



## Effect of zinc oxide nanoparticles on some antioxidant biomarkers and enzymes of broiler chickens during starter phase

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### ABSTRACT

The study investigated the effects of zinc oxide nanoparticles on some of the antioxidant biomarkers and enzyme serum in broiler chickens during starter stage (1–21d). A total of 240 one-d-old broilers (Ross-308) were randomly divided into four groups, with 60 birds in each group and 4 replicates with 15 birds in each experimental pen. The experimental diets were T1, Control (basal corn-soybean diet supplemented with 36.27 mg Zn/kg from zinc oxide; T2, T3 and T4, basal corn-soybean diets supplemented with 30, 60 and 90 mg ZnO-NPs/kg, respectively. Dietary ZnO-NPs supplementation significantly increased total antioxidant capacity (TAC), super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzyme activity in test groups compared to the control. However, the serum enzyme activity of lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in the birds fed ZnO-NPs had not significantly altered in comparison to control group. However, a decreasing trend was observed in the activity of ALT and AST enzymes in the birds fed 60 and/or 90 mg ZnO-NPs. Also, the MDA concentration was numerically decreased compared to the control group. In conclusion, results of study suggest that dietary zinc oxide nanoparticles @ 90 mg per kg of basal diet improved antioxidative status and activity of some enzymes used as body health indicator in the broilers during starter period (1–21 d).

**Key words:** Antioxidative, Blood, Broiler, Enzyme, Nanoparticles

Zinc is one of the essential trace element that plays an important role in nutrient metabolism. Zinc-containing enzymes participate in the synthesis and/or breaking down of carbohydrates, lipids, proteins, and nucleic acids and encompass all known classes of enzymes. Also, zinc participate as a co-factor in the metalloenzymes that are important for regulation of antioxidative status (Prasad *et al.* 2011). Zinc is a part of around 250–300 enzymes from all six-enzyme classes (Prasad *et al.* 2011). Zinc is an integral part of superoxide dismutase (SOD) enzyme that participates in the antioxidant defense system (Bartlett *et al.* 2003). Adding ZnO nanoparticles in the diets improved performance traits of broiler viz. body weight gain, feed conversion ratio, etc at the starter period (Ahmadi *et al.* 2013). Similarity, nanochelating technology is a new field that gives the ability to design and manufacture nanostructures via the self-assembly method (US8288587 B2 in the US Patent). However, literatures survey also revealed that nonomaterials caused oxidative stress by means of producing free radicals in the animal body (Choi *et al.* 2010, Wang 2008). Therefore, the present study was carried out to investigate the effects of different levels of zinc oxide nanoparticles on the antioxidant status and some

of specific enzyme activities of broiler chickens during starter stage (1–21d).

### MATERIALS AND METHODS

**Birds and housing:** This research was carried out under the protocol approved by the Faculty of Agriculture, Department of Animal Science, Shabestar Branch, Islamic Azad University, Iran. A total of 240 one-day-old male broilers (Ross-308) provided by Behparwar Poultry Breeding Company, Urmia, Iran (Average initial mean weight, 44.2±0.3 g). Birds were housed in wire-floored pens (150×100×60 cm). The lighting program was provided based on Ross 308 hybrid guide (Ross 308). Each pen was equipped with a cylindrical hand feeder and an automatic water supply system so the birds had *ad lib.* access to feed and water throughout the study. The temperature, humidity and ventilation in the pens were controlled according to the recommendations for commercial hybrid broiler chickens (Ross 308).

**Diet and experimental design:** The birds were fed four experimental diets that consisted of 4 replicates with 15 birds in each replicate. The birds were individually wing-banded and distributed in a completely randomized design (CRD). The four treatments (T1-T4) comprised of corn-soybean meal-based diets supplemented with 0, 30, 60 and 90 mg Zn/kg as ZnO-NPs (average particle size 20 nm),

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respectively. The basal diets (Table 1) were formulated to meet all nutrients for all the experimental birds during starter period (NRC 1994). Feed and water were provided in plastic feeders and waterer to minimize environmental Zn contamination throughout the entire feeding trial. For uniform mixing of ZnO-NPs to the basal diet, dicalcium phosphate (DCP) was used as a carrier to dilute ZnO-NPs powder for blending the ration to achieve the desired experimental levels. The ZnO-NPs was obtained from the US Research Nano-material Inc (Houston, TX 77084, USA). ZnO-NPs characteristics are presented in Table 2.

**Determination of Zn in the basal diet:** Around 5 g sample of the basal diet was precisely ground and digested at 120°C by 5 ml of concentrated HNO<sub>3</sub> acid for one hour in a Tecator digestion system (Tecator Digestion Unit). The digested sample was cooled and then 3 ml of 70% HClO<sub>4</sub> was added for further digestion at 200°C and the process continued until the solution become colourless. A sample blank (as control) was also run along with test sample at the same time. The digested sample was carefully passed through a sieve and filtered into a volumetric flask. The contents of digestion tube were repeatedly washed with deionized water to obtain a complete extract of the minerals. Zinc concentration in the basal diet was determined by atomic absorption spectrophotometry (Jahanian *et al.* 2008).

**Data collection:** At the end of the trial (21d), four birds (one bird per replicate) from each experimental treatment were randomly selected and blood sample was collected

Table 1. Ingredient and nutrient composition of the basal diet (As-fed basis)<sup>1</sup>

Ingredient	(%)
Corn grain	58.31
Soybean meal (44% CP)	37.00
Soybean oil	2.60
Dicalcium phosphate (DCP)	1.70
Limestone	1.31
Premix vitamin <sup>2</sup>	0.30
Premix mineral <sup>2</sup>	0.30
Common salt	0.25
DL-Methionine	0.18
L-Lysine HCl	0.05
Total	100
<i>Nutrient composition</i>	
ME (kcal/kg)	2983.4
Crude protein	22.48
L-Lysine	1.16
DL-Methionine	0.54
Methionine + Cystine	0.94
Zn (mg/kg)	36.2

<sup>1</sup>Basal diet supplied 36.27 mg of Zn/kg as zinc oxide (ZnO, 72%); and without ZnO-NPs (Analyzed). <sup>2</sup>Provided per kilogram of diet: vitamin A, 15.00 mg; cholecalciferol, 3.93 mg; vitamin E, 3 mg; 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; 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.

from brachial vein. The serum was separated using a centrifuge (3000×g, 15 min at 4°C) and then immediately transferred to laboratory for determining different parameters.

**Antioxidant assay:** The antioxidant enzymes were measured by using commercial kits (Randox, Pars Azmon Co. Tehran, Iran) and Auto Diagnostic Analyzer (Alcyon 300, USA). For measuring activity of SOD, a commercial kit was used (Aebi 1983). Measurement of the enzyme was expressed in the production of superoxide radicals resulted by xanthine and xanthine oxidase and then reacted with 2-(4-iodophenyl)-3-(4-nitrophenol) 5-phenyltetrazolium chloride (INT) to form a red colour, and then was read at 505 nm. The amount of GPx activity was determined by using a commercial kit by measuring the rate of oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. Based on definition, one unit of SOD activity clarifies equal to the amount of enzyme necessary to produce 50% inhibition in the INT reduction rate. Total antioxidant capacity (TAC) was measured by using commercial kits (Randox, Pars Azmon Co. Tehran, Iran) and Auto Diagnostic Analyzer (Alcyon 300, USA). Malondialdehyde (MDA) was determined by a selective third-order derivative spectrophotometric method previously reported (Botsoglou *et al.* 1994).

**Serum enzyme assay:** Alanine aminotransferase (ALT; Bowers and Macomb 1966), aspartate aminotransferase (AST; Bergmeyer and Walefeld 1974), alkaline phosphatase (ALP; Bowers and Macomb 1966), and lactate dehydrogenase (LDH, Bergmeyer and Walefeld 1974) were measured by using commercial kits (Randox, Pars Azmon Co. Tehran, Iran) and Auto Diagnostic Analyzer (Alcyon 300, USA). The level of enzyme activity was assayed by an automatic biochemical analyzer (Alcyon 300, USA).

**Statistical analysis:** Data were analyzed by the mixed model procedure of the SAS (2003) for completely randomized design (CRD) with different levels of ZnO-NPs supplementation in the basal diets as the factors examined. Linear and quadratic effects were determined utilizing regression relationship for equally spaced treatments.

## RESULTS AND DISCUSSION

Results indicated that supplementation of ZnO-NPs in the diet had significantly ( $P < 0.05$ ) increased level of total antioxidant capacity (TAC), SOD ( $P < 0.05$ ) and GSH-Px ( $P < 0.05$ ) in the birds fed with 60 and 90 mg ZnO-NPs/kg basal diet in comparison to control treatment. The highest (1.65 mmol/l) level of TAC was observed in the birds fed diet supplemented with 90 mg (T4) ZnO-NPs per kg basal diet. However, the concentration of MDA numerically ( $P > 0.05$ ) decreased in the birds fed ZnO-NPs compared to control (Figs 1–4). ZnO-NPs levels had not significantly ( $P > 0.05$ ) effected the activity of different enzymes (AST, ALP and ALT); although a decreasing trend of enzyme activity in comparison with control treatment in the bird fed diets containing ZnO-NPs was observed (Fig 5).

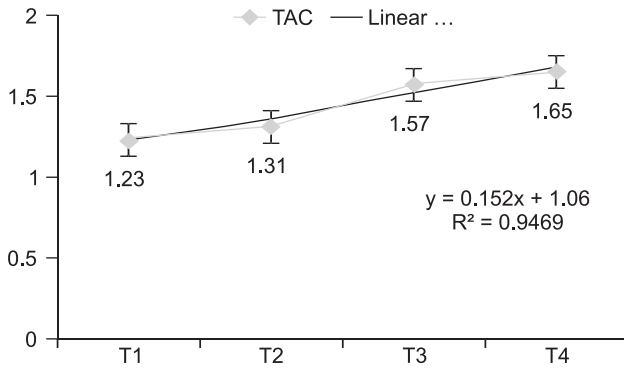


Fig. 1. Effect of ZnO-NPs on the TAC (mmol/l) concentration.

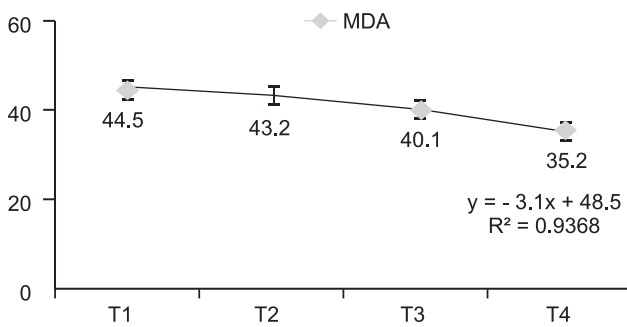


Fig. 2. Effect of ZnO-NPs on the MDA (nmol/l) concentration.

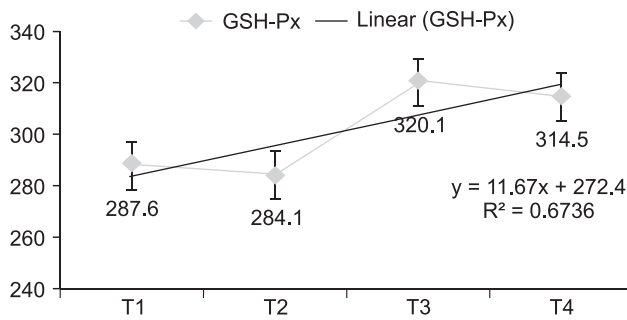


Fig. 3. Effect of ZnO-NPs on the GSH-P<sub>x</sub> (IU/g Hb) concentration.

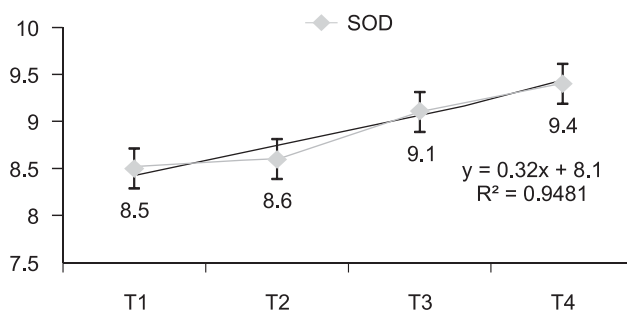


Fig. 4. Effect of ZnO-NPs on the SOD (IU/g Hb) concentration.

Zhao *et al.* (2014) indicated that dietary inclusion of zinc in the form of ZnO nanoparticles with a graded level of 60 mg/kg significantly increased the activity of antioxidant enzymes such as GSH-P<sub>x</sub> and SOD which is in agreement with our study results. However, Berg and Shi (1996) reported that a negative correlation exist between ZnO-NPs supplementation in the diet and the concentration of MDA

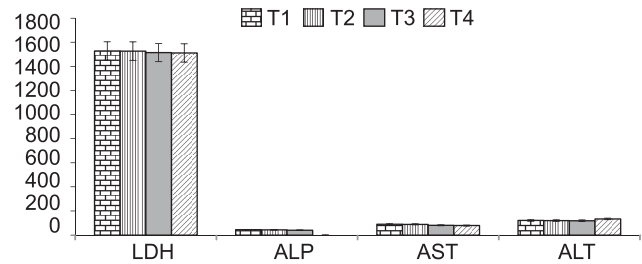


Fig. 5. Effect of ZnO-NPs on the serum enzyme activity (UI/l).

in rats. Literature indicated that nonmaterial in the animal body induce oxidative stress by means of lipid peroxidation that increase concentration of MDA and impair antioxidant defense system (Wijnhoven *et al.* 2009, Asharani and Wu 2008, Chen 2008).

There are several enzyme systems that catalyze reactions to neutralize free radicals and reactive oxygen species (ROS) produced during oxidative stress condition. Glutathione peroxidase (GSH-P<sub>x</sub>), Superoxide dismutase (SOD) and total antioxidant capacity (TAC) are the important antioxidant parameters that represents the ability to protect the body opposite produced H<sub>2</sub>O<sub>2</sub> in the body cells during oxidative stress (Rather *et al.* 2011). There is an interrelationship between the activities and the intracellular levels of these metabolites, protecting themselves from oxygen toxicity (Grazioli *et al.* 1998). The concentration of malondialdehyde generally serves as an indicator of ROS mediated injury and the concentration of MDA in blood and tissues is generally used as biomarker of lipid peroxidation (Ahmadi and Rahimi 2010). Oxidative stress is the major cause of reduction in growth rate and economical traits in broilers and increased incidence of infectious and metabolic diseases in poultry; which can be minimized by the use of anti-stress compounds (Sen 1995).

The findings of our study were contradictory with report of Fazilati (2014) who investigated the effect of different levels of zinc oxide nanoparticles (25–200 ppm) on the serum enzyme activity in rats and reported that dietary ZnO-NPs had significantly increased the activity of ALT, AST and ALP enzyme in male rats. Sharma *et al.* (2009) indicated that exposure time and concentration of nanoparticles used are the most important factors affecting on farm animal. It had also been suggested that exposure to ZnO-NPs led to negative effects on the cells likely DNA mutation, genotoxic potential by means of lipid peroxidation and oxidative stress in the body. Generally, the observed incompatibility in different reports may be due to the dosage of nanoparticles, time of exposure, animal species and experimental method (Ahmadi and Rahimi 2010). It is believed that a healthy diet improves the exogenous antioxidant concentration and reduces the risk of tissue damage. In our earlier study, ZnO-NPs supplementation at 90 mg per kg of basal diet significantly improved the growth performance and blood parameters in broiler chickens during the starter phase (Ahmadi *et al.* 2013). In conclusion, results suggest that dietary supplementation of 90 mg of ZnO-NPs per kg of

basal diet improved antioxidative status in the broiler chicks during starter period (1–21 d) and had a positive effect on the health of broilers.

#### REFERENCES

- Aebi H E. 1983. Catalase. *Methods of Enzymatic Analysis*. Verlag Chemie, Weinheim, pp 276–286.
- Ahmadi F and Rahimi F. 2010. The effect of different levels of nanosilver on performance and retention silver in edible tissue of broilers. *World Applied Science Journal* **12**: 1–4.
- Ahmadi F, Yahia E, Jamshid G G K and Nasser M S. 2013. The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period. *International Journal of Biosciences* **3**(7): 23–29.
- Asharani P V, Wu Y L, Gong Z and Valiyaveetil S. 2008. Toxicity of silver nanoparticles in zebrafish models. *Journal of Nanotechnology* **19**: 255–59.
- Bartlett J R and Smith M O. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. *Poultry Science Journal* **82**: 1580–88.
- Berg J M and Shi Y. 1996. The galvanization of biology: A growing appreciation for the roles of zinc. *Science Journal* **271**: 1081–85.
- Bergmeyer H and Walefeld M. 1978. The clinical method to determine of TGO, ET and TGP without phosphate of pyridoxal. *Clinical Chemistry Act* **24**: 58–71.
- Botsoglou N A, Florou-Paneri P, Christaki E, Fletouris D J and Spais A B. 2002. Effect of dietary oregano essential oil on performance of chickens and iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science* **43**(2): 223–30.
- Bowers G N J and Mc-Comb R B. 1966. A continuous spectrophotometer method for measuring the activity of serum alkaline phosphatase. *Clinical Chemistry Journal* **12**: 73–79.
- Chen X and Schluesener H. 2008. Nanosilver: a nanoparticle in medical application. *Toxicology Letters* **176**(1): 1–12.
- Choi J E, Kim S, Ahn J H, Youn P, Kang J S, Park K and Yid D Y. 2010. Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. *Aquatic Toxicology* **100**: 151–59.
- Duncan D B. 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1–42.
- Fazilati M. 2013. Investigation of toxicity properties of zinc oxide nanoparticles on liver enzymes in male rats. *Plagia Research Library* **3**: 97–103.
- Grazioli V, Schiavo R, Casari E, Marzatico F, Rodriguez R and Baena P. 1998. Gaetani: Antioxidant enzymatic activities and lipid peroxidation in cultured human chondrocytes from vertebral plate cartilage. *FEBS Letters* **431**: 149–53.
- Jahanian R, Hasan N M and Abbas R. 2008. Improved broiler chick performance by dietary supplementation of organic zinc sources. *Asian Australasian Journal of Animal Science* **21**(9): 1348–54.
- National Research Council. 1994. *Nutrient Requirements of Poultry*. 9<sup>th</sup> Ed., National Academy Press, Washington DC.
- Prasad T N V and Elumalai E K. 2011. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine* **27**: 439–42.
- Rather M A, Sharma R and Aklakur M. 2011. Nanotechnology: a novel tool for aquaculture and fisheries development. A prospective mini-review. *Fisheries and Aquaculture Journal* **16**: 1–5.
- SAS Institute. 2003. *SAS Statistics User's Guide*. Statistical Analytical System. 5th rev. ed. Carry, NC, SAS Institute Inc.
- Sen C K. 1995. Oxidants and antioxidants in exercise. *Applied Physiology Journal* **79**(3): 675–86.
- Sharma V, Shukla R K, Saxena N, Parmar D, Das M and Dhawan A. 2009. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. *Toxicology Letters* **185**: 211–18.
- Wang Z L. 2008. Splendid one-dimensional nanostructures of zinc oxide: a new nonmaterial family for nanotechnology. *ACS Nano* **2**(10): 1987–92.
- Wijnhoven S, Herberts C, Hagens W, Oomen A, Heugens E and Roszek B. 2009. Nano silver-a review of available data and knowledge gaps. Report 360003001/2008. National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.
- Zhao C Y, Shu-Xian T, Xi-Yu X, Xian-Shuai Q and Jia-Qiang P. 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. *Biological Trace Element Journal* **160**: 361–67.