INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 7, p. 23-29, 2013

RESEARCH PAPER OPEN ACCESS

The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period

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Key words: Zinc oxide nanoparticles, performance, serum lipids, broiler chickens.

doi: http://dx.doi.org/10.12692/ijb/3.7.23-29 Article published on July 25, 2013

Abstract

The aim of current study was to investigate the effect of different levels of zinc oxide nanoparticles (ZONPs) on growth Performance, digestive organs and serum lipids concentration in broiler chickens during starter phase (1-21d). Three hundred one-d-old broilers (Ross- 308) were randomly divided into 5 treatments including 75 birds. All treatments were replicated 4 times, using 15 birds in each pen. The experimental diet was T1) basal diet (control, without ZONPs), T2, T3, T4 and T5 supplementation basal diet with 30, 60, 90 and 120 mg/kg of ZONPs respectively. This study was lasted 21 d and birds accessed as *ad libitum* to feed and water throughout research. Live body weight (LBW) and feed intake (FI) measured as weekly and feed conversion ratio (FCR) calculated at the final of research. On d 21, 4 birds in each group were selected as randomly, blood samples collected from bronchial vein and after centrifuging (3000 rpm for 15 min at 4°C), serum removed and stored in -20 °C until analysis. The results revealed that ZONPs had significantly affected on body weight gain ($P<0.05$) and feed intake ($P <0.05$) among treatments including ZONPs and control group from 1 to 21 d. The feed intake was significantly decreased at levels of 60 and 90 mg/kg of ZONPs. As well, feed conversion ratio had significantly decreased ($P<0.05$) at the level of 60 mg/kg of ZONPs compared with control and others groups. ZONPs had resulted in low density lipoproteins (LDL) ($P>0.05$), triglyceride (TG) ($P>0.05$) and cholesterol ($P>0.05$), as well as increased high density lipoproteins (HDL) ($P<0.05$), respectively compared to control treatment. In conclusion, ZONPs can improve growth performance especially at the levels of 30 to 90 mg/kg of diet in broiler chickens.

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Introduction

After prohibit of antibiotics as growth promoters (GPA) in poultry nutrition in the worldwide, researcher had attempt that find alternative that to have positive characteristics without negative side effect (Iji *et al*., 2001). Therefore, nutritionist finds other alternatives with natural origin for example: medicine plant, probiotic, prebiotic, organic acid etc. In recently years, researchers have used from nanotechnology science and those products as additives in broiler nutrition to achieve positive effects in poultry production, some of products is: nanosilver, nanoselenium and zinc oxide nanoparticles (Ahmadi and Rahimi 2010; Sawosa *et al*., 2007).

Zinc is one of the essential elements that need to growth and different physiological processes (Walravens, 1979). Zinc is a part of liver enzymes as cofactor including alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), and aspartate aminotransferase (AST); as well it is typically found in large quantities in liver enzymes (Bennett et al., 2001). Decreasing level of zinc in the plasma has been resulted impaired physiological duties and variety of liver diseases including cirrhosis and hepatitis (Halifeoglu et al., 2004; Cesur et al., 2005). Zinc is required for the activity of over from 250 to 300 enzymes and participates in many enzymatic and metabolic functions in the body of animals (Prasad and Kucuk, 2002).

Zinc is one of the structural component of wide variety proteins and dependent enzymes like superoxide dismutase (SOD) that act as essential component of antioxidant defense system (Bao and choct, 2009). It has been indicated that Zn increases the synthesis of different of metallothionein, which a cystine-rich protein that acts as a free radical scavenger (Oteiza *et al.,* 1996). As well as, other essentional responsibility zinc element is participating in growth, metabolism pathways, physiological and biosynthetic processes within the body of poultry and animals (Cesur *et al.,* 2005). The above functions are primarily duty of zinc as catalysts or coenzyme factors in the body enzyme systems within cells. Zinc has an important role in the organism because of that is cofactor in more than 200 enzymes (Powell, 2000).

One of the most significant functions of zinc is related to its antioxidant role and its participation in the antioxidant defense system (Powell, 2000).

Zn enzymes are included in the synthesis and/or breaking down of carbohydrates, lipids, proteins, and nucleic acids and encompass all known classes of enzymes (Jahanian *et al.,* 2008). They are also constituents of hundreds of proteins involved in intermediary metabolism, hormone secretion pathways, and immune defense systems (Sunder *et al.,* 2008). Little information is available regarding the effects of zinc oxide nanoparticles on performance and blood parameters.

Therefore, our study had two main purposes: First, we decide to investigate the effect of ZONPs on performance traits and serum lipids on broilers. Secondly, on the condition that ZONPs had significantly positive affected on mention parameters? Which level is the optimum to practical broiler nutrition?

Material and methods

Birds and housing

A total of 300 one-day-old male broilers (Ross-308) with an initial BW of 43.3 ± 1.12 g were purchased from a local commercial hatchery (Poultry Breeding Company, Uremia, Beh Parvar, Iran). The chickens were allocated in house windowless and wire-floored pens (100 cm × 90 cm× 70 cm). House temperature was maintained at 33°C during the first 3 d of life and then was reduced gradually according to age until reaching 24°C at 21 d. Chicks received a continue lighting program (23D:1L) throughout study (21 d). Birds had free accessed to feed and water throughout the trial. There were five dietary treatments, with each treatment being applied to four pens of 15 chicks. All chicks were inoculated base on the program of vaccination of local veterinary organization, Kurdistan, Iran (Newcastle and inactivated infectious bursal disease vaccine on 7 and 21 d). All chicks used in this experiment were under current and approved animal care protocols through the Islamic Azad University (Shabestar, Iran), Animal Care Committee.

Basal diet and assay zinc

The basal diet (Table 1) was formulated to meet completely nutrient requirements broiler based on NRC (1994) recommendations. The dietary treatments were based on corn grain- soybean meal and balanced with others nutrients. We have analyzed basal diet for zinc contain by atomic absorption spectrophotometery (Perkin Elmer, Precisely Analyst 200, Absorption spectrophotometer). Samples of basal diet (four samples removed to high precision) were dried at 105 °C for 12 h; all samples were then dry-ashed at 550 °C for 16 h, solubilized in HCl, and filtered through 42 Whatman filter paper. Diets were analyzed for contents of Zinc (Method 967.02; AOAC International, 2000).

Experimental Diets

The ZONPs was provided by the US Research Nonmaterial's, Inc (Houston, TX 77084, USA). The product was a white powder with a measured ZONPs content of purity ≥99.99% and size of nanoparticles was 35 to 45 nm (median size was 40 nm). To provide experimental diets and increasing precision during mixing ZONPs in basal diet, first removed amount of ZONPs based on experimental levels and then finely mixed to five kg from basal diet. Experimental diets were: T1) basal diet (control, without ZONPs), T2, T3, T4 and T5 supplementation basal diet with 30, 60, 90 or 120 mg/kg ZONPs respectively.

Sampling collection

On d 21, four chickens were randomly selected from each replicate for both bloods sampling analysis and removed digestive organs to determine indices growth weight. As well as to decrease the direct effect of feeding on serum lipids before sampling, chicks were given a feed withdrawal period of 12 h.

Statistical analysis

The data were subjected to one way ANOVA as a completely randomized design using the general linear models (GLM) procedure of SAS software (SAS Institute, 2003). Significant differences among the means were determined by using Duncan's multiple-range test (Duncan, 1995) at $P<0.05$.

Results and discussion

Growth performance

The results related to the effect of ZONPs on performance traits at 21 d presented in Table 3. Live body weight (LBW: 1124.2 g) in birds fed on diet including 60 and/or 90 (LBW: 1191.5) mg/kg of ZONPs had significantly ($P<0.05$) higher than control (T1), T2 and T5. The lowest LBW (940.95 g) observed in control treatment. Feed intake was significance ($P<0.05$) difference among treatment, the lowest (1228.5 g) and highest (1391.3 g) feed intake observed in T1 (control) and T4 treatment group, respectively. Results in the present study is agreement with reports of Huang *et al.* (2007) and Hudson *et al.* (2004), they reported different levels of zinc had significantly affected on body weight gain of broilers, and finding this study is adversely with results of Pimental *et al.* (1991) reported that additive zinc no significant affect on feed intake, feed conversion ratio and live body weight of broiler chickens. One possible explanation for this difference may be related to new physico-chemical properties of ZONPs. Mortality was low (1.86%) and no observed any significant effect among treatment (data not shown). Most of the mortality (>40%) occurred in birds fed diet including 120 mg ZONPs per kg diet. On reason to explanation is that mention level (120mg/kg) was induced toxicity in broilers. Wang *et al.* (2007) that despite being an essential trace element, in fact is toxic at a level not much higher than the requirement.

Digestive and visceral organs

As shown in Table 4, the results indicated that ZONPs no significant effect on digestive and visceral organs compared with control and others treatment. Of course, weight of abdominal fat and small intestine in birds fed supplementation diet with 60 and/or 90 mg of ZONPs/kg was lower than other treatments (P>0.05). We have observed significantly (P<0.05) increased liver weight (as percentage of LBW) in birds fed diet supplementation with 90 mg ZONPs/kg in comparison to other treatments. This finding was consistent with previous reports that none of visceral organs were significantly affected by the level of zinc in the diet (Mengheri *et al*., 1988). An explanation for increasing liver weight may be due to positive effect of ZONPs on better digestion and absorption of nutrient in gastrointestinal tract (GIT) and or probably to be higher bioavailability zinc in the form of nanoparticles. As well as, the mention reason caused that zinc retention was higher in liver of broiler after absorption and inters to portal of blood. Sharma *et al.* (2012) reported that no obvious difference was observed in the organ weights (liver, kidney and brain) of control mice and ZnO nanoparticles treated mice.

Table 1. Ingredient composition and calculated analysis of the basal diet (%) [1]

Ingredient (%)	value
Corn grain	60.83
Soybean meal (48 % CP)	32.55
Soybean oil	2.60
Limestone powder	1.20
Dicalcium phosphate (DCP)	1.40
Common salt (NaCl)	0.25
Vitamin Premix[2]	0.25
Trace mineral Premix[3]	0.25
DL-Methionine	0.21
L-Lysine HCL	0.05
Threonine	0.41
Total	100.00
Calculated analysis	
ME (kcal/kg)	3017
CP (%)	21.86
Calcium (%)	0.93
Available phosphorus (%)	0.45
Lysine (%)	1.21
Arginine (%)	1.48
Methionine (%)	0.54
Met+Cys	0.86
Calculated Zn (mg/kg)	26.12

[1]Basal diet supplied 26.15 mg of Zn per kg diet and without ZONPs.
[2]Provided per kilogram of diet: vitamin A, 15,000 IU; cholecalciferol, 3,930 IU; vitamin E, 30 IU; vitamin K, 3.0 mg; thiamin (as thiamin mononitrate), 2.4 mg; riboflavin, 9.0 mg; vitamin B6, 4.5 mg; vitamin B12, 0.021 mg.
[3]Calcium pantothenate, 30 mg; niacin, 45 mg; folic acid, 1.2 mg; biotin, 0.18 mg; choline (as choline chloride), 700 mg; Cu, 8 mg; Mn, 100 mg; Fe, 80 mg; I, 0.35 mg; Se, 0.15 mg. Zn; 26.12 mg.

Table 2. Effect of dietary ZONPs on live body weight, feed intake, and feed/gain of broilers at d 21[1]

Growth performance	Experimental diets						
	T1 Control	T2 30 mg	T3 60 mg	T4 90 mg	T5 120 mg	SEM	P-value
LBW (g)	940.95[b]	1094.2[a]	1124.2[a]	1191.5[a]	1014.8[b]	128.2	0.013
FI (g)	1228.5[b]	1252.9[b]	1322.3[a]	1391.3[a]	1276.6[b]	136.3	0.022
FCR(g/g)	1.30[a]	1.14[c]	1.17[c]	1.16[c]	1.25[b]	0.03	0.019

[a-c] Means in a row with different superscripts are significant (P<0.05). [1]Each value represents the mean of four pens with 15 chicks per pen.

Serum lipids

The mean of total cholesterol (T- COL), triglyceride (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) in broilers of different groups is presented in Table5. Our research showed decreases (P>0.05) of triglycerides values, total cholesterol (P>0.05), LDL-cholesterol (P>0.05) and increase (P<0.05) of HDL-cholesterol values in birds fed 60 or 90 mg of ZONPs/kg in comparison to the control at d 21. As well as, the highest concentration of HDL-C and LDL-C observed in the serum of broilers that fed diets including 90 and 120 mg of ZONPs/kg of diet, respectively. Our results is in contrast with Malcoln-calis *et al.* (2000) they reported that serum cholesterol concentrations were not altered by added zinc (0.26, 0.25, and 0.27 mg/mL for 20, 100, and 200 mg of added zinc/kg; respectively. Another reason to explain mention results may be due to induce oxidative stress and per oxidative membrane lipids cells of birds that fed diet including 120 mg/kg of ZONPs (T5). Syama *et al.* (2013) reported that Liver tissue exposed to three different concentrations of nanoparticles showed significant increased lipid peroxides formation. In conclusion, the finding present study indicated that the supplementation of ZONPs in broiler diets could improve growth performance and some parameters of blood serum that this condition could be leaded to improve performance traits and health of broiler chickens. ZONPs supplementation exhibited significant effects with the optimum responses at the level of 60 from 90 mg/kg. As well as, based on results this trial we can proposal supplementation diets with levels of 60 to 90 mg/kg ZONPs in practical nutrition of broiler chickens.

Table 3. Effect of dietary ZONPs on the growth index of digestive and visceral organs at d 21 of broilers age[1]

Organs growth index (% LBW[2])	Experimental diets						
	T1 Control	T2 30 mg	T3 60 mg	T4 90 mg	T5 120 mg	SEM	*P-value*
Abdominal fat pad	2.17	2. 16	2.09	2.10	2.16	0.18	0.732
Small intestine weight	2.23	2. 25	2.22	2.19	2.21	0.43	0.902
Small intestine length (Cm)	189	186	191	192	193	16.43	0.802
Liver	2.8^{b}	2.9^{b}	2.9^{b}	3.5^{a}	2.6^{b}	0.13	0.042
Pancreas	0.64	0.63	0.66	0.67	0.65	0.12	0.788
Gizzard	2.21	2.22	2.21	2.19	2.20	0.18	0.774
proventriculus	1.09	1.08	1.06	1.07	1.06	0.15	0.701
Heart	0.98	1.0	1.01	0.98	0.97	0.11	0.896

[a-b] Means in a row with different superscripts are significant (P<0.05).

[1]Each value represents the mean of 4 pens with 15 chicks per pen.

[2]LBW= Live body weight

Table 4. Effect of dietary ZONPs on the serum lipids concentration at d 21of broiler age[1]

Serum lipids	Experimental diets						
	T1 Control	T2 30 mg	T3 60 mg	T4 90 mg	T5 120 mg	SEM	*P-value*
Total cholesterol (mg/dL)	135.25	135.00	135.25	138.75	149.5	13.81	0.603
TG (mg/dL)	56.00	54.25	46.75	55.75	59.00	5.50	0.958
LDL-C (mg/dL)	46.54	47.09	46.76	46.71	48.27	4.09	0.772
HDL-C (mg/dL)	87.17^{b}	89.03^{a}	89.73^{a}	92.61^{a}	83.04^{b}	4.23	0.041
VLDL (mg/dL)	21.34	22.07	20.19	20.48	23.16	3.24	0.613

[a-b] Means in a row with different superscripts are significant (P<0.05).

[1]Each value represents the mean of four pens with 15 chicks per pen.

Acknowledgment

This article is adopted from Ph.D. thesis in Poultry Science, Islamic Azad University, Shabestar Branch (thesis supervisor: Dr. Y. Ebrahimnezhad). The authors gratefully acknowledge the support of Eng. Pourahmad, also especially thank for Eng. Doulgari to help us during analysis of paraclinical examination.

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