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ORIGINAL ARTICLE

BONE INDICES AND MINERAL INTERACTION IN ORGANS AND BONES OF BROILER CHICKENS FED DIET SUPPLEMENTED DIFFERENT ZINC TYPES

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

A 42-day trial was carried out to assess the influence of dietary supplementation of three zinc types and their combinations on bone indices and interaction with other minerals in the tissues and bones of broiler chickens. Two hundred and sixteen (216) Arbor Acres day-old broiler chicks were divided into six groups to consist of 36 birds and assigned to six treatments, which include zinc-methionine (Zn-met), zinc-oxide (ZnO), nano zinc (NZn), NZn + Zn-met, NZn + ZnO and control (antibiotics). Each treatment was replicated thrice with 12 birds. Data collected were subjected to one-way analysis of variance. Phosphorus was the only mineral that varied in the liver. Heart and meat mineral content were not significantly ($P > 0.05$) affected except for zinc and phosphorus respectively. Supplementation of nano-zinc alone increased bone weight, and bone robusticity was higher generally in birds fed diets fortified with zinc. Zn retention was markedly reduced in birds supplemented with Zn-met and NZn + ZnO. Sole supplementation of nano-zinc significantly elevated phosphorus (37.96 mg/dl) in bones, while Cu content was higher in all zinc-supplemented groups except the ZnO group. This study concludes that nano-zinc supplementation increased tibia bone length and weight, while bone ash and calcium were not affected by different zinc types and their combinations.

Keywords: antibiotics; bone morphometry; mineral retention; zinc types; zinc supplementation

INTRODUCTION

Broiler health is a major concern arising from its impact on their growth, welfare, and productivity. Skeletal issue is another common problem in broilers stemming from rapid growth rates exceeding skeletal development, nutritional imbalance, or infectious agents. Birds with deformed limbs have walking problems, and their sitting on the bedding also affects the quality of meat and the profitability of poultry production [1]. In addition to this situation, broiler chickens with weak legs have worse performance because they have reduced feed intake [2]. This often leads to problems of bone breakage during handling and transportation and during processing at slaughterhouse [3].

To address some of these problems, there has been increased research into the adoption of various alternatives to synthetic drugs, such as antibiotics, to minimize the risk associated with their use on both broilers and consumers. The use of minerals such as zinc, calcium, copper, selenium, etc., has been used for the improvement of broiler health and productivity.

Zinc, a nutritionally indispensable trace element, plays three major biological roles in the body: as a catalyst, and as a structural and regulatory ion [4]. It has a catalytic, coactive, or structural role in a wide variety of enzymes that regulate many physiological processes, including metabolism and growth [5]. Burrell et al. [6] opined that the National Research Council (NRC) recommendation of 40 mg/kg of zinc in broiler diets may have only considered growth performance as a criterion. However, higher levels of Zn may need to be supplemented during the entire period of production but not exceeding 70 mg/kg [7]. It is well known that a wide range of metals, including zinc, possess antimicrobial activity [8].

Different forms of zinc supplementation, including inorganic (e.g., zinc sulfate, zinc oxide), organic (e.g., zinc amino acid complexes), and nano-sized zinc, are used in broiler chicken diets to enhance mineral retention, bone development, and meat quality. Minerals from inorganic sources interact between mineral cations and other dietary components when fed to animals [9], leading to the formation of insoluble complexes (phytates) with much lower availability to animals. Organic or chelated forms of microminerals may have higher bioavailability than inorganic sources, mainly because of their lower interaction with other dietary constituents. Recently, ZnO nanoparticles (ZnNPs with sizes within 1–100 nm) are getting more attention for use in the nutrition of livestock to address dietary requirements and to promote animal growth due to the larger surface area, higher surface activity, catalytic efficiency, and stronger adsorbing quality compared with the conventional use of Zn [10].

Swain et al. [10] reported that minerals are transported directly to target organs and are easily absorbed and distributed into organs and tissues of broiler chickens. Also, bones function as a reserve of most of the trace minerals in broilers, including Zn; thus bone characteristics such as tibia bone index, bone strength, bone mineral concentration [11], and bone ash have often been used as sensitive indicators of bone status and response to both macro- and trace-mineral supplementation [12].

Overall, the strategic supplementation of different zinc forms and their combinations in broiler diets is crucial for optimizing mineral retention in organs, enhancing bone morphometry and mineralization, and improving meat quality, thus supporting productive performance and health in poultry production systems. Hence, this study was conducted to investigate the possible influence of the three aforementioned zinc types and their proportionate combinations in the diet on bone morphometry and mineral retention in some selected organs and bones of broiler chickens.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Directorate of University Farms of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The farm area lies within latitude 7°13’49’’N, longitude 3°26’11.98’’E, and altitude 76 m above the sea level. The vegetation is located within the derived savannah zone of southwestern Nigeria. The area is characterised by a humid climate, a mean annual rainfall of 1037 mm and a temperature of about 30.4°C [13].

Source of test ingredients

The different zinc types (organic, inorganic, and nano-zinc) were acquired from a reputable pharmacy in Abeokuta, Ogun State. Feed-grade inorganic zinc oxide (95% purity and Zn content not less than 76.3%) was manufactured by Guangdong Guanghua Sci-Tech Co., Ltd.; nano-zinc (100 nm) was produced by TATA Chemicals, India; and the zinc-methionine (contains 12% zinc and 27.3% methionine) was manufactured by Zinpro Corporation, USA.

Experimental design and management of birds

A total of 216 Arbor Acres day-old broiler chicks were acquired from a reliable hatchery. The pen was cleared and disinfected prior to the arrival of the birds. Birds were raised under intensive management using a deep litter system over a period of 42 days. The day-old chicks were allocated into 6 treatments, and each treatment group was subdivided into three (3) replicates with 12 birds each. The treatments include supplementation of 60 mg of nano-zinc (NZn), zinc oxide (ZnO), zinc methionine (Zn-met), NZn + ZnO (30 mg each), NZn + Zn-met (30 mg each) in the diets and the control. Brooding was carried out for 2 weeks with extended daylight (about 5 hours) provided for chicks to feed well and a heat source (charcoal pot) to maintain the environmental temperature suitable for chicks' growth. The birds were fed commercial diets at both starter (22% crude protein, 3000 Kcal/kg metabolisable energy, 5.0 g/kg crude fat, 4.3% crude fibre, 1.2% calcium,, 0.45% phoshorus, 0.56% methionine and 1.2% lysine) and finisher (18% crude protein, 3150 Kcal/kg metabolizable energy, 5.5 g/kg crude fat, 3.0% crude fibre, 1.2% calcium, 0.44% phoshorus, 0.5% methionine and 1.2% lysine). Medications (vitamins and coccidiostat) and vaccines (Gumboro and Lasota) were given as at when due during the study. Antibiotics (enrofloxacin) was applied in water for the control treatment only according to the manufacturer's recommendation, while various zinc types served as alternatives to antibiotics in the respective treatment groups.

Data collection

Mineral retention in blood, muscle, and organs

On the 42nd day, one bird was selected from each replicate. Blood samples (2 ml) were collected from each bird via jugular vein into plain bottles, after which the birds were slaughtered. A small piece of the breast muscle (5 g) and organs such as the liver, heart, and spleen were removed from each bird and stored in airtight plastic containers to determine mineral content [14]. The concentrations of Zn, Cu, P, and Ca were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Bone morphometry

The left tibia bones of the slaughtered birds were collected, labeled, and soaked in boiling water (100°C) for 10 mins to facilitate removal of adhering muscles and connective tissues. The width of the bone was measured with vernier calipers, which was expressed in centimeters (cm). The tibia weight (g), tibia length (cm), and tibia robusticity index were obtained as described by Mutus et al. [15]. The bone index was obtained as described by Seedor et al. [16].

Tibia index=(Tibia weight)/(Tibia length)

Robusticity index=(Bone length)/((Bone weight)1/3

Bone mineral analysis

The left tibia bones were air-dried and taken to the laboratory for analysis of the ash content and some minerals (Ca, P, Cu, and Zn). Tibia samples were subjected to oven-drying at 105°C for 24 h, cooled in a desiccator, and weighed. Thereafter, bone samples were ashed in a muffle furnace at 600°C for 24 h in crucibles according to the method recommended by AOAC [17]. Ash content of the tibia and femur was expressed as a percentage of dry bone weight. For mineral content analysis, ash samples were subjected to digestion using a mixture of nitric (65% HNO_3) and perchloric ($HClO_4$) acid at 2:1 v/v [18]. Following digestion, samples were allowed to cool at room temperature. The content of calcium (Ca), phosphorus (P), copper (Cu), and zinc (Zn) in digested samples was determined as described by Skoog et al. [19] using atomic absorption spectrophotometry.

Data analysis

Data collected were subjected to one-way analysis of variance in a Completely Randomized Design using SPSS (version 23.0). Significant differences among means were separated using Duncan's multiple range test as contained in the software at a 5% level of significance ($P < 0.05$).

RESULTS

Effect of dietary supplementation of different zinc sources and their combinations on mineral retention in some organs and meat of broiler chickens

The effect of dietary supplementation of different zinc sources and combinations on mineral retention in some organs and meat of broiler chickens is shown in Table 1. Results showed there was no significant (P > 0.05) difference in mineral composition of the spleen. All mineral content of liver determined was not significantly (P > 0.05) affected except (P < 0.05) phosphorus. Phosphorus was least in the organic (2.77 mg/dl) zinc group and highest in birds fed a diet supplemented with 30 mg/kg each of nano + inorganic (4.23 mg/dl). Heart and meat mineral content were not significantly (P > 0.05) affected except for zinc (Zn) and phosphorus (P), respectively. The highest values for Zn were observed in birds fed a diet supplemented with organic (90.67 µg/dl) zinc, inorganic (87.00 µg/dl) zinc, and the control birds (84.27 µg/dl), while the least value was recorded in the nano + inorganic (52.47 µg/dl) group for heart. Phosphorus content for meat was highest in birds fed a diet supplemented with inorganic (3.80 mg/dl) zinc and organic zinc (3.53 mg/dl), while the least value was recorded at 60 mg/kg nano-zinc (2.53 mg/dl) group.

Effect of dietary supplementation of different zinc sources and their combinations on bone morphometry of broiler chickens

The effect of dietary supplementation of different zinc sources and combinations on bone morphometry of broiler chickens is shown in Table 2. The result revealed that different zinc supplementation in the diet of broiler chickens had a significant (P < 0.05) effect on the bone length, weight, and robusticity index. Highest values for bone length were recorded in nano and organic groups (10.09 cm and 10.01 cm, respectively), while the least value was recorded in inorganic (9.29 cm) group. The highest value for weight was observed in birds fed a diet supplemented with nano-zinc (7.47 g), and the least value was observed in birds fed a diet supplemented with inorganic (5.50 g) zinc. The robusticity index was highest in birds fed a diet supplemented with nano + inorganic (5.42 cm/g) zinc and the least recorded in control (5.01 cm/g) birds.

Table 1. Mineral composition in some organs and meat of broiler chickens fed diets supplemented with different zinc sources and their combinations

	Zinc types and combination supplementations							
Parameters	**NZn**	**Zn–met**	**ZnO**	**NZn+ Zn-met**	**NZn + ZnO**	**Control**	**SEM**	**P-value**
Spleen								
Ca (mg/dl)	12.63	12.97	11.70	12.20	12.20	12.40	0.21	0.67
Zn (µg/dl)	63.03	84.37	71.17	71.07	85.20	76.17	3.02	0.24
Cu (µg/dl)	238.73	258.33	214.10	235.40	307.90	272.77	15.12	0.61
P (mg/dl)	4.13	3.93	3.50	3.53	3.13	3.70	0.16	0.63
Liver								
Ca (mg/dl)	11.20	10.33	10.93	12.13	17.77	11.70	1.01	0.32
Zn (µg/dl)	79.13	92.83	64.93	86.90	66.63	79.63	4.86	0.57
Cu (µg/dl)	161.80	187.57	187.57	212.17	244.23	200.57	13.34	0.67
P (mg/dl)	3.40^{ab}	2.77^{b}	3.67^{ab}	3.93^{ab}	4.23^{a}	3.37^{ab}	0.17	0.03
Heart								
Ca (mg/dl)	11.50	10.80	10.60	12.17	11.7	11.77	0.22	0.32
Zn (µg/dl)	78.93^{ab}	90.67^{a}	87.00^{a}	74.43^{ab}	52.47^{b}	84.27^{a}	4.04	0.04
Cu (µg/dl)	210.50	187.57	187.57	281.60	193.93	207.73	12.69	0.25
P (mg/dl)	3.23	3.67	4.07	4.10	4.27	3.40	0.15	0.26
Meat								
Ca (mg/dl)	10.60	11.07	10.40	10.70	10.70	10.63	0.18	0.97
Zn (µg/dl)	79.57	88.47	85.97	64.30	76.77	74.60	4.44	0.73
Cu (µg/dl)	181.20	182.83	185.90	203.87	210.00	172.33	7.34	0.73
P (mg/dl)	2.53^{c}	3.53^{a}	3.80^{a}	3.40^{ab}	3.47^{ab}	2.77^{bc}	0.13	0.02

^{abc}Means within a row with different superscripts differ significantly (P < 0.05)

Effect of dietary supplementation of different zinc sources and their combinations on bone minerals of broiler chickens

The effect of dietary supplementation of different zinc sources and combinations on bone minerals of broiler chickens is shown in Table 3. All parameters measured were significantly ($P < 0.05$) affected except ($P > 0.05$) calcium and ash. Zinc (Zn) and phosphorus (P) content were least in the organic (28.25 and 0.86 mg/100mg) group, respectively. The highest P content was observed in nano (37.96 mg/100mg) group, and the highest Zn content recorded in inorganic (1.62 mg/100mg) group. The highest Cu was recorded in birds fed a diet supplemented with 30 mg/kg each of nano + organic (0.63 mg/100mg) and birds in the organic (0.62 mg/100mg) group. The least Cu was observed with birds in inorganic and control (0.47 and 0.49 mg/100mg, respectively) groups.

DISCUSSION

Zn concentration in organs and tissues is often used as an indicator of the Zn status and storage in the body of animals as well as the level of Zn consumed by birds, because low levels are considered an early symptom of zinc deficiency [20]. Swain et al. [10] reported that minerals are transported directly to target organs and are easily absorbed and distributed into organs and tissues of broiler chickens. Mineral retention, particularly of calcium and copper was not significantly influenced in all organs and meat assessed in this study. This suggests that zinc types and supplementation did not interfere with calcium and copper availability, absorption, and retention in broiler chickens. In a related study, Ayoola et al. [21] reported similar Ca retention in organs of broiler chickens fed diets supplemented with different levels of nano-zinc.

Zinc retention only varied in the heart, and the variation did not give a clear picture of the influence of zinc supplementation because the control with no zinc supplementation had similar retention as some of those groups supplemented. In a similar study, Zhang et al. [22] recorded no significance in zinc retention in the liver of broiler chickens when zinc supplementation was varied between 0 and 120 g/kg. They added that the variation recorded in the breast muscle was not consistent with the dosages of supplementation at both 21 and 42 days of age.

Birds provided a diet with Zn-met had the least retention, whereas birds fed a mixture of nano-zinc and ZnO showed better phosphorus retention in the liver. Furthermore, compared to the use of nano-zinc, phosphorus re-

Table 2. Bone morphometry of broiler chickens fed diets supplemented with different zinc sources and their combinations

	Zinc types and combination supplementations							
Parameters	NZn	Zn–met	ZnO	NZn+ Zn-met	NZn+ ZnO	Control	SEM	P-value
Bone length (cm)	10.09^{a}	10.01^{a}	9.29^{b}	9.84^{ab}	9.87^{ab}	9.68^{ab}	0.10	0.02
Width (cm)	0.78	0.71	0.70	0.70	0.66	0.79	0.02	0.20
Weight (g)	7.47^{a}	7.13^{ab}	5.50^{b}	6.47^{ab}	6.07^{ab}	7.20^{ab}	0.25	0.02
Total bone index (g/cm)	0.74	0.71	0.59	0.66	0.61	0.74	0.02	0.16
Robusticity index (cm/g)	5.17^{ab}	5.21^{ab}	5.25^{ab}	5.29^{ab}	5.42^{a}	5.01^{b}	0.15	0.02

^{ab}Means within a row with different superscripts differ significantly(P<0.05)

Table 3. Bone minerals (Ca, P, Cu and Zn) of broiler chickens fed diet supplemented with different zinc sources and their combinations

	Zinc types and combination supplementations							
Parameters	NZn	Zn–met	ZnO	NZn+ Zn-met	NZn+ ZnO	Control	SEM	P-value
Calcium (mg/100mg)	114.67	111.64	118.98	103.37	118.01	121.35	2.35	0.28
Phosphorus(mg/100mg)	37.96^{a}	28.25^{b}	31.55^{ab}	31.29^{ab}	28.25^{b}	30.67^{ab}	1.17	0.04
Zinc (mg/100mg)	1.17^{ab}	1.01^{b}	1.62^{a}	1.20^{ab}	0.86^{b}	1.17^{ab}	0.08	0.02
Copper (mg/100mg)	0.55^{ab}	0.62^{a}	0.47^{b}	0.63^{a}	0.51^{ab}	0.49^{b}	0.02	0.03
Ash (g/100g)	28.3	27.67	27.6	27.47	26.1	27.79	0.78	0.99

^{ab}Means within a row with different superscripts differ significantly (P < 0.05) tention in meat showed better retention when conventional sources of zinc (ZnO and Zn-met) were utilized alone or in conjunction with Zn nanoparticles. According to this study, zinc has a crucial role in enhancing phosphorus absorption, distribution, and retention in tissues and organs. The noteworthy zinc result from the heart indicates a reduction in or lack of the antagonistic action between divalent metals, allowing zinc to have a fuller synergetic, beneficial influence on mineral absorption and meeting the zinc needs of broilers [23].

Bone characteristics like tibia bone index, bone strength, bone mineral concentration, and bone ash have frequently been used as sensitive indicators of bone status and response to both macro- and trace-mineral supplementation. Bones function as a reserve of most of the trace minerals in broilers, including Zn [12]. Additionally, a prominent sign of the adequate intake of minerals in a chicken diet is the quality of the bone [24].

In this study, dietary zinc supplementation resulted in an increase in tibia weight, length, and robusticity index, which is indicative of a moderate tibia bone width and density. According to Seedor et al. [16], a larger ratio of bone weight to bone length denotes greater bone density and enhanced bone strength. This ratio depicts the principle of bone mineralization [25]. The zinc supplementation in the form of nano-zinc-alone demonstrates its ability to increase tibia bone index due to improved bone length and weight, even without any significant differences in tibia bone density.

In comparison to birds given inorganic zinc supplements, the bioavailability of nano-zinc particles, organic zinc, and their mixtures was shown to be higher. This may be explained by the fact that different forms of zinc and their combinations were easily taken up by the digestive system and used to improve the bone length and weight of birds. Bone appears to be a functional store of zinc that could be quickly mobilized for any urgent need, as evidenced by the positive relationship between the robusticity index and weight and length. This suggests that different forms of zinc and their combinations supplemented in diet favour the formation of stronger bone and that Zn can be utilized when deficiency occurs [26].

The results of this study are consistent with the report of Cufadar et al. [27], which indicates that different sources of zinc exhibited similar results at various inclusion levels (20, 40, 60, 80, and 100 mg/kg) in diets, although tibia Ca and P were not negatively affected. The findings of this study contradict those of Idowu et al. [28], which reported a significant increase in Ca concentration in the tibia of birds supplemented with conventional (organic and inorganic) zinc sources. According to Ao et al. [23], increased Zn binding affinity for Ca would limit its retention in the tibia. This claim contradicts this study's findings because the tibia's calcium retention was not. Zinc sulphate and zinc methionine chelate-supplemented birds showed notably greater zinc reserves, which is compatible with the much higher zinc deposition in the tibia seen in these birds [29].

The bone ash found in this investigation is consistent with the findings of Kumar et al. [30], who found no significant difference in the percentage of tibia ash between various zinc sources (ZnS, ZnM, and HME 100) given at the same amount. Additionally, Sahraei et al. [24] found that when conventional (organic and inorganic) sources were added to broiler chickens' diet at three dosage levels (100, 150, and 200 mg/kg), the zinc concentration in the tibia ash did not change. This is explained by the fact that zinc (Zn) is a necessary component of the enzyme system that produces vitamin D3, which controls the retention of tibia ash and the absorption of minerals (P, Cu, and Ca) in broiler chickens [31].

CONCLUSIONS

This study concludes that different zinc types and their combinations did not influence zinc retention in organs except the heart, with no consistency. Phosphorus abundance, however, varied significantly in liver and meat. Bone length and weight were increased by nano-zinc supplementation, while bone ash and calcium were not affected by different zinc types and their combinations. Hence, any of the zinc types studied can be safely incorporated at the considered dosage into the diet of broiler chickens without any negative implication on mineral retention in organs. Also, nano zinc supplementation has a superior tendency to improve bone length and weight, which could possibly enhance better bone strength and minimize the chances of skeletal problems in broiler chickens.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

The research was approved by the Animal Ethics and Welfare Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta. The Approval Reference number is FUNAAB/COLANIM/AEWC/2025/002.

Conflict of Interest

There is no conflict of interest whatsoever as regards this study among the authors.

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Generative AI Statement

There was no use of generative AI in the writing of this manuscript.

Authors' Contributions

The idea of this research was originally conceived and mapped out by Ayoola Akeem A. and developed by all authors. Ajayi Oluwatosin D. and Oke Oluwaseun C. collected the field data. Ekunseitan Deji A. and Sanda Adeyinka J. assisted with analysis of data and interpretation of results. Ayoola Akeem A. and Oluwatubosun Olawale B. were involved in the preparation of the manuscript for publication.

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