

Growth performance, immune organs weight changes and caecal microbial population of broiler chickens fed supplemental nano and organic sources of zinc-based diets

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Abstract

Zinc application in the diet of broiler chickens supports growth performance, bone development and immune function, however, the sources or types of zinc influence its absorption and utilization. A 42-day trial was conducted with 216 1-day-old broiler chicks to assess their performance, immune organ weight changes and caecal microbial population when fed diet supplemented with different zinc types and their combinations. The chicks were divided into six groups and randomly assigned to zinc dietary treatment groups which includes control (C) diet, diets supplemented with 60mg/kg Nano zinc (NZn), 60mg/kg Zinc- Methionine (Zn-M), 60mg/kg Zinc Oxide (ZnO), 30mg/kgNZn+30mg/kg Zn-M and 30mg/kg NZn + 30mg/kgZnO. All treatment groups except control were given antibiotics. Data on growth performance, immune organs (thymus, bursa of fabricus and spleen) weights and caecal microbial load were collected and subjected to One-way Analysis of Variance using SPSS version 23. Results showed that performance at starter phase and overall performance did not vary with the supplementation of different zinc types and their combinations. There was significant ($P<0.05$) increase in spleen size among the zinc supplemented groups particularly the Zn-M group (0.07%). Total bacteria count of the caeca digesta was statistically similar ($P>0.05$) among all treatment groups. The prevalence of selected and identified bacteria in the caeca varied from present to highly present across the various treatment groups. This study concludes that zinc sources had no varied significant influence on growth performance (feed intake, weight gain and feed conversion ratio), bacterial load, however spleen size increased among the zinc supplemented groups particularly the Zn-M group.

Keywords; Zinc sources, lymphoid organs, caecal bacteria and performance

Performance de croissance, modifications du poids des organes immunitaires et population microbienne cœcale chez des poulets de chair nourris avec des régimes supplémentés par des sources de zinc nanoparticulaire et organique



Résumé

L'application de zinc dans l'alimentation des poulets de chair soutient la performance de croissance, le développement osseux et la fonction immunitaire. Cependant, les sources ou types de zinc influencent son absorption et son utilisation. Un essai de 42 jours a été réalisé avec 216 poussins de chair âgés de 1 jour pour évaluer leur performance, les modifications du poids des organes immunitaires et la population microbienne cœcale lorsqu'ils sont nourris avec un régime supplémenté par différents types de zinc et leurs combinaisons. Les poussins ont été répartis en six groupes et assignés au hasard à des groupes de traitement alimentaire au zinc comprenant : un régime témoin (C), des régimes supplémentés avec 60 mg/kg de Zinc Nano (NZn), 60 mg/kg de Zinc-Méthionine (Zn-M), 60 mg/kg d'Oxyde de Zinc (ZnO), 30 mg/kg de NZn + 30 mg/kg de Zn-M et 30 mg/kg de NZn + 30 mg/kg de ZnO. Tous les groupes de traitement, sauf le témoin, ont reçu des antibiotiques. Les données sur la performance de croissance, le poids des organes immunitaires (thymus, bourse de

Fabricius et rate) et la charge microbienne cœcale ont été collectées et soumises à une Analyse de Variance à un facteur en utilisant SPSS version 23. Les résultats ont montré que la performance en phase de démarrage et la performance globale ne variaient pas avec la supplémentation des différents types de zinc et de leurs combinaisons. Il y a eu une augmentation significative ($P < 0,05$) de la taille de la rate parmi les groupes supplémentés en zinc, particulièrement le groupe Zn-M (0,07 %). Le dénombrement total des bactéries dans le digesta cœcal était statistiquement similaire ($P > 0,05$) parmi tous les groupes de traitement. La prévalence des bactéries sélectionnées et identifiées dans le cœcum variait de "présente" à "très présente" selon les différents groupes de traitement. Cette étude conclut que les sources de zinc n'ont pas eu d'influence significative variable sur la performance de croissance (consommation alimentaire, gain de poids et indice de consommation), ni sur la charge bactérienne ; cependant, la taille de la rate a augmenté parmi les groupes supplémentés en zinc, en particulier le groupe Zn-M.

Mots-clés : Sources de zinc, organes lymphoïdes, bactéries cœcales, performance.

Introduction

The ever-increasing human population and high demand for animal protein continuously calls for increasing livestock production. Poultry production particularly broilers have been an established enterprises consistently supplying animal protein. Farmers are always interested in increasing the profit margin by raising broilers to table size within the shortest possible time as well as minimize losses due to incidence of diseases. Antibiotic growth promoters have been extensively used to achieve the two purposes (Cromwell, 2002; WHO, 2017); however, various shortcomings (residue in livestock products, development of antibiotics resistance by organisms, cost, e.t.c) have been reported which has necessitated search and use of various alternatives (Ayoola *et al.*, 2023). Several researches have experimented different alternatives to antibiotics such as probiotics, prebiotics, symbiotics, herbs, essential oils, minerals etc (Lee *et al.*, 2022; Wickramasuriya *et al.*, 2022). The use of minerals has gained interest from researchers and feed producers as alternative to antibiotics growth promoters (Yang *et al.*, 2017; Foltz *et al.*, 2017; Dukare *et al.*, 2020; Ayoola *et al.*, 2023). Various minerals are available with different efficacy as influenced by the mineral types, forms, sources and route of administration. Zinc is one the commonest

minerals that have been used extensively in broiler production (Zhao *et al.*, 2014; Yang *et al.*, 2017; Foltz *et al.*, 2017; Zakaria *et al.*, 2017).

Zinc, an essential trace element for poultry contributes to the maintenance of growth performance, skeletal development and immune function of chickens (Ao *et al.*, 2007; Liu *et al.*, 2011). It ensures normal growth, health, fertility, development of bones and feathers and regulates appetite in broilers (Shao *et al.*, 2014). The National Research Council's (NRC) recommendation that 40 mg/kg of feed be given to broiler chicks seems to be based only on growth performance (Burrell *et al.*, 2004). Higher Zn levels, but not exceeding 70 mg/kg, might need to be supplemented for the duration of production (Chitithoti *et al.*, 2012).

Zn is added to broiler diets in inorganic feed-grade forms, such as zinc sulfate (ZnSO₄) (Rossi *et al.*, 2007) or zinc oxide (ZnO) (Wojciech *et al.*, 2007). Organic Zn sources, such as Zn methionine (Zn-Met) and Zn amino acid complex (ZnAA), have been used in the poultry feed industry owing to their potentially higher Zn bioavailability (Salim *et al.*, 2010) when compared with the inorganic forms. Nano-zinc sources such as Zinc oxide nanoparticles (ZnOPs) represent a good alternative to the conventional zinc sources in

livestock feeding, specifically as an enhancer of broiler growth performance (Badawi and Behairy, 2017; Bami *et al.*, 2018). Researches tailored towards assessing the efficacy of combinations of various forms of zinc in the diet of broilers are very few, hence this study was conducted to compare the individual and combined use of these zinc sources in the diet of broiler chickens as they influence their performance, immune organs and caecal microbial population.

Materials and Methods

Experimental site

The experiment was carried out at the Teaching and Research Farm, 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 76mm above the sea level. The vegetation is located within the derived savannah zone of South-Western Nigeria. The area is characterised with humid climate, a mean annual rainfall of 1037mm and temperature of about 30.4°C (Google Earth, 2023).

Source of test ingredients

The different dietary zinc sources (zinc oxide, zinc methionine and zinc oxide nanoparticles) were acquired from a reputable store in Abeokuta, Ogun State.

Experimental birds and management

A total of 216 1-day old Abor-Acre broiler chicks were acquired from a reliable hatchery. The pens were cleared and disinfected prior to the arrival of the birds. Birds were raised under intensive management using deep litter system over an experimental period of 42 days. Brooding was carried out for two weeks with extended daylight (about 5 hours) provided for chicks to feed well and heat source (charcoal pots) to maintain the environmental temperature suitable for chick's growth. Birds were fed *ad libitum* with commercial feed and fresh water daily. Medications (vitamins and

coccidiostat) and vaccines (Gumboro and Lasota) were given as at when due during study. Antibiotics (Enrofloxacin) was applied in water for the control treatment only according to the manufacturer's recommendation.

Experimental diets

The birds were fed commercial diets at both starter (22%CP and 3000 Kcal/kg ME) and finisher (18%CP and 3150 Kcal/kg ME).

Experimental design

The day-old chicks were allocated into 6 treatments and each treatment group was subdivided into three replicates with 12 birds each. The treatment groups include, the control, Control diet + 60mg/kg Zinc oxide nanoparticles, Control diet + 60mg/kg Zinc methionine (organic), Control diet + 60mg/kg Zinc oxide (inorganic), Control diet + 30mg/kg Zinc oxide nanoparticles + 30mg/kg Zinc methionine and Control diet + 30mg/kg Zinc oxide nanoparticles + 30mg/kg Zinc oxide.

Data collection

Body weight and weight gain

Birds were weighed at the commencement of the experiment and subsequently on weekly basis.

Average body weight(g)

$$= \text{Total weight of birds (g)} / \text{Total number of birds}$$

Weight gain (g)

$$= \text{Final weight (g)}$$

$$- \text{Initial weight (g)}$$

Average daily weight gain

Average Daily Weight Gain

$$= (\text{Final weight} - \text{Initial weight} / \text{Number of days})$$

Feed intake and average feed intake

It is the total feed consumed by the birds in each group. The amount of feed given to birds and leftover was measured weekly to determine the feed intake.

$$\begin{aligned} \text{Feed Intake}(g) & \\ &= \text{Feed given}(g) \\ &\quad - \text{Feed leftover}(g) \end{aligned}$$

$$\begin{aligned} \text{Average feed intake per birds} & \\ &= (\text{Feed intake} / \text{number of birds per replicate}) \end{aligned}$$

Feed conversion rate

This is the amount of feed converted to flesh in the birds. It was calculated as:

$$\begin{aligned} \text{Feed conversion ratio} & \\ &= \text{Total feed intake}(g) / \text{Total weight gain}(g) \end{aligned}$$

Mortality percentage

This measured the number of deaths recorded in birds. It was calculated as the total number of dead birds divided by the total number of birds expressed in percentage.

$$\begin{aligned} \text{Mortality}(\%) & \\ &= \frac{\text{Total number of dead birds}}{\text{Total number of birds per replicates}} \times 100 \end{aligned}$$

Immune response (lymphoid organs weight)

Two birds were selected from each replicate and sacrificed by cutting the carotid artery of the neck and allowed to bleed for 2 minutes for lymphoid organ weight determination. The spleen, thymus and bursa of fabricius were removed from the carcasses and weighed using the sensitive scale to determine the immune organ weight. Immune organ index was calculated using the weight of the immune organs and live body weight of respective birds.

$$\begin{aligned} \text{Immune organ index} & \\ &= \frac{\text{Weight of immune organ}}{\text{live body weight}(g)} \times 100 \end{aligned}$$

Microbial count

The caeca of the slaughtered birds for each replicates were cut open and its content emptied into sample bottle with clear labeling for identification and taken into the laboratory for analysis. Total bacterial count of the representative sample from the caeca of each bird was carried out on a nutrient agar by plate

count method. Serial dilutions were made and spread uniformly on nutrient agar plates which were incubated at 37°C for 48 hours. Different colonies obtained were strained using grams strain and subsequently count was done for different bacteria types and estimated in log₁₀CFU/ml as described by Wynn *et al.* (2013).

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 was separated using Duncan multiple range test as contained in the software at 5% level of significance.

Results

Effect of dietary supplementation of different zinc types and their combinations on growth performance of broiler chickens at starter phase

Effect of dietary supplementation of different zinc types and their combinations on the growth performance of broiler chickens at starter phase (0–4weeks) is presented in Table 1. All parameters measured were not significantly (P>0.05) different.

Effect of dietary supplementation of different zinc types and their combinations on growth performance of broiler chickens at finisher phase

Effect of dietary supplementation of different zinc types and their combinations on overall growth performance of broiler chicken at 42nd day (6weeks) is presented in Table 2. All parameters measured were not significantly (P>0.05) different.

Effect of dietary supplementation of different zinc types and their combinations on the immune organs of broiler chickens

Effect of dietary supplementation of different zinc types and their combinations on the immune organs of broiler chickens is presented on Table 3. All parameters measured were not

significantly ($P>0.05$) different with the exception ($P<0.05$) of spleen. Spleen size was least in nano-zinc and control birds (0.04% and 0.04%) and highest in birds fed dietary supplementation of organic zinc (0.07%).

Effect of dietary supplementation of different zinc types and their combinations on microbial load and prevalence of some bacteria in broiler chickens

Effect of dietary supplementation of different zinc types and their combinations on microbial load and prevalence of some bacteria in broiler chickens is presented in Table 6. The prevalence of some of the selected species varied with different zinc types and their combinations. Total bacteria count across treatment were 1.50 (nano), 1.77 (organic), 1.67 (inorganic), 1.40 (nano and organic), 1.87 (nano and inorganic) and 1.40 (control). *Staphylococcus aureus* varied from present to moderately present in birds supplemented different zinc types but highly present in the control group. *Streptococcus faecalis* was reduced across the treatment groups except in birds fed inorganic zinc and the control birds. *Klebsiella sp* was also moderately present across the groups.

Table 1: Effect of dietary supplementation of different zinc types and their combinations on growth performance of broiler chickens at starter phase

Parameters	Zinc Types and their Combinations					Control	SEM	P-Value
	NZn	Zn-Met	ZnO	NZn+Zn-Met	NZn+ ZnO			
Initial body wt(g)	42.28	42.31	42.32	42.33	42.44	42.53	0.06	0.87
Final body wt(g)	1418.06	1417.64	1341.03	1414.9	1361.8	1334.72	16.9	0.51
Total body wt gain(g)	1375.78	1375.33	1297.42	1371.28	1319.36	1292.2	17.02	0.51
Feed intake (g)	1939.17	1976.56	1843.56	1893.94	1859.17	1883.78	17.85	0.26
Feed conversion Ratio	1.42	1.44	1.43	1.38	1.41	1.46	0.01	0.77
Mortality(%)	0.00	0.00	2.78	2.78	0.00	0.00	0.63	0.57

Table 2: Effect of dietary supplementation of different zinc types and their combinations on growth performance of broiler chickens at finisher phase

Parameters	Zinc Types and their Combinations						SEM	P-Value
	NZn	Zn-Met	ZnO	NZn+Zn-Met	NZn+ ZnO	Control		
Initial body wt(g)	42.28	42.31	42.32	42.33	42.44	42.53	0.06	0.87
Final body wt(g)	2359.72	2355.56	2145.83	2347.12	2323.89	2251.67	35.13	0.33
Total body wt gain(g)	2317.45	2309.14	2097.84	2297.86	2150.72	2206.32	37.00	0.45
Feed intake(g)	4238.08	4617.54	4390.00	4505.94	4150.43	4260.22	65.33	0.31
Feed conversion Ratio	1.83	2.01	2.10	1.96	1.95	1.94	0.09	0.69
Mortality(%)	0.00	8.33	11.13	13.89	2.78	5.56	1.81	0.23

^{abc}Means within row with different superscript differs significantly (P<0.05)

Table 3: Immune organs of broiler chickens fed diet supplemented with different zinc types and their combinations

Parameters (%)	Zinc Types and their Combinations						SEM	P-Value
	NZn	Zn-Met	ZnO	NZn+Zn-Met	NZn+ZnO	Control		
Thymus	0.21	0.15	0.2	0.14	0.15	0.18	0.02	0.86
Bursa	0.18	0.12	0.13	0.09	0.16	0.13	0.01	0.46
Spleen	0.04 ^b	0.07 ^a	0.05 ^{ab}	0.05 ^{ab}	0.06 ^{ab}	0.04 ^b	0.00	0.04

^{abc}Means within row with different superscript differs significantly (P<0.05)

Table 4: Microbial load and prevalence of some bacteria as influenced by dietary supplementation of different zinc types and their combinations

Parameters	Zinc Types and their Combinations						SEM	P-Value
	NZn	Zn-Met	ZnO	NZn+Zn-Met	NZn+ ZnO	Control		
TBCx10 ⁵ (cfu/mL)	1.50	1.77	1.67	1.40	1.87	1.40	0.80	0.45
<i>Escherichia coli</i>	++	+++	+++	++	++	+++		
<i>Pseudomonas sp</i>	+++	+++	+++	++	+++	+		
<i>Streptococcus faecalis</i>	++	+	+++	++	++	+++		
<i>Staphylococcus aureus</i>	+	++	+	++	++	+++		
<i>Klebsiella sp</i>	++	+++	++	+++	+	+		

TBCx 10⁵ cfu/ml = Number of viable bacteria x serial dilution ratio/ volume of inoculum used

+: Present

++: Moderately present

+++ : Highly present

Discussion

The overall growth performance of broiler chickens was not affected irrespective of the zinc types at both starter and the finisher phase of production; this is in accordance with other studies (Jondreville *et al.*, 2007; Salim *et al.*, 2008; Schlegel *et al.*, 2010). There are also reports (Foltz *et al.*, 2017; Yang *et al.*, 2017; Zakaria *et al.*, 2017) that zinc addition on broiler production performances, average (daily intake, average weight gain, feed conversion ratio and mortality) were not significantly affected by different zinc types.

The absence of any significant difference in performance between groups fed diets with or without supplemental Zn could be due to the fact that Zn content of basal diets (about 30mg/kg) is adequate to maintain the growth of broiler chickens necessitating no further replenishments in diets (Stahl *et al.*, 2015). However, in contrast to this study, there are certain reports of significant effects of different Zn sources on growth parameters (Liu *et al.*, 2011; Rajendran, 2013; Savari *et al.*, 2015; Gold *et al.*, 2018) of broiler chickens.

Lymphoid organs (bursa of fabricius, thymus and spleen) are part of the structure and function of the immune system in broilers that can protect the body from attack by micro-organisms (Kidd *et al.*, 2019). The significance of spleen size among other parameters measured indicates that primary lymphoid organs (thymus and bursar of fabricus) were not influenced by the supplementation of various zinc types. This further implies that production of B- cells and T- cells in bursa of fabricus and thymus, respectively were not affected.

According to Gheisari *et al.* (2010), dietary supplementation of the trace minerals did not significantly affect lymphoid organ weights. Nevertheless, the relative weights of bursa of Fabricius and spleen in group fed diet

containing high levels of zinc, manganese and copper were higher than the other groups. Bartlett and Smith (2003) explained that Zn addition to broiler diets did not affect the relative weight of lymphoid organs because zinc consumed by broilers was used to support the development of organs related to the immune system. Smith and Hunt (2004) reported that the function of spleen is to support the development of lymphocytes, which have a crucial function in cellular and humoral immunity.

According to Zhang *et al.* (2014) and Lewis *et al.* (2019), birds with heavier immune organs usually show stronger immunity. In addition, as stated by Moller and Erritzoe (2000), the mass size of the spleen is closely related to the level of humoral immunity and a large spleen size will result in high humoral immunity and *vice versa*. The significant increase in spleen size in birds supplemented only organic zinc (zinc-methione) could be as a result of role of methionine in avian immune functions. Swain and Johri (2000) Stated that methionine promotes antibody production and cell-mediated responses in broiler chickens. Wu *et al.* (2004) reported that the weight and relative weight of spleens were decreased in methionine deficient birds, which indicated that the development of the spleens was impacted by methionine deficiency. The smaller spleen size noted in birds supplemented only nano zinc and control can not be adduced to poor immune response to pathogenic challenges since the birds did not present any obvious signs of infection. Also, mortality recorded at both starter and finisher phase did not reflect any incidence of infection. Zinc has an important function in protecting from infection of pathogen (Zhang *et al.*, 2012) and can effectively eliminate diseases by inducing the expression of antibacterial substances such as antibacterial peptides

(Talukder *et al.*, 2011). This result showed no marked difference in the presence of selected bacteria (*Escherichia coli*, *Pseudomonas sp.*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Klebsiella sp.*) species evaluated between the control diet and groups on diets supplemented with different zinc types and their combinations. This result is in agreement with Lee *et al.* (2005) who reported that there was no significant difference in the abundance of selected bacteria species in the gut microbiota of broiler chickens between the control group and the groups fed different zinc types. Moreover, the experiment was conducted in floor pens allowing for re-infection and increased pathogenicity before immunity developed; this factor could explain the presence of bacteria (El-Maddawy, 2022). The similar total bacteria load recorded in this study depicts that zinc supplementation regardless of type or combination could elicit antibacterial effect in broiler chickens. It also suggests that the dosage adopted can sufficiently compete favourably in terms of efficacy with antibiotics in broiler chickens.

Conclusion

The dietary supplementation of various zinc types and their combinations investigated in this study indicates that growth performance of broiler chickens did not differ significantly. The immune system of the birds was not markedly affected as lymphoid organs had similar weight except spleen which was significantly heaviest in birds supplemented zinc-met singly. Also, the bacteria inhibiting or depopulation traits of the different zinc types and their combinations were found similar even comparable with the antibiotics used. There are varying degrees in prevalence of selected bacteria (*Escheria coli*, *Pseudomonas sp.*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Klebsiella spp.*) in the caeca of broiler chickens.

The result of this study suggests that any of the zinc types can be used either solely or combined as alternative to antibiotics for improved broiler performance and reduced caecal bacterial load. However, the sole use of Zinc-methionine has the tendency to elicit better immune status in the birds.

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