most of the broiler's grains-based diets thus impairs zinc and calcium absorption (Abd-Elsamee et al. 2012; Yan et al. 2016).

Zinc oxide NPs are involved in a wide range of applications such as nutrition, drug delivery, antibacterial, batteries and fertilizers (Ismail et al. 2019; Samy et al. 2019; Menazea et al. 2021). Zinc oxide NPs also have been known as food and feed additives, and theyt were entitled by USFDA as GRAS (generally recognized as safe) (Pulit-Prociak et al. 2016). Green methods for nanomaterials synthesis are the lasted trend in nanotechnology because

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high value compounds are synthesized from wastes to minimize the negative effect of wastes on the climate. Using agro-industrial wastes such as fruit peels which could be extracted and use this extract as both reducing and capping reagent to synthesis nanomaterials (Begum et al. 2018; Ismail et al. 2019; Samy et al. 2019; Menazea et al. 2021).

Many studies of poultry feeding focused on decreasing the amount of minerals through enhancing their availability. Nano minerals have unique properties such as increasing reactivity, absorption and utilization compared to the traditional minerals (Rajendran 2013). Structure of nano minerals shows new properties, such as more specific surface, catalytic action and powerful adsorbing capacity (Sirelkhatim et al. 2015). With a view to find sources of zinc highly bioavailability and low-price, researchers focused on sources of nano-Zn in livestock diets (Lee et al. 2017). Nanoparticles can effectively achieve the mineral requirements in the animal body, improve growth performance and feed efficiency. Sahoo et al. (2014) found that addition of nano-Zn as feed additives to broiler diets exhibited better results on performance and enhancing the immune status compared with other Zn sources. Swain et al. (2015) reported that nano minerals have major prospect as mineral supplements for birds even at low levels than the traditional inorganic sources. Successfully using low level of nano-dicalcium phosphate in chicks diet compared with the high level of conventional dicalcium phosphate have been reported in previous studies (Samy et al. 2015; Hassan et al. 2016; Mohamed et al. 2016). Sahoo et al. (2016) reported that dietary supplementation of nano-zinc oxide enhanced ($P<0.05$) broiler performance and health status. Thus, zinc nanoparticles can be considered as good alternative feed additive in poultry diets (Sagar et al. 2018). Kumar et al. (2021) reported that dietary nano Zinc at a lower level (55 ppm) shows the same growth performance as common $ZnSO_4$ at 110 ppm. Therefore, the aim of this study was to investigate the effect of Nano Zinc oxide (Green and Chemical) and traditional Zinc (Oxide and Sulphate) on broiler performance, tibia bone measurements and some physiological parameters.

MATERIALS AND METHODS

The authors confirm that the ethical policies of the journal have been adhered to and the appropriate ethical review committee approval has been received. The authors followed EU standards for the protection of animals used for scientific purposes. This study was performed at Poultry Nutrition Research Unit (PNRU), Agriculture College, Cairo University, Giza, Egypt.

Zinc sources (oxide, sulphate, chloride and nitrate) and polyethylene glycol were obtained from chemical traditional company, in addition to orange peel wastes from juice factories. Nano zinc oxide, using green or chemical method, was synthesized using Sol-Gel methods (Ismail et al. 2019; Samy et al. 2019; Menazea et al. 2021).

Chick growth experiment was conducted to investigate the comparative study between four sources of zinc: nano zinc oxide (green and chemical) and traditional zinc (oxide and sulphate) from 1-35 day of age. The 240 a-day-old male chicks (Arbor Acres) were assigned into 4 treatment groups (4 groups x 6 replicate x 10 chicks each). Four diets were prepared, diets 1 and 2 contained zinc in traditional form as zinc oxide (ZnO) and zinc sulphate (ZnSO4), respectively. Whereas diets 3 and 4 contained zinc in nano form as green synthesized zinc oxide (GZnO NPs) and chemically synthesized zinc oxide (CZnO NPs), respectively. The diets were prepared to cover all the nutrient needs of Arbor Acres chicks except zinc which was added from the different sources of zinc according to the strain guide (90mg). Formulation of all experimental diets in different periods are shown in Table 1.

The initial weights of chicks in all replicates were close to each other about 42g. Chicks were reared in a warm climate and fed on the experimental diets during starter (1-14d), grower (14-28d) and finisher (28-35d) periods. The chicks were supplied feed and water *ad libitum*. Vaccination program against avian flu, New Castle, IB and IBD was carried out throughout the trial period.

After an overnight fast, the chicks were weighed, and feed intake was recorded for each replicate weekly. Body weight gain (BWG) and feed conversion ratio were calculated.

At 35 days of age, 3 chicks per treatment were fasted for 6h and samples of blood were collected through the vein. The blood samples were centrifuged for 10 min at 2000rpm and the serum was stored for later analysis. Blood serum analysis was fulfilled for aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, creatinine, alkaline phosphatase (ALP) using commercial kits (Bio Diagnostic, Cairo, Egypt) in automatic titration spectrophotometer with a high-performance readout (FlexorEL200 Biochemical Analyzer). Total T_3 and T_4 hormone in blood serum were measured using ELISA kits (My Bio Source Inc, San Diego, CA) and ratio of T_3/T_4 was calculated.

Right tibia bone was removed and prepared for the different measurements. Adhering flesh of tibia bone was removed and then extracted with diethyl ether. The weight, length and width of tibia bones were measured and then oven dried at 105˚C for 3h. Breaking strength of tibia bones were measured on apparatus Digital Force Gauge according to Mašić et al. (1985). Tibia bones were ashed in a muffle furnace at 600˚C for 6h for obtaining ash percentage. Radiographs were taken on the tibia bones to study the effect of different treatments on tibia bone mineral concentration using X-ray (Mohamed et al. 2016).

Densitometric Analysis of the Tibia Bone

Bone mineral density (BMD) and bone mineral content (BMC) of tibia were determined using method of dual-energy X-ray absorptiometry (DXA), Norland Excell Plus Densitometer (Norland, Fort Atkinson, WI, USA) equipped with Norland Illuminatus Small Subject Scan software v. 4.3.1. dedicated to small animals (Puzio et al. 2014). Bone mineral density and concentration measurements are reported as grams per square centimeters and grams, respectively.

One way analysis of variance was applied for statistically analyzed data using General Liner Model of SAS (SAS 2000). The differences among means were detected using Duncan's new multiple range test at ($P<0.05$) (Duncan 1955).

RESULTS AND DISCUSSION

High Resolution Transmission Electron Microscopy

The morphology of synthesized ZnO NPs was examined using High Resolution Transmission Electron Microscopy (HRTEM). As illustrated in Fig. 1A, HRTEM image shows that green ZnO NPs have infinitesimal very small size ranged from 2-5 nm. In addition, this image indicated that most of the ZnO NPs are spherical in shape with average particle size of 3nm. Whereas in Fig. 1B, the chemical ZnO NPs has 30-40nm. In addition, this image indicated that the ZnO NPs are hexagonal shape.

Growth Performance

The results of four dietary zinc sources: nano zinc oxide (green and chemical) and traditional zinc (oxide and sulphate) on performance parameters of broiler chicks are shown in Table 2. Results revealed that replacing traditional zinc with nano-zinc in diets has led to an improvement in poultry performance in the starter, grower and overall periods. The highest BWG was observed in broilers fed on nano-zinc diets (GZnO NPs and CZnO NPs) being 1929 and 1919g, respectively, compared to 1767 and 1750g for birds fed on traditional zinc sources (ZnO and ZnSO4) respectively. There were no significant differences between the two sources of zinc in the traditional form as well as between the two sources of zinc in the nano form. No significant differences were recorded in BWG among all treatments in the finisher period. No significant differences among all treatments in feed intake (FI) and feed conversion ratio (FCR) in the starter period. The worst FCR was recorded for traditional ZnO, while was significantly enhanced with those fed other treatments during the grower, finisher and overall period. The greatest values of FCR were observed with GZnO NPs group. In addition, all chicks' groups consumed higher FI than those fed ZnSO4 during grower and overall period. Whereas in the finisher period the highest FI recorded for those fed ZnO in traditional source while, the lowest FI for those fed green ZnO NPs.

The obtained results of growth performance enhancement by nano zinc were supported by both Ahmadi et al. (2013) and Sahoo et al. (2016) those reported that broiler fed diets containing nano zinc oxide gave significant ($P<0.05$) higher growth performance compared to traditional source. Kim et al. (2017) found that nano-zinc improved poultry performance, feed utilization and feed cost. Fathi et al. (2016) and Hafez et al. (2017) reported that using nano-zinc in broilers diets significantly increased BWG and improved FCR without affecting feed intake. The beneficial effects of nano zinc on growth performance may be related to the role of zinc in metabolism of energy and protein (Tabatabaie et al. 2007), increasing nutrients digestibility and improved feed intake utilization. The higher values of the improvement in growth performance parameters for nano-zinc sources may be due to nano-zinc has higher bioavailability and faster absorption in the intestine (Zhao et al. 2014; Sahoo et al. 2016). Improvement of growth performance in birds fed diet with nano-zinc may be due to the enhancement of the intestinal parameters (Hafez et al. 2017). Sagar et al. (2018) reported that using green nano-zinc in birds diets significantly enhanced performance and immunity. From these results, it could be concluded that the enhancement in broiler performance with nano zinc addition, could be related to the nano materials are highly small size, which lead to a major surface area and thus improve absorption.

Table 1: Formulation and nutrient composition of starter, grower and finisher diets

Ingredients	Starter	Grower	Finisher
Yellow corn	51.82	56.58	61.88
Soybean meal (44%)	36.2	29	25.2
Corn Gluten meal (60%)	5	6.14	5
Soybean oil	3	4.2	4.5
Dicalcium phosphate	1.3	1.5	1.3
Limestone	1.6	1.6	1.3
Vit and Min Mix (1)	0.3	0.3	0.3
NaCl	0.3	0.3	0.3
L-lysine HCl	0.28	0.26	0.17
Dl-methionine	0.13	0.08	0.05
Therionine	0.07	0.04	0
Total	100	100	100
Calculated Composition%(2)			
Crude protein	23.02	21.03	19.08
ME (Kcal/kg)	3007	3159	3237
Lysine%	1.44	1.25	1.06
Methionine%	0.52	0.45	0.39
Methionine+Cystine%	0.91	0.81	0.72
Therionine%	0.94	0.83	0.71
Tryptophane%	0.32	0.27	0.24
Valine%	1.1	1	0.91
Calcium%	1.01	1.03	0.87
Nonphytate P%	0.53	0.52	0.46

(1) Vitamin - mineral mixture supplied per kg of diet: Vit A, 12000IU; Vit D, 2200IU; Vit E, 10 mg; Vit K3, 2mg; Vit B1, 1mg; Vit B2, 4mg; Vit B6, 1.5mg; Vit B12, 10g; Niacin, 20mg; Pantothenic acid, 10mg; Folic acid, 1mg; Biotin, 50g; Choline chloride, 500 mg; Copper, 10mg; Iodine, 1mg; Iron, 30mg; Manganese, 55mg and Selenium, 0.1mg: (2) According to NRC 1994.

Tibia Bone Characteristics

The average weight (g), length (mm), width (mm), breaking strength (kgf) and ash percentage of tibia bone of broilers fed different dietary zinc sources are presented in Table 3. Birds fed nano zinc sources had the highest improvement in tibia measurements than birds fed the traditional zinc sources. Whereas all tibia bone measurements did not differ ($P>0.05$) between GZnO NPs and CZnO NPs treatments.

Tibia bone mineral density (BMD) and bone mineral concentrate (BMC) of broilers fed the different dietary zinc sources have been shown in Table 4. Birds fed nano zinc sources gave the best values in BMD and BMC compared to those fed traditional sources of zinc. Effect of different sources of zinc on BMD and BMC at 35 days of age has been illustrated in radiographs in Fig. 1 and 2. Also, the best BMD of tibia was obtained in the birds fed nano ZnO (GZnO NPs or CZnO NPs). On the other hand, birds fed zinc sulphate in diet showed comparable BMD and BMC as those fed zinc oxide as traditional form.

These results reflect the values obtained for tibia length, width, breaking strength and ash since the dietary GZnO NPs and CZnO NPs recorded the highest values compared to the other traditional form. Therefore, it could be concluded that using nano zinc form instead of conventional form did improve all the measured bone parameters.

Usually, tibia breaking strength and their content of ash are used as indicators of bone health and response to mineral supplementation (Ma et al. 2018). Tibia breaking

Table 2: Performance of broilers fed with the different dietary zinc sources

Parameters	Zinc source				SEM	Significant
	ZnO	$ZnSO_4$	GZnO NPs	CZnO NPs		
Starter period						
BWG (g)	242b	249b	280a	278a	5.68	**
FI (g)	269	270	269	305	7.08	NS
FCR (g/g)	1.11	1.09	0.96	1.10	0.03	NS
Grower period						
BWG (g)	657b	677b	785a	787a	22.33	*
FI (g)	1107a	1000b	1146a	1156a	22.79	*
FCR (g/g)	1.69a	1.51b	1.46b	1.47b	0.03	*
Finisher period						
BWG (g)	834	824	864	858	11.12	NS
FI (g)	1467a	1333b	1222c	1345b	28.82	**
FCR (g/g)	1.76a	1.63ab	1.42b	1.57ab	0.04	*
Overall period						
BWG (g)	1767b	1750b	1929a	1914a	31.10	*
FI (g)	2843a	2603c	2638bc	2807ab	39.40	*
FCR (g/g)	1.61a	1.50b	1.38c	1.47bc	0.03	**

a-c Means within a row with different alphabets differ significantly (P<0.05).

Table 3: Tibia bone parameters of broilers fed with the different dietary zinc sources

Parameters	Zinc source				SEM	Significant
	ZnO	$ZnSO_4$	GZnO NPs	CZnO NPs		
Weight (g)	9.70b	8.03c	11.83a	12.22a	0.52	**
Length (mm)	90.18b	87.79c	96.85a	96.70a	1.23	**
Width (mm)	8.04b	7.56b	8.98a	9.14a	0.23	**
Breaking strength (kgf)	10.33bc	8.46c	16.08a	14.70ab	1.17	*
Ash %	39.87b	42.05ab	44.90a	43.97a	0.69	*

a-c Means within a row with different alphabets differ significantly (P<0.05).

Table 4: Tibia bone mineralization of broilers fed with the different dietary zinc sources

Parameters	Zinc sources				SEM	Significant
	ZZnO	ZnSO4	GZnO NPs	CZnO NPs		
BMD (g/cm^2)	0.17b	0.20b	0.27a	0.26a	0.02	***
BMC (g)	0.06b	0.07b	0.13a	0.12a	0.02	***

a-c Means within a row with different alphabets differ significantly (P<0.05).

Table 5: Thyroid hormones (T3, T4) levels and T3/T4 ratio of broilers fed with the different dietary zinc sources

Treatment	Zinc sources				SEM	Significant
	ZnO	ZnSO4	GZnO NPs	CZnO NPs		
T3 (ng/mL)	3.88	4.38	4.26	3.34	0.16	NS
T4 (ng/mL)	18.37	17.94	17.16	18.13	0.97	NS
T3/T4	4.76	4.16	4.14	5.43	0.35	NS

a-c Means within a row with unlike superscripts differ significantly (P<0.05).

Table 6: Some biochemical parameters of broilers fed with the different dietary zinc sources

Parameters	Zinc sources				SEM	Significant
	ZnO	ZnSO4	GZnO NPs	CZnO NPs		
Creatinine (mg/dL)	0.51	0.54	0.57	0.61	0.03	NS
ALT (U/L)	13.81ab	8.94c	9.92bc	16.52a	1.08	**
Albumin (g/dL)	1.47ab	1.62a	1.55a	1.35b	0.04	*
AST (U/L)	197.71a	156.18b	153.19b	175.57ab	6.23	**
ALP (IU/L)	109.03c	114.65c	154.22a	135.21b	5.66	**

a-c Means within a row with different alphabets differ significantly (P<0.05): AST: Aspartate amino transferase; ALT: Alanine amino transferase, ALP: alkaline phosphatase.

strength has been significantly correlated with collagen content. Minerals are necessary for collagen formation, so, hortage of zinc can damage collagen formation, resulting impair of tibia bone characteristics (Rath et al. 2000). Wang et al. (2002) reported that supplemented zinc leads to promote the anabolic impact of insulin-like growth factor I on osteoblasts that effect on growth of the bone. Supplementation of zinc in nano form resulted in stronger tibia than the same supplementation dose from inorganic zinc. Mohamed et al. (2016) found that mineral in nano form gave the superior effect in bone mineral density and other bone characters than the traditional form. Nano zinc sources are less reactive than inorganic forms of zinc causing more bioavailability and saving of zinc for collagen synthesis and osteoblast growth, it may explain the improvement of tibia breaking strength with nano zinc groups (Nguyen et al. 2021).

Some Physiological Blood Parameters

The results of different dietary zinc sources on thyroid hormones are displayed in Table 5. Different dietary treatments did not affect the metabolic hormones (T3, T4) levels and their ratio T3/T4.

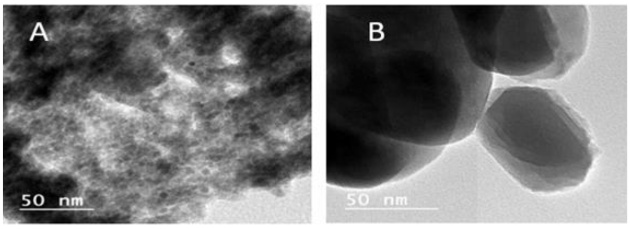

Fig. 1: The HRTEM of ZnO NPs synthesized by A: green Sol-Gel method and B: Chemical method

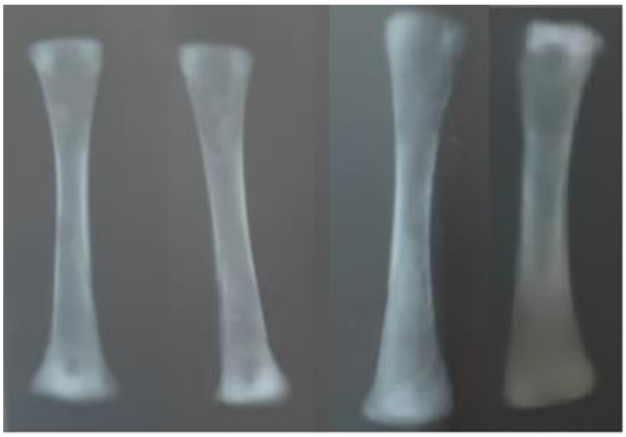

Fig. 2: Radiographic images were taken on tibia to study the effect of different treatments on tibia bone mineral density

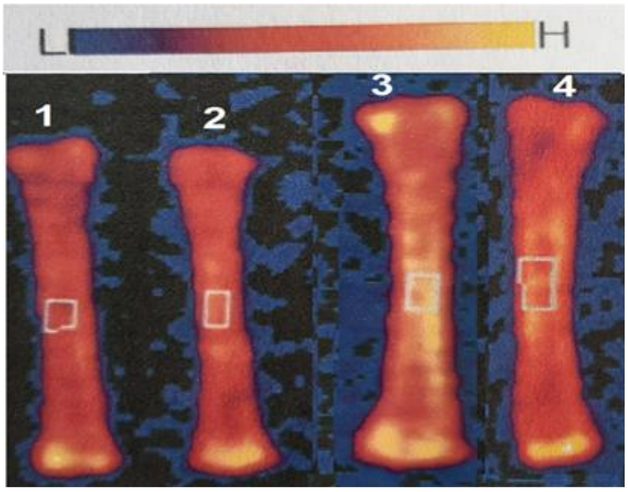

Fig. 3: Radiographic image of the effect of different sources of zinc on tibia bone scans using dual energy X-ray absorptiometry at 35 days of age.

Dietary zinc sources supplementation and some blood biochemical are shown in Table 6. The results of the liver function (ALT, AST and albumin) showed significant differences between treatments, but all values were within the normal range. In addition, no significant effect of treatments was observed on the kidney function (creatinine). Also, ALP shows slightly increase in the birds fed nano form of zinc compared to the traditional form but this was within the normal range.

Some blood parameters ALT, Albumin, AST and ALP were affected by the different sources of zinc except for creatinine. This is in agreement with Feng et al. (2010) and Fawzy et al. (2016**)** who reported that the increases in albumin and globulin levels with zinc oxide addition in broilers diet may be related to increase muscle mass. Also, zinc is necessary for growth and protein synthesis; it enhances the availability of proteins, carbohydrates and lipids, and many biochemical actions (Suttle 2010; Tahir et al. 2021). Sharideh et al. (2015) reported that dietary supplementation of zinc oxide increased activity of ALT and AST. On the other hand, Ahmadi et al. (2014) reported that ALT and AST levels did not affect with supplementation of nano-zinc oxide in broiler diets. Fathi et al. (2016) found that addition of nano-zinc oxide to broiler diet significantly increased ALP, but had no effect on AST, ALT and creatinine levels. The increase in ALP enzyme in chicks fed with nano- zinc oxide as compared to other groups may be related to the action of vitamin D3, which has effects on the intestine and bone, improving calcium absorption into the extracellular fluid and possibly stimulating the ALP production in the epithelial cells. Although the values of blood parameters measurements in chicks fed on Zn sources were different, but they were within the normal range value.

Conclusion

From these results it could be concluded that nano zinc sources (GZnO NPs and CZnO NPs) proved to be better sources than traditional zinc sources (ZnO and ZnSO4) for improving growth performance and bone parameters of broiler. Based on these results it could be concluded that using zinc in nano form in practical nutrition of broiler chicks may be better approach instead of traditional form to enhance broiler productive performance.

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Author's Contribution

All authors contributed equally to study design methodology, interpretation of results, and writing of the manuscript.

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