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RESEARCH ARTICLE

Impact of Different Levels of Silver Nanoparticles (Ag-NPs) on Performance, Oxidative Enzymes and Blood Parameters in Broiler Chicks

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ABSTRACT

This research was carried out to investigate the effects of Nano-silver (Ag-NPs) on traits of productivity, oxidative stress state and some of blood Parameters in broiler chicks. A total of 240, one-day-old male broiler chicks (Ross 308) were allocated in a completely randomized design into four groups containing 60 birds, four replicates and 15 birds in each experimental pen. Basal diet supplemented with different levels of Ag-NPs (Diameter 14±0.8 nm) was given throughout research (1-42 days). Experimental diets included: 0 (Control), 20, 40, and 60 ppm Ag-NPs/kg diet. At the end of study period, one bird with closest weight to the mean of any treatment was selected. Blood samples were collected, centrifuged and removed serum stored at -20°C until analysis. Then birds slaughtered and spleen, thymus, and bursa of Fabricius removed and their relative weights calculated. The results showed that nano-silver has no significant effect on growth performance but a decrease was found in feed efficiency. Relative bursa weight had significantly decreased in compared with control treatment ($P<0.05$). Lowest weight among treatments observed in treatment that supplemented with 60 ppm nano-silver. Ag-NPs had significantly effected on oxidative stress enzymes activity among treatment in comparison to control group ($P<0.01$). Blood parameters such as ALT, AST, ALP, TP, Albumin, Gama globulin, triglyceride, and cholesterol were significantly affected by using nano-silver in broiler chicks ($P<0.05$).

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INTRODUCTION

In the recent years, nanotechnology had rapid progress in the most of different scientific branches and showed the effects on all parts of human, animal, environmental, and industrial life. One of the substances used in nano-formulation is silver nano-particle. It has been used since ancient times for jewelry, utensils, monetary currency, dental alloy, photography, explosives, etc (Chen and Schlesinger, 2008). Until the introduction of antibiotics, it was also used for its antiseptic activity, specifically in the management of open wounds and burns. Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and clean swimming pool water (Agency for Toxic Substances and Disease Registry, 1990). Particle morphologies include spheres, cubes, wires and multi facets, normally within a size range of <100 nm. Silver nano particles have been considered as antibacterial made by human and could be used as an additive instead of antibiotics due to their antibacterial properties and their adaptability to biological systems (Kermanshahi *et al*., 2006). Sawosza *et al.* (2007) studied the effect of different levels of colloidal Ag-NPs (0, 5, 15 and 25 mg/kg) on intestinal microbial flora and duodenal morphology in Quails. The result of this study showed that the effect of silver nano-particles on the number of *E. coli* and other intestinal bacteria were not significant. Grodzik and Sawosza (2006) evaluated effect of silver nanoparticles on the fetal growth and morphology of bursa of fabricius. They showed that silver nano-particles with concentration of 10 ppm have no effect on the growth of chicken embryos, but the number and size of the lymph follicles were decreased (Grodzik and Sawosza, 2006). Silver nanoparticles have been shown to damage liver cells (Hussein *et al*., 2005). The toxic effect or heavy metal poisoning is defined as "any functional or morphologic change in the body produced by an ingested, injected, inhaled or absorbed drug, chemical, or biological agent" (Hussein, 2005). Colloidal silver is a colloidal state of silver-containing particles in

water with 1 nm to 1 micron silver or silver-containing particles. Nano silver has a more active surface area and better porosity than commercial silver (Alt *et al*., 2004). The objective of this study was to investigate the effects of silver nanoparticles on performance, oxidative enzyme activities, and blood parameters in broiler chicks.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments: A total of 240 one-day-old male broiler chicks (Ross 308) were housed in an environmentally controlled broiler house throughout study (42 days). The floor was covered with about 5 cm layer of wood shavings. Pens measured 1×1.5× 0.75 m. The poultry farm was equipped with automatic feeders, drinker, and automatic heating and ventilation systems. Birds were allocated in a completely randomized design (CRD) and each treatment was replicated in 4 experimental pens and 15 birds in each pen. Diets contained adequate levels of nutrients as recommended by the National Research Council (NRC, 1994). The composition and nutrient content of the basal diet are presented in Table 1. Diet of birds was supplemented with 0 (Control), 20, 40 and 60 ppm Ag-NP/kg (Nanostructured & Amorphous Materials, Inc, USA) of feed. Birds were free accessed *ad libitum* to feed and water intake throughout the research.

Table1: Ingredient and nutrient composition of basal diets

Ingredient	Starter 1-21d	Grower 22-42d
Corn	61.55	52.00
meal (48% CP)	34.50	36.00
Soybean oil	2.50	1.50
Salt	0.45	0.44
Limestone	1.32	1.06
Dicalcium phosphate (DCP)	1.75	1.68
DL-Methionine	0.18	0.17
Vitamin-mineral mix[1]	0.25	0.25
Calculated nutrients		
AMEn (Kcal/kg)	3050.0	2950.0
CP %	22.37	21.87
Ca %	1.01	1.00
Available P %	0.45	0.45
TSAA %	0.90	0.89
Lysine %	1.30	1.28
AP (%)[2]	0.58	0.56

[1]Supplied the following per kilogram of diet:: 11,025 IU of vitamin A; 3,528 IU of vitamin D3; 33 IU of vitamin E; 0.91 mg of vitamin K; 2 mg of thiamin; 8 mg of riboflavin; 55 mg of niacin; 18 mg of Ca pantothenate; 5 mg of vitamin B6; 0.221 mg of biotin; 1 mg of folic acid; 478 mg of choline; 28 μg of vitamin B12; 75 mg of zinc; 40 mg of iron; 64 mg of manganese; 10 mg of copper; 2 mg of iodine; and 0.3 mg of selenium; [2] Available phosphorous.

Performance traits: At the end of study period, one birds with closest weight (4 birds per treatment) to the mean of any treatment selected and after sampling blood from brachial vein, the birds immediately slaughtered and lymphoid organs as spleen, thymus and bursa of Fabricius, small intestine and abdominal fat removed, and relative weights calculated. Also, growth performance as feed intake (FI), body weight gain (BWG) recorded weekly during study. At the final of research (42d), feed conversion ratio (FCR) and feed efficiency (FE) were determined by the following formula:

Feed Conversion Ratio = Feed intake (g) ÷ Live body wt (g)

Feed efficiency (FE) = Live body wt (g) × 100 ÷ Feed intake (g)

Blood sample collection: At 42 d of age under the condition of *ad libitum* feeding, a blood sample was collected from the brachial vein into heparinized syringes from 4birds per treatment (one bird per replicate). Blood samples were collected on ice and centrifuged, and plasma was stored at -20°C until analysis. The concentrations of plasma metabolites were measured using standard kits (Sigma Chemical Co, St. Louis, MO 63178-9916, USA).

Blood parameters analysis: At the end of study, 4 birds each group with closest mean weight to treatment were selected. Blood samples were centrifuged (3000 rpm, 10 min) and the plasma was separated. Erythrocytes are susceptible to oxidative stress as a result of the high polyunsaturated fatty acid content of their membranes (Cicha *et al*, 1999). Whole heparinized blood was assayed for GPx activity. GPx (Glutathione peroxidase) activity was determined via a commercially available enzyme kit (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK). Erythrocyte haemolysate was used for SOD (Superoxide dismutase) and CAT (catalase) activity. CAT activity was determined using the method of Aebi (1984). SOD activity was determined using the commercially available enzyme kit (Ransod, RANDOX/ SD-125 supplied by Randox Laboratories). Lipid peroxidation was measured by the thiobarbituric acid method (Yagi, 1984). This method evaluates oxidative stress by measuring malondialdehyde (MDA), the last product of lipid breakdown caused by oxidative stress.

Immune Organs: Immediately after blood sampling, birds slaughtered, and then immune organs such as spleen, thymus and bursa of Fabricius were removed, cleansed of adhering material, and those related weight was calculated by the following formula, the organs weight were expressed as percentage of live body weight.

Related organ weight = organ weight (g) × 100 ÷ Live body weight (g)

Statistical Analysis

The experiment was performed as a completely randomized design (CRD) with four replicates of 15 chicks assigned to each of four dietary treatments. Data were subjected to statistical analysis using the general linear models procedure of SAS (Anonymous, 2000). Variable means for treatments showing significant differences in the one-way ANOVA were compared using Duncan's multiple-range test. All statements of significant difference at were based on the $P<0.05$ level of probability. Using the model:

$Yij = \mu + T_i + \varepsilon_{ij}$ Where

Y is a single observation; μ is the general mean; T is the effect of different levels of Ag-NPs

(i= 0, 20, 40, and 60 ppm); and ε is the error.

RESULTS AND DISCUSSION

Growth performance: The effect of diet inclusion of different levels of Ag-NPs on FI, FCR, and LBW of

Table 2: Effects of different levels of Ag-NPs on performance of broiler chickens (42 d)

Performance traits	basal diet (without Ag-Ps)	Basal diet+ 20 ppm	Basal diet+ 40 ppm	Basal diet+ 60 ppm
Body weight gain (g)	2284.4±567.4	2128.4±437.1	2018.4±503.0	2033.4±492.4
Feed intake (g)	4758.6±717.4	4787.4±667.2	4745.7±602.1	4812.4±591.4
Feed conversion ratio (g/g)	2.08±0.74	2. 24±0.44	2.35±0.34	2.30±0.31
Feed efficiency (%)	48.05±2.36	44. 47±2.25	42.52±2.64	42.25±2.44
Small intestine (%LBW)	2.23±0.20[b]	2. 29±0.38[b]	2.49±0.20[a]	2.51±0.14 [a]
Abdominal fat (%LBW)	2.17±0.54[b]	2. 56±0.44[a]	2.69±0.61[a]	2.88±0.59[a]

[a-b] Means in the same column with no common superscripts differ significantly (P<0.05); LBW= Live Body Weight

broilers are presented in table 2. There was no significant effect of dietary treatment on performance traits of chickens in the present experiment in comparison with control treatment, but a decrease trend in feed conversion ratio (feed: gain) was observed. The results of current study shown that Ag-NPs had significantly increased the weight of small intestine (SI) and abdominal fat of broiler compared with control group (P<0.05). This effect may be result of affected Ag-NPs on organisms in the gut (gut microflora). Nano-silver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria (Burrell *et al*., 1999; Yin *et al*., 1999), including antibiotic-resistant strains (Wright *et al*., 2002; Percival *et al*., 2007). Gram-negative bacteria include genera such as *Acinetobacter, Escherichia*, *Pseudomonas, Salmonella*, and *Vibrio.* Based on studies having shown that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria (Sondi and Salopek-Sondi 2004; Morones *et al*., 2005).

Immune organ: The results are shown in table 3. Changes of relative weight of bursa were significantly lower in 20, 40, and 60 ppm treatment than in comparison with control treatment. A decreasing trend lymphoid organ weight coincides observed with raising concentration of nanosilver in the other treatment groups compared with control. The lowest weight related to 60 ppm treatment at 42 d of age. The reason for this condition may be due to the antimicrobial properties of Ag-NPs that affected microbial population and probably changed the proportion between pathogen and non-pathogen organisms in the gut. According to Sondi and Salopek-Sondi (2004) silver nano-particles affect some compounds of bacterial membrane leading to structural changes, dissipation of the proton motive force and in consequence to the death of microorganisms.

Blood parameters: Effects of nanoparticle on blood parameters are presented in table 4. Adding Ag-Nps to diet of broiler chicks ALT, AST, ALP, TP, albumin, gamma-globulin, triglyceride, and cholesterol were significantly affected (P<0.05). These effects may be related to oxidative stress that caused peroxidation of fat and release of free radicals in the body. Research showed function of mitochondria (Braydich-Stolle, 2005; Hussein, 2005; Johnston *et al*., 2010; Mahmoudi *et al*., 2011) that exposure to silve nano-prarticles significantly decreased the mitochondria which seem to be sensitive targets to cytotoxicity of silver nano-particles. In the study with BRL 3A liver cell line, depletion of GSH level and increased ROS was found in association with mitochondrial perturbation, suggesting that oxidative stress might mediate the cytotoxicity of silver nano-particles. Recently, it has been found that Ag+ seems to perturb mitochondria through interactions with thiol groups of the mitochondrial inner membrane. As a result of the experimental studies by Gopinath *et al*. (2008), it is known that nanomaterials can pass through cell membranes easily and cause severe toxic effects on human health. They have concluded that silver nanoparticles in higher concentrations (> 44.0 μg ml-1) are necrotic to cells, leading to rapid cell membrane rupture.

Table 3: Effects of Ag-NPs on relative weight of immune organs (%LBW) at 42 days of broiler age

Treatment (ppm)	Bursa of Fabricius	Spleen	Thymus
0 (Control)	0.169±0.008a	0.131±0.006	0.588±0.024
20	0.166±0.006a	0.130±0.004	0.590±0.021
40	0.160±0.006b	0.132±0.008	0.589±0.022
60	0.158±0.004b	0.137±0.006	0.584±0.020

[a-b]Means in the same column with no common superscripts differ significantly (P<0.05); LBW= Live Body Weight

Oxidative enzyme: The results of measurements of CAT, SOD, GSH, and MDA are presented in Table 5. The different levels of silver nanoparticles had significant effect on oxidative enzymes. MDA concentration at control treatment was the lowest in comparison with other groups, 20, 40 and 60 ppm (P<0.01). CAT, GPx and SOD activities were significantly increasing in comparison with control group (P<0.01). Lovric *et al* (2005) reported that after absorption of nanosilver from GIT, entered to blood systemic circulation, therefore this particles can, potentially, interact with different metabolites such as: plasma proteins, coagulation factors, platelets and red and white blood cells. For this reason, nanoparticle silver perhaps induces oxidative stress and adversely affects the structure and physiology of the cells, Oxidative metabolism, fat membrane structure, and function (Mager and Kruijff, 1995; Iwagami, 1996). Hussein, (2005) studied the toxicity of different sizes of silver nano-particles on rat liver cell line (BRL 3A) (ATCC, CRL-1442 immortalized rat liver cells).

In conclusion the results of this study showed that:

1) the addition of Ag-NPs had a negative stimulating effect on blood parameters and relative weight of immune organs specially bursa of Fabricius therefore, this situation may decrease the immune response of broiler due to decreased serum globulin, gamma globulin.

2) Ag-NPs in this research acted as factors of induce oxidative stress by means of change of concentration of related enzymes such as MAD, SOD and etc.

3) Ag-NPs may cause decrease of immune system by means of effect on the gut of microorganism at the point of view those of type and population (indirect effect on immune system).

4) This study showed that the addition of Ag-NPs to diets did not improves performance in comparison with control treatment such as body weight, feed intake, feed conversion ratio and feed efficiency of broilers through a 42-d trial period.

Table 4: Effects of silver nano-particle on blood parameters of broilers at 42 days

Blood parameters	Treatment (ppm)			
	0 (Control)	20	40	60
Total Protein(g/dl)	3.89±0.41a	3.56±0.31a	3.01±0.35b	2.98±0.37b
Albumin (A)(g/dl)	1.82±0.21a	1.76±0.11a	1.79±0.23a	1.68±0.31b
Globulin (G) (g/dl)	2.14 ±0.23a	2.09 ±0.21a	1.92 ±0.05b	1.94 ±0.26b
Triglyceride*	132±3.85a	145±3.25b	144±2.98b	137±3.05 c
cholesterol*	112±11.70b	116±10.23b	117±11.09b	132±9.93a
ALT (U/l)	6.02±0.43a	5.96±0.39a	5.39±0.41b	5.33±0.37b
AST (U/l)	502.12±24.09a	91.09±28.09a	483.45±30.12b	476.92±31.24b
ALP (U/l)	2187.95±398.3a	2043.31±402.8b	2013.09±414.1b	1989.43±376.3c

Values in the same row not a common superscript differ significantly (P <0.05);
*(mg/dl)

Table 5: The effect of Ag-NPs on the concentrations of MDA and oxidative enzymes activities of broilers (Mean± SEM)

Treatment (PPM)	CAT (U/mgHb)	SOD (U/gHb)	GPx (U/gHg)	MDA (nmol/gHb)
0 (Control)	2.45±0.58a	2278.50±624.59c	35.14±1.74a	1008.04±548.70c
20	2.15±0.18b	2295.16±426.81b	26.71±1.94b	1416.16±498.60b
40	1.89±0.17c	1373.50±393.98b	18.26±1.74c	2257.36±591.73a
60	1.72±0.17c	2271.73±283.87a	14.48±1.74c	2512.36±530.34a

[a-c] Means within a column with different subscripts differ significantly (P<0.01).

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