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The effect of dietary silver nanoparticles on performance, immune organs, and lipid serum of broiler chickens during starter period

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Abstract

This research was carried out to investigate the effect of silver nanoparticles (SNPs) on growth performance, immune organs, and serum lipids of broilers from 1 to 21 days of age. A total of 240 one-day-male broilers (Ross 308) distributed in four groups of 60 birds, including 4 replicates and 15 birds in each pen. Birds were fed on experimental diets including: T1 (control) without SNPs, T2, T3 and T4 supplementation basal diet with 4, 8 or 12 mg SNPs per kg of diet, respectively. At 21 d about 5 ml bloods (4 birds per groups) was removed from bronchial vein of four birds per replicate. Serum removed by centrifuged and stored at -20°C till the start of analysis. After blood sampling, birds were slaughtered and then visceral organs removed. Although the weight of selected organs was calculated regarding the total live body weight of each birds. The results indicated that SNPs have no significant effects on the performance ($P \geq 0.05$), there is a relative increased weight in SI and liver compared to control group ($P \leq 0.05$). Considering the abort points, the bursa weight was decreased compared to control treatment ($P \leq 0.05$). In addition TG, LDL, VLDL, and uric acid increased significantly ($P \leq 0.05$) in all treated than the control, as well as, HDL had significantly ($P \leq 0.05$) decreased compared with control. Relative weight of bursa Fabricius decreased ($P \leq 0.05$) in birds fed supplementation diet with levels of SNPs compared with control, especially in T4 birds. In conclusion, the SNPS no suitable alternatives as growth performance, Therefore, because of the mention changes may lead to negative effect on performance, immune response and health of broiler chickens.

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Introduction

Nanotechnology is one of the newest scientific branches. This technology deals with structures ranging from 1 to 100 nm. Silver (Ag) is one of the substances that have been used in nano formulation. This element has been used since ancient times for jewellers, utensils, monetary currency, dental alloy, photography, explosives (Oberdörster, 2010). These characteristics mainly attributed to increase the surface area to volume ratio, which potentially results in high reactivity (Hansen *et al.,* 2008). Silver nanoparticles have been used as nonmaterial's most frequently in consumer products because of antimicrobial properties (Hansen et al. 2008). Silver in the form of Ag^+ ions has toxic effects on many pathogens, including bacteria, viruses, and fungi (Lok *et al.* 2007).

Sawosa (2007) studied the effect of different levels of colloidal SNPs (0, 5, 15, and 25 mg/kg diet) on gut micro flora and duodenal morphology in quails. The results of mention study indicated that the effect of silver nanoparticles on the number of *E. coli* and other intestinal bacteria were not significant. Grodzik and Sawosza (2008) had investigated the effect of silver nanoparticles on the embryo broiler growth and morphology of bursa of Fabricius. Their research results showed that silver nanoparticles with concentration of 10 mg had not affect on the growth of embryo chicken, but the number and size of the lymph follicles decreased.

Ahmadi and Rahimi (2011) reported the birds fed diet supplemented with powder of silver nanoparticles had significantly decreased performance compared to control group. Therefore, the aims of current study was to examine the effects of silver nanoparticles with different levels on growth performance, weight of visceral organs, and some blood parameters of broilers from 1 to 21 days of broiler age.

Material and methods

Birds, housing

A total of 240 one-day-old male chicks (Ross 308) with initial mean body weight (45.5g±0.29) purchased from a local commercial hatchery (Behparvar Broiler Co, Rasht, Iran) used in study. Birds had distributed to four groups of 60 birds, including 4 replicates and 15 birds in each pen at the Poultry Farm of Kurdistan Branch, Islamic Azad University. Birds were housed in the experimental pens (120 cm×150 cm×70 cm) with individual feed and water trough and all pens bedded with a wood-shavings litter. Chickens had free access to water and feed throughout study. The temperature inside the poultry house was maintained at 35°C during the first 3 days, between 28 and 33° C during the specific number of days, and about 25°C in the whole study.

Table 1. Ingredients and composition basal diet (as-fed basis).

Ingredients (%)	Starter(1-21d)
Corn	60.00
Soybean meal (48%)	35.00
Soybean oil	2.00
Salt	0.17
Limestone	1.20
Dicalcium phosphate (DCP)	1.21
DL-Methionine	0.15
Vitamin and Mineral premix[1]	0.50
Calculated nutrients	
ME_n (kcal/kg)	3050
CP	22.17
Ca	1.01
Available phosphorus	0.45
Methionine + cysteine (%)	0.85
Lysine	1.36
Arginine	1.27

[1]Supplied per kilogram of diet respectively: 37.5 mg of $ZnSO_4 \cdot H_2O$, 37.5 mg of MnO, 37.5 mg of $FeSO_4 \cdot 7H_2O$, 3.75 mg of $CuSO_4 \cdot 5H2O$, 0.83 mg of KI, and 0.23 mg of $NaSeO_3$. I, 0.7, 0.6 mg; Se, 0.3, 0.3 mg; vitamin A, 8,000, 6,000 IU; vitamin D3, 1,000, 500 IU; vitamin E, 30, 20 IU; menadione, 0.5, 0.5 mg; thiamine, 2.0, 2.0 mg; flavin, 8.0, 5.0 mg; niacin, 35, 30 mg; pyridoxine, 3.5, 3.0 mg; vitamin B12, 0.01, 0.01 mg; pantothenic acid, 10.0, 10.0 mg; folic acid, 0.55, 0.55 mg; biotin, 0.18, 0.15mg; choline chloride, 1, 1 g; flavomucin, 0.1, 0 g; antioxidant, 0.4,05 g.

Experimental Diets

The four groups of birds were fed experimental diets supplemented with different levels of SNPs. Experimental diets were: T1) control (basal diet, without SNPs); T2) basal diet + 4 mg SNPs/kg basal diet; T3) basal diet + 8 mg SNPs/kg basal diet, and T4) basal diet + 12 mg SNPs/kg basal diet. The concentration of stock SNPs used in current study was 0.8 percent silver. Basal diets were formulated to meet or exceed nutrient requirement of broiler based on recommendation of NRC (1994). Composition of the basal diet is shown in Table 1. Also due to the small amount of stock powder in this basal diet it was mixed with the respective amounts of nanosilver of stock powder and nanosilver as a small batch, then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed. The analysis of silver nanoparticles stock is shown in Table 2.

Sample collection

At the final part of the research (on the day 21), four birds has been selected from each replicate for blood sampling and then slaughtering. Visceral organs such as fat pad, small intestine, liver, pancreas, gizzard, bursa Fabricius, spleen, and Thymus have been removed and their relative weight calculated as percentage of live body weight. The blood samples (16 samples per treatment) have been taken from the brachial vein. Blood samples were centrifuged (2500×*g* for 10 min at 4° C), and then serum stored at -20°C until analysis. The concentrations of serum metabolites were measured using standard kits (Pars Azmoon Co., Tehran, Iran).

Statistical analysis

A completely randomized experimental design was applied with four groups of 60 bird that including four replicates, (15 birds in each replicate). Experimental data were submitted to analysis of variance (ANOVA) and using the General Linear Models (GLM) procedure of the SAS (2009) statistical programs after their normality was verified. The results were expressed as the mean values and errors when the differences, Duncan's multiple range tests performed. Mean values were considered significantly different at P≤0.05.

Results and discussion

Growth performance

Based on the performance results are shown in the Table 3, ANOVA did not detect any significant effect of SNPs levels on performance traits such as gain body weight (LBW), feed intake (FI), and feed conversion ratio (FCR). The experiment diets were formulated using a single basal diet and then supplemented basal diet with different levels of SNPs, thereby reducing any potential errors in feed mixing, which supports any possible differences detected. These results are in line with the works of Ahmadi and Kurdistani (2010) who observed that birds group fed diet supplemented with different levels of colloidal nanosilver (5, 10, and 15 mg SNPs/kg diet) presented lower FI and LBW. No significant (P≥0.05) differences in mortality were found among treatments throughout the experiment (data no shown).

Table 2. Composition of silver nanoparticles of stock.

Name	**Nano silver Powder**				
Appearance	White Powder				
Composition stock (%)					
[$Ag_{0.15}$ $Na_{0.45}$ $H_{0.4}$ $Zr_2(PO4)_3$]	99.99				
	Ag	Na	H	Zr	PO4
	0.8	7.2	6	20	66
PH	7.2				
Melting point (Centigrade)	250				
Size (µm)	25- 40				
Density (g/cm&sup3)	D50≤2.0				
Water (%)	1.4				

Visceral organs

Based on the results related the effect of SNPs on relative visceral organs weight are shown in the Table 4. SNPs significantly (P≤0.05) increased relative weight of small intestine (SI) and liver of birds. These results can be explained due to antimicrobial effect of SNPs. Therefore, silvernanoparticles can change number and type of

microorganism and for this reason the increase of internal toxin. Ahmadi and Kurdistan (2010) and Keller et al. (2008) reported that nanosilver had acted as an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria including antibiotic-resistant strains such as *Acinetobacter, Escherichia, Pseudomonas, Salmonella,* and *Vibrio*. Therefore, this situation could be indirectly affected on digestion and absorption of nutrients in SI and relative weight of it. Yildirimer *et al.* (2011) reported that after absorption of nanosilver from GIT, it entered to blood systemic circulation, therefore this particle can potentially interact with different metabolites such as: plasma proteins. Oberdörster *et al.* (2010) showed that silver of nanoparticles induced oxidative stress; therefore, this condition probably has negative effects on metabolic pathway and therefore may be impaired utilisation of nutrients by birds. The results at this study indicate that SNPs had significantly affected on relative weight of liver (P≤0.05). This can be explained due to accumulate of silver metal in liver. Jia *et al.* (2008) and Savolainen *et al.* (2010) indicated that nanoparticles after absorption from GIT could enter in the blood stream, and then distributing in different organs especially liver and kidney.

Table 3. Effect of silver nanoparticles on growth performance of broilers[1].

Treatments	n*	Growth performance		
		LBW (g)	FI (g)	F/G (g/g)
T1. Control	16	915	1234	1.37
T2. 4 ppm/kg	16	908	1242	1.39
T3. 8 ppm/kg	16	876	1213	1.41
T4. 12ppm/kg	16	821	1183	1.44
SEM		0.40	0.15	0.16

[1]Means with different superscripts in the same column differ ($P \leq 0.05$). *The means represent four pens per treatment.

Immune organs

Results are shown in Table 5. Data shown that SNPs had significantly decreased (P≤0.05) relative weight of bursa Fabricius compared to control treatment. Lowest bursa weight was observed in birds groups that fed on T4 diet (12 mg) at the end of study. There were no significant effects of SNPs levels on relative spleen and thymus weights. Bursa is one of the organs related to immune system with B-lymphocyte production. In poultry, the bursa of Fabricius is the primary lymphoid organ responsible for the establishment and maintenance of the B-lymphocyte compartment. Therefore, it could be changed by using silver nanoparticles. Tang *et al.* (2011) showed that silver nanoparticles can anchor and penetrate to the cell wall of Gram-negative bacteria and impaired chain electron of transmissions and caused cell death.

Serum lipid

Based on the results, Triglyceride (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) in comparison with control treatment were increased (P≥0.05), and HDL decreased (Table 6). Also, it has been found that Ag+ seems to disturb mitochondria due to interactions with thiol (-SH) groups of the mitochondrial inner membrane. Landsiedel *et al.* (2010) have concluded that silver nanoparticles in higher concentrations (>44.0 µg/ml) are necrotic to cells, leading to rapid cell membrane rupture. Yildirimer *et al.* (2011) demonstrated that nanoparticles of silver induces oxidative stress and adversely affects the structure and peroxidation of lipid in the cell membranes and function, for this reason, fat membrane structure ruptured and this condition probably changed concentration of different serum of lipid.

Conclusion

Results current trial revealed that silver nanoparticles no suitable an alternatives as growth performance. As well as SNPs had caused that decreased relative immune organs of weight and increased LDL of serum. Therefore, this changes may be induced negative effects on immune response and overall health of broilers.

Table 4. Effect of silver nanoparticles on visceral organs of broilers[1]

Treatments	n*	Visceral organs (% LBW)					
		Liver	Heart	Gizzard	Pancreas	Fat pad	SI
T1. Control	16	0.90c	1.23	1.23	0.38	1.35	1.34d
T2.4 pm/kg	16	1.13b	1.24	1.24	0.36	1.38	1.72c
T3.8 pm/kg	16	1.18b	1.21	1.21	0.37	1.44	2.05ab
T4. 2ppm/kg	16	1.26a	1.18	1.19	0.35	1.45	2.34a
SEM		0.40	0.15	0.15	0.66	0.16	1.64

[1]Means with different superscripts in the same column differ ($P \leq 0.05$)

*The means represent four pens per treatment.

Table 5. Effects of silver nanoparticles on relative weight of immune organs of broiler age[1]

Treatments	n*	Bursa Fabricius	Spleen	Thymus
T1. Control	16	0.23a	0.13	0.58
T2.4 pm/kg	16	0.19b	0.13	0.59
T3.8 pm/kg	16	0.18b	0.13	0.58
T4. 2ppm/kg	16	0.14c	0.13	0.58
SEM		0.81	0.67	0.61

[1]Means with different superscripts in the same column differ ($P \leq 0.05$).

*The means represent four pens per treatment.

Table 6. Effect of silver nanoparticles on the lipid of serum of broiler[1]

Treatments	N*	Cholesterol (mg/dL)	TG (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
T1. Control	16	59.23	131.24c	19.11b	37.1c	92.3a
T2. 4 ppm/kg	16	63.09	139.15ab	19.62b	36.6c	91.6a
T3. 8 ppm/kg	16	71.21	144.8a	23.35a	44.3b	82.2b
T4. 12ppm/kg	16	78.35	147.27a	24.09a	48.9a	84.7b
SEM		0.51	3.82	1.21	4.89	8.36

[1]Means with different superscripts in the same column differ ($P \leq 0.05$).

*The means represent four pens per treatment.

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