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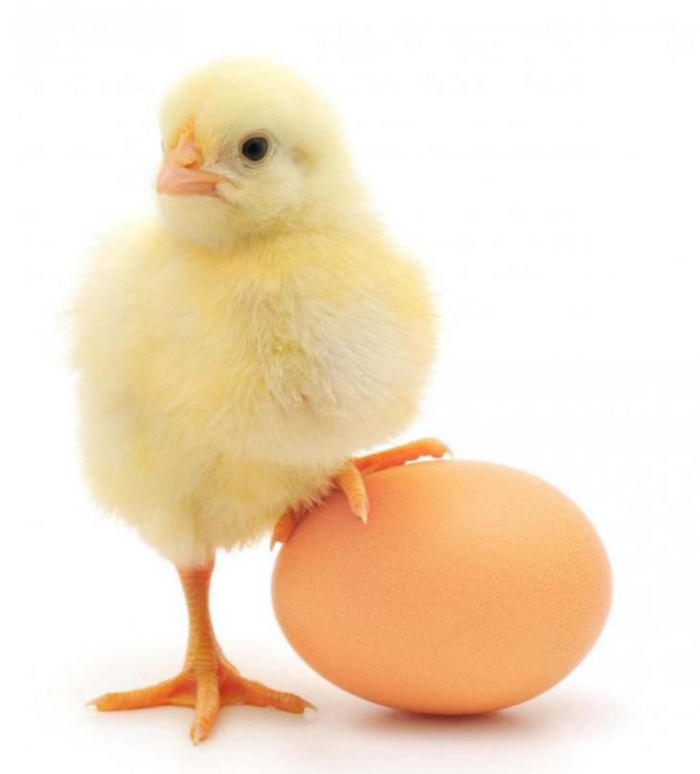

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Effect of Dietary Nanosilver on Broiler Performance

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Abstract: This study was carried out to investigate the effect of silver nanoparticles (AgNPs) on broiler growth performance, carcass traits, blood constitutes and counts of *E. coli* and *lactobacillus*. A total of 180 seven day old un-sexed broiler chicks (Hubbard) were allocated into six groups each group containing three replicates (10 birds in each replicate). Basal control diet was supplemented with different levels of AgNPs (2, 4, 6, 8 and 10 ppm/kg) throughout growth trial period (7-35 days). The results showed that the heaviest final body weight and the highest body weight gain recorded by adding 4 ppm AgNPs/kg. There were no significant differences in overall feed intake at different levels of nanosilver. The best feed conversion ratio (1.5) obtained by using 4 ppm AgNPs/kg compared with all treatments studied. Serum total lipids were significantly decreased in all treatments compared to control. Cholesterol was significantly decreased at 2, 4 and 6 ppm AgNPs/kg diet compared to control. All levels of AgNPs had significantly decreased AST except 6 ppm AgNPs. Total serum antioxidant capacity significantly increased in all supplemented levels of dietary AgNPs compared to control, while 4 ppm AgNPs recorded the highest value. In addition, nanosilver had increase the European production efficiency index (EPEI) in all treatments compared to control and 4 ppm AgNPs recorded the best EPEI compared to all treatments. Broilers fed different levels of AgNPs had decreased the number of harmful bacteria represented as *E. coli* compared to control and had no effect on microflora represented as *lactobacillus*. It could be concluded that the best productive performance of broiler occurred by supplementing 4 ppm AgNPs/kg in broiler diets. More studies should be done in this new area of researches in the future.

Key words: Silver nanoparticles, performance, blood constituents, bacteria count, broiler

INTRODUCTION

Nanotechnology plays a major role in the areas of research in poultry science. The future challenges of the poultry science research include: increasing the feed efficiency, controlling the macro and micro-nutrient absorption, tackling the diseases, targeting drug delivery, safety growth promoter, modifying the contents of the egg like full protein egg and cholesterol free egg, reducing the energy and protein wastage in unproductive physiological purposes thereby, increasing the feed efficiency and reducing the price of the poultry meat. It can be achieved through the application of nano-biotechnology and nano immunology to poultry research (Kannaki and Verma, 2006).

Silver compounds appear as a potential alternative of some feed additives such as organic acids, oligosaccharides, plant extracts, etc. The major concerns about the safe use of an additive in animal feeding are its effective role as antimicrobial, acting selectively over potential pathogens but not over symbiotic microbial communities (Fondevila *et al.*, 2009).

It has been shown that the continuous usage of antibiotics as growth promoters encourage the retention in animal tissues and that the human consumption of such animal products would potentially increase processes of antibiotic resistance. Movements of social pressure towards food security were claiming for a strict control and against their usage in animal feeding. So, the European community banned using of antibiotics as growth promoters since 2006. Therefore, nutritionists trying to substitute those with different feed additives materials such as nanosilver, organic acids and probiotic (Leeson, 2007).

Andi *et al.* (2011) recorded a significant improvement in the weight gain, feed intake and feed conversion ratio of broilers fed diets contain silver nanoparticles. Marwa (2012) studied the effect of nanosilver on reproductive of laying quail in drinking water with level of 20 ppm and found highly significant effect of the interaction between age at mating and the administration of nanosilver on live body weight of quail at all growing period. On contrary, Ahmadi and Kurdestani (2010) reported that silver in the form of nanoparticles (5, 15 and 25 ppm/kg) had no effect on weight gain of broilers. However, Ahmadi (2009) reported that the level of 900 ppm AgNPs had more significant effect on live body weight than 300, 600 ppm AgNPs/kg of broiler diet.

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According to Hong *et al.* (2014), nanosilver is considered as a potential additive to animal feed. Nanosilver is an effective killing agent against a broad spectrum of gram positive and gram negative bacteria (Burrell *et al.*, 1999), including antibiotic resistance strains (Wright *et al.*, 1999). Sawosz *et al.* (2007) studied the effect of different levels of colloidal AgNPs (0, 5, 15 and 25 mg/kg diets) on intestinal microbial flora and duodenal morphology in Quails and they found that the effect of silver nanoparticles on the number of *E. coli* and other intestinal bacteria were not significant. Hassanabadi *et al.* (2012) showed that nanosilver improved intestinal microflora, increased *lactobacillus* bacteria and decreased *E. coli* in broiler chicks.

Therefore, this study aimed to evaluate the effects of silver nanoparticles on growth performance, organs weight, bacteriological studies and some blood constituents.

MATERIALS AND METHODS

This study was carried out with 180 Hubbard broiler chickens (7 days old) at different levels of silver nanoparticles (0, 2, 4, 6, 8 and 10 ppm /kg of diet) within 18 replicates. In each replicate, 10 chickens were kept with an average weight of 125 g. Each treatment divided to three replicates. Chicks were fed corn soybean meal based mash diet as nutrient requirements were covered according to strain catalog of Hubbard. Ingredients and chemical composition of basal diet were presented in Table 1. All management factors being temperature, light, water, ventilation and vaccination were similar for all treatments. Body weight (BW) and feed intake (FI) were recorded biweekly and average body weight gain (BWG), feed conversion ratio (FCR) and European production efficiency index (EPEI) were calculated. At the end of the experiment (35 day), three birds from each treatment were randomly taken and slaughtered to evaluate carcass characteristics. Blood samples were collected in heparinized tube from 3 birds/group and plasma was separated to determine serum content of total protein, albumin, globulin, cholesterol, total lipids, AST, ALT and total antioxidant capacity, using commercial kits. The definition and count of the gastrointestinal tract microbial count were measured according to Yoon *et al.* (2007).

The obtained data were statistically analyzed using linear models procedure described in SAS users guide (SAS, 1990). Differences among treatment means were tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Body weight and body weight gain: The effect of diet supplemented with different levels of AgNPs on body weight (BW) and body weight gain (WG) are shown in Table 2. There were no significant differences between all treatments in initial body weight (BW1) and BW3. While, BW4 had significantly increased for all treatments except T6 compared with control group. The heaviest final body weight (BW4) and the highest body weight gain (BWG3) recorded in T3 (4 ppm AgNPs/kg). The overall body weight gain (OBWG) showed significantly increase for all treatments compared with control group except T6 (10 ppm). The best BWG recorded with T3 (4 ppm) being 1857 g.

All levels of AgNPs for all periods studied increased body weight and body weight gain compared with control. The same trend obtained by Andi *et al.* (2011) who reported that weight gain was significantly increased by using nanosil (ionic silver and H_2O_2) in days of 1-35 and 1-42 days. Sawosz *et al.* (2007) demonstrated that when Japanese quail supplemented with 5, 15 and 25 mg AgNPs/liter in drinking water, the body weight was increased at 25 ppm/kg and increased lactic acid bacteria.

Table 1: Composition and calculated analysis of starter grower and finisher diets

Ingredients	Starter grower (1-28 d)	Finisher (29-35 d)
Yellow corn	57.88	62.05
Soybean meal 44%	29.79	26.25
Corn Gluten meal 60%	5.00	4.00
Soy bean oil	3.29	4.26
Limestone	1.12	0.99
Di-Calcium phosphate	1.70	1.46
Vitamin. premix *	0.30	0.30
DL-Methionine	0.18	0.16
L-lysine (HCl)	0.24	0.13
Na Cl	0.50	0.40
Total (%)	100.0	100.0
Calculated analysis **		
CP (%)	21.00	19.00
ME (k cal/kg)	3078	3178
Calcium (%)	0.92	0.80
Available phosphorus (%)	0.45	0.40
Lysine (%)	1.25	1.06
Methionine (%)	0.54	0.50
Methionine and Cystine (%)	0.92	0.83
Sodium (%)	0.21	0.17

*Each 3 kg contains: Vit A 12 000 000 IU, Vit D_3 2 000 000 IU, Vit E 10 g, Vit K_3 2 g, Vit B_1 1 g, Vit B_2 5 g, Vit B_6 1.5 g, Vit B_{12} 10 mg, Nicotinic acid 30 g, Pantothenic acid 10 g, Folic acid 1 g, Biotin 50 mg Choline chloride 250 g, Iron 30 g, Copper 10 g, Zinc 50 g, Manganese 60 g, Iodine 1 g, Selenium 0.1 g, Cobalt 0.1 g and carrier ($CaCO_3$) to 3 kg

**According to Feed Composition Tables for Animal and Poultry Feedstuff Used in Egypt (2001)

Table 2: Effects of dietary supplementation of silver nanoparticles (AgNPs) on body weight (BW) and body weight gain (BWG) of broiler chicks

Treatments traits	T1	T2	T3	T4	T5	T6
BW1 (7 d)	125	125	125	125	125	125
BW2 (14 d)	341^{b}	369^{a}	362^{ab}	376^{a}	361^{ab}	359^{ab}
BW3 (28 d)	1249	1311	1282	1299	1288	1261
BW4 (35 d)	1738^{c}	1960^{a}	1982^{a}	1930^{ab}	1973^{a}	1833^{bc}
BWG1 (7-14 d)	217^{b}	243^{ab}	236^{ab}	250^{a}	236^{ab}	234^{ab}
BWG2 (15-28 d)	907	943	920	923	928	903
BWG3 (29-35 d)	489^{c}	655^{a}	701^{a}	639^{a}	686^{a}	572^{b}
OBWG (7-35 d)	1613^{c}	1835^{a}	1857^{a}	1805^{ab}	1848^{a}	1708^{bc}

a,b and c Means in the same row with no common superscripts differ significantly ($p<0.05$)

T1: Control, T2: 2 ppm AgNPs/kg, T3: 4 ppm AgNPs/kg, T4: 6 ppm AgNPs/kg, T5: 8 ppm AgNPs/kg and T6: 10 ppm AgNPs/kg

Table 3: Effects of dietary supplementation of silver nanoparticles (AgNPs) on feed intake (FI) and feed conversion ratio (FCR)

Treatments traits	T1	T2	T3	T4	T5	T6
FI1 (g) (7-14 d)	352	333	331	363	328	353
FI 2 (g) (15-28 d)	1388^{b}	1422^{b}	1418^{b}	1387^{b}	1423^{b}	1489^{a}
FI 3 (g) (29-35 d)	889^{c}	1129^{a}	1084^{a}	1054^{ab}	1138^{a}	933^{bc}
OFI (g) (7-35 d)	2629^{b}	2883^{a}	2883^{a}	2803^{a}	2890^{a}	2775^{ab}
FCR 1 (7-14 d)	1.62^{a}	1.37^{c}	1.40^{bc}	1.44^{bc}	1.39^{bc}	1.51^{ab}
FCR 2 (15-28 d)	1.53^{b}	1.51^{b}	1.54^{b}	1.50^{b}	1.53^{b}	1.65^{a}
FCR 3 (29-35)	1.82^{a}	1.73^{ab}	1.55^{b}	1.65^{ab}	1.66^{ab}	1.63^{b}
OFCR (7-35 d)	1.66^{a}	1.53^{c}	1.50^{c}	1.53^{c}	1.53^{c}	1.60^{b}
*EPEPI	299^{c}	359^{a}	374^{a}	357^{a}	368^{a}	328^{b}

a, b and c Means in the same row with no common superscripts differ significantly (p<0.05). *EPEI: European production efficiency index

T1: Control, T2: 2 ppm AgNPs/kg, T3: 4 ppm AgNPs/kg, T4: 6 ppm AgNPs/kg, T5: 8 ppm AgNPs/kg and T6: 10 ppm AgNPs/kg

Table 4: Effects of dietary silver nanoparticles (AgNPs) on carcass and relative weight of organs at 35 days of broiler age

Treatment traits	T1	T2	T3	T4	T5	T6
LBW (g)	1738^{c}	1960^{a}	1982^{a}	1930^{ab}	1973^{a}	1833^{bc}
Carcass (%)	67.92^{ab}	67.74^{ab}	68.53^{a}	68.22^{ab}	67.44^{ab}	66.33^{b}
Liver (%)	3.11	2.28	2.27	2.05	2.36	2.01
Heart (%)	0.60	0.55	0.52	0.52	0.50	0.46
Gizzard (%)	1.42^{ab}	1.37^{ab}	1.48^{a}	1.42^{ab}	1.23^{b}	1.36^{ab}
Spleen (%)	0.12	0.13	0.13	0.13	0.14	0.14
Abdominal fat (%)	1.18^{b}	1.46^{ab}	1.47^{ab}	1.35^{ab}	1.49^{ab}	1.68^{a}

a,b,c and d mean in the same row, within each period with different superscript are significantly (p<0.05) different

T1: Control, T2: 2 ppm AgNPs/kg, T3: 4 ppm AgNPs/kg, T4: 6 ppm AgNPs/kg, T5: 8 ppm AgNPs/kg and T6: 10 ppm AgNPs/kg

Also, Ahmadi (2009) reported that the level of 900 ppm AgNPs had significant increase in live body weight compared with control group. On the other hand, Ahmadi (2011) showed that nanosilver had no significant effect on growth performance compared with control group by using nanosilver at levels of 20, 40 and 60 ppm/kg of diets. In this connection, Ahmadi and Rahimi (2011) studied the effect of nanosilver on broiler performance using levels 4, 8 and 12 ppm in drinking water and showed that nanosilver had no significant effect on body weight gain. Felehgari *et al.* (2013) investigated effects of nanosilver on performance and digestive organs of broiler during starter period at levels 25 and 50 ppm and showed that nanosilver had no significant effect on live body weight compared with control.

The positive response results may be due to the antibacterial properties of nanosilver affecting microbial populations without inducing resistance and increasing anabolic activity that may lead to the stimulation of development and growth of animals and increase the rate of metabolism, so, this may lead to the improvement of the growth of broiler (Usama, 2012).

Feed intake and feed conversion ratio: The effects of nanosilver on feed intake and feed conversion ratio are shown in Table 3. The feed intake was increased by addition of nanosilver. There were no significant differences in FI 1 (7-14 d) and FI 2 (15-28 d) between all treatments studied except T6 (10 ppm) in FI 2 which recorded the significantly highest feed intake (1489 g). While, T6 and T1 in FI 3 (29-35 d) and OFI (7-35 d) recorded the lowest FI compared with other treatments. The worst feed conversion ratios (FCR) were observed in control treatment for FCR1, FCR2 and OFCR. There were no significant differences in OFCR between T2, T3, T4 and T5 although, they significantly recorded best feed conversion ratio compared with control (T1) and (T6). Numerically, T3 (4 ppm) obtained the best FCR (1.5) compared with all treatments studied. Same results were obtained by Andi *et al.* (2011) who showed that feed intake increased and feed conversion ratio improved in total period (1-42 days) by using nanosilver in broiler diets. Also, Ahmadi (2009) studied the effect of different levels of nanosilver (300, 600 and 900 ppm/kg of diet) on broiler performance and showed that 900 ppm of nanoparticle improved feed conversion ratio than other treatments and increased feed intake. In contrast, Ahmadi (2011) found that dietary supplementation of AgNPs at levels 20, 40 and 60 ppm decreased trend in feed conversion ratio of broiler chicks compared with control group.

In this regard, Hassanabadi *et al.* (2012) studied the effect of nanosilver supplementation with 0.5, 1, 1.5 ppm/kg of diet in starter and 1, 2, 3 ppm/kg of diet in grower compared with vaccine on broiler chicks performance and they found that mean feed intake and feed conversion ratio were the same for birds fed nanosilver and the group that received vaccine. On contrary, Felehgari *et al.* (2013) found that 25 and 50 ppm of nanosilver used in broiler starter diet had no significant effect on feed intake and FCR compared with control. Also, Ahmadi (2011) showed that the addition of AgNPs (20, 40 and 60 ppm/kg) didn't improves feed intake and feed conversion ratio of broiler through 42 day trial period. These results may be due to the effect of ionic silver on harmful bacteria in intestine and resulted in healthy hindgut and better absorption of nutrients (Andi *et al.*, 2011).

The European production efficiency Index (EPEI) was calculated and appears that nanosilver had increased the EPEI in all treatments that received nanosilver. Numerically, T3 (4 ppm) recorded the best EPEI, being 374, compared to all treatments.

Carcass characteristics: The effect of dietary AgNPs on carcass weight percentage and relative weight of organs are presented in Table 4. There was no significant effect observed between all treatments on carcass weight percentage expect T3 (4 ppm) which recorded highest weight and T6 (10 ppm) being the lowest. The same trend was observed for gizzard percentage.

On the other hand, adding of AgNPs had no effect (p<0.5) on liver, heart and spleen percentage. There was no significant effect for AgNPs supplemented levels on abdominal fat% (p<0.05) except T6 (10 ppm) was recorded the highest value of abdominal fat compared

Table 5: Effect of dietary nanosilver (AgNPs) on blood constituents of broiler

Treatments trait	T1	T2	T3	T4	T5	T6
Total protein (g/dl)	3.816^{ab}	3.887^{ab}	3.668^{b}	4.030^{ab}	4.375^{a}	3.988^{ab}
Albumin (mg/dl)	3.007^{ab}	3.009^{ab}	2.788^{b}	3.053^{ab}	3.030^{ab}	3.160^{a}
Globulin (mg/dl)	0.809^{b}	0.878^{b}	0.880^{b}	0.976^{ab}	1.345^{a}	0.828^{b}
A/G ratio	3.72^{ab}	3.43^{ab}	3.17^{b}	3.13^{ab}	2.25^{a}	3.82^{a}
Total lipid (g/dl)	293.16^{a}	238.26^{b}	228.46^{bc}	222.22^{bc}	227.35^{bc}	206.25^{c}
Cholesterol (mg/dl)	96.00^{a}	69.67^{c}	72.81^{c}	83.58^{b}	94.68^{a}	92.06^{a}
ALT (U/L)	33.5	33.00	32.67	30.33	27.67	23.17
AST (U/L)	86.50^{a}	69.00^{b}	67.00^{b}	62.00^{b}	79.00^{a}	60.00^{b}
Total antioxidant capacity (Mm/L)	0.153^{d}	0.223^{c}	0.311^{a}	0.275^{b}	0.283^{b}	0.223^{c}

a, b, c and d mean in the same row, within each period with different superscript are significantly ($p<0.05$) different

ALT: Alanine aminotransferase enzyme, AST: Aspartate aminotransferase enzyme

T1: Control, T2: 2 ppm AgNPs/kg, T3: 4 ppm AgNPs/kg, T4: 6 ppm AgNPs/kg, T5: 8 ppm AgNPs/kg and T6: 10 ppm AgNPs/kg

Table 6: Effects of AgNPs on total *lactobacilli* and *E. coli* on broiler

Treatment	Total *Lactobacillus*	*E. coli*
T1	*More than 300 cfu/10 g sample	More than 300 cfu/10 g sample
T2	More than 300 cfu/10 g sample	7.5×10^{6} cfu/10 g sample
T3	More than 300 cfu/10 g sample	3.5×10^{6} cfu/10 g sample
T4	More than 300 cfu/10 g sample	3.5×10^{5} cfu/10 g sample
T5	More than 300 cfu/10 g sample	3.5×10^{5} cfu/10 g sample
T6	More than 300 cfu/10 g sample	3.5×10^{5} cfu/10 g sample

*More than 300 cfu / 10 g sample: means un countable plate

T1: Control, T2: 2 ppm AgNPs/kg, T3: 4 ppm AgNPs/kg, T4: 6 ppm AgNPs/kg, T5: 8 ppm AgNPs/kg and T6: 10 ppm AgNPs/kg

to control group. Similarly, Ahmadi and Rahimi (2011) reported that the levels of AgNPs 4, 8 and 12 ppm had significantly increased the weight of small intestine and abdominal fat compared with control and had no effect on weight of liver and gizzard. Also, Ahmadi and Kurdestani (2010) recorded lower bursa weight and increased spleen and thymus weight. Felehgari *et al.* (2013) recorded that AgNPs significantly increased small intestine and liver but had no effect on heart, gizzard, proventriculus and pancreas. While Andi *et al.* (2011) showed that AgNPs had negative effects on liver weight relative to live body weight.

These results may be due to the effect of nanosilver on microbial population and probably changes in the proportion between pathogen and non-pathogen organisms in cecum.

Blood constituents: The effects of dietary nanosilver on blood constituents are presented in Table 5. Serum total protein was increased by increasing AgNPs levels with, T5 recorded the best total protein value followed by T4. The same trend was obtained with serum globulin. The highest value of albumin was obtained by using 10 ppm AgNPs (T6) while, T3 recorded the lowest value. Serum total lipids was significantly decreased in all treatments compared to control.

Cholesterol was significantly decreased in T2, T3 and T4 compared to control. All levels of AgNPs had significantly effect on AST except T5. Total serum antioxidant activity significantly increased in all levels of dietary AgNPs compared to control, with T3 recorded the highest value followed by T5 and T4. The same result obtained by Ahmadi and Kurdestani (2010) who studied the effect of different levels of nanosilver (5, 15 and 25 ppm) in drinking water and showed that nanosilver has significantly effect on total of oxidative stress enzyme in comparison to control treatment. However, in *ovo* AgNPs injection of broiler eggs had no effect on blood biochemical parameters in blood serum (Sikorska *et al.*, 2010). Ahmadi (2011) showed that ALT, AST, Albumin and cholesterol were significantly affected by using nanosilver (20, 40 and 60 ppm/kg of diet) in broiler chicks. On the other hand Andi *et al.* (2011) found that AgNPs had no effect on ALT and AST. Also, Sawosz *et al.* (2009) reported that the nanoparticles of silver had no effect on activity of enzymes, AST, ALT and cholesterol in quail serum. These effects may be related to oxidative stress that caused peroxidation of fat in the body (Ahmadi, 2011).

Total count: Results of the influence of AgNPs on cecum microbial (total *lactobacillus* and *E. coli*) content in broiler are shown in Table 6. Broiler fed different levels of AgNPs had decreased the number of harmful bacteria represented as *E. coli* compared to control and had no effect on microflora represented as *lactobacillus*. This study agree well with Fondevila *et al.* (2009) who studied AgNPs supplementation levels of 0, 25, 50 and 100 ppm *in vitro* and found that the proportion of coliforms ileal content reduced whereas no affect was observed on *lactobacillus* proportion. Also, Blomberg *et al.* (1993) found that metallic silver nanoparticles would reduce the viability of organisms with a potentially harmful effect, such as coliforms, whereas it does not affect *lactobacilli*, which positively compete against pathogens proliferation and reduce their virulence.

On the other hand, Sawosz *et al.* (2007) investigated that 25 mg/kg of AgNPs supplemented in quail drinking water had significantly increased number of gram-positive bacteria (*Lactobacillusspp, Leuconostoclactis Actinomycesnaeslundii*) compared to control birds.

The mechanism of the inhibitory effects of AgNPs was higher in case of Gram negative bacteria. This might be due to the thickness of the peptidoglycan layer in Gram-positive bacteria cell wall which may prevent to some extent. Yoon *et al.* (2007) observed a higher effect of silver nanoparticles on *Bacillus subtilis* than on *Es-cherichia coli*, suggesting a selective antimicrobial effect, possibly related to the structure of the bacterial membrane, although Singh *et al.* (2008) assumed higher sensitivity of Gram-negative bacteria to treatment with nanoparticles.

It could be concluded that the addition of nanosilver to broiler diets improve the performance. The best level was 4 ppm/kg diet. More studies should be directed to investigate more advantages of using nanosilver in poultry feeding.

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