

## Effects of Zinc Oxide Nanoparticles Supplementation on Mortality due to Ascites and Performance Growth in Broiler Chickens

Research Article

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Received on: 25 Jun 2015

Revised on: 21 Aug 2015

Accepted on: 31 Aug 2015

Online Published on: Jun 2016

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### ABSTRACT

Six hundred 1-d old male broilers (Ross 308) were assigned into four experimental groups; each was composed of 5 replications of 30 birds including control (0), 10, 20 and 40 mg/kg nano-ZnO in the basal diets. Birds were exposed to low ambient temperature (15 to 18 °C) to induce ascites. Blood parameters including; activity of aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH) and levels of protein, glucose, hemoglobin, hematocrit, white blood cell (WBC), red blood cell (RBC) and malondialdehyde (MDA) were determined. Mortalities were inspected to determine cause of death and diagnose of ascites. At the end of the experiment (day 42), 2 chickens from each replicate were randomly selected, slaughtered and ascites index calculated. Average body weight gain (ABWG) and average feed intake (AFI) were measured weekly and weekly average feed conversion ratio (AFCR) was calculated. Results showed that 40 mg/kg nano-ZnO significantly reduced MDA level in plasma and liver. Moreover, 20 and 40 mg/kg nano-ZnO had decreased significantly mortality due to ascites and ascites index. It is also, nano-ZnO in all levels had significantly increased ABWG. Birds in the 40 mg/kg nano-ZnO group had lower AFRCR. It was concluded that nano-ZnO improved performance and reduced mortality due to ascites, and 40 mg/kg nano-ZnO is the optimal level in diets.

**KEY WORDS** ascites, blood parameters, broilers, growth performance, zinc oxide nanoparticles.

### INTRODUCTION

Ascites or pulmonary hypertension syndrome (PHS) is a most common metabolic syndrome associated of rapidly growing tissues in broiler chickens. This syndrome is characterized by chronically elevated pulmonary blood pressure, right ventricular hypertrophy, systemic hypoxia, ultimately, congestive heart failure and for that reason death (Cisar *et al.* 2004). During the development of ascites, broilers exert hematological changes, like Hematocrit, hemoglobin, and red blood cell counts (RBC) all increase dramatically (Balog *et al.* 2003). It is proposed that ascites might be associated with oxidative stress induced by reactive oxygen species (ROS) (Ruiz-Feria, 2009). Oxidative

stress occurs with depletion of tissue antioxidants and outweighs antioxidant protection within cells (Iqbal *et al.* 2002). A high pulmonary arterial pressure associated with hypoxia can induce ROS production and endothelial damage by impairing endothelial nitric oxide (NO) synthesis. The ROS causes a loss of NO bioavailability by shortening its half-life, so that, reducing the potential for endothelial vasodilatation and subsequently, incidence high pulmonary arterial pressure. Moreover, the reactions of ROS like superoxide anion with NO leads to production of peroxy-nitrite, a powerful oxidant agent for endothelial damage (Ruiz-Feria, 2009). There are diverse reports of the protective efficiency of commonly used antioxidants on ascites prevalence in broilers. These works were limited to the in-

investigation of several nutritional and medicinal additives include vitamins (C and E), coenzyme Q10, L-carnitine and uric acid (Ruiz-Feria, 2009; Nakamura *et al.* 1996; Geng *et al.* 2004a; Geng *et al.* 2004b; Roch *et al.* 2000; Stinefelt, 2003). The most common synthetic antioxidants that are used in poultry industry include butylated hydroxyanisole and butylated hydroxytoluene, have been restricted recently, because of their possibility carcinogenicity causing liver bump and changing liver enzyme activities (Rajani *et al.* 2011).

Zinc (Zn) is a vital mineral for animals that plays a central role in stability of biomembranes and protein as it helps in balancing ROS production and scavenging because of its presence in antioxidant enzymes such as superoxide dismutase (SOD) (Burmana *et al.* 2013). Furthermore, it had been showed that Zn acts as an antioxidant to prevent cell membrane from damage due to free radicals, however the mechanism was not clear. (Cunningham-Rundles *et al.* 1990).

Moreover, it is reported that, Zn is an essential component in Cu-Zn-SOD complex and dietary Zn levels positively correlate with Cu-Zn-SOD activity in cells. It has been demonstrated that Cu-Zn-SOD is involved in cellular scavenging of free radicals and moderate oxidative stress (Prasad, 2008). Compared to Zn oxide form, nano-ZnO form of zinc has a higher chemical activity and undergoes oxidation reactions with a variety of organic compounds. Also in addition, the permeability of nano-ZnO can help avoid adverse gastrointestinal reactions and improve the absorption of nutrients in small intestine (Zhao *et al.* 2014). The primary objective of this study is to assess the possible role of nano-ZnO on growth performance, ascites incidence and mortality in broilers under induced ascites.

## MATERIALS AND METHODS

### Experimental design

Six hundred 1-d-old male broiler chickens (Ross 308) were allocated randomly in to 4 experimental groups with 5 replicates each and 30 chicks per replicate (per cage). The floor was covered with 5 cm layer of wood shavings. Cages measured 2 × 1.5 × 2 m. All chicks were fed a basal corn-soybean meal diet, including 22.04% crude protein (CP) and 3100 kcal/kg of ME (1 to 21 d), or 20.26% CP and 3050 kcal ME (22 to 42 d) (Table 1). Birds had free access to feed and water, with 23 hour light per day throughout the experimental period. From d 7, diets were supplemented with 0, 10, 20 and 40 mg/kg of nano-ZnO. The nano-ZnO is provided by the US Research Nonmaterial's, Inc. (Houston, TX 77084, USA). The product is a white powder with a measured nano-ZnO content of purity ≥ 99%.

The sizes of the nano-ZnO are 35 to 45 nm with average 40 nm.

**Table 1** Composition of experimental diets

Item	Starter (0 to 21 d)	Grower (22 to 42 d)
<b>Ingredients (%)</b>		
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*	0.5	0.5
Salt	0.25	0.25
DL-methionine	0.2	0
L-lysine	0	0.03
Total (%)	100	100
analyzed composition		
ME (kcal/kg)	3100	3050
CP (%)	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

\* Supplied per kilogram of diet: vitamin A: 11000 IU; vitamin D<sub>3</sub>: 5000 IU; vitamin E: 40 IU; vitamin K: 4 mg; Riboflavin: 5 mg; vitamin B<sub>6</sub>: 4 mg; vitamin B<sub>12</sub>: 0.011 mg; Niacin: 50 mg; Biotin: 0.01 mg; Thiamine: 3 mg; Zinc: 80 mg; Manganese oxide: 100 mg; Selenium: 10 mg and Iron sulfate: 80 mg.

### Growth performance assay and ascites index

Body weight gain (BWG) and feed intake (FI) were measured and weekly feed conversion ratio (FCR) was calculated. On days 42, eight birds were selected and slaughtered after 8 h of feed deprivation. Hearts were removed and pericardium, marginal adipose tissues and atriums were removed. The left and right ventricles were separated and their individual weights were measured. The right ventricle/total ventricle (RV/TV) as a simple measure of ascites incidence was determined (Arab *et al.* 2006).

### Ascites induction

The experimental ascites were induced using cold temperature model (Daneshyar *et al.* 2009). All birds were raised under 32 °C and 30 °C during the first and second week of age (respectively and then the house temperature was decreased to 15 °C during wk 3 and maintained between 10 and 15 °C for the rest of the study. Mortalities were recorded daily and all of the dead birds inspected for judgment of ascites. Diagnosis of ascites generally depends on observation of the following one or several symptoms including right ventricle hypertrophy, cardiac muscle laxation, swollen and colloidal fluid in the abdominal cavity (Geng *et al.* 2004b).

## Sampling and measurement

### Blood biochemical parameters and organ index

On 42<sup>nd</sup> day, eight birds were selected and weighted after 8 h of feed deprivation. Whole blood samples (2 mL) were collected by vein puncture into heparinized anticoagulation tubes for the measurement of red blood cell (RBC), white blood cell (WBC), hematocrit and hemoglobin (Sysmex KX-21 N Automatic blood analyzer, Kobe, Japan). Another set of blood samples (2 mL) were immediately collected into non-anticoagulant tubes to obtain serum for the determination of glucose, total protein, lactate dehydrogenase (LDH), aspartateamino transferase (AST) and alanine amino transferase (ALT) enzyme activities (Autolab, PM 4000, Autoanalyzaer Medical System, Rome, Italy).

### Malondialdehyde (MDA) concentration in liver and serum

At 42<sup>nd</sup> day, eight birds per experimental group were slaughtered. Whole blood samples were drawn from slaughtered birds.

Liver removed ground and apportioned into a set of subsample immediately after slaughter. The MDA content of liver sample was determined as a measure of lipid oxidative susceptibility. The determination of MDA concentration was based on colorimetric assay of thiobarbituric acid reactive substances as described by [Botsoglou et al. \(1994\)](#). MDA concentration in serum via determination lipid peroxidation in serum was measured by the thiobarbituric acid method ([Yagi, 1984](#)).

### Statistical analysis

The study was conducted based on a completely randomized design (CRD) with four treatments and five replicates per treatment. The data were analyzed using the GLM procedure of SAS ([SAS, 2002](#)). Differences between treatments means were evaluated by Tukey's test at a significance level of 5%.

## RESULTS AND DISCUSSION

### Growth performance

Table 2 displays that of nano-ZnO supplementation could improve the growth performance of broilers under induced ascites. These results showed that at day 42, the body weight gain (BWG) of broilers fed 40 mg/kg nano-ZnO was significantly higher and (FCR) was significantly lower than

that of the control birds ( $P < 0.05$ ).

### Ascites index and mortality due to ascites

In the present study, it was clearly demonstrated that nano-ZnO supplementation at 20 and 40 mg/kg nano-ZnO, significantly reduced ascites mortality and ascites index (right ventricle/total ventricle weight) in broilers (Table 3).

### Serum enzymes activities and blood parameters

Results related to the effect of different levels of nano-ZnO on several serum enzymes (AST, ALT and LDH) activities and blood parameters were presented in Tables 4 and 5. This data indicated that the supplementation diet with nano-ZnO had no significantly ( $P > 0.05$ ) affects the ALT, AST and LDH activities. Blood parameters had not significantly affected by nano-ZnO supplementation ( $P > 0.05$ ).

### Malondialdehyde concentration in serum and liver

The effect of different level of nano-ZnO on MDA concentration in serum and liver is presented in Table 6. As shown in this table, levels 20 and 40 mg/kg nano-ZnO, compared to other group, significantly ( $P < 0.05$ ) reduced MDA in plasma and liver.

### Growth performance parameters

Zinc is an essential microelement for the growth performance of broilers so that, its deficiency can cause appetite reduction, growth falling and abnormalities of the skin and its appendages in broilers ([Brooks et al. 2013](#); [Sahraei et al. 2013](#); [Petrovic et al. 2010](#)). In conformity with these results, some researchers have reported that dietary zinc supplementation increases growth rate, and improves feed efficiency in broiler chicks ([Sandoval et al. 1998](#); [Roberson and Edwards, 1994](#); [Zhao et al. 2014](#); [Feng et al. 2010](#)).

It is also, [Ghiasi et al. \(2011\)](#), reported that addition of ZnO supplement (100 ppm) to feed has significant effect on the small intestine lucine aminopeptidase activity that lead to improving intestinal digestion and absorption of nutrients in broilers. Moreover, it is conceivable that zinc oxide supplementation in broilers reduces labile active molecules such as iron (redox active molecule) by improving the antioxidant defense system in intestine mucosal cell ([Ghiasi et al. 2011](#)). It is also, suggested that, zinc oxide in broiler can increase the concentration of sucrase in small intestine and result in increased carbohydrate absorption ([Karamouz et al. 2011](#)).

**Table 2** Effects of different levels of zinc oxide nanoparticles on performance of broiler chickens (42 d)

Performance traits	Treatments (mg/kg nano-ZnO)				±SEM	P-value
	0	10	20	40		
Body weight gain (g)	2595 <sup>b</sup>	2665 <sup>a</sup>	2669 <sup>a</sup>	2675 <sup>a</sup>	17.23	0.0001
Feed intake (g)	5017 <sup>b</sup>	5195 <sup>a</sup>	5072 <sup>b</sup>	49940 <sup>b</sup>	47.44	0.0014
Feed conversion ratio (g/g)	1.93 <sup>ab</sup>	1.94 <sup>a</sup>	1.90 <sup>bc</sup>	1.86 <sup>c</sup>	0.019	0.0009

The means within the same row with at least one common letter, do not have significant difference ( $P > 0.05$ ).

SEM: standard error of the means.

**Table 3** The effect of zinc oxide nanoparticles on mortality due to ascites and RV/TV of broiler chickens

Treatments (mg/kg nano-ZnO)	Mortality due to ascites	RV/TV
0	14.00 <sup>a</sup>	0.36 <sup>a</sup>
10	12.00 <sup>a</sup>	0.33 <sup>a</sup>
20	6.00 <sup>c</sup>	0.26 <sup>b</sup>
40	4.00 <sup>c</sup>	0.24 <sup>b</sup>
±SEM	0.45	0.04
P-value	0.001	0.0001

The means within the same column with at least one common letter, do not have significant difference ( $P > 0.05$ ).

RV/TV: right ventricular weigh/ total ventricular.

SEM: standard error of the means.

**Table 4** The effect of zinc oxide nanoparticles on blood parameters of broiler chickens

Treatments (mg/kg nano-ZnO)	Protein (mg/dL)	Glucose (mg/dL)	Hemoglobin (g/dL)	Hematocrit (%)	White blood cell ( $\mu\text{L}/10^3$ )	Red blood cell ( $\mu\text{L}/10^4$ )
0	3.80	229.25	7.20	29.22	153.02	2.17
10	4.25	228.00	7.68	30.27	157.22	2.23
20	3.90	222.00	7.55	30.20	156.32	2.26
40	3.80	218.25	8.70	34.02	163.17	2.53
±SEM	0.25	8.17	0.46	1.62	2.95	0.12
P-value	0.5958	0.1388	0.2114	0.3203	0.0669	0.2097

SEM: standard error of the means.

#### Ascites index and mortality due to ascites

Ascites index (RV/TV), that is, the ratio of right ventricle weight to total ventricle weight and it is suggested being a sensitive indicator of prior exposure of the heart to increased pulmonary arterial pressures (Geng *et al.* 2004b).

**Table 5** Effects of zinc oxide nanoparticles on the plasma activities of ALT, AST and LDH of broiler chickens

Treatments (mg/kg nano-ZnO)	LDH (U/L)	AST (U/L)	ALT (U/L)
0	3150	294	6.81
10	3200	417	5.96
20	3050	395	5.87
40	2910	388	5.37
±SEM	215	49	3.09
P-value	0.120	0.329	0.088

LDH: lactate dehydrogenase; AST: aspartate amino transferase and ALT: alanine amino transferase.

It is agreed that broiler with an RV/TV  $< 0.27$  without fluid in the abdomen were regarded as normal status, whereas birds with an RV/TV  $\geq 0.30$  and fluid accumulation were regarded as having pulmonary hypertension (Cawthon *et al.* 2001). The present study shows that nano-ZnO supplementation significantly decreases RV/TV compared with the control ( $P \leq 0.05$ ), with 40 mg/kg nano-ZnO appearing more effective in decreasing RV/TV than other levels. This further supported the idea that nano-ZnO may offer some protection to chicks' cardiac myocytes by improving antioxidant status (Powell, 2000).

Moreover, the results of these experiments strongly suggests that nano-ZnO had additive effects on improving cardiopulmonary performance and reducing pulmonary hypertension, and these may have been mediated by reductions in

oxidative stress, reduced MDA in serum and liver, (Table 6) and an increased availability of nitric oxide.

#### Serum enzymes activities and blood parameters

The finding of the current study is adverse with Fazilati (2013), who reported that zinc oxide nanoparticles (25-200 mg) had significantly increased ( $P < 0.05$ ) activity of ALT and AST enzymes in serum male rats. Possible reason for these differences is probably related to using doses and time of animal exposed, as, it has been reported that, level above 50 mg/kg of nano-ZnO induce the oxidative stress and increase the plasma level of ALT and AST (Sharma *et al.* 2009).

But, these results confirms Ahmadi *et al.* (2014) who reported that different levels of nano-ZnO in dietary feed has no significantly effects on ALT and AST activities in serum in broilers.

#### Malondialdehyde concentration in serum and liver

Increased MDA concentration is an important index for lipid peroxidation and oxidative damage caused by ROS in cell (Nielsen *et al.* 1997). Zinc is considered a cofactor and component of more than 240 enzymes that play a central role in oxidative processes and protected cells from oxidative damage by reduction in the formation of  $\cdot\text{OH}$  from  $\text{H}_2\text{O}_2$  and  $\text{O}_2^-$  through the antagonism of redox-active transition metals. Generally, the antioxidation effect of zinc is in increasing bird sensitivity to some oxidative stresses (Powell, 2000). Cunningham-Rundles *et al.* (1990) showed that Zn acts as an important antioxidant to reduce cell membrane damage due to free radicals, although the mechanism was not specified.

In current study, nano-ZnO significantly reduced MDA concentration in liver tissue (Table 6).

**Table 6** Effects of different levels of zinc oxide nanoparticles on concentrations MDA in Serum and liver of broiler chickens

Treatments (mg/kg nano-ZnO)	MDA in plasma (nmol/mL)	MDA in Liver (nmol/mL)
0	4.27 <sup>a</sup>	3.17 <sup>a</sup>
10	4.12 <sup>a</sup>	3.22 <sup>a</sup>
20	3.42 <sup>b</sup>	2.43 <sup>b</sup>
40	3.10 <sup>b</sup>	2.17 <sup>b</sup>
±SEM	0.21	0.19
P-value	0.0157	0.002

MDA: malondialdehyde.

The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

SEM: standard error of the means.

The liver is not only play central role by detoxification, but also by the storage of antioxidants like T-AOC, so that, liver injury may lead to a reduction in T-AOC and can lead to oxidative stress. Data from present study, suggests that 20 and 40 mg/kg nano-ZnO has a significant effect on decreasing MDA in serum and liver tissues relative to the control, could be related to increased T-AOC resource of cells. Total antioxidant capacity (T-AOC) in the cells mainly, liver and serum, contributes to balance of active oxygen, and T-AOC is an potent parameter reflecting the status of all the antioxidants in serum and body fluids (Zhao *et al.* 2014).

## CONCLUSION

In summary, nano-ZnO used in the present study was effective in reducing RV/TV, mortality due to ascites and MDA concentration in serum and liver tissue. It also improves body weight and feed conversions ratio for 40 mg/kg level. Nano-ZnO may have balancing effects on cardiopulmonary performance by increasing of nitric oxide bioavailability through a greater substrate for nitric oxide synthesis and probably by reducing the losses of nitric oxide associated with oxidative stress and reducing endothelial damage by ROS. As only four dosage levels were tested in the present study for nano-ZnO, investigation of different dose levels is warranted for future studies.

## ACKNOWLEDGEMENT

I thank the anonymous reviewers for their comments. The current research was partially supported by Research Grant from Payam Noor University.

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