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The Impact of Silver Nano Particles on Growth Performance, Lymphoid Organs and Oxidative Stress Indicators in Broiler Chicks

Farhad Ahmadi and Ardashir Hafsi Kurdestany

Islamic Azad University, Sanandaj Branch, Kurdistan, Iran

Abstract: This research was carried out to evaluate effects of different levels of nanosilver (Ag-NPs) on growth performance, lymphoid organs and oxidative stress indicators in broiler chicks. A total of 240, one-day-old male broiler chick (Ross 308) was allocated in a completely randomized design (CRD) with four groups and 15 birds in each experimental pen. Birds of Water intake were supplemented with 0 (Control) or 5, 15 and 25 ppm Ag-NPs (Diameter 14±9.8 nm) throughout this research (42 days). Body weight, Feed intake recorded as weekly recorded during research and calculated overall growth performance at the final of study. At the end of the research, one bird with closest weight to any of treatment weight selected. Take of blood samples from birds and removed serum by centrifuge. Serum stored at-20°C until analysis. Immediately after slaughtered of birds, lymphoid organs such as spleen, thymus and bursa of Fabricus removed and relative weights was expressed as percentage of live body weight. Results showed that, nano-silver had no effect on performance. Weight of bursa had significantly decreased in comparison with control treatment ($p<0.05$) and, lowest weight among treatments observed in treatment that supplemented with 25 ppm nanosilver. Ag-NPs, had significant affect on concentration oxidative stress indicators activity among treatment in comparison to control group ($p<0.05$). It was concluded that Ag-NPs: 1) No positive effect on performance of broilers, 2) As one of the factors induced oxidative stress and, 3) It is may be decreased immune system. In addition, investigation of these effects need to further research.

Key words: Broiler chick · Performance · Oxidative stress · Lymphoid organs

INTRODUCTION

In the recent years, nanotechnology had rapid progress and had the most effect 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 [1]. 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 [2]. Particle morphologies include spheres, ods, cubes, wires and multi facets, normally within a size range of <100 nm. Silver nanoparticles 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 [3].

Sawosza *et al*. [4] studied the effect of different treatments (0, 5, 15 and 25 mg/kg) of on the performance of intestinal microbial flora and morphological assessment of duodenal in *Quail*. 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 and no significant effect on the number of white blood cells. Silver nanoparticles have been shown to damage liver cells [5]. 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. 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 [6]. The aim of this research was to investigate effect of silver nano-particles on growth performance, oxidative stress activities and immune organs weight in Broiler Chicks.

Corresponding Author: Farhad Ahmadi, Islamic Azad University, Sanandaj Branch, Kurdistan, Iran
Tel: +98 871 6387112, E-mail: farhad_ahmadi@iausdj.ac.ir.

MATERIALS AND METHODS

Birds management and experimental Design: A total of 240 one-day-old male broiler chicks (Ross 308) were housed in an environmentally controlled broiler house until 42 days of age. The floor was covered with a bout 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 [7]. The composition and nutrient content of basal diet were presented in table 1. Water intake of birds was supplemented with 0 (control), 5, 15 and 25 ppm nanosilver. Birds were free accessed *ad libitum* to feed and water intake that supplemented with different levels of nano silver throughout the research.

Biochemical analysis: At the end of study (42 d) from any treatment, 1 bird with closest mean weight to treatment selected. Blood samples were centrifuged (3000g, 10 min) and the plasma was separated. Erythrocytes were washed three times with both 140 nM NaCl and phosphate buffer (7.4pH) [8]. The erythrocyte suspension was haemolysed with mercaptoethanol and used to measure haemolysate MDA and SOD, GSH-Px, CAT, activities. Drapper and Hadley.M. [9]. Method was used to measure the MDA level of erythrocytes. Williams, *et al*. [10] method was used to detect SOD activity [8] method to detect CAT activity. Paglia and Valentine, W.N. [11] method to detect GSH-Px activity. Obtained results were calculated as U/gHb, U/gHb, U/mgHb and nmol/gHb, respectively, for SOD, GSH-Px, CAT and MDA level. Erythrocytes are susceptible to oxidative stress as a result of the high polyunsaturated fatty acid content of their membranes. Whole heparinised blood was assayed for GPx activity. GPx activity was determined using a commercially available enzyme kit (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK). Erythrocyte haemolysate was used for SOD and CAT activity. CAT activity was determined using the method of Aebi, [8]. SOD activity was determined using the commercially available enzyme kit (Ransod, RANDOX/SD-125 supplied by Randox Laboratories).

Table 1: Dietary ingredients and chemical composition diets

Ingredient (%)	Starter (1-21d)	Grower (22-42d)
Corn	60.30	61.20
Wheat middling	2.00	2.00
Soybean meal	30.50	30.003.00
Corn gluten meal	3.20	3.00
DCP	1.40	2.50
Limestone	0.35	0.68
Salt	0.25	1.25
NaHCO3	0.10	0.16
Soybean oil	1.30	0.25
Premix [1]	1.00	1.20
Total	100.00	100.00
Component Nutrients		
ME (MJ/kg)	12.40	12.74
CP (g/kg)	210.0	200.0
Ca (g/kg)	8.71	8.53
Available phosphorus (g/kg)	4.12	4.21
TP (g/kg)	6.42	6.04
Lys (g/kg)	12.02	11.41
Met (g/kg)	4.72	4.43
Met +cys	0.89	0.75

[1]Supplied the following per kilogram of diet: vitamin A, 25,000 IU; vitamin D, 5,000 IU; vitamin E, 12.5 IU; vitamin K, 2.5 IU; vitamin B1, 1.0 mg; vitamin B2, 8.0 mg; vitamin B6, 3.0 mg; vitamin B12, 15 µg; folic acid, 250 µg; nicotinic acid, 17.5 mg; calcium pantothenate, 12.5 mg; Fe, 80 mg; Cu, 10 mg; Mn, 80 mg; Se, 0.15 mg; I, 0.35 mg. TP=Total Phosphorous

Immune organs weight: Immediately after blood sampling, Birds and slaughtered, immune organs such as spleen, thymus and bursa fabricius were removed, cleansed of adhering material and those related weight was calculated by the following formula and The organs weight was expressed as gram of organ per 100 g of body weight.

$$\text{Relative weight of organ} = \text{organ weight (g)} \times 100 / \text{body weight (g)}$$

Statistical analysis: Data were analyzed by SAS 9.2[12] version for Windows. The differences between groups were determined with variance analysis (one-way analysis of variance [ANOVA]. When the differences were significant, Duncan's multiple range tests was performed with $p<0.05$ were considered significant. Data were expressed as means±SEM.

RESULTS AND DISCUSSION

Growth performance: The results related to, Feed intake (FI), Live Body weight (LBW) and Feed Conversion Ratio (FCR) showed in Table 2. The results showed that nanosilver no significant affected on FI, LBW and FCR

Table 2: Effect of nanosilver on means of LBW, FI and FCR at 42 days*

Treatment (ppm)	LBW(kg)	FI(kg)	FCR
Control (without Ag-NPs)	2.66	5.12	1.92
5ppm	2.54	5.16	2.03
10ppm	2.59	4.98	1.92
25ppm	2.58	5.08	1.96

(FI)=Feed intake; (LBW) = Live Body Weight; (FCR) = Feed Conversion Rate; (SE) = Standard error

experimental treatment (2, 3 and 4) in comparison to control(without Ag-NPs) treatment. It was agreement with other research, Ahmadi, j. [13] showed that nanosilver with different levels no significant effect on economic traits such as feed conversion ratio, feed intake.

Oxidative stress indicators: As showed in table 2, while erythrocyte MDA level was incrassating at control treatment in comparison to other groups,5, 15 and 25 ppm ($p<0.05$). CAT, GSH-Px and SOD activities were significantly difference in comparison to control group ($p<0.05$) and among other treatment. After absorption nanosilver from GIT and entered to blood systemic circulation, the particles can, potentially, interact with different metabolites like plasma proteins, coagulation factors, platelets and red and white blood cells [14]. Silver nanoparticles in most studies are suggested to be non-toxic. But due to their small size and variable properties they are suggested to be hazardous to the environment Braydich-stolle *et al*. [15].

Hussain *et al*. [5] studied the toxicity of different sizes of silver nano-particles on rat liver cell line (BRL 3A) (ATCC, CRL-1442 immortalized rat liver cells). The authors found that after an exposure of 24 h the mitochondrial cells displayed abnormal size, cellular shrinkage and irregular shape. Braydich-Stolle *et al*. [15] reported the toxicity of silver nano-particles on C18-4 cell, a cell line with spermatogonial stem cell characteristics. From the study, it was concluded that the cytotoxicity of silver nano-particles to the mitochondrial activity increased with the increase in the concentration of silver nano-particles. Recently, reported that nano-particles and nano-materials such as manganese, copper and silica generate free radicals and oxidative stress [16]. The results of researches showed that silver nano-particles can damage to different organs and tissue such as liver cells [5]. In general, the liver is able to actively remove compounds from the blood and transform them to chemical forms that can easily be excreted. It is a logical assumption that ingested silver nano-particles might have impact on the liver, since the liver serves as the ?rst check point for everything absorbed through GIT before becoming systemic. Researches showed function of mitochondria [5, 15], that exposure to silve nano-prarticles signi?cant decreased the mitochondria; seem to be sensitive targets of 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 these effects of Ag+ could be completely blocked by sulphydryl reagents, e.g. reduced glutathione (GSH), the results clearly suggest that mitochondria are under oxidative stress when the cells are exposed to Ag+ [17]. In rat models, copper toxicity has been seen to accompany MDA generation [18, 19]. Excessive copper accumulation in the liver has also been shown to depress superoxide dismutase (SOD) and Se-GSH peroxidase activities and to result in high MDA in the serum and liver homogenates due to the lipid peroxidation induced by copper overload [20] Reactive oxygen species (ROS) formation, GSH oxidation and lipid per oxidation have also been seen to be induced by copper [21].

Immune organs weight: The results showed in Table 4. Changes of relative Weight of bursa was significantly lower in 5, 15 and 25 ppm treatment than in control treatment and observed a decreasing trend Lymphoid

Table 3: Effect of nanoparticle silver on erythrocytes CAT, SOD, GSH-Px activities and MDA of level broiler chicks (least- square mean±SEM)

Treatments (PPM)	CAT(U/mg Hb)	SOD(U/g Hb)	GSH-Px (U/g Hb)	MDA(µmol/g Hb)
Control (without Ag-NPs)	2.45 ± 0.58^{b}	2278.50 ± 424.59^{a}	43.69 ± 8.04^{b}	1.92 ± 0.15^{c}
5 ppm	2.28 ± 0.42^{b}	2195.31 ± 446.80^{a}	44.21 ± 7.81^{b}	2.01 ± 0.11^{c}
10 ppm	2.94 ± 0.19^{a}	1925.21 ± 346.07^{b}	48.30 ± 9.28^{a}	2.61 ± 0.09^{a}
25 ppm	2.62 ± 0.27^{a}	1925.51 ± 336.25^{b}	47.73 ± 3.87^{a}	2.13 ± 0.08^{b}

a-c: Means within a column with different subscripts differ significantly ($p<0.05$).

Table 4: Effects of silver nanoparticle on immune organ weight (%LBW) at 42 days of age

Treatment (ppm)	Bursa of Fabricius	Spleen	Thymus
Control (without Ag-NPs)	0.169±0.008[a]	0.131±0.006	0.588±0.024
5 ppm	0.166±o.006[a]	0.130±0.004	0.590±0.021
15 ppm	0.160±0.006[b]	0.132±0.008	0.589±0.022
25 ppm	0.158±0.004[b]	0.137±0.006	0.584±0.020

a-b Means in the same column with no common superscripts differ significantly (P<0.05).

organ weight coincide with raising concentration of nanosilver in the treatment. The lowest weight related to 25 ppm treatment at 42 d of age. In the present investigation, it has been hypothesized that the antimicrobial properties of colloidal solutions of Ag-NPs, when used in poultry nutrition, may affect microbial population and probably changes the proportion between pathogen and non-pathogen organisms in caecum. Grodzik and Sawosza [22] evaluated effect of silver nano-particles on the fetal growth and morphology bursa of fabricius. They showed that Silver nano-particles with concentration of 10 ppm no effect on the growth of chicken embryos, but the number and size of the lymph follicles was decreased.

Yoon *et al*. [23] demonstrated susceptibility constants of *E. coli* and *Bacillus subtilis* to silver nano-particles. Furthermore, silver nano-particles showed inhibitory effects on *E.coli* and *Staphylococcus aurous* [24]. Silver nano-particles as agents carrying easy available oxygen can especially reduce strictly anaerobic microorganisms and consequently negitive effect on growth Bursa Fabricius. Matsumura *et al*. [25] reported that the possible action of silver zeolite might be due to the intake of silver ions by bacterial cells when they come in contact with silver zeolite, which inhibits their cellular functions and damages the cell. Secondly, it can be due to the generation of reactive oxygen molecules, which inhibit the respiration. It is logical and considerable that exists of microorganisms in GIT necessary to growth and development of bursa and healthy broiler. Therefore, may be trend decreasing weight of bursa related to control were due to effects of nanosilver on count and variety of gut microflore.

It was concluded that to understand affect of silver nanoparticle on performance, immune system, oxidative stress state and 0ther traits that are important in poultry production, we need to further study. Also, authors was carried out a study about the rate of retention silver in different organs such as liver, pancreas, lung, heart, feces, bursa fabricius, spleen and etc (not published). Overall, the results of this study showed that nanosilver hasnot positive effect on performance. So, we purpose that don't necessary study to investigation effect of Nano-Ag on growth performance in broiler chicks.

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