Effects of zinc oxide nanoparticles on antioxidant status, serum enzymes activities, biochemical parameters and performance in broiler chickens

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Abstract This research was conducted to investigate the effect of zinc oxide nanoparticles (nano-ZnO) in diets on the performance, antioxidant status, enzymes activity and some hematological parameters of broiler chickens. A total of 600 one–day-old male Ross 308 broilers were allocated to four treatment groups with five replications of 30 each from day 1 to 42 day of age. The birds were fed continuously with diets supplemented with 0 (control), 10, 20 and 40 mg/kg of nano-ZnO. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were measured from 1 to 42 d of age. Triglycerides, high density lipoproteins (HDL), activity of alkaline phosphatase (ALP), aspartate transferase (AST), alanine transferase (ALT), creatine kinase (CK), total antioxidant capacity (T-AOC),superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GPx), were measured at 42 d of age. Broilers receiving 10mg/kg nano-ZnO had significantly ($P<0.05$) greater ADG and birds in the 20 mg/kg nano-ZnO group had significantly ($P<0.05$) lower AFCR than other groups. Serum concentrations of HDL, cholesterol, SOD and ALP activity were significantly ($P<0.05$) increased at 20 mg/kg nano-ZnO. Moreover, Serum concentration of MDA was significantly ($P<0.05$) reduced at 20 mg/kg nano-ZnO. Other biochemical parameters were not significantly ($P>0.05$) affected by treatments. In conclusion, the results of this study showed the inclusion of 20 mg/kg nano-ZnO improved the growth performance and antioxidant status in broilers.

Keywords: zinc oxide nanoparticles, growth performance, antioxidant status, biochemical parameters, broilers

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Introduction

Zinc (Zn) is an important mineral with diverse functions in mammals and birds such as in nutrient metabolism, as a component of numerous metalloenzymes, appetite control, regulation of the immune system, oxygen free radical scavenging, and in transcription factors. Furthermore, Zn enzymes are involved in the synthesis and/or breaking down of carbohydrates, lipids, proteins, and nucleic acids, and encompass all known classes of enzymes (Liu et al., 2011). Zinc is capable of reducing post ischemic injury to a variety of tissues and organs through a mechanism that might involve the antagonism of copper reactivity. It is also reported that Zn is a necessary part of a superoxide dismutase enzyme in the antioxidant defense system. Although the evidence for the antioxidant properties of Zn is compelling, the mechanisms are still unclear. In poultry, Zn deficiency leads to decreased feed intake and collagen formation, in turn leading to lesions on the skin, delayed wound healing, long bone malformation, and poor feathering (Park et al., 2004).

The NRC (1994) requirement for Zn in broiler chickens is 40 mg/kg. In the broiler industry, it is a common practice to formulate diets containing 100–120 mg supplemental Zn kg^{-1} (Feng et al., 2010). Therefore, Zn has been used extensively as a feed additive in poultry diets. It has been reported that the effect of Zn varies from different sources, organic or inorganic; on the production performance (Zhao et al., 2014).The bioavailability of organic Zn is higher than that of inorganic Zn, but the application of organic Zn in animal diets is limited due to its higher cost (Zhao et al., 2014). In animal production, to meet the needs of animals, the added concentration of inorganic Zn is 20-to 30-fold higher than the normal requirement, due to the low utilization rate of inorganic Zn (Bratz et al., 2013). However, high dietary Zn can lead to an excess in the stool, causing environmental pollution. Furthermore, high Zn supplementation may affect the balance of other trace elements in the body and reduce the stability of vitamins and other nutrients, and long-term application can cause Zn residue in the animal body (Zhao et al., 2014).

Recently, the development of nanotechnology and its related products has rapidly progressed in different scientific areas; in fact, this branch of science has fundamentally affected human, animal, environment, and industrial lives. In this regard, Zinc Oxide (ZnO) nanoparticles have attracted a great deal of attention because nano-formulation particulates exhibit novel distinguishing qualities such as size, shape, large surface area, high surface activity, high catalytic efficiency, and strong adsorbing ability (Wijnhoven et al., 2009).

Compared with ZnO, nano-ZnO has a stronger chemical activity and participates in oxidation reactions with a variety of organic compounds. In addition, the permeability of nano-ZnO can also help prevent adverse gastrointestinal reactions and improve the absorption of medicine (Zhao et al., 2014). However, it is unclear whether nano-ZnO can improve growth performance, mortality and antioxidant status of broilers. Therefore, the main objective of the present research was to investigate the effects of nano-ZnO on serum enzymes activity, growth performance, blood parameters, and antioxidant status in broilers from 1 to 42 days of age.

Materials and methods

Birds and diets

A total of 600one-day-old male Ross 308 broiler chicks were randomly allocated to four treatments in a completely randomized design. Each treatment consisted of five replicates (cage) of 30 birds each. Birds were reared in floor pens and in an environmentally controlled house with a 23:1 light:dark cycle. The experimental birds had *ad libitum* access to water and mash diets (Table 1). Four experimental treatments were based on supplementing the diet with 0, 10, 20 or40 mg/kgnano-ZnO. The nano-ZnO was provided by the US Research Nonmaterial's, Inc. (Houston, TX 77084, USA). The product was a white powder with a measured nano-ZnO content of purity ≥99%. The sizes of the nano-ZnO were 35 to 45 nm with an average of 40 nm.

Growth performance

Body weight of birds and feed consumption were determined on the pen basis at 42 d of age, and average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were determined from 1 to 42 d of age. Feed intake was adjusted for mortalities, with

Table 1. Composition of experimental diets

Item	Starter (0 to 21 d)	Grower (22 to 42 d)
Ingredients (%)		
Corn	54.47	59.25
Soybean meal (44% protein)	22.5	20.75
Corn gluten meal	7	8
Fish meal	6.16	3
Soybean oil	6	5.7
Dicalcium phosphate	1.72	1.22
Limestone	1.2	1.3
Vitamin and mineral premix[1]	0.5	0.5
Salt	0.25	0.25
dl-Methionine	0.2	0
l-Lysine	0	0.03
Total	100	100
Calculated analysis		
ME (kcal/kg)	3100	3050
Crude protein(%)	22. 4	20.26
Calcium (%)	0.9	0.9
Available phosphorus (%)	0.4	0.35
Arginine (%)	1.3	1.3
Lysine (%)	1.14	1
Methionine (%)	0.53	0.4
Methionine + cystine (%)	0.9	0.75

1:Supplied per kilogram of premix: vitamin A, 11,000 IU; vitamin D3, 5,000 IU; vitamin E, 40 IU; vitamin K, 4 mg; riboflavin,5 mg; vitamin B6,4 mg; vitamin B12, 0.011mg; niacin, 50 mg; biotin, 0.01 mg; thiamine, 3 mg; zinc 80 mg; manganese oxide, 100 mg; selenium , 10 mg; iron sulfate 80 mg.

their ADG included in the calculation of FCR.

Blood sampling

At 42d of age, two birds per replicate (10 birds/treatment) were randomly selected and Blood samples were collected from the wing vein. Blood samples (1ml/bird) for serum metabolites were collected into tubes containing no anti-coagulant and then centrifuged (10 min, 3,000 rpm).

The serum were removed and stored at –20°C until further analysis. Serum concentrations of cholesterol, triglyceride, HDL and the ALP, CK, AST, and ALT enzyme activities were measured using an autoanalyzer (Autolab, PM 4000, Auto-analyzer Medical System, Rome, Italy) according to the instructions in the kits (Sigma Chemical Co, St. Louis, MO 63178-9916, USA).

Antioxidant parameters

Activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) were measured in 2 mL whole blood, washed and centrifuged (748 g for 10 min) three times with 0.9% NaCl (Hosseini Vashan et al., 2015). The washed-centrifuged erythrocytes volume was made up to 2.0 mL with cold redistilled water. Then, the lysate was prepared based on the instruction in the kits manual RANSEL and RANDOX (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK) to determine the activity of GPx and SOD, respectively. The absorbance was read spectrophotometrically (Alcyon300, USA.) at 340 and 505 nm for GPx and SOD, respectively. Serum MDA concentration was measured in accordance with Yagi. (1984) by spectrophotometer at 520 nm, and expressed as nmol/mL TBARS (thiobarbituric acid reaction substances) index.

Statistical analysis

Data were analyzed as a completely randomized design using the GLM procedure (SAS 9.1 institute 2002) and mean separation was performed by the Tukey's test ($P<0.05$).

Results and discussion

Growth performance

The effects of treatments on broiler growth performance are presented in Table 2. The birds receiving nano-ZnO had significantly ($P<0.05$) higher body weight gain and lower feed intake and feed conversion ratio compared with the control broilers. Zn is a known essential micronutrient for the growth of broilers. Zn deficiency results in reduced appetite depressed growth, and abnormalities of the skin and its appendages (Brooks et al., 2013; Sahraei et al., 2013, Petrovic et al., 2010). Consistent with these results, a number of researchers documented that dietary zinc supplementation increased growth rate, and improved feed efficiency in broiler chicks (Sandoval et al., 1998; Roberson and Edwards, 1994; Zhao et al., 2014; Fazilati, 2013). In the present study it was observed that appropriate levels of nano-ZnO (20 mg/kg) can promote body weight gain and provide a better feed conversion ratio compared with other groups. However the higher concentration of nano-ZnO (40 mg/kg nano-ZnO) inhibited body weight gain and increased the feed conversion ratio, where these effects were exacerbated with prolongation of the dietary regimen. Researchers (Zhao et al., 2014) suggested that appropriate concentrations of nano-ZnO are better than conventional organic Zn (ZnO) for improving the efficiency of feed utilization and growth performance in broilers, but excess nano-ZnO may have a toxic effect and thus inhibit broiler growth. They further proposed that compared with conventional ZnO, nano-ZnO has many desirable properties including strong chemical activity, oxidation reactions, and permeability. It has also been suggested that Zn is required by the fetus to support cell proliferation and tissue differentiation of developing organs (Hostetler and Kincaid, 2004). Additionally it has been suggested that Zn enhances pituitary hormone function (Ruth and Donald, 2000) and has many desirable prope-

Table 2. Effects of zinc oxide nanoparticles on performance of broiler chickens (42d)

Treatments (nano-ZnO in diet, ppm)	Body weight gain (g)	Feed intake (g)	Feed conversion ratio (g/g)
0	2760^{d}	5684^{c}	2.05^{a}
10	3105^{b}	5736^{a}	1.84^{c}
20	3191^{a}	5715^{b}	1.79^{d}
40	2988^{c}	5720^{b}	1.90^{b}
SEM	83.71	10.32	0.051
P-value	0.001	0.001	0.001

^{a,b}: Within columns, means with common superscript are not different ($P>0.05$).

rties, such as strong chemical activity, oxidation reactions, and permeability (Zhao et al., 2014).

Biochemical and enzymatic parameters

Table 3 shows 20 mg/kg nano-ZnO tended to increase the serum cholesterol. These results are consistent with those of Roberson and Edwards, 1994, who reported that there is a significant elevation in the serum HDL and cholesterol for the subjects in the zinc supplemented group. These researchers suggested that increased level of HDL and cholesterol is probably due to improvement in calories and fat intake after zinc supplementation. Additionally, it has been reported that zinc-deficient diets are accompanied by decreased plasma total cholesterol, LDL, HDL, and triglyceride concentrations. This can be due to diminished absorption of dietary lipids in addition to decreased intake of fat and calories intake (Wu and Sun, 2004). It is also congruent with the findings of (Hazim et al., 2011) that showed that supplementation zinc of diet in broilers increased plasma total cholesterol. These researchers suggested that an increase in the amount of cholesterol ingested slightly increases the plasma cholesterol concentration. Moreover, a change of cholesterol levels in blood plasma may be due to the zinc's role in enzyme action in that zinc forms an integral part of several enzymes (metalloenzymes) that are severed in lipid digestion and absorption (Hazim et al., 2011).

Table 4 show 20 mg/kg nano-ZnO increased ALP activity, but had no significant effect on ALT, CK and AST activates. This finding is inconsistent with (Fazilati, 2013) who reported that nano-ZnO (25-200 mg) showed significantly increase ($P<0.05$) activities in the ALT and AST enzymes in male rats. One reason to explain these differences may be related to the doses and time the animal was exposed to the nano-ZnO. It has been reported that levels above 50 mg/kg of nano-ZnO induce oxidative stress and increase the plasma level of ALT and AST (Sharma et al., 2009).

Ahmadi et al., 2014, reported nano-ZnO had no significant effects on ALT and AST activities in serum of broilers. Some researchers have also concluded Zn supplementation increased ALP activity in plasma (Levengood et al., 2000; Peretz et al., 2001). The significant increase in serum ALP activity in birds by 20 mg/kg of nano-ZnO as compared to other group may be attributed to the action of vitamin D_3, which has several effects on the intestine, kidneys and bones, increasing absorption of calcium into the extra cellular fluid and possibly promoting the formation ALP in the epithelial cells (Guyton and Hall, 2006). In addition, increased ALP activity may be attributed to increased concentrations of cholesterol by nano-ZnO (Table 3). Zaghari et al., (2009) reported that progesterone injection of broiler breeder pullets (20 week of age) affects serum glucose, triglycerides and cholesterol concentrations of hens. Therefore, the increase in the corticosteroids hormones secretion, epinephrine and norepinephrine leads to elevated ALP activity, but the mechanism is not totally clear (Al-Darraji, 2008).

Table 3. Effects of zinc oxide nanoparticles on serum lipid parameters of broiler chickens

Treatments (nano-ZnO in diet, ppm)	Cholesterol (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)
0	106.25^{c}	60.35^{c}	35.25
10	127.00^{b}	59.85^{c}	34.25
20	133.75^{a}	71.10^{a}	38.75
40	123.25^{b}	64.35^{b}	37.00
SEM	6.86	3.40	3.66
P-value	0.014	0.037	0.310

a,b: Within columns, means with common superscript are not different ($P>0.05$).

Table 4. Effects of zinc oxide Nanoparticles on serum activities of ALT, AST, ALP and CK of broiler chickens

Treatments (nano-ZnO in diet, ppm)	CK (U/L)	ALP (U/L)	AST (U/L)	ALT (U/L)
0	4020	1420^{b}	271	4.75
10	4388	1305^{b}	359	7.50
20	3973	1825^{a}	255	4.00
40	5655	1555^{ab}	349	6.25
SEM	1068	220	49	3.09
P-value	0.4068	0.04746	0.3297	0.0886

CK : Creatine kinase, ALP: Alkaline phosphatase, AST: Aspartate amino-transferase, ALT: Alanine amino-transferase.

a,b: Within columns, means with common superscript are not different ($P>0.05$).

Table 5. Effect of zinc oxide nanoparticles on the concentrations of MDA, T-AOC and antioxidant enzymes in broiler chickens

Treatments (nano-ZnO in diet, ppm)	MDA (nmol/ml)	SOD (U/gHb)	GPx (U/gHg)	T-AOC (nmol/ml)
0	3.17^{a}	3111^{b}	222	0.98
10	3.12^{a}	3210^{b}	196	0.81
20	2.42^{b}	3373^{a}	169	0.88
40	2.70^{ab}	2876^{c}	160	0.71
±SEM	0.21	254	16	0.15
P-value	0.015	0.021	0.112	0.711

MDA: Malondialdehyde, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, T-AOC: Total antioxidant activity.
^{a,b}:Within columns, means with common superscript are not different (P>0.05).

Antioxidant status and oxidative enzyme

There were significant differences were observed in the SOD activity and level of MDA in serum (Table 5). 20mg/kgnano-ZnO significantly (P<0.05) reduced MDA and increased SOD activity in the serum. MDA is an important index of lipid peroxidation and oxidative damage caused by ROS (Nielsen at al., 1997). Zn is considered a cofactor and it is a component of more than 240 enzymes and can influence oxidative processes. Generally, the chronic effect of antioxidation results in increased sensitivity to certain oxidative stresses (Powell, 2000).Researchers (Cunningham-Rundles et al., 1990) showed that Zn acts as an antioxidant to reduce cell membrane damage due to free radicals, although the mechanism was not specified in their study. The total antioxidant capacity (T-AOC) in the body contributes mainly to the dynamic balance of active oxygen, where T-AOC is an integrative parameter reflecting the status of all the antioxidants in serum and body fluids. Hepatic injury may lead to a reduction in T-AOC (Zhao et al., 2014). Moreover, it has been reported that Zn is an essential component in Cu-Zn-SOD, and dietary Zn levels positively correlate with Cu-Zn-SOD activity. It has been showed that Cu-Zn-SOD is involved in the cellular scavenging of free radicals and ROS (Prasad, 2008; Ozturk and Gumuslu, 2004). Here 20 mg/kg nano-ZnO had a significant effect on Cu-Zn-SOD activity in serum, while higher concentrations of nano-ZnO (40 mg/kg) were not associated with a significant growth in Cu-Zn-SOD activity in serum suggesting that excess nano-ZnO does not contribute to biological function (Zhao et al., 2014). These findings are consistent with those of previous reports (Duzguner and Kaya, 2007; Zhao et al., 2014) who reported that appropriate concentrations of nano-ZnO may stimulate Cu-Zn-SOD activity, and that enhanced Cu-Zn-SOD will suppress the generation of ROS and thus decrease MDA.

Conclusion

Addition of 20 mg/kg nano-ZnO to the broiler diet improved the growth performance and reduced serum concentration of cholesterol and malondialdehyde.

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چکیده این تحقیق برای بررسی اثرات نانوذرات اکسید روی بر عملکرد، وضعیت آنتی اکسیدانی، فعالیت آنزیمی و برخی فراسنجه‌های خونی در جوجه‌های گوشتی انجام شد. تعداد ۶۰۰ قطعه جوجه گوشتی یک‌روزه نر سویه راس ۳۰۸ به چهار تیمار آزمایشی با ۵تکرار و ۳۰ جوجه در هر تکرار از روز ۱ تا روز ۴۲ اختصاص یافتند. پرندگان به طور مرتب با جیره‌های مکمل شده با مقادیر ۰ (شاهد)، ۱۰، ۲۰ و ۴۰ میلی‌گرم در کیلوگرم نانوذرات اکسید روی تغذیه شدند.میانگین افزایش وزن، میانگین خوراک مصرفی و ضریب تبدیل خوراک از روز ۱ تا ۴۲ اندازه‌گیری شدند. تری‌گلیسرید، کلسترول، لیپوپروتئین با دانسیته بالا (HDL)، فعالیت آنزیم‌های آلکالین فسفاتاز(ALP)، آسپارتات ترانسفراز(AST)، آلانین ترانسفراز(ALT)، کراتین کیناز(CK)، کل ظرفیت آنتی‌اکسیدانی (T-AOC)، فعالیت آنزیم سوپراکسید دیسموتاز(SOD)، گلوتاتیون پراکسیداز(GPx) و سطح مالون دی‌آلدئید (MDA) پلاسما نیز در روز ۴۲ اندازه‌گیری شدند. نتایج نشان داد، پرندگان دریافت کننده ۱۰ میلی‌گرم نانوذرات اکسید روی دارای افزایش وزن روزانه بیشتر و پرندگان دریافت کننده ۲۰ میلی‌گرم نانوذرات اکسید روی کم‌ترین ضریب تبدیل خوراک را در مقایسه با سایر گروه‌ها داشتند ($P<0.05$). علاوه بر این، کلسترول، HDL، ALP و SOD با سطح ۲۰ میلی‌گرم نانوذرات اکسید روی به طور معنی‌داری افزایش یافت($P<0.05$). هم‌چنین، سطح MDA سرم همزمان با تجویز سطح ۲۰ میلی‌گرم نانوذرات اکسید روی به طور معنی‌داری کاهش یافت. سایر فراسنجه‌های خونی به طور معنی‌داری تحت تاثیر تیمارهای آزمایشی قرار نگرفتند ($P>0.05$). نتیجه‌گیری این‌که، نتایج حاصل از این مطالعه نشان داد که تجویز ۲۰ میلی‌گرم در کیلوگرم خوراک نانوذرات اکسید روی سبب بهبود عملکرد و وضعیت آنتی‌اکسیدانی در جوجه‌های گوشتی شد.