Iranian Journal of Applied Animal Science

Gastrointestinal Microbial Population Response and Performance of Broiler Chickens Fed with Organic Acids and Silver Nanoparticles Coated on Zeolite under Heat Stress Condition

Research Article

A. Abbasi[1], S.R. Hashemi[1*], S. Hassani[1] and M. Ebrahimi[2]

[1] Department of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran
[2] Department of Food Industry, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran

Received on: 20 Dec 2017
Revised on: 26 Apr 2018
Accepted on: 30 Apr 2018
Online Published on: Dec 2018

*Correspondence E-mail: hashemi711@gau.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

The aim of this study was to evaluate gastrointestinal microbial population response and performance of broiler chickens fed organic acids and silver nanoparticles coated on zeolite under heat stress condition. In this experiment, 375 one-day old broiler chicks (Cobb 500) were randomly divided into 5 treatments containing 5 replicates with 15 birds in each replicate. Five dietary treatments were compared: (1) basal diet (control), (2) basal diet containing 1% zeolite, (3) basal diet containing 1% of zeolite coated with 0.5% silver nanoparticles, (4) basal diet containing 1 g/kg organic acids and (5) basal diet containing 1% zeolite-coated with 0.5% silver nanoparticles and 1 g/kg organic acids. Feed intake and body weight were recorded in the course of the whole experiment for each treatment, and the feed conversion ratio were calculated subsequently. On days 21 and 42 of the experiment, one chicken in each replicate slaughtered to enumerate gastrointestinal microbial population. The results of the experiment indicate that diet containing nanosilver with organic acid decreased broiler body weight compared with the control and zeolite treatment at 21-42 days of age (P<0.05). Also feed conversion ratio was significantly higher in the nanosilver coated on zeolite group than in the control, zeolite and organic acids groups in the whole experimental period (P<0.05). However, there was no significant difference between the experimental treatments on the responses of gastrointestinal microbial population (P>0.05). In conclusion, the present results showed, although silver nanoparticles and organic acids did not have particular effect on performance parameters and increasing the number of useful intestinal microbial population (lactic acid), yet they did not have destructive effects on outcome either. Therefore, these additives can be used in broilers diet.

KEY WORDS gastrointestinal, heat stress, organic acid, performance, silver nanoparticle.

INTRODUCTION

Poultry industry plays a critical role in providing jobs for millions of people across the world and it is considered as one of the main suppliers of human's animal protein (Morêki, 2008). This industry is rapidly growing, especially in tropical and subtropical regions, with extensive areas of Asia, Africa and South America which contains the majority population of the world and located in such climatic condition (Lee *et al.* 2007). Broilers keep their body temperature constant in a portion of environment temperature called the thermal comfort zone (21-23 ˚C), with the least expenditure of energy. Temperatures higher than thermal comfort zone will cause adverse heat effects or heat stress

(Hashemi *et al.* 2007). High environmental temperature reduces feed intake, live weight and feed use efficiency, and thereby, decreases the performance of broilers (Daneshyar *et al.* 2015). In addition, heat stress has destructive effects on health and physiology of birds, which can lead to changes in body composition. Digestive system in particular reacts to stressors especially heat stress. Heat stress changes the protective and normal population of the gastrointestinal tract (Bailey *et al.* 2004). Gastrointestinal microflora plays a key role in the production efficiency and health of birds. An imbalance gastrointestinal tract ecosystem attenuates useful microorganisms and increases pathogens chance to create colony formation. Factors such as heat stress, digestive disorders, diet changes, and the use of antibiotics cause imbalancement in gastrointestinal microbial flora population (Lin *et al.* 2011). Feed additives are able to alleviate the negative consequences of heat stress. Therefore, have an important role in poultry industry.

In the recent years, nanotechnology has received a great attention within scientific and industrial communities in many countries including Iran. Nanotechnology, as a powerful new technology, has the ability to create massive revolution in feed supply and agricultural systems at global scale by improving diet quality and consequently health and growth performance. Studies showed substances which are smaller than few nanometers have different properties than initial substance; including large surface area, more solubility and higher mobility (Buzea *et al.* 2007).

Materials with dimensions less than 100 nm referees as nanoparticles. There are various types of nanoparticles such as Ag, Au and Zn. Among metal nanoparticles silver nanoparticles have further antibacterial activity compared to others (Lioyd, 2003). Many studies have identified silver nanoparticles antibacterial effect against a wide range of gram-positive, gram-negative and even antibiotic-resistant bacteria for example, (Shameli *et al.* 2011) examined the antimicrobial ability of silver nanoparticles embedded in the zeolite pores, and found that silver nanoparticles have bactericidal effect on gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. Silver reduces anaerobic microorganisms and also increases the microorganism's population with ability to live in the reduced oxygen pressure, especially *Lactobacillus* (Grudzien and Sawosz, 2006). In addition, experiments performed on nanosilver showed this product reduced the feed intake and improved feed conversion ratio in broiler chickens (Fondevila *et al.* 2008; Naghizadeh *et al.* 2011). In a study by Andi *et al.* (2011), the presence of silver nanoparticles improved weight gain, feed intake and feed conversion ratio.

On the other hand, organic acids are feed additives which by decreasing intestine pH, decrease harmful microorganisms, leading to reduced susceptibility to pathogens, improved immune system and high resistance to diseases (Waldroup and Kanis, 1995). According to the reports of Parks *et al.* (2001), diets containing propionic acid in turkey poults significantly reduced the enterobacteria and increased mortality. Moreover, it was reported that addition of organic acids in poultry diets improves growth performance and feed conversion ratio likely due to maintaining digestive health (Gornowicz and Dziadek, 2002). It has been reported that at levels 5000 and 10000 ppm, formic acid significantly improves broiler's feed conversion ratio. (Garcia *et al.* 2007). Organic acids also have ability to improve performance in poultries, and can provide healthier food for humans (Levic *et al.* 2008). The aim of this study is to investigate gastrointestinal microbial population response and performance of broiler chickens fed with organic acids and silver nanoparticles coated on zeolite under heat stress condition.

MATERIALS AND METHODS

Experimental conditions

This experiment performed at the Poultry Research Station, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran. For experiments, 375 one-day old broiler chicks (Cobb 500) were randomly divided into 5 treatments containing 5 replicates with 15 birds in each replicate. Five dietary treatments were compared: (1) basal diet (control), (2) basal diet containing 1% zeolite, (3) basal diet containing 1% of zeolite coated with 0.5% silver nanoparticles, (4) basal diet containing 1 g/kg organic acids and (5) basal diet containing 1% zeolite-coated with 0.5% silver nanoparticles and 1g/kg organic acids. The acidifier (Biotronic®) manufactured by BIOMIN in Austria, and consists of formic acid and propionic acid. The basal diet prepared for starter and growth periods, and composition of the experimental diets prepared according to the requirements prescribed in Cobb 500 manual and set up using UFFDA software (Table 1). Birds had *ad libitum* access to feed and water for all treatment groups and continuous lighting program was provided during experiments. For all treatments room temperature was set at 32 ˚C on first day and decreased gradually to reach about 23 ˚C by 20'th day of period. From day 35 to 42, birds were exposed to heat exposure (high temperature, 34±1 ˚C, 70±5% RH) for 4 hours each day from 12 to 16 pm.

Performance assessments and statistical analysis

Feed intake and body weight were recorded in the course of the whole experiment for each treatment, and the feed conversion ratios were calculated subsequently. On day 21 and 42 of the experiment, ileum and cecum of the slaughtered birds (n=1 for each replicate) aseptically removed, put into sterile stomacher bags (Spiral Biotech Inc., Norwood, MA), and kept on ice. Fresh ileum and cecum contents were diluted 10-fold by weight in 0.9% normal saline and mechanically homogenized and inoculants serially diluted up to 10^{-9}. Subsequently, dilutions of 10^{-7}, 10^{-8}, and 10^{-9} were inoculated (100 µL of each dilution) onto appropriate selective agar media to determine lactic acid bacteria and total anaerobic bacteria, respectively, on de Man Rogosa and Sharpe agar (MRS agar, 110660, Merck, Darmstadt, Germany) and plate count agar (PCA agar, 1.05463, Merck, Darmstadt, Germany) media. All dilutions were inoculated onto selective agar in triplicate. An anaerobic condition was prepared using the bilayered pour plate culture to grow total anaerobic bacteria, which grow in anaerobic conditions (Ashayerizadeh *et al.* 2007). The media were dispensed into the plates near to flame under the hood. The plates were incubated at 37 ˚C for 48 hours. Then, the population of colonies was counted under a colony counter. Finally, Bacterial colonies were counted and averaged. Data have been expressed as log10 colony-forming units/g digesta (Ashayerizadeh *et al.* 2007). Analysis of variance was performed to evaluate the performance characteristics and the microbial population in a completely randomized design using general linear method (GLM) procedure by using SAS (2005), software.

Data were initially checked for normality and homogeneity of variance using Bartlett and Kolmogorov-Smirnov tests. Data analyzed by one-way analysis of variance (ANOVA) and mean comparison was done by Duncan's multiple range tests at 5% level with values of $P < 0.05$ being considered significantly different.

RESULTS AND DISCUSSION

The effects of dietary treatments on performance characteristics including body weight, feed intake and feed conversion ratio in broiler chickens, are shown in Table 2. The results of experiment indicate that diet containing nanosilver with organic acid decreased broiler body weight compared with the control and zeolite treatment at 21-42 days of age (P<0.05). Moreover, feed conversion ratio was significantly higher in the nanosilver coated on zeolite group than in the control, zeolite and organic acids groups in the whole experimental periods (P<0.05). Ileum and cecum microbial population enumeration results on d 21 and 42 are presented in Table 3. The population enumeration of lactic acid bacteria and total anaerobic bacteria in the ileum and cecum showed there is no significant difference between treatments (P>0.05).

Table 1 Composition (% as fed) and analysis of the basal diet

Ingredients (%)	Starter (1-21 d)	Grower (22-42)	Starter (1-21)	Grower (22-42)
Corn	53.7	56.84	50.6	59.96
Soybean meal	39.52	33.68	39.95	33.25
Organic acid	0	0	1	1
Silver nanoparticles coated on zeolite	0	0	0/5	0/5
Soybean oil	3	4.11	3.69	3.41
Dicalcium phosphate	1.47	1.09	1.47	1.09
Limestone	1.19	1.28	1.18	1.29
Salt (NaCl)	0.43	0.32	0.43	0.32
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[1]	0.25	0.25	0.25	0.25
DL-methionine	0.13	0.05	0.13	0.05
L-lysine	0.06	0.13	0.06	0.13
Chemical analysis				
Metabolizable energy (ME) (kcal/kg)	2950	3050	2950	3050
Crude protein (CP) (%)	21.2	19.06	21.2	19.06
Ca (%)	0.92	0.86	0.92	0.86
P (%)	0.41	0.33	0.41	0.33
Na (%)	0.18	0.14	0.18	0.14
Lys (%)	1.01	0.95	1.01	0.95
Met (%)	0.47	0.36	0.47	0.36
Cys (%)	0.36	0.37	0.36	0.37
Arg (%)	1.45	1.27	1.45	1.27
Thr (%)	0.84	0.74	0.84	0.74

[1] Supplied per kg of diet: vitamin A: 1500 IU; vitamin E: 10 IU; Cholecalciferol: 200 IU; Riboflavin: 3.5 mg; Pantothenic acid: 10 mg; Niacin: 30 mg; Cobalamin: 10 µg; Choline chloride: 1000 mg; Biotin: 0.15 mg; Folic acid: 0.5 mg; Thiamine: 1.5 mg and Pyridoxine: 3.0 mg.
Supplied per kg of diet: Iron: 80 mg; Zinc: 40 mg; Manganese: 60 mg; Iodine: 0.18 mg; Copper: 8 mg and Selenium: 0.15 mg.

This study evaluated how organic acids and silver nanoparticles dietary supplementation can impact growth performance parameters and intestinal microbial population in broiler chickens, although some impacts were observed but no significant differences was found between the treatments. In this respect, several studies suggested that the use of silver nanoparticles has not any significant effect on broiler performance traits such as feed intake, body weight and feed conversion ratio (Ahmadi *et al*. 2013; Hassanabadi *et al*. 2012).

Moreover, it has been reported that colloidal silver nanoparticles at 30, 45 and 60 ppm has no effect on the broiler chickens performance on day 21 and 42 of the rearing period (Saki and salari, 2013). Ahmadi and Rahimi (2011), have also stated that use of 4, 8 and 12 ppm silver nanoparticles in the diet has negative impact on broiler chickens performance. In contrast, (Ahmadi, 2009) reported with 900 ppm nanosilver in broilers diet feed conversion ratio improves. Andi *et al.* (2011), reported that feeding chickens with silver nanoparticles, increases weight and feed intake and also improves feed conversion ratio. Naghizadeh and Karimi-Torshizi (2013), also demonstrated that the birds receiving the silver nanoparticles by 50 ppm had the lowest feed intake and the best feed conversion ratio (FCR) compared to control group. In heat stress condition, the production of free radicals increases in chicks' body (Sahin *et al*. 2001).

On the other hand, silver nanoparticles may be associated with protein thiol and oxidant enzymes which are responsible for neutralizing oxidative stress and balancing the production of reactive oxygen species (ROS) in energy metabolism. These particles affect the antioxidant defense mechanism (reducing defensive capability) and cause ROS accumulation (Chen and Schluesener, 2008). Free radicals have high affinity to react with important biomolecules, such as nucleic acid, fatty acid and proteins which cause damage to the membrane, enzymes, and other cell structures in different tissues of birds' body (Solhi-Oskouyi, 2016).

In addition, it has been reported that addition of organic acids to the feed in order to inhibit microorganisms, improves broiler performance through improved digestion and absorption of food in intestine (Dhawale, 2005). Abdel-Azeem *et al.* (2000) and Abdo and Zeinb (2004), reported, use of organic acid supplement improves body weight gain of broilers. It could be due to an improved feed intake, high digestion and absorption of food, increase beneficial intestinal microflora, reduces toxins production and incidence infections while balancing immune response of poultries. Abdel-Fattah *et al.* (2008) also showed that the use of acetic acid, citric acid and lactic acid improves weight gain, feed intake and feed conversion ratio.

On the other hand, the results of other studies showed that the use of organic acids in broiler diets has no significant effect on broiler performance traits such as feed intake, body weight gain and feed conversion ratio (Barbosa Fascina *et al*. 2012; Gunal *et al.* 2006). In addition, Lee *et al.* (1993) reported that growth stimulating compounds such as probiotics, organic acids and antibiotics are ineffective on broilers performance. Lesson *et al*. (2005) observed that 0.4% of butyric acid reduced feed intake. In a research by Ghazalah *et al.* (2011), reported that the levels of 0.25%, 0.5% and 0.75% acetic acid in broiler chickens diet will increase daily weight of broiler chickens. Vander Sluis *et al*. (2002), showed that consumption of organic acids reduces feed speed through digestive system and helps to improve absorption. However, in the current experiment, this factor along with thermal stress seems to be effective in decreasing feed intake and ultimately leads to weight loss in birds. The mentioned factors, along with the effect of nanosilver on increasing the accumulation of ROS and the destruction of some enzymes in a coherent manner, meaningfully reduced the weight gain of birds under thermal stress compared to control treatment.

Nanosilver is an antibacterial compound that affects the composition of bacterial membranes and causes structural change and death of microorganisms. It can cause bacteria death by disrupting the respiratory enzymes and electron transport system, as well as by binding to the surface of bacteria and changing in the structure of the membrane (Percival *et al.* 2005). In a study by Hassanabadi *et al.* (2012), reported with 0.5, 1.0 and 1.5 ppm of silver nanoparticles in early period, alongside 1, 2 and 3 ppm in growth period improves microflora in broilers. This impact consequently increases the number of lactobacilli colonies and reduces the population of *E. coli* in intestinal content. In another study, Grudzien and Sawosz (2006) reported that silver nanoparticles reduced anaerobic microorganisms and also increased the population of microorganisms that have the ability to live in the presence of reduced oxygen pressure, especially lactobacilli. Pineda *et al.* (2012) stated adding 10 and 20 mg/kg silver nanoparticles in chickens drinking water on days 7 to 36 have no significant effect on gut microflora, such as the total population of anaerobic bacteria, lactic acid bacteria, lactose-negative bacteria, coliforms, Enterococci and *Clostridium perfringens*. Sawosz *et al.* (2007), reported silver nanoparticles have no effect on negative-gram bacteria numbers in quail's cecum.

Diet acidification can lead the beneficial bacteria predominance such as Lactobacillus over the pathogens occurring in intestinal contents (Ghazalah *et al.* 2011). In the study by Engberg *et al.* (2000), it has been showed that use of organic acids in broiler diets reduces coliforms population in crop and cecum.

Table 2 Effect of treatments on performance of broiler during the experimental period

Treatment	C	Z	N	A	NA	SEM	P-value
Body weight (g)							
1 to 21 d	687.91	695.55	691.48	687.31	697.20	10.20	0.84
21 to 42 d[1]	1562.13[a]	1537.47[a]	1519.99[ab]	1552.21[ab]	1461.96[b]	27.08	0.043
1 to 42 d	2272.09	2246.12	2211.37	2262.72	2194.57	38.81	0.127
Feed intake (g)							
1 to 21 d	1122.27	1141.21	1138.32	1152.69	1139.11	14.62	0.721
21 to 42 d[1]	3760.28	3701.26	3691.28	3588.53	3642.56	43.24	0.143
1 to 42 d	4781.46	4715.78	4751.21	4755.63	4729.54	54.08	0.227
Feed conversion ratio (g/g)							
1 to 21 d	1.632	1.643	1.648	1.671	1.637	0.013	0.398
21 to 42 d[1]	2.41	2.40	2.44	2.35	2.49	0.028	0.053
1 to 42 d	2.109[b]	2.082[b]	2.192[a]	2.103[b]	2.154[ab]	0.021	0.048

[1] Birds were exposed to heat exposure (34±1 ˚C, 70±5% RH) for 4 hours from d 35 to day 42 of trail.
C: control; Z: basal diet containing 1% zeolite; N: basal diet containing 1% of zeolite-coated with 0.5% silver nanoparticles; A: basal diet containing 1 g/kg organic acids and NA: basal diet containing 1% of zeolite-coated with 0.5% of silver nanoparticles and 1 g/kg organic acids.
[1] Birds were exposed to heat exposure (34±1 ˚C, 70±5% RH) for 4 hours from d 35 to day 42 of trail.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effect of treatments on ileum and cecum microbial population of broilers in d 21 and 42 of breeding[1]

Treatment	d 21 of breeding						
	C	Z	N	A	NA	SEM	P-value
Cecum							
Lactic acid	8.9	8.7	9.0	8.8	8.8	0.20	0.77
Total anaerobic bacteria	9.0	9.4	8.9	9.2	8.9	0.24	0.33
Ileum							
Lactic acid	8.2	8.3	8.4	8.0	8.0	0.19	0.20
Total anaerobic bacteria	8.7	9.0	8.4	8.4	8.3	0.25	0.33
	d 42 of breeding						
Cecum							
Lactic acid	8.8	8.9	8.9	8.8	8.4	0.19	0.55
Total anaerobic bacteria	8.2	8.0	8.3	8.4	9.0	0.38	0.69
Ileum							
Lactic acid	7.5	7.7	7.7	7.2	7.3	0.30	0.90
Total anaerobic bacteria	8.2	8.0	8.1	8.4	8.2	0.31	0.81

[1] Birds were exposed to heat exposure (34±1 ˚C, 70±5% RH) for 4 hours from d 35 to day 42 of trail.
C: control; Z: basal diet containing 1% zeolite; N: basal diet containing 1% of zeolite-coated with 0.5% silver nanoparticles; A: basal diet containing 1 g/kg organic acids and NA: basal diet containing 1% of zeolite-coated with 0.5% of silver nanoparticles and 1 g/kg organic acids.
[1] Birds were exposed to heat exposure (34±1 ˚C, 70±5% RH) for 4 hours from d 35 to day 42 of trail.
SEM: standard error of the means.

According to the results of Byrd *et al.* (2001), the addition of propionic acid and formic acid in the diet effectively decreased the number of coliform and salmonella bacteria in poultry. On the other hand, Akbari *et al.* (2004) showed that addition of acetic acid as an organic acid in drinking water of broilers does not affect the number of total aerobic bacteria and coliform counts in ileal contents. Furthermore, Izat *et al.* (1990) concluded that addition of formic acid to broiler diets has no significant effect on the bacteria in the cecum.

It is believed that antibacterial effects of organic acids occur mainly in initial parts of the gastrointestinal tract of chickens (crop and gizzard) because high density of these acids are removable only from crop and gizzard (Hume *et al.* 1993). In normal conditions the value of pH in crop is about 5.5 (Akbari *et al.* 2004).

Because of high pH in crop compared to pKa of most organic acids, a lot of acid is quickly cleaved into respective proton (H^+) and anion after entering the crop. Because of having charge, the ionized form is unable to cross the cell membrane, and therefore, it cannot exert bactericidal effect. Therefore, the feed that moves to the next part of the digestive system can still have considerable amounts of microbial contamination. These bacteria can be colonized in gastrointestinal tract and affect performance negatively. In addition, Burkholder *et al.* (2008), expressed that thermal stress significantly reduces bird's intestinal microbial population. According to a report from Song *et al.* (2014), in chickens exposed to heat stress compared to those with thermal comfort zone, the count of lactobacilli and bifidobacteria is decreased, and the number of coliforms and *Clostridium perfringens* is increased.

CONCLUSION

In conclusion, the present results showed, although silver nanoparticles and organic acids did not had particular effect on performance parameters and increasing the number of useful intestinal microbial population, yet they did not had destructive effects on outcome either. So these additives can be used in broilers diet.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude and appreciation to Dr D. Davoodi from Department of Nanotechnology, Agricultural Biotechnology Research Institute of Iran (ABRII) who helped us for technical and logistical support of nanomaterial. We would also like to thank Poultry Research Station at Gorgan University of Agricultural Science and Natural Resources for their assistance and providing facilities to perform this research.

REFERENCES

Abdel-Azeem F., El-Hommosany Y.M. and Nematallah G.M. (2000). Effect of citric acid in diets with different starch and fiber levels on productive performance and some physiological traits of growing rabbits. *Egyptian J. Rabbit Sci.* **10,** 121-145.

Abdel-Fattah S., Sanhoury A., Mednay N. and Abdel-Azim F. (2008). Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *Int. J. Poult. Sci*. **7,** 215-222.

Abdo M. and Zeinb A. (2004). Efficacy of acetic acid in improving the utilization of low protein-low energy broiler diets. *Egypt. Poult. Sci*. **24,** 123-141.

Ahmadi F. and Rahimi F. (2011). Factors affecting quality and quantity of egg production in laying hens: A review. *World Appl. Sci. J*. **12,** 372-384.

Ahmadi F., MohammadiKhah M., Saman J., Zarneshan A., Akradi L. and Salehifar P. (2013). The effect of dietary silver nanoparticles on performance, immune organs, and lipid serum of broiler chickens during starter period. *Int. J. Biosci*. **3,** 95-100.

Ahmadi J. (2009). Application of different levels of silver nanoparticles in food on the performance and some blood parameters of broiler chickens. *World Appl. Sci. J*. **7,** 24-27.

Akbari M.R., Kemanshahi H. and Kelidari G.H.A. (2004). Investigating the effect of acetic acid in drinking water on performance, growth and microbial population of ileum in broiler chickens. *J. Agric. Sci. Technol. Natur. Res*. **3,** 139-147.

Andi M.A., Mohsen H. and Farhad A. (2011). Effects of feed type with / without nanosil on cumulative performance, relative organ weight and some blood parameters of broilers. *Glob. Vet*. **7,** 605-609.

Ashayerizadeh O., Dastar B., Shams Shargh M. and Khomeiry M. (2007). Evaluation of intestinal microbial population and the response of young broiler chickens to diets supplemented with Roxarsson, Avilamycin and Gold Formicin. *J. Agric. Sci. Technol. Natur. Res.* **43,** 545-553.

Bailey M.T., Lubach G.R. and Coe C.L. (2004). Prenatal stress alters bacterial colonization of the gut in infant monkeys. *J. Pediatr. Gastroenterol. Nutr*. **38,** 414-421.

Barbosa Fascina V., Roberto Sartori J., Gonzales E., Barros de Carvalho F., Mailinch Gonçalves Pereira de Souza I., do Valle Polycarpo G., Cristina Stradiotti A. and Cristina Pelícia V. (2012). Phytogenic additives and organic acids in broiler chicken diets. *R. Bras. Zootec.* **41,** 2189-2197.

Burkholder K.M., Thompson K.L., Einstein M.E., Applegate T.J. and Patterson J.A. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. *Poult. Sci.* **87,** 1734-1741.

Buzea C., Blandino I. and Robbie K. (2007). Nanomaterials and nanoparticles. Sources and toxicity. *Biointerphases*. **2,** 17-172.

Byrd J.A., Hargis B.M., Caldwell D.J., Bailey R.H., Herron K.L., Mcreynolds J.L., Brewer R.L., Anderson R.C., Bischoff K.M., Callaway T.R. and Kubena L.F. (2001). Effect of lactic acid administration in the drinking water during pre-slaughter feed with drawal on *Salmonella* and *Campylobacter*. *Poult. Sci*. **80,** 278-283.

Chen X. and Schluesener H.J. (2008). Nanosilver: A nanoproduct in medical application. *Toxicol. Lett*. **176,** 1-12.

Daneshyar M., Pirmohamadi A. and Farhoomand P. (2015). Effect of *Thymus vulgaris* and *Menthapulegium* powders on performance, carcass characteristics and some blood parameters of broilers under heat stress condition. *J. Iranian Vet*. **4,** 12-25.

Dhawale A. (2005). Better eggshell quality with a gut acidifier. *J. Poult. Int*. **44,** 18-21.

Engberg R.M., Hedemann M.S., Lesser T.D. and Jensen B.B. (2000). Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poult. Sci*. **79,** 1311-1319.

Fondevila M., Herrer R., Casallasa M.C., Abecia L. and Duchab J.J. (2008). Silver nanoparticles as a potential antimicrobial additive for weaned pigs. *Anim. Feed Sci. Technol*. **150,** 259-269.

Garcia V., Catala-Gregori P., Hernandez F. Megias M.D. and Madrid J. (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. *J. Appl. Poult. Res.* **16,** 555-562.

Ghazalah A.A., Atta A.M., Elkoub K., Moustafa M.E.L. and Shata F.H. (2011). Effect of dietary supplementation of organic acids on perforamance, nutrients digestibility and health of broiler chicks. *Poult. Sci*. **10,** 176-184.

Gornowicz E. and Dziadek K. (2002). The effect of acidifying preparations added to compound feeds on management conditions of broiler chickens. *Ann. Anim. Sci*. **1,** 93-96.

Grudzien M. and Sawosz E. (2006). The influence of silver nanoparticles on chick embryo development and bursa fabricius morphology. *Anim. Feed Sci*. **15,** 111-115.

Gunal M., Kaya G.O., Karahan N. and Sulak O. (2006). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tis sue of broilers. *Int. J. Poult. Sci*. **5,** 149-155.

Hashemi S.R., Dastar B., Hassani S. and Jafari Ahangari Y. (2007). Growth performance, body temperature and blood proteins in broilers in response to betaine supplement and dietary protein level under heat stress. *J. Agric. Sci. Natur. Res.* **2,** 138-147.

Hassanabadi A., Hajati H. and Bahreini L. (2012). The effects of nano-silver on performance, carcass characteristics, immune system and intestinal microflora of broiler chickens. Pp. 55-65 in Proc 3rd Int. Vet. Poult. Cong., Tehran, Iran.

Hume M.E., Corrier D.E., Ivie G.W. and Deloach J.R. (1993). Metabolism of propionic acid in broiler chicks. *Poult. Sci.* **72,** 786-793.

Izat A.L., Tidwell N.M., Thomas R.A., Reiber M.A., Adams M.H., Colberg M. and Waldroup P.W. (1990). Effect of buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. *Poult. Sci*. **69,** 818-826.

Lee D.N., Liu S.R., Chen Y.T., Wang R.C., Lin S.Y. and Weng C.F. (2007). Effects of diets supplemented with organic acids and nucleotides on growth, immune responses and digestive tract development in weaned pigs. *J. Anim. Physiol. Anim. Nutr*. **91,** 508-518.

Lee S.J., Kim S.S., Suh O.S., Na J.C., Lee S.H. and Chung S.B. (1993). Effect of dietary antibiotics and probiotics on the performance of broiler. *J. Agric. Sci*. **35,** 539-548.

Lesson S., Namkung H., Antongiovanni M. and Lee E.H. (2005). Effect of butyric acid on the performance and carcass yield of broiler chickens. *Poult. Sci*. **84,** 1418-1422.

Levic J., Siniša M., Djuragi O.B. and Slavica S. (2008). Herbs and organic acids as an alternative for antibioticgrowth- promoters. *J. Arch. Zootech*. **11,** 5-11.

Lin S.Y., Hung A.T.Y. and Lu J.J. (2011). Effects of supplement with different level of *Bacillus coagulans* as probiotic on growth performance and intestinal microflora populations of broiler chickens. *J. Anim. Vete. Adv*. **10,** 111-114.

Lioyd J.R. (2003). Microbial reduction of Metals and Radionuclides. *FEMS Microbial. Rev*. **27,** 412-425.

Morêki J.C. (2008). Feeding strategies in poultry in hot climate. *Poult. Today*. **601,** 1-5.

Naghizadeh F. and KarimiTorshizi M.A. (2013). Evaluation of nanosilvers efficiency as an antibiotic substitute on performance and morphometric parameters of broiler chicks. *Iranian J. Anim. Sci*. **44,** 255-262.

Naghizadeh F., KarimiTorshizi M.A. and Rahimi S. (2011). Comparison of nanosilver and in feed disinfectants on layer performance and intestinal microflora and yolk cholesterol. *J. Anim. Prod*. **13,** 49-58.

Parks C.W., Grimes J.L., Ferket P.R. and Fairchild A.S. (2001). The effect of mannan oligosaccharides, mambermycins and virginiamycin on performance of large white male market turkeys. *Poult. Sci*. **80,** 718-723.

Percival S.L., Bowler P.G. and Russell D. (2005). Bacterial resistance to silver in wound care. *J. Hosp. Infect*. **60,** 1-7.

Pineda L., Chwalibog A., Sawosz E., Engberg C.R., Elnif J., Hotowy A., Sawosz F., Gao Y., Ali A. and Sepehri Moghaddam H. (2012). Effect of silver nanoparticles on growth performance, metabolism and microbial profile of broiler chickens. *Arch. Anim. Nutr*. **66,** 416-429.

Sahin K., Sahin N., Onderci M., Yaralioglu S. and Küçük O. (2001). Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. *Vet. Med. Czech*. **46,** 140-144.

Saki A.A. and Salary J. (2013). Intravenous injection of silver nanoparticles and thyme and savory extracts on the 17th day of embryonic development and its effect on performance and blood parameters of broiler chicks on 14 and 21 days of breeding period. *J. Anim. Sci*. **101,** 71-78.

SAS Institute. (2005). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Sawosz E., Binek M., Grodzik M., Zielinska M., Sysa P. and Szmidt M. (2007). Influence of hydrocolloidal silver nanoparticles on gastrointestinal microflora and morphology of enterocytes of quails. *Arch. Anim. Nutr*. **61,** 444-451.

Shameli K., Ahmad M.B., Zargar M., Yunus W.M.Z.W. and Ibrahim N.A. (2011). Fabrication of silver nanoparticles doped in the zeolite framework and antibacterial activity. *Int. J. Nanomed*. **6,** 331-341.

Solhi Oskouyi A. (2016). Effect of silver nanoparticles coated on zeolite and organic acids on performance, the morphology of the intestine, gastrointestinal microbial population and carcass characteristics in broiler chickens under heat stress conditions. Ph D. Thesis. Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran.

Song J., Xiao K., Ke Y.L., Jiao L.F., Hu C.H., Diao Q.Y., Shi B. and Zou X.T. (2014). Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poult. Sci*. **93,** 581-588.

Vander Sluis A.A., Dekker M., Skrede G. and Jongen W.M. (2002). Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *J. Agric. Food Chem*. **50,** 7211-7219.

Waldroup A. and Kanis W. (1995). Performance characteristics and microbiological aspects of broiler fed diets supplemented with organic acids. *J. Food Prot*. **58,** 482-48.