Performance, Intestinal Microbial Population, Immune and Physiological Responses of Broiler Chickens to Diet With Different Levels of Silver Nanoparticles Coated on Zeolite

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Research

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Abstract

Background: The aim of this study was to evaluate the effects of different levels of silver nanoparticles coated on zeolite on performance, gastrointestinal microflora, heterophile to lymphocyte ratio and humoral immune system responses of broiler chickens. Three hundred seventy five Cobb 500 in a completely randomized design were distributed to 5 experimental diets: control diet, 1% zeolite, 0.25%, 0.50% and 0.75% of silver nanoparticles coated on 1% zeolite. Performance traits were recorded and Blood samples were taken to determine antibody response against Newcastle on d 14, infectious bursal disease (Gumboro) on d 21 and Serum immunoglobulins (IgY, IgM, and IgA) were determined on d 42. Heterophile to lymphocyte ratio was evaluated on days 28, 35 and 42. At the end of d 21 and d 42.

Results: The result showed that feed conversion ratio was significantly improved in silver nanoparticle treatments compare with the control group ($p < 0.05$). Total anaerobic bacteria and the population of *E.coli* in ileum and cecum were decreased ($p < 0.05$). The highest number of lactobacillus was observed in NS50 ($p < 0.05$). Antibody response against Gumboro was decreased as well as the amount of IgY antibody in silver nanoparticles treatments. Significant differences were observed in heterophile to lymphocyte ratio on d 28 and 35 and the highest amount was observed in NS50 group ($p < 0.05$).

Conclusions: In conclusion, using silver nanoparticle dietary coated on zeolite could improve gut microflora, but silver nanoparticles could also have harmful side effects on the immune mechanism of broiler chickens.

Background

Intensive poultry farming and industrial poultry production has been achieved from the second half of the 20th by shortening the productive cycle and increasing the production capacity. In the past 50–60 years, the most important goal of broiler breeding is to increase profitability of broiler meat production and most achieved in poultry have been on performance traits. High intensity poultry production requires fast growing strains, usually at high stocking densities. With this type of husbandry, flocks are highly susceptible to disease. Studies with wild birds showed that increased productive effort reduces specific immune responses, parasite resistance, and parent survival (Van der Klein et al. 2015). It was determined that genotypes with higher body weight have weaker antibody response than lower body weight line of broilers (Boa-Amponsem et al. 2000).

On the other hand, gastrointestinal tract and the gut microbial ecosystem with 10^7 to 10^{11} bacteria per each gram play vital roles in nutrition absorption, development of immunity, and disease resistance (Pickard et al. 2017). Alterations in the microbial community may have adverse effects on feed efficiency, productivity, and health of chickens (Wu and Wu 2012). It was also observed that the gut microbial population can promote the migration of mast cells into the intestine (Kunii et al. 2011). Gut microbial population was also found to be the major driving force to produce mucosal IgA (Crabbé et al. 1968).

During the last few decades, antibiotics have been widely used as growth promoters to improve feed efficiency and animal growth through the modulation of gut microbial population and the host's immune system. The ban on antibiotics for growth promotion within the European Union in 2006, caused more interests in finding alternatives to antibiotics in poultry production (Torok et al. 2011).

Therefore, any factor and approach that can improve this microbial ecosystem could be considered as immunomodulators and also, one of several approaches to increase immune responsiveness in high intensity production in poultry is to supplement rations with feed additives (Rauw 2010; Hashemi and Davoodi 2012). The common feed additives used in poultry industry are herbal plants, antioxidants, emulsifiers, binders, enzymes, pH control agents, probiotics and prebiotics (Hashemi and Davoodi 2014).

Recently, using silver as an alternative antimicrobial agent has been noticed by researchers. Metallic silver compounds and silver ions have been known to have unique antibacterial properties for a long time. It has been used since ancient times to treat burns, wounds and bacterial infections (Rai et al. 2009). Nanotechnology is one of the newest branches of science that make it possible to manufacture silver in the nano size (1-100 nm). Nanoparticles are more reactive than larger particles because of the larger surface area and exposing more atoms on the surface of them. Furthermore, silver nanoparticles (Ag-NPs) have unique biological properties and broad-spectrum antibacterial activity against microorganisms (Lara et al. 2010). Because of these strong abilities of silver nanoparticles to eliminate bacteria, and disability of pathogens to resist against these particles, it is supposed that Ag-NPs may affect microbial populations of gastrointestinal tract and hereby may improve immune system and productive purposes (Varner et al. 2010).

In addition to antimicrobial properties, Ag-NPs are capable to carry oxygen molecules at the cellular level. The oxygen fits and accumulates in the octahedral holes of Ag-NPs may increase anabolic activity and subsequently stimulate growth and development (Pineda et al. 2012). Also, it has been reported the use of the Ag-NPs in poultry have great potential (Sawosz et al. 2007) but there are only a few investigations regarding the use of silver nanoparticles and zeolite in poultry nutrition.

Also, zeolites are crystalline aluminosilicates with physicochemical effects such as permitting ion exchange, absorption, diffusion, molecular sieving, dehydration, reversible dehydration and catalysis that caused using of these products in animal nutrition (Eleroglu and Yalçın 2005). Mallek et al (2012) reported that supplementing zeolite to broiler diets has positive effects on performance, organoleptic parameters and mainly increased level of Omega 3 fatty acid. Besides, silver nanoparticles coated on zeolite were demonstrated to use as feed additives in several studies because of their positive effects on meat quality, feed conversion ratio and hepatic enzymes of broiler chickens (Hashemi et al. 2014).

Thus the aim of this experiment was to study the effects of silver nanoparticles coated on zeolite on microbial community composition of ileum and cecum, heterophile to lymphocyte ratio as an index of stress, humoral immunity and morphometric analysis of the gut in broiler chickens.

Methods

Diet and feeding

A total of 375 one-day-old (Cobb 500) broiler chicks (half male and half female) were obtained from a local commercial hatchery. Broilers were vaccinated for infectious bronchitis (on d 1), Newcastle disease (on d 7), and Gumboro live vaccine (on d 14). Chickens were randomly allocated in 5 experimental treatments for 6 weeks. Each treatment arranged in 5 replicated of 15 broilers each. All procedures were approved by the ethics committee for animal experiments at the Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The temperature was regulated at $32\pm1^{\circ}\text{C}$ during the first week and it decreased 1°C every 3 days. The temperature was fixed at $23\pm1^{\circ}\text{C}$ for the rest of the experiment. The experimental treatment diets were:

1. Basal diet without any addition (C).
2. Basal diet containing 1% zeolite (Z).
3. Basal diet containing 1% zeolite coated with 25 ppm Ag-NPs (NS25).
4. Basal diet containing 1% zeolite coated with 50 ppm Ag-NPs (NS50).
5. Basal diet containing 1% zeolite coated with 75 ppm Ag-NPs (NS75).

The zeolite that used in this experiment obtained from well-defined zeolitic stratigraphic units from the region of Semnan province, Iran. Zeolitic rock was pulverized and sieved to make particles in the size of 1-2 mm. distilled water was used to wash and remove contaminations from the particles and subsequently dried at 105°C all over the night in the oven. The chemical formula of pure zeolite was $(\text{K}_2, \text{Na}_2, \text{Ca}, \text{Mg})_3 \text{Al}_6\text{Si}_{30}\text{O}_{72} \cdot 24\text{H}_2\text{O}$.

Nanosized silver particles (Ag-NPs) with a maximum diameter of 50 nm was coated on zeolite and received as a gift from Dr. D. Davoodi at Agricultural Biotechnology Research Institute of Iran (ABRII), (Karaj, Iran). Briefly, the preparation of coating method on zeolite with desired properties was that the zeolite was first stirred in distilled water with a stirrer, and then, the prepared nanosilver (20-50 nm) with the desired percentage added to the mixture after adjusting the pH and Stirring was continued for a further 30 min at 15°C . The stabilizers were gradually added to the mixture and stirred until the resulting mixture color became brown. After settling of the precipitate dried at ambient temperature.

Zeolites were analyzed by X-ray Fluorescence (XRF) technique to determine their chemical composition and the XRF data collected on a PHILIPSPW1480 XRF spectrometer with Rh tube (Nikpey et al. 2013) (Table 1). The morphology studied by Field emission scanning electron microscopy (FESEM, Mira, 3-XMU) and the materials composition investigated by energy dispersive X-ray spectroscopy (EDX) and elemental mapping analyses at Razi Metallurgical Research Center, Iran (Figure 1). To measure the silver (Ag) content of zeolite we used the method described by Kulthong et al (2010). Briefly, 0.2-0.3 g of sample digested in 5 mL of 14.4 M HNO_3 by a microwave digestion system to dissolve all the silver content. The microwave irradiation cycles were 250 W (5 min), 400 W (5 min), and 600 W (5 min). Then the digested

sample was cooled and diluted up to 25 mL with deionized water to enable quantification of silver by a graphite furnace atomic absorption spectroscopy or GFAAS (Perkin Elmer Analyst 300, Waltham, MC).

The basal diet was in mash form and formulated for starter (1 to 21 day), and grower (22 to 42 day) periods. The composition and ingredients of basal diets are shown in table 2. Birds had free access to feed and water.

Sample collection and measurement

Feed consumption was recorded on a weekly basis, by monitoring the feed offered and amounts remained at feeders at the end of the week. Individual bird weight was also recorded weekly. Feed conversion ratio (FCR) was calculated as the following: $FCR = \text{average feed consumed} / \text{average live weight}$. On d 21 and 42, two birds of each replication were killed. The gastrointestinal tract was removed and immediately the contents of the cecum and ileum were diluted 10-fold using sterile Phosphate Buffer Solution (BPS) (pH 7.0) and homogenized on a vortex for 3 min. the dilution continued serially for each sample from 10^{-1} to 10^{-9} and then subsequently plated to the enumeration of bacterial groups.

Total anaerobes, *Lactobacillus* and *Escherichia coli* were enumerated respectively using duplicated plate count agar (PCR), MRS agar and Mac Conkey agar according to tuohy et al (2002). Anaerobic incubation was achieved using sealed anaerobic jars. The plates were incubated at 37°C for 24 to 72 h and then the numbers of colonies were counted. The results of the experiment were expressed as $\log_{10}$ colony-forming units per each gram of digested content of the intestine.

A total of 50 birds [10 birds per treatment] was used for determining the proportion of heterophile to lymphocyte (H/L) as an index of stress on d 28, 35 and 42. Blood samples (1-2 ml) were collected from the wing vein and immediately transferred to heparinized containers. Two drops of blood samples placed on the slide and a blood smear were made. After drying stained with Wright-Giemsa stain. Samples were examined under a compound microscope (Nikon, Eclipse E200-LED) (Carroll et al. 1996). Standard avian guidelines for manual hematology were used to identify cells. Heterophils and lymphocytes were counted to a total of 100 cells.

The titer of antibody activity against Newcastle disease measured by conventional Hemagglutination Inhibition (HI) assay on d 14 (7 d after postimmunization). HI assay was done according to the procedure of OIE and the titers of antibodies against Gumboro were measured on d 21 (7 d after postimmunization) using a commercial Enzyme linked immuno sorbent assay (ELISA) kit (Razi Company; Arak, Iran). To measure the amount of plasma immunoglobulin, blood samples were collected from the wing vine on d 42 and centrifuged at 2000 rpm for 10 min. Blood plasma stored at -20°C for further analysis. The concentrations of immunoglobulin Y, M and A (IgY, IgM and IgA) were measured by commercial ELISA kits (Kamiya Biomedical Company).

Statistical analysis

All data were initially checked for normality and homogeneity of variance using Bartlett and Kolmogorov-Smirnov tests. All bacteria enumeration data were transformed to $\log_{10}$ CFU/ml. Statistical analyses were subjected to ANOVA using the General Linear Model procedure (GLM) (SAS 2003). When significant differences occurred ($p < 0.05$), means were separated by Tukey's Studentized range test (SAS 1989).

Results

The effect of dietary treatments on performance traits including body weight and feed intake indicates that diet containing silver nanoparticles and zeolite have not significant difference with the control group ($p > 0.05$). Moreover, feed conversion ratio was significantly higher in silver nanoparticle treatments compare with the control group ($p < 0.05$). The population of total anaerobes bacteria of ileum on d 21 did not show any significant difference between treatments, while it significantly decreased on day 42 in NS75. The total anaerobes bacteria of cecum on d 21 and 42 days of age significantly affected by experimental diets, and decreased compared to the control group. Moreover, the number of *E. coli* bacteria was also decreased in both ileum and cecum of broiler chickens on d 21 and 42. The population of *Lactobacillus* of ileum was affected by experimental treatments. At 21 and 42 days of age, the highest total count of *Lactobacillus* sp. was observed in NS50 treatment ($p < 0.05$).

The ratio of H/L on day 28 increased in both NS50 and NS75. It was also increased on d 35 in NS50 significantly.

Experimental treatments did not have any significant effect on the titer of antibody against Newcastle but Ag-NPs significantly decreased antibody against Gumboro compared to the control diet. Moreover, the concentration of IgY and IgA of plasma significantly decreased by increasing the level of Ag-NPs while the amount of IgM did not show any significant difference.

Discussion

Many studies have demonstrated the antibacterial activity of Ag-NPs even in low concentrations (Rai et al. 2009). The mechanisms of action of Ag-NPs on bacteria are not totally investigated yet but it is known that silver binds with sulfur-containing proteins of the bacterial cell wall and cell membrane and inhibits the respiration process (Slavin et al. 2017). Moreover, silver ions interact with the phosphorus containing compounds like DNA and inhibit replication (Silver et al. 2006). It has also been attributed that Ag-NPs can inactive phosphomannose isomerase enzyme. This enzyme catalyzes the conversion of mannos-6-phosphate to fructose-6-phosphate which is an important intermediate of glycolysis as the most common pathway in sugar catabolism of bacteria. Moreover, in the *E. coli* bacteria, some holes in the cell membranes were observed after contact with Ag-NPs (Choi and Hu 2008).

In addition, Pineda et al (2012) reported that *in ovo* injection of silver nanoparticles reduced feed intake and body weight gain in chickens however, no concurrent effect on feed conversion ratio was observed.

Although there is no negative effects on feed intake has been reported as a result of silver nanoparticle supplementation in the feed or via the drinking water (Pineda et al. 2012; Ahmadi 2012).

In agreement with other researches, Sawosz et al (2007) reported that when a diet supplemented with 25 mg/kg Ag-NPs the number of gram-positive bacteria (*Lactobacillus* spp, *Leuconostoc lactis*, *Actinomyces naeslundii*) significantly increased compared to the control group in quails. The number of *Streptococcus bovis* colonies in samples taken from quails receiving 25 mg/kg Ag-NPs increased. However, Ag-NPs did not influence the population of *Enterococcus faecium*. There was no significant effect on the number of *E. coli* and other Enterobacteriaceae.

Exact mechanism of nanoparticle synthesis in biological systems was not completely described. The mechanism of intracellular and extracellular production differs from one organism to another.

Therefore, as it was observed in this study the population of anaerobic bacteria was decreased which are sensitive to oxygen concentration while the colonies of lactic acid bacteria (non-obligatory anaerobic), increased in competition with anaerobes (Singh et al. 2008). Moreover, it was shown that gram-negative bacteria are more sensitive to treatment with nanoparticles (Coccini et al. 2013) that can explain the reason for significant reduction in *E. coli*. Moreover, in this study, the amount of H/L ratio increased significantly in NS50 (Days 28 and 35 of age) and NS75 (Day 35 of age).

It has been shown that nanoparticles can induce oxidative stress. There are several key factors involved in inducing reactive oxygen species (ROS) by nanoparticles: 1) pro-oxidant groups that exist on the surface of nanoparticles; 2) redox cycle on the surface of nanoparticles due to transition metal-based NP; and 3) interactions of particle cells. ROS have strong bactericidal activities but they may also hurt body cells as well and induce apoptosis in the body tissues (Coccini et al. 2013).

It was reported that silver nanoparticles can cause apoptosis via the mitochondrial pathway by generating ROS and activating c-Jun N-terminal kinase (JNK) (Hsin et al. 2008). Accumulation of ROS can cause inflammatory response, perturbation and destruction in mitochondria.

Lovric et al (2005) reported that Ag-NPs entered blood systemic circulation after absorption from GIT. These particles are capable to interact with different metabolites like plasma proteins, platelets, red and white blood cells, and coagulation factors. In this way, silver nanoparticles can induce oxidative stress by changing the structure and physiological characteristics of cells.

Ahmadi (2012) showed that 20, 40 and 60 ppm of Ag-NPs significantly increase the activity of oxidative enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Moreover Ag-NPs can cause damage to different organs and tissues like liver cells.

It was demonstrated that stressors, such as food or water deprivation, temperature extremes, constant light, and exposure to novel social situations increase a number of heterophils while decrease lymphocytes. The mechanisms of these changes in the number of immune cells of birds are not clearly

known, but it may be due to the effects of stress on the adrenal corticotrophic hormone and corticosterone and cytokines.

The results of this study indicated that Ag-NPs is a stressor factor in young chickens (d 28 and 35) but it did not affect the H/L ratio at the age of 42 d. Toxicity effects of Ag-NPs were studied in the zebrafish embryonic model. The results showed that all sizes of colloidal Ag-NPs (3, 10, 50 and 100 nm) could induce toxicity and the rate of mortality increased by size reduction of particles (80%, 64%, 36%, and 3% respectively). Overall, there are other different parameters that can be responsible for toxicity of Ag-NPs, such as, concentration, stability and chemistry.

Similar to the results of this study in humoral immunity parameter, Pineda et al (2012) reported that the concentration of plasma IgY reduced by using 10 and 20 ppm of Ag-NPs.

Vadalasetty et al (2018) also showed that the concentration of IgY and IgM were lower in the chickens received Ag-NPs compared to the control group. The reason for reduction in the levels of IgY and IgA in this study might be due to the changes in microbial colonization occurred by Ag-NPs received from the diet. Ag-NPs may impair intestinal transportation of nutrients such as sugar, amino acids, trace elements and vitamins and the deficiency of nutrients can decrease antibody formation.

In addition, this event might be due to the effects of Ag-NPs on lymphoid organs such as bursa and spleen (Elkloub et al. 2015). Grodzik and Sawosz (2006) determined that 10 ppm of Ag-NPs had no effect on chickens' growth but reduced the number and size of the lymph follicles in the bursa of Fabricius.

Bursa is one of the most important organs related to the immune system by producing B-lymphocyte. It is the first lymphoid organ that is responsible for the establishment and maintenance of B-lymphocyte compartment. Therefore using Ag-NPs can influence the amount of antibodies by affecting the quantity and the quality of B-lymphocytes.

Castillo et al (2008) showed that Tiopronin monolayer-protected Ag-NPs doesn't have any proinflammatory effects on macrophages while it can inhibit the secretion of IL-6 by specific TLRs located on the cell membrane or in the endocytic compartments.

Furthermore, Ag-NPs could significantly inhibit phytohemagglutinin that induce cytokine production.

Conclusions

In conclusion, the results of the current study investigated that silver nanoparticles improved the gastrointestinal microflora properly. Despite its potential effect on digestive microbial biodiversity and function, other effects of nanosilver related to host physiological statuses, such as the immunological status and stress factors may negatively be influenced by silver nanoparticles.

Declarations

Ethics approval and consent to participate

This study obtained ethical clearance (Approval No: 97/20/1655) from the institutional animal care and use committee of Gorgan University of Agricultural Sciences and Natural Resources.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: S. R. Hashemi

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Methodology: D. Davoodi, A. Ashayerizadeh and B. Dastar

Data analysis: S. Hassani

Writing original draft: N. Bolandi and S. R. Hashemi

Writing review and editing: S. R. Hashemi

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Tables

Table 1. The summarized chemical composition of the used zeolite by means of X-ray Fluorescence (XRF) ¹ technique.	
Constituents	% by weight
SiO ₂	68.95
Al ₂ O ₃	11.14
Fe ₂ O ₃	0.97
CaO	4.83
Na ₂ O	0.95
K ₂ O	0.90
MgO	0.79
TiO ₂	0.201
MnO	0.011
P ₂ O ₅	0.012
SO ₃	0.068
L.O.I ²	10.64
Si/Al	4.81
Ag ³	< 5ppm

Semnan natural zeolite-rich tuffs.

¹ XRF PHILIPS PW1480 XRF spectrometer with Rh tube.

² LOI: loss on ignition.

³ The silver content was measured by a graphite furnace atomic absorption spectroscopy (GFAAS).

Table 2. Composition and analysis of the basal diet (as fed basis)				
Zeolite diet		Control diet		
(22-42)	(1-21)	(22-42)	(1-21)	Ingredients (%)
57.84	51.60	59.69	53.70	Yellow corn
33.68	39.95	33.25	39.52	Soybean meal (44% CP)
4.11	3.69	3.41	3.00	Soybean oil
1.00	1.00	0	0	Zeolite (Coated zeolite)
1.09	1.47	1.09	1.47	Dicalcium phosphate
1.28	1.18	1.29	1.19	Limestone
0.32	0.43	0.32	0.43	Salt
0.25	0.25	0.25	0.25	Vitamin premix ¹
0.25	0.25	0.25	0.25	Mineral premix ²
0.05	0.13	0.05	0.13	DL-Methionine
0.13	0.05	0.13	0.06	L-Lysine
<i>Chemical analysis</i>				
3050	2950	3050	2950	ME (Kcal/kg)
19.06	21.2	19.06	21.2	CP (%)
0.86	0.92	0.86	0.92	Ca (%)
0.33	0.41	0.33	0.41	Pa (%)
0.14	0.18	0.14	0.18	Na (%)
0.95	1.01	0.95	0.01	Lys (%)
0.36	0.47	0.36	0.47	Met (%)
0.37	0.36	0.37	0.36	Met + Cys (%)

¹Supplied per Kg of diet: Vitamin A, 1,500 IU; Cholecalciferol, 200 IU; Vitamin E, 10 IU; Riboflavin, 3.5 mg; Pantothenic acid, 10 mg; Niacin, 30 mg; Cobalamin, 10 µg; Choline chloride, 1,000 mg; Biotin, 0.15 mg; Folic acid, 0.5 mg; Thiamine 1.5 mg; Pyridoxine 3.0 mg.

²Supplied per Kg of diet: Iron, 80 mg; Zinc, 40 mg; Manganese, 60 mg; Iodine, 0.18 mg; Copper, 8 mg; Selenium, 0.15 mg.

Table 3. Effect of different treatments on body weight, feed intake and feed conversion ratio in broilers			
Treatments	Body weight (g)	Feed intake (g)	Feed conversion ratio
From d 1 to 21			
C	812.38	1270.00	1.56
Z ¹	816.12	1243.92	1.52
NS25 ²	817.82	1199.68	1.46
NS50 ³	836.36	1198.67	1.43
NS75 ⁴	831.04	1238.25	1.49
SEM ⁵	21.11	39.48	0.04
<i>P-value</i>	.9117	.6719	.3136
From d 22 to 42			
C	1376.82	3297.14	2.39 ^a
Z ¹	1507.44	3384.23	2.24 ^b
NS25 ²	1477.59	3105.34	2.10 ^{bc}
NS50 ³	1440.98	3102.57	2.09 ^c
NS75 ⁴	1434.01	3124.82	2.18 ^{bc}
SEM ⁵	45.19	96.30	0.04
<i>P-value</i>	.3459	.0772	.0012
From d 1 to 42			
C	2189.21	4567.1	2.08 ^a
Z ¹	2323.55	4628/0	1.99 ^b
NS25 ²	2295.41	4204.9	1.87 ^c
NS50 ³	2277.34	4211.04	1.84 ^c
NS75 ⁴	2265.05	4363.1	1.92 ^{bc}
SEM ⁵	50.28	104.43	0.02
<i>P-value</i>	.43	.05	.00

¹Diet containing 1% Zeolite; ²Diet containing 1% Zeolite coated with 25 ppm nanosilver; ³Diet containing 1% Zeolite coated with 50 ppm nanosilver; ⁴Diet containing 1% Zeolite coated with 75 ppm nanosilver; ⁵Standard error of means.

Values with different superscripts in the same column for each section are significantly different ($p < .05$).

Table 4. Effect of treatments on microflora of broilers on d 21 and 42 (log cfu/ g)					
treatments	Cecum		Ileum		
	<i>E. coli</i>	Total anaerobes	<i>E. coli</i>	Total anaerobes	<i>Lactobacillus</i> sp.
Day 21 of age					
C	9.23 ^a	7.93 ^a	7.73 ^a	8.28	7.21 ^c
Z ¹	8.73 ^{ab}	8.40 ^a	6.28 ^b	7.50	7.78 ^{bc}
NS25 ²	8.44 ^b	8.16 ^a	5.88 ^b	7.58	8.14 ^{ab}
NS50 ³	8.31 ^b	5.73 ^b	5.70 ^b	7.17	8.71 ^a
NS75 ⁴	8.12 ^b	8.28 ^a	5.10 ^b	7.60	8.15 ^{ab}
SEM ⁵	0.221	0.470	0.420	0.313	0.244
<i>P-value</i>	.019	.012	.003	.215	.015
Day 42 of age					
C	7.83 ^a	9.38 ^a	7.41 ^a	8.47 ^a	8.31 ^c
Z ¹	6.96 ^{ab}	8.14 ^b	6.47 ^{ab}	8.04 ^a	8.70 ^{bc}
NS25	6.77 ^{ab}	8.06 ^b	6.63 ^{ab}	7.87 ^a	9.13 ^{ab}
NS50	6.25 ^{bc}	8.02 ^b	6.25 ^{ab}	7.57 ^a	9.52 ^a
NS75	5.14 ^c	7.61 ^b	4.93 ^b	4.34 ^b	8.76 ^{bc}
SEM	0.470	0.204	0.523	0.342	0.220
<i>P-value</i>	.010	< .0001	.041	< .0001	.014

¹Diet containing 1% Zeolite; ²Diet containing 1% Zeolite coated with 25 ppm nanosilver; ³Diet containing 1% Zeolite coated with 50 ppm nanosilver; ⁴Diet containing 1% Zeolite coated with 75 ppm nanosilver; ⁵Standard error of means.

Values with different superscripts in the same column for each section are significantly different ($p < .05$).

Table 5. Effect of treatments on H/L ratio, antibody titer against Newcastle (HI) and Gumboro of broilers					
treatments	H/L ratio Day 28 of age	H/L ratio Day 35 of age	H/L ratio Day 42 of age	Newcastle Day 14 of age	Gumboro Day 21 of age
C	0.24 ^b	0.27 ^b	0.33	3.70	427.50 ^a
Z ¹	0.25 ^b	0.30 ^b	0.33	4.10	418.50 ^a
NS25 ²	0.25 ^b	0.31 ^{ab}	0.33	4.10	396.25 ^{ab}
NS50 ³	0.30 ^a	0.34 ^a	0.36	4.50	372.25 ^b
NS75 ⁴	0.29 ^a	0.30 ^{ab}	0.35	4.10	372.75 ^b
SEM ⁵	0.011	0.015	0.010	0.321	11.009
<i>P-value</i>	.0004	.021	.160	.454	.0088

¹Diet containing 1% Zeolite; ²Diet containing 1% Zeolite coated with 25 ppm nanosilver; ³Diet containing 1% Zeolite coated with 50 ppm nanosilver; ⁴Diet containing 1% Zeolite coated with 75 ppm nanosilver; ⁵Standard error of means.

Values with different superscripts in the same column for each section are significantly different ($p < .05$).

Table 6. Effect of treatments on the amount of immunoglobulins of plasma (mg/ml) on d 42			
treatments	IgA	IgM	IgY
C	0.480 ^a	0.091	0.552 ^a
Z ¹	0.472 ^a	0.083	0.508 ^a
NS25 ²	0.350 ^b	0.078	0.324 ^b
NS50 ³	0.280 ^{bc}	0.070	0.342 ^b
NS75 ⁴	0.234 ^c	0.073	0.256 ^b
SEM ⁵	0.043	0.061	0.032
<i>P-value</i>	.0002	.360	< .0001

¹Diet containing 1% Zeolite; ²Diet containing 1% Zeolite coated with 25 ppm nanosilver; ³Diet containing 1% Zeolite coated with 50 ppm nanosilver; ⁴Diet containing 1% Zeolite coated with 75 ppm nanosilver; ⁵Standard error of means.

Values with different superscripts in the same column for each section are significantly different ($p < .05$).

Figures

Figure 1

Field emission scanning electron microscopy (FESEM) micrographs for morphology studied and energy dispersive X-ray spectroscopy (EDX) for elemental map-ping analyses and materials composition of the Ag-NPs coated on zeolite.

Figure 1

Field emission scanning electron microscopy (FESEM) micrographs for morphology studied and energy dispersive X-ray spectroscopy (EDX) for elemental map-ping analyses and materials composition of the Ag-NPs coated on zeolite.