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

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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 (Lloyd, 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 pouls 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 1 g/kg organic acids. The acidifier (Bioticron®) 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 20th 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>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Starter (1-21)</th>
<th>Groover (22-42)</th>
<th>Starter (1-21)</th>
<th>Groover (22-42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>53.7</td>
<td>56.84</td>
<td>50.6</td>
<td>59.96</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>39.52</td>
<td>33.68</td>
<td>39.95</td>
<td>33.25</td>
</tr>
<tr>
<td>Organic acid</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Silver nanoparticles coated on zeolite</td>
<td>0</td>
<td>0</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3</td>
<td>4.11</td>
<td>3.69</td>
<td>3.41</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.47</td>
<td>1.09</td>
<td>1.47</td>
<td>1.09</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.19</td>
<td>1.28</td>
<td>1.18</td>
<td>1.29</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.43</td>
<td>0.32</td>
<td>0.43</td>
<td>0.32</td>
</tr>
<tr>
<td>Vitamin premix¹</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mineral premix¹</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.13</td>
<td>0.05</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>L-lysine</td>
<td>0.06</td>
<td>0.13</td>
<td>0.06</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**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, Enterococi 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 shown that use of organic acids in broiler diets reduces coliforms population in crop and cecum.
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.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C</th>
<th>Z</th>
<th>N</th>
<th>A</th>
<th>NA</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 21 d</td>
<td>687.91</td>
<td>695.55</td>
<td>691.48</td>
<td>687.31</td>
<td>697.20</td>
<td>10.20</td>
<td>0.84</td>
</tr>
<tr>
<td>21 to 42 d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1562.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1537.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1519.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1552.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1461.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.08</td>
<td>0.043</td>
</tr>
<tr>
<td>1 to 42 d</td>
<td>2272.09</td>
<td>2246.12</td>
<td>2211.37</td>
<td>2262.72</td>
<td>2194.57</td>
<td>38.81</td>
<td>0.127</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 21 d</td>
<td>1122.27</td>
<td>1141.21</td>
<td>1138.32</td>
<td>1152.69</td>
<td>1139.11</td>
<td>14.62</td>
<td>0.721</td>
</tr>
<tr>
<td>21 to 42 d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3760.28</td>
<td>3701.26</td>
<td>3691.28</td>
<td>3588.53</td>
<td>3642.56</td>
<td>43.24</td>
<td>0.143</td>
</tr>
<tr>
<td>1 to 42 d</td>
<td>4781.46</td>
<td>4715.78</td>
<td>4751.21</td>
<td>4755.63</td>
<td>4729.54</td>
<td>54.08</td>
<td>0.227</td>
</tr>
<tr>
<td>Feed conversion ratio (g/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 21 d</td>
<td>1.632&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.643</td>
<td>1.648</td>
<td>1.671</td>
<td>1.637</td>
<td>0.013</td>
<td>0.398</td>
</tr>
<tr>
<td>21 to 42 d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.41</td>
<td>2.40</td>
<td>2.44</td>
<td>2.35</td>
<td>2.49</td>
<td>0.028</td>
<td>0.053</td>
</tr>
<tr>
<td>1 to 42 d</td>
<td>2.109&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.082&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.192&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.103&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.154&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.021</td>
<td>0.048</td>
</tr>
</tbody>
</table>

<sup>1</sup>Birds were exposed to heat exposure (34±1 °C, 70±5% RH) for 4 hours from d 35 to day 42 of trial.

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.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

**Table 3** Effect of treatments on ileum and cecum microbial population of broilers in d 21 and 42 of breeding<sup>1</sup>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d 21 of breeding</th>
<th>d 42 of breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Z</td>
<td>N</td>
</tr>
<tr>
<td>Cecum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>8.9</td>
<td>8.7</td>
</tr>
<tr>
<td>Total anaerobic bacteria</td>
<td>9.0</td>
<td>9.4</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>8.2</td>
<td>8.3</td>
</tr>
<tr>
<td>Total anaerobic bacteria</td>
<td>8.7</td>
<td>9.0</td>
</tr>
<tr>
<td>Ceum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>8.8</td>
<td>8.9</td>
</tr>
<tr>
<td>Total anaerobic bacteria</td>
<td>8.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>7.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Total anaerobic bacteria</td>
<td>8.2</td>
<td>8.0</td>
</tr>
</tbody>
</table>

<sup>1</sup>Birds were exposed to heat exposure (34±1 °C, 70±5% RH) for 4 hours from d 35 to day 42 of trial.

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.

**SEM:** standard error of the means.
CONCLUSION

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, yet they did not have 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.

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