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Dietary supplementation with zinc oxide nanoparticles improves growth performance and gut microbiota of broiler chickens reared in the tropics

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Abstract
This study evaluated the effect of zinc oxide (ZnO) on growth performance, serum biochemistry, and gut microbial counts of broiler chickens reared in a tropical environment. A total of 120 one-day-old broiler chicks were assigned using a completely randomized design to four equal groups, with three replicates, containing 10 chicks per replicate. The control group was fed basal diet without ZnO, while the second, third and the fourth groups were fed diets supplemented with 0.60, 90.00, and 120.00 mg of zinc oxide/kg diet, respectively. The diets were formulated to be isocaloric and isonitrogenous, i.e., CP = 23.21%, ME = 2850 kcal/kg for the starter, and CP = 20%, ME = 3009 kcal/kg for the finisher phase. In the starter and finisher phases, dietary treatments had substantial effects on total feed intake, daily feed intake, daily body weight gain, and feed conversion ratio. Broilers fed diets supplemented with 90 mg or 120 mg ZnO/kg had a better weight gain and feed conversion ratio than the control treatment in both phases. Dietary ZnO had no effect on total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP) in the blood. Diets with low or no dietary ZnO showed an increase in *Escherichia coli* and *Lactobacillus spp.* in the ileum. Chickens fed high doses of dietary ZnO had relatively low cecal total bacterial counts and *Lactobacillus* bacterial counts. Supplementing broiler diets with ZnO improved growth performance and intestinal bacterial count.

Keyword: zinc oxide, growth, microbiota, chickens
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Introduction
Globally, the essential trace minerals receiving particular attention in poultry nutrition are copper, iron, manganese, zinc, cobalt, iodine, and selenium. However, most feedstuffs used in poultry feed formulations contain inadequate concentrations of trace minerals (TM), hence they are typically included in broiler diets as inorganic salts or in organic forms. The inorganic forms tend to dissociate when exposed to the low pH of the upper digestive tract, making them susceptible to the antagonism of several feedstuffs and nutrients, which reduces their availability for utilization and consequently, increases their excretion in the environment (Opoola, 2020). Zinc is an important nutrient for broilers (Baltaci *et al*., 2018) and plays three important roles in the body to facilitate biological functions: as a catalyst, regulator, and structural constituent (Baltaci *et al*., 2018). It is commonly included in animal diets as zinc oxide, zinc sulphate, or as organically-bound zinc. The zinc content in broiler diets, as recommended by the National Research Council (NRC 1994), is 40 mg/kg of diet, which can be supplemented using inorganic or organic forms.

Zinc (Zn) is an important nutrient in poultry and its deficiency has been linked to various disorders, in addition to depressed growth and performance. Zinc is now recognised to play a major role in antioxidation, growth and development, production, immunity, antimicrobial properties, and

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stress-related issues (Zaghari *et al*., 2015; Naz *et al*., 2016). Additionally, supplementation of ZnO can improve growth, reduce feed conversion ratio, augment immunity, enhance antioxidant capacity, increase endocrine secretion, and interact with other minerals in the gut. This indicates that ZnO addition may be more important for broiler production in the tropics in order to minimize the negative effects associated with high temperature conditions.

The dose of ZnO needed to improve the immune response of broilers differs across reports, with some studies reporting the required dose to be greater than the NRC (1994) recommendation to enhance antibody production. In the tropics, early investigations of the Zn requirement of broiler chickens were based on purified diets and there was a tendency to ignore the mineral contents of the feed ingredients because of the uncertainty or inaccuracy of the data from books and the lack of real data on local ingredients. There is little information about zinc oxide bioavailability in feed ingredient content for broiler chicken production. Based on limited data, it seems that the bioavailability of ZnO also varies substantially between feed ingredients. Some reports suggest that the supplemental zinc oxide or the requirements of broiler chickens should be determined on the basis of the diets used for broilers. It is also important to consider that fact that in practical diets, many plant ingredients such as soybean, contain components like phytate that complex with Cu, Zn, and other minerals, affecting their bioavailability. This current study was conducted to determine the effect of zinc oxide supplementation in broiler diets on production performance, gut health, and immune response.

Materials and Methods

The experiment was conducted at the Poultry Unit of the teaching and research farm of Kabba College of Agriculture, Ahmadu Bello University. Kabba is a town in Kogi State, in mid-west Nigeria, in the northern Guinea Savannah Zone, located at latitude 7º49′43″ N, longitude 6º04′23″ E at an altitude of 436 m above sea level. The climate is characterized by well-defined dry and wet seasons with a mean annual rainfall of ~1311 mm. Onset of wet season begins in late April or early May, peaks between July and August, and ends in mid-October. This is followed by the Harmattan season, which consists of cold and dry weather. Temperatures are typically between 24 °C and 36 °C throughout the year, but, rarely, can drop to 15 °C or can rise to as high as 39 °C during the hot season.

A total of 120, 1-d-old mixed sex broiler chicks (40.00 ± 0.3 g), were distributed in a completely randomized design. There were four dietary treatments with three replicates of ten chicks. The four treatments (T1–T4) received maize-soybean meal-based diets supplemented with 0.0, 60, 90, and 120 mg/kg of zinc oxide, respectively. Birds were housed in wire-floored pens (150×100×60 cm^3). The lighting program was 23L: 1D per day. The birds had unrestricted (*ad libitum*) access to feed and water throughout the feeding trial. Day-old broilers were vaccinated against Infectious Bronchitis and NCD (New Castle Disease; spray vaccination) in the hatchery. During the experiment, the broilers were vaccinated against NCD at 20 days of age, according to commercial practice.

Two diets were formulated with zinc oxide added to the diets (Table 1). Experimental diets were fed in a two-phase feeding system, with the starter phase starting from day 1 to day 24 post-hatch and the finisher phase starting at day 28 until day 48 post-hatch. Treatment 1 was the basal diet (positive control) without zinc oxide. Treatments 2, 3, and 4 consisted of the basal diet supplemented with 60, 90, and 120 mg/kg zinc oxide, respectively. The basal diets were formulated to meet or exceed NRC (1994) recommendations for the total requirement of nutrients. The zinc oxide used in this experiment was manufactured by Shandong YingLang Chemical Co., Ltd, in China and purchased from Agricare in Ibadan, Oyo State, Nigeria. Dicalcium phosphate was used as a carrier to dilute the concentration of zinc oxide to a suitable level for blending the ration to achieve the desired content for dietary formulation. Water and feed were supplied *ad libitum*. The experimental diets were fed to the birds directly after they were placed in the cages. All the diets were chemically analyzed according to AOAC (1990) methods for their proximate composition (Table 2)

Table 1 Ingredients contained in the basal diet for broilers on an as-fed basis and the calculated nutrient content of the two rations

INGREDIENTS	BASAL DIET (STARTER)	BASAL DIET (FINISHER)
Maize	56.80	59.45
Soyabean cake	11.83	11.00
Groundnut cake	24.74	21.99
Palm oil	2.00	2.50
Bone meal	2.50	3.00
Limestone	1.00	1.00
Common Salt	0.30	0.30
Methionine	0.21	0.21
Lysine	0.32	0.30
Vit-min-Premix[1]	0.30	0.25
Total	**100.00**	**100.00**
Calculated Nutrient		
ME (kcal/kg)	2850	3009
Crude Protein (%)	23.21	20.00
Crude Fibre (%)	3.73	4.73
Ether Extract (%)	5.84	4.01
Calcium (%)	1.27	1.34
Phosphorus (%)	0.59	0.55
Methionine (%)	0.58	0.57
Lysine (%)	1.28	1.14

[1]Vitamin mineral premix provide per kg of diet. Vit. A, 13,340 i.u; Vit. D_3, 2680 i.u; Vit. E, 10 i.u; Vit. K, 2.68 mg; Calcium pantothenate, 10.68 mg; Vit. B_{12}, 0.022 mg, Folic acid, 0.668 mg; Choline chloride, 400 mg; Chlorotetracyline, 26.68 mg; manganese, 13 mg; iron, 66.68 mg; Zinc, 53.34 mg; Copper, 3.2 mg; Iodine, 1.86 mg; Cobalt, 0.268 mg; Selenium, 0.108 mg

Table 2 Formulated proximate feed analyses

Parameter	Starter feed	Finisher feed
Dry matter	94.13	93.26
Crude protein	22.87	21.11
Crude fibre	7.03	8.45
Ether extract	4.93	5.45
Ash	5.81	6.81
NFE	46.51	48.56

NFE = nitrogen free extract

The initial and final weights of birds were taken at the beginning and at the end of the experiment, in both the starter and finisher phases, respectively. The body weight gain was obtained by subtracting the initial weight from the final weight. Daily feed intake was determined by the difference between daily feed offered and daily feed leftover; total feed intake was obtained by cumulative addition of the daily feed intake. Feed conversion ratio (FCR) was computed by dividing daily feed intake by daily weight gain as feed intake/ weight gain.

After slaughter on day 49, ~2.5 cm of the ileum was sectioned and pooled samples of the cecal contents were collected in separate sterile dishes. Collected samples were immediately put on ice, transferred to the laboratory to determine microbial population, and serially diluted. Then 10 µl of each dilution was spotted on each plate containing plate count agar and MacConkey agar and incubated at 37 °C for 48 h to count total aerobic and *Coliforms* bacteria, respectively (Behnamifar *et al*., 2015). The results were read and expressed as a colony-forming unit (CFU) per gram of cecal contents. De Man–Rogosa–Sharpe agar was used for *Lactobacilli*, which was cultivated in a 3% CO_2 atmosphere at 37 ºC for 48 h (Guban *et al*., 2006). After incubation, the bacteria were counted in Petri dishes, and the number of bacteria in the initial volume was calculated using the formula:

$$\text{Number of bacteria} = \text{Number of colonies} \times (1/\text{Dilution factor}) \times \text{Cultured volume} \qquad (1)$$

The logarithms to base 10 of the obtained values were used in the CFU/g for later analyses.

Two millilitres of blood samples were collected from three birds per replicate at the end of the finisher phase. The blood samples were collected into a sterilized sample bottles containing no anticoagulant and were allowed to clot and then centrifuged. The serum was separated and stored at -20 °C at the Clinical Pathology Laboratory of the Ahmadu Bello Teaching Hospital for the determination of parameters related to liver function; total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP), according to the methods described by Lamb (1991).

Data were analysed according to a completely randomized design using the general linear model procedure of SAS (2003). Means were separated using Duncan's multiple range tests. Differences were considered to be significant at P <0.05.

Results and Discussion

Performance results for body weight at 24 d of age and total feed intake, daily feed intake, daily body weight gain, and feed conversion ratio of the broilers measured from 1–24 d of age are presented in Table 3. Performance results were affected by dietary treatment (P <0.05). Chickens fed 90.00 and 120.00 mg/kg dietary ZnO had the best results in terms of final weight, weight gain, and feed conversion ratio (FCR). Final weight and weight gain increased as the levels of ZnO increased up to the highest level tested in this experiment. On average, body weight of broilers at 24 d of age was 1047 g. This body weight at 24 d of age was 38.92% below the performance objectives for Ross 308 male broilers (1553 g) (Aviagen, 2019). A percentage difference formula was used to obtain the value of 38.92%; a percentage difference equals the absolute value of the change in value, divided by the average of the two numbers, multiplied by 100.

The improved body weight and body weight gain observed in the current study are similar to the findings of Batal *et al*. (2001), Hidayat *et al*. (2020), and Opoola *et al*. (2022), who reported an improved body weight, body weight gain, and feed conversion ratio showing linear effects (P <0.05) in broiler chicks fed dietary ZnO compared to the birds fed the control diet. Rossi *et al*. (2007) reported that the body weight gain and carcass yield were not influenced by the addition of increasing levels of dietary organic ZnO in broiler diets. The improved growth performance may be due to the vital role of the trace element in the molecular structure of enzymes and proteins, serving as a co-enzyme and activator, which are primarily involved in physiological functions (Prasad, & Lall, 2022). Moreover, Zn also improves broiler growth by increasing the activity of the insulin-like growth factor and growth hormone genes and plays a role in appetite regulation (Ibrahim & El-Mandrawy, 2017).

Over the past 20 years, there have been inconsistent results of the inclusion of dietary ZnO in relation to growth performance. The lack of consistent effects of dietary zinc on performance of birds may be due to the amount and sources of zinc present in the basal diet (Leeson & Summers, 2005). Total feed intake and daily feed intake was not affected by dietary ZnO across the treatment groups (P <0.05). These results are similar to those of Saleh *et al*. (2018) and Abdel-Wareth *et al*. (2022), who reported no marked difference in chicks fed Zn-supplemented diets over the control treatment. However, the average total and daily feed intakes across the treatments were above the NRC (1994) feed intake recommendations. This may be related to the role of zinc in inducing appetite (Berger, 2002). An improved feed efficiency was observed with chicks fed dietary ZnO compared to those not fed ZnO. This report agrees with the findings of Hess *et al*. (2001) and Nollet *et al*. (2007), who reported improved feed efficiency of broilers when diets were supplemented with ZnO. However, Burrell *et al*. (2004) and Rossi *et al.* (2007) reported that progressive addition of Zn in an inorganic or organic form and in combination as a complex to the basal diet did not affect the feed efficiency of broilers.

Table 4 show the results for body weight, daily body weight gain, total feed intake, daily feed intake, and feed conversion ratio of the broilers measured from 28–48 d of age. Dietary treatments had effects on body weight, body weight gain, total feed intake, daily feed intake, and feed conversion ratio (P <0.05). A low FCR value observed in chickens fed dietary ZnO implies better feed efficiency, thus demonstrating the efficiency of ZnO in supplying sufficient Zn to the broilers. Dietary treatments with supplemental ZnO had an effect (P < 0.05) on the BWG and FCR of 28–48-d-old broilers compared to birds fed the control diet without ZnO supplementation; all the dietary ZnO-supplemented treatments were similar in terms of body weight and body weight gain. This report is similar to the findings of Khawanda, (2022) and Ogbuewu, (2023) who reported an increased body weight gain of broiler chickens fed diets supplemented with Zn. This is contrary to the reports of Burrell *et al*. (2004), Yu *et al*. (2005), and Zaghari *et al*. (2022), who found an overall improvement in body weight of male broilers at 21 d of age, but not at 42 d and 49 d. Yusof *et al*. (2023) reported that dietary supplementation with ZnO had no effect on growth performance parameters.

The observed increase in total and average daily feed intake for chickens fed the diet without ZnO compared to chickens fed dietary ZnO may due to the fact that chickens on low ZnO diets would tend to consume more feed to meet the daily zinc requirement. Again, it was observed that chickens fed ZnO-supplemented diets has similar results in terms of FCR. This result is similar to the findings of Zaghari *et al*. (2022), who reported that at 28 d, chickens who received 125 mg/kg Zn from Hizox had better feed efficiency (P <0.05).

The results of the intestinal bacterial counts of broiler chickens fed varying levels of dietary ZnO are presented in Table 5. Dietary treatments had a marked effect on *Escherichia coli* (EC), *Lactobacilli* spp*.,* and *Bacillus* spp*.* (B) counts (P <0.05). The concentration of *E. coli* in the ileum and caecum was higher in birds without ZnO compared to those fed ZnO (P <0.05). However, it was observed that the concentration of *E. coli* in the caecum of birds was lower in chickens fed 90 and 120 mg/kg dietary ZnO. The concentration of *Lactobacilli* spp. in the ileum and caecum was higher in broiler chickens fed 90 mg dietary ZnO. Furthermore, the concentration of *Bacillus spp.* in the ileum and caecum was higher in birds fed 120 mg ZnO compared to treatments with lower levels of ZnO (P <0.05). The supplementation of ZnO decreased the ileal and caecal concentration of *Salmonella* spp. This is in line with the findings of Mahmoud *et al*. (2020), El-Shenawy *et al*. (2022), and Qu *et al*. (2023), who reported that supplementation of diet with Nano Zinc reduced *E. coli* spp. and *Salmonella* spp*.* colonization. The reduction in the *E. coli* spp. and *Salmonella* spp. load in the current study may be attributed to the activity of the ZnO in modulating the intestinal tract of the birds, thereby competitively excluding pathogenic microbes. Furthermore, zinc nanoparticles are characterized by antimicrobial activity, especially against foodborne pathogenic bacteria, such as *E. coli*, *Listeria monocytogenes*, *Salmonella*, and *Staphylococcus aureus* (Jones *et al*., 2008). Previous studies have stated that the major processes of nanoparticle elements are disruption and penetration of the bacterial cell membrane (Wu *et al*., 2010). Microbial load for the pathogenic bacterium, *E. coli*, in the caecum was not statistically significant and no particular trend was observed. There was an increase in the populations of *Lactobacillus* spp. and *Bacillus* spp. with supplementation of ZnO in the ileum and caecum. This agrees with a statement made by El-Shenawy *et al*. (2022), who said that 'bacteria in the intestine increase in diversity and density in the more distal parts of the intestine with the largest number of bacteria species in the caecum'. *Lactobacillus* spp. and *Bacillus* spp. are gram-positive bacteria which produce antimicrobial peptides, proteins, and bacteriocins that selectively inhibit microbial growth and have a positive effect in modulating pathogenic bacteria. The high *Lactobacillus* count of broiler chicken groups fed on both ZnO-supplemented diets indicated that ZnO can be considered as a treatment for gram-positive and gram-negative bacteria (Arabi *et al*., 2012; Mahmoud *et al*., 2020). The higher concentrations of ZnO (90 mg and 120 mg) showed the highest antibacterial activity against the total bacterial count. Some previous studies have suggested that increasing bacterial cell permeability increases bacterial death (Zaghari *et al*., 2015; Siddiqi *et al*., 2018).

Liver blood tests are designed to detect abnormalities, for example, inflammation or liver cell damage, in the liver. Results related to the effect of different dietary levels of ZnO on selected serum enzymes are presented in Table 6. Dietary treatments had no effects on total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate (ALP) (P >0.05). This present result is similar to the findings of Ahmadi *et al.* (2014), who reported no effect of ZnO on serum chemistry of broiler chickens (P >0.05). This result is contrary to the findings of Hatab *et al*. (2022), who observed the highest concentration of serum enzymatic activity for ALT and AST in ZnO treated groups of chicks. Reasons for these differences are probably related to dose, timing, and the age of animal exposed.

Table 3 Effect of dietary ZnO on performance of broiler chicks (1–24 d)

	ZnO (mg/kg)				
Parameters	0.00	60.00	90.00	120.00	SEM
Initial weight (g)	40.01	40.00	40.00	40.00	0.01
Final weight (g)	933.67^{b}	932.00^{b}	1121.00^{a}	1203.28^{a}	46.81
Weight gain (g)	893.66^{b}	892.00^{b}	1081.00^{a}	1163.78^{a}	45.78
Avg. daily gain (g)	37.24^{b}	37.17^{b}	45.04^{a}	48.49^{a}	1.78
Total feed intake (g)	2094.33	2134.50	2142.43	2144.33	21.95
Daily feed intake (g/b/d)	87.36	88.94	89.26	89.36	1.45
FCR	2.35^{b}	2.42^{b}	1.98^{ab}	1.84^{a}	0.03

a, b, = Means with different superscript on the same row differ significantly (P <0.05)
SEM = Standard error of mean
FCR = Feed conversion ratio

Table 4 Effect of dietary ZnO on performance of broiler finisher chickens (28–48 d)

	ZnO (mg/kg)				
Parameters	0.00	60.00	90.00	120.00	SEM
Initial weight (g)	875.00	873.67	876.00	879.67	16.09
Final weight (g)	2311.30^{b}	2740.00^{a}	2936.76^{a}	2769.30^{a}	90.62
Weight gain (g)	1438.30^{b}	1866.30^{a}	2060.30^{a}	1840.70^{a}	91.70
Av. daily gain (g)	71.92^{b}	93.32^{a}	103.01^{a}	92.04^{a}	7.67
Total feed intake (g)	4226.67^{a}	4057.83^{b}	4042.43^{b}	4044.33^{b}	18.34
Daily feed intake (g/b/d)	211.33^{a}	202.89^{b}	202.12^{b}	202.22^{b}	1.78
FCR	2.95^{b}	2.22^{a}	1.96^{a}	2.19^{a}	0.22

a, b, = Means with different superscript on the same row differ significantly (P <0.05)
SEM = Standard error of mean
FCR = Feed conversion ratio

Table 5 Intestinal bacterial count of broiler chickens fed diets containing varying contents of ZnO

	ZnO (mg/kg)					
Parameters	Location	0.0	60	90	120	SEM
EC (×10^2 CFU/g)	Ileum	5.77^{a}	5.00^{ab}	3.90^{b}	3.96^{b}	0.62
	Caecum	3.56	3.17	3.76	2.21	1.40
LB (×10^2 CFU/g)	Ileum	12.88	14.09	11.99	14.98	1.52
	Caecum	2.00	3.65	2.50	3.90	1.45
B (×10^2 CFU/g)	Ileum	4.30^{b}	4.47^{b}	4.37^{b}	5.27^{a}	0.14
	Caecum	2.30	2.31	2.37	2.36	1.23

a, b, = Means with different superscript on the same row differ significantly (P <0.05)
SEM = Standard error of mean
EC = *Escherichia coli*; LB = *Lactobacilli* spp.; B = *Bacillus* spp.

Table 6 Serum biochemistry of broiler chickens fed varying contents of dietary ZnO

	ZnO				
Parameters	0.00	60.00	90.00	120.00	SEM
Total Protein	6.75	4.30	5.50	4.63	1.88
AST	40.00	46.30	55.33	45.33	9.01
ALT	26.30	34.47	36.67	55.33	13.82
ALP	63.23	57.60	41.83	51.53	12.90

SEM = standard error of mean
AST = aspartate aminotransferase
ALT = alanine aminotransferase
ALP = alkaline phosphate

Conclusion and Recommendations

ZnO is an essential trace element in poultry, implicated in aspects of both physiology and performance. The results obtained in the current study indicate that dietary inclusion of ZnO has positive effects on growth performance, feed efficiency, microbial count, and health status in broiler chickens. Moreover, dietary ZnO supplementation appears to alleviate the adverse effects of *E. coli*, *Lactobacilli* spp., and *Baccillus* spp. on broiler chicken growth, immune response, and intestinal microbial count. Birds fed 90 and 120 mg/kg dietary ZnO had the best results in terms of weight gain and feed conversion ratio. However, further investigations are required on the type, dose, and duration of Zn supplementation in poultry feed formulated for broiler chickens reared under tropical environments.

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Authors' contributions:

Conceptualization and design of study, EO (ORCID ID 0000-0002-1244-4720); methodology, formal analysis, and data curation, EO, MOJ, ORY, APO; original draft preparation, EO, MOJ; review and editing, EO, MOJ, and OO. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

References

Ahmadi, F., Ebrahimnezjad. Y., Ghiasi Ghalehkandi, J. & Maheri, S.N., 2014.The effect of dietary ZnO nanoparticles on the antioxidant state and serum enzymes activity in broiler chickens during starter stage. International Conference on Biological, Civil, and Environmental Engineering (BCEE-2014) March 2014, 17–18, Dubai (UAE).

Abdel-Wareth, A.A.A., Hussein, K.R. & Ismail, Z.S.H., 2022. Effects of ZnO nanoparticles on the performance of broiler chickens under hot climatic conditions. Bio. of Tra. Ele. Res. 12, 5218–5225. DOI: 10.1007/s12011-022-03095-9.

AOAC (1990). Association of Official Method of Analysis,18th Edition. Association of Analytic Chemists. Washington D.C. U.S.A

Arabi, F., Imandar, M., Negahdary, M., Imandar, M., Noughabi, M.T., Akbari-Dastjerdi, H. & Fazilati, M., 2012. Investigation anti-bacterial effect of ZnO nanoparticles upon life of *Listeria monocytogenes*. Anna. of Bio. Res. 7, 3679-3685. https://link.springer.com/article/10.1007/s11250-020-02229-2#ref-CR12.

Aviagen. Broiler Nutrition Specification Ross 308; Aviagen: Huntsville, AL, USA, 2019.

Baltaci, S.B., Mogulkoc, R., Baltaci, A.K., Emsen, A. & Artac, H., 2018. The effect of zinc and melatonin supplementation on immunity parameters in breast cancer induced by DMBA in rats Arch. Physio. Biochem; 124, 247–252.

Batal, A.B., Parr, T.M. & Baker D.H., 2001. Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poult. Sci. 80, 87–90. DOI: 10.1093/ps/80.1.87

Behnamifar, A., Rahimi, S., Karimi, D., Torshizi, M.A., Hasanpour, S. & Mohammadzade, Z., 2015. Effect of thyme, garlic, and caraway herbal extracts on blood parameters, productivity, egg quality, hatchability, and intestinal bacterial population of laying Japanese quail. Iran. J. Vet. Med. 9(3), 179–187. DOI: https://www.doi.org/10.22059/IJVM.2015.55286

Berger, L.L., 2002. Zinc: Nutritional and pharmacological roles. Salt and Trace Minerals, Salt Institute for the Animal Nutrition Professional, Suite 600, Alexandria, VA. 34(3), 1–3.

Burrell, A.L., Dozier, W.A., Davis, A.J., Compton, M.M., Freeman, M.E., Vendrell, P.F. & Ward, T.L., 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. Br. Poult. Sci. 45, 225 –263.

El-Shenawy, A., Salim, A.A. & Gouda, M.Y., 2022. Effects of Nano Zinc on growth performance, health status, and cecal microbiota in broiler chickens challenged with *Salmonella Kentucky*. Wrld. Vet. J. 12 (1), 105–122. DOI: https://dx.doi.org/10.54203/scil.2022.wvj14

Guban, J., Korver, D.R., Allison, G.E. & Tannok GW., 2006. Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of *Lactobacillus salivarius* and reduced bile salt deconjugation in the ileum of broiler chickens. Poult. Sci. 85, 2186–2194. DOI: www.doi.org/10.1093/ps/85.12.2186

Hatab, M.H., Rashad, E., Saleh, H.M., El-Sayed, E.R. & Taleb, A.M.A., 2022. Effects of dietary supplementation of myco-fabricated ZnO nanoparticles on performance, histological changes, and tissues Zn concentration in broiler chicks. Sci. Rep. 12, 18791 (2022). doi.org/10.1038/s41598-022-22836-3

Hidayat, C., Sumiati T., Jayanegara, A. & Wina E., 2020. Effect of zinc addition on the immune response and production performance of broilers: A meta-analysis. Asian-Australasian J. Anim. Sci. 33, 465–479. doi:10.5713/ajas.19.0146

Hess, J.B., Bilgili, S.F., Parson, A.M. & Downs, K.M., 2001. Influence of complexed zinc products on live performance and carcass grade of broilers. J. App. Ani. Res. 19, 49–60.

Hooge, D.M., Mathis, G.F., Lumpkins, B., Ponebˇsek, J &, Moran D., 2012. Dose-responses of broiler chicks, given live coccidian vaccine on day of hatch, to diets supplemented with various levels of Farmatan R _ (Sweet chestnut wood tannins) or BMD R _ /Stafac R _ in a 42-day pen trial on built-up litter. Intl. J. Poult. Sci. 11, 474–481.

Ibrahim,D., Ali, H. & El-Mandrawy, S., 2017. Effects of different zinc sources on performance, bio distribution of minerals, and expression of genes related to metabolism of broiler chickens Zagazig Vet. J., 45,292–304, doi.org/10.21608/zvjz.2017.7954

Jones, N., Ray, B., Ranjit, K.T. & Manna, A.C., 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiology Letter 279(1), 71–76. https://www.doi.org/10.1111/j.1574-6968

Khawanda M.M., 2022. Effect of stocking density and vitamin E or zinc supplementation on growth, physiology, gene expression, and economic efficiency of growing broiler chicks. Trop Anim Health Prod. 26, 54(6), 03. doi: 10.1007/s11250-022-03382-6

Lamb, G.N., 1991. Manual of Veterinary Laboratory Techniques. Ciba-Geig Y, Kenya. Pp. 96–107.

Mahmoud, U.T., Abdel-Mohsein, H.S., Mahmoud, M.A. & Ohtsuka., 2020. A. Effect of ZnO nanoparticles on broilers' performance and health status. Trop. Anim. Hlth. Prod. 52, 2043–2054. DOI: https://www.doi.org/10.1007/s11250-020-02229-2.

National Research Council. Nutrient Requirements for Poultry, 9th ed.; National Academy Press, Washington, DC, USA, 1994.

Naz, S., Idris, M., Khalique, M.A., Zia-Ur-Rahman, I.A., Alhidary, M.M., Abdelrahman, R.U., Khan Chand, Farooq, U. & Ahmad S, 2016. The activity and use of zinc in poultry diets. *Wld Poult. Sci. J.* 72, 159–167. http://dx.doi.org/10.1017/S0043933915002755.

Nollet, L., Van der Klis, J.D., Lensing. M., Spring. P., 2007. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. Journal of Appl. Poult. Res. 16, 592–597.

Ogbuewu, I.P., Modisaojang-Mojanaga, M.M.C., Mokolopi, B.G. & Mbajiorgu, C.A., 2023. A meta-analysis of responses of broiler chickens to dietary zinc supplementation: Feed intake, feed conversion ratio and average daily gain. Biol Trace Elem Res. 201(5), 2491–2502. doi: 10.1007/s12011-022-03320-5

Opoola E., 2020. Effect of silicon oxide supplementation in broiler chickens' drinking water on performance and litter quality under tropical environment. Nig. J. Ani. Prod. 47(4), 132–138.

Opoola, E., Atte, P.O., Lawal, A.N., Oyebanji, O.A. & Olaniyan G., 2022. Effects of vitamin C and zinc supplementation in drinking water on growth performance of broiler chickens reared in the tropics. Nig. J. Ani. Sci. 24(3), 71–78.

Prasad, S. & Lall, R., 2022. Zinc–curcumin based complexes in health and diseases: An approach in chemopreventive and therapeutic improvement J. Trace Elem. Med. Biol., 73, Article 127023. Doi.org/10.1016/J.JTEMB.2022.127023

Qu, J., Zuo, X., Xu, Q., Li, M., Zou, L., Tao, R., Liu, X., Wang, X., Wang, J., Wen, L. & Li R., 2023. Effect of two particle sizes of nano ZnO on growth performance, immune function, digestive tract morphology, and intestinal microbiota composition in broilers. Animals (Basel). 13(9), 1454. DOI: 10.3390/ani13091454

Rossi, P., Rutz, F., Anciuti, M.A., Rech, J.L. & Zauk, N.H.F., 2007. Influence of graded levels of organic zinc on growth performance and carcass traits of broilers. J. Appl. Poult. Res.16, 219–225.

Saleh, A.A., Ragab, M.M., Ahmed, E.A., Abudabos, A.M. & Ebeid, T.A., 2018. Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties, and immune response in broiler chickens under high ambient temperature. J. Appl. Ani. Res. 46(1), 820–827. https://doi.org/10.1080/09712119.2017.1407768

SAS Institute. 2003. SAS/STAT User's Guide: Statistics: Release 9.1 Ed.; SAS Institute Inc.: Cary, NC, USA.

Siddiqi, K.S., Rahman, U., Tajuddin, A. & Husen, A. 2018., Properties of ZnO nanoparticles and their activity against microbes. Nanoscale Research Letters. 13(1), 141. https://www.doi.org/10.1186/s11671-018-2532-3.

Wasti, S., Sah, N. & Mishra, B., 2020. Impact of heat stress on poultry health and performances, and potential mitigation strategies. Anim. 10, 1266.

Wu, B., Huang, R., Sahu, M., Feng, X., Biswas, P. & Tang, Y.J., 2010. Bacterial responses to Cu-doped TiO_2 nanoparticles. Sci. Total. Environ. 408(7), 1755–1758. https://www.doi.org/10.1016/j.scitotenv.2009.11.004.

Yusof, H.M., Rahman, N. A., Mohamad, R., Zaidan, U.H., Arshad, M.A. & Samsudin, A.A., 2023. Effects of dietary ZnO nanoparticles supplementation on broiler growth performance, zinc retention, liver health status, and gastrointestinal microbial load. J. Trace Elem. Min. 4, 100072. https://doi.org/10.1016/j.jtemin.2023.100072

Yu, Z.P., Le, G.W. & Shi, Y.H., 2005., Effect of zinc sulfate and zinc methionine on growth, plasma growth hormone concentration, growth hormone receptor, and insulin-like growth factor-1 gene expression in mice. Clinical Exp. Pharm. Physio., 32, 273–278.

Zaghari, M., Avazkhanllo, M. & Ganjkhanlou, M., 2015., Re-evaluation of male broiler zinc requirement by dose response trial using practical diet with added exogenous phytase. J. Agr. Sci. Tech. 17, 333–343.

Zaghari, M., Mehrvarz, H., Hajati, H. & Moravej H., 2022. Evaluation of an innovative Zn source on feed efficiency, growth performance, skin and bone quality of broilers suffering heat stress. Animals (Basel). 24, 12(23), 3272. doi:10.3390/ani12233272.