Effects of Probiotics and Antibiotic Supplementation on Serum Biochemistry and Intestinal Microflora in Broiler Chicks

Fahimeh Hashemzadeh¹, Shaban Rahimi¹*, Mohammad Amir Karimi Torshizi¹, Ali Akbar Masoudi²

¹. Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
². Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

*Corresponding author email: Rahimi_S@modares.ac.ir

ABSTRACT: A study was conducted to compare Gram positive (Lactobacillus rhamnosus and Berevibacillus laterosporus) and Gram negative probiotics’ bacteria (E. coli Nissle 1917) with antibiotic (Virginiamycin) on serum biochemistry and intestinal microflora in broiler chicks. Total of 140, one-day old male Ross 308 broiler chicks were subjected to a 42-day experimental period. The chicks were divided into five experimental groups; Lactobacillus rhamnosus, Berevibacillus laterosporus, E.coli Nissle 1917 in drinking water, virginiamycin antibiotic treatment in feed, the last group considered as control. Each group with equal numbers included 4 replicates (10 chicks per replicate). Usage of treatments had no significant effect on serum concentration of total protein and albumin (P>0.05), but significant effect was observed in uric acid and glucose level in serum. Treatments had no significant effect of on calcium and phosphorous levels in serum (P>0.05). Usage of probiotics enhanced the level of serum high-density lipoproteins (P<0.01), and reduced the level of cholesterol (P<0.01). Treatments had no significant effect on serum triglyceride level (P>0.23), but they had significant effect on intestinal selected microflora (P<0.01). It seems that probiotics have potential to be a reliable alternative to growth promoter antibiotics in poultry production. In general, using of both Gram negative and Gram positive probiotics bacteria in drinking water had positive effect on biochemical parameters profiles in serum and population of intestinal microflora.

Key word: Antibiotic; Serum biochemistry; Intestinal microflora; E. coli Nissle 1917; Lactobacillus rhamnosus, Berevibacillus laterosporus.

INTRODUCTION

Although cholesterol is an important basic block for body tissues; hypercholesterolemia is a well-known major risk factor for coronary heart diseases (Glu and Oner, 2006). World’s health organization has predicted that, by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the world (WHO, 2009). Probiotic bacteria with active bile salt hydrolase or products containing them have been suggested to lower cholesterol levels through interaction with host bile salt metabolism (De Semet et al., 1998). In several studies have shown that using of probiotic has the ability to reduce cholesterol in blood (Grunewald, 1982; Taranto et al., 1998 and Xiao et al., 2003).

Probiotics are defined as live microbial food supplements, which beneficially influence human and animals health (FAO/WHO 2001). Most microorganisms recognized to date as probiotics are Gram-positive, with Lactobacillus, Bacilli and Bifidobacteria being the main species used as commercial sources of probiotics (Marco et al. 2006). However, few Gram negatives are also used as probiotics. The best example of this group is Escherichia coli Nissle 1917 (EcN), which has been used in Germany for many years in the treatment of chronic constipation and colitis (Schutz, 1989).

The objective of the present study was to evaluate the effect of some Gram positive (Lactobacillus rhamnosus and Berevibacillus laterosporus) and Gram negative (E coli Nissle 1917) probiotic bacteria in drinking...
water of broiler chickens in comparison with virginiamycin antibiotic on some biochemical parameters in sera and intestinal microflora of these birds from 1 to 21 days of age.

MATERIALS AND METHODS

Animals and diets
Total of 140, one-day old male broiler chicks (Ross 308), with the same average weight were subjected to a 21-day experimental period. The chicks were randomly divided into five experimental groups receiving following treatments; Lactobacillus rhamnosus 7 log CFU/mL of drinking water (L), Brevibacillus laterosporus (Tarbiat Modares University, Tehran, Iran) 7 log cfu/mL of drinking water (B), E. coli Nissle 1917 (Mutaflor, Germany) 7 log cfu/mL of drinking water (EcN), Virginiamycin antibiotic 15 mg/kg of diet (V) and control group with no additives in drinking water or feed (C). Each group with equal numbers included 4 replicates (10 chicks per replicate). The probiotic properties of the used Gram positive bacteria were confirmed already (Karimi Torshizi et al., 2006; Bagherzadh Kasmani et al., 2012).

The basal diet was formulated to meet or exceed nutrients recommended by the NRC (1994) for broiler chickens based on soybean meal and corn (without supplementing antibiotics, coccidiostats or growth promoters).

Biochemical parameters of sera samples
At the end of the experiment (day 21), two birds were randomly selected from each replicate (8 chicks per each treatment group) and blood samples were collected from the brachial wing vein. The collected blood samples were centrifuged at 3000 rpm for 10 min and the sera were decanted into aseptically treated vials and stored at –20˚C until further analysis. Serum samples were analyzed for total protein, albumin, uric acid, glucose, cholesterol, high-density lipoproteins, and triglycerides concentrations by using commercial diagnostic kits (Pars Azmun, Tehran, Iran), using a microplate reader (Stat Fax 3200, Awareness Technology Inc. Palm City, FL, USA).

Bacterial enumeration
Conventional microbiological techniques using selective agar media were used for analysis. Samples of ileal contents from the bleeded birds were homogenized in buffered peptone water, and serial of decimal dilution were prepared. Following selective agar media were used for enumeration of target bacterial groups - total aerobes (plate count agar - Merck), lactic acid bacteria (MRS agar - Merck), and coli bacilli (Mac Conkey agar - Merck). Results were expressed as log₁₀ colony forming units per gram of ileum digesta (log₁₀ cfu/g).

Statistical Analysis
The data of experiment were analyzed in a completely randomized design model; the comparison of means was carried out through least significant differences (LSD) test. All statistical analyses were done using SAS program (SAS Institute, 1998).

RESULTS AND DISCUSSION
The amount of serum total protein, albumin, uric acid and glucose levels are shown in Table 1. The treatments had no significant effect on total protein and albumin in serum (P>0.05), but significant effect was observed in uric acid and glucose. The highest level of uric acid was observed in control group that had no significant difference with other groups except E. coli Nissle 1917 and B. laterosporus (P<0.05). A significant increase in glucose level was recorded in virginiamycin treatment which had no significant difference with other groups except control (P<0.01).

Djouvinov et al. (2005) and Aluwong et al. (2012) also didn’t observed significant effect on total protein and albumin level with supplementing rations with probiotics. Decreased uric acid in probiotics received groups is in agreement with Kamgar et al. (2013) and Newaj-Fyzul et al. (2007) findings.

Results of this experiment revealed that there was a significant decrease in uric acid level in probiotic groups, indicating beneficial effect of the probiotic on the kidney function. On the other hand, certain probiotic microorganisms can utilize urea, uric acid and creatinine and other toxins as its nutrients for growth (Salim et al., 2011). Using of antibiotic and probiotic has enhanced the serum glucose level in broilers; which is agree with results of Azza et al. (2012).
The result of this experiment for cholesterol, high-density lipoproteins (HDL) and triglyceride are presented in Table 2. The highest level of cholesterol was observed in control and virginiamycin groups and the lowest in E. coli Nissle 1917 (P<0.01). In general, usage of probiotic had enhancing effect on HDL; all groups that received probiotic bacteria in drinking water had the highest concentration of HDL in their blood. Treatments had no significant effect on serum triglyceride level (P>0.05).

Table 1. Effect of virginiamycin and probiotics on serum glucose, albums and total proteins levels of broiler chickens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total protein (g/dl)</th>
<th>Albumin (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.89</td>
<td>3.91</td>
<td>4.82ab</td>
<td>77.5c</td>
</tr>
<tr>
<td>B. laterosporus</td>
<td>4.72</td>
<td>3.98</td>
<td>4.53ab</td>
<td>81.5bc</td>
</tr>
<tr>
<td>E. coli Nissle 1917</td>
<td>4.96</td>
<td>3.93</td>
<td>4.37ab</td>
<td>80.72bc</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>4.85</td>
<td>3.86</td>
<td>4.64ab</td>
<td>82.95a</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>5.06</td>
<td>3.94</td>
<td>4.56ab</td>
<td>80.72bc</td>
</tr>
<tr>
<td>SEM</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04</td>
<td>0.67</td>
</tr>
<tr>
<td>P-value</td>
<td>0.37</td>
<td>0.22</td>
<td>0.04</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Means with different superscripts are statistically different (P<0.05).

In the present study, serum cholesterol content tended to decrease in the probiotic supplemented groups (P>0.05). Reduction in circulating cholesterol with supplemental probiotic agrees with the results of other researchers (Onifade et al., 1997 and 1999; Jouybari et al., 2009). Also, blood cholesterol levels of layers fed yeast supplemented diets were low compared to the control (Saadia et al., 2010). Probiotics could contribute to the regulation of serum cholesterol concentrations by deconjugation of bile acids. Since the excretion of deconjugated bile acids is enhanced and cholesterol is its precursor, more cholesterol molecules are spent for the recovery of bile acids (De Smet et al., 1994).

There was no significant effect of treatment on calcium and phosphorous levels in serum; however, usage of feed additive had positive effect on level of these minerals in blood. It has been reported that probiotics increase the calcium absorption from intestinal tract. Fermentation products as a result of probiotics’ activity may increase the absorption surface by accelerating proliferation in enterocytes (Scholz-Ahrens et al., 2007). Furthermore short chain fatty acids and the other products of some probiotic bacteria decrease the gastrointestinal (GI) pH. Therefore, calcium solubility increases and this may be related to increased expression of calcium channels in intestinal mucosa. Vinderola et al. (2007) observed that supernatant from milk fermented by Lactobacillus helveticus R389 enhanced expression of TRPV6 channels in the duodenum. Enhanced expression of Ca channels indicates improvement of dietary Ca uptake capacity.

Influence of various experimental groups on the microbial population in the GI tract is reported in Table 3. Treatments had significant effect on the microbial population of the selected bacteria in GI tract. In the case of total aerobic bacteria the order of counts was observed in control, Lactobacillus rhamnosus, Virginiamycin and Berevibacillus laterosporus groups, respectively. The lowest number of total aerobic bacteria was recovered in E. coli Nissle 1917 group (P<0.01). The highest population of Lactobacillus rhamnosus was observed in the groups that consumed Lactobacillus rhamnosus in drinking water which had no significant difference with E. coli Nissle 1917 and antibiotic groups (P<0.01). The lowest number of colibacilli bacteria was observed in Berevibacillus laterosporus group that had no significant difference with E. coli Nissle 1917 and Lactobacillus rhamnosus groups.

Table 2. Effect of virginiamycin and probiotics on serum glucose, albumins, total proteins, calcium and phosphorus levels of broiler chickens

<table>
<thead>
<tr>
<th>Treatments</th>
<th>cholesterol (mg/dl)</th>
<th>high-density lipoproteins</th>
<th>triglycerides (mg/dl)</th>
<th>calcium (mg/dl)</th>
<th>phosphorus (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>159a</td>
<td>25.5c</td>
<td>54</td>
<td>8.02</td>
<td>4.78</td>
</tr>
<tr>
<td>B. laterosporus</td>
<td>136ab</td>
<td>29.7ab</td>
<td>52</td>
<td>8.26</td>
<td>5.09</td>
</tr>
<tr>
<td>E. coli Nissle 1917</td>
<td>135a</td>
<td>31.5ab</td>
<td>53</td>
<td>8.57</td>
<td>4.85</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>140a</td>
<td>30.7ab</td>
<td>56</td>
<td>8.65</td>
<td>4.60</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>159a</td>
<td>23.3b</td>
<td>58</td>
<td>8.32</td>
<td>5.03</td>
</tr>
<tr>
<td>SEM</td>
<td>2.72</td>
<td>0.22</td>
<td>0.04</td>
<td>0.23</td>
<td>0.10</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Means with different superscripts are statistically different (P<0.01).
aerobic bacteria the order of counts was observed in control, Lactobacillus rhamnosus, Virginiamycin and Berevibacillus laterosporus groups, respectively. The lowest number of total aerobic bacteria was recovered in E. coli Nissle 1917 group (P<0.01). The highest population of Lactobacillus was observed in the groups that consumed Lactobacillus rhamnosus in drinking water which had no significant difference with E. coli Nissle 1917 and antibiotic groups (P<0.01). The lowest number of colibacilli bacteria was observed in Berevibacillus laterosporus group that had no significant difference with E. coli Nissle 1917 and Lactobacillus rhamnosus groups. The maximum population of colibacilli was observed in antibiotic group (P<0.01).

Gut microflora has significant effects on host nutrition, health, and growth performance of chickens (Barrow, 1992) by interacting with nutrient utilization and the development of gut system of the host. Reduced ileal colibacilli populations were noticed in chickens given a diet supplemented with lactobacilli strains, but the populations of other kinds of bacteria were not affected (Watkins and Kratzer, 1984). In contrast, Murry et al. (2006) reported that birds supplemented with botanical probiotic containing lactobacilli had higher lactobacilli but lower colibacilli compared to the control birds.

Table 3. Effect of virginiamycin and probiotics on population of intestine microflora in broiler chickens (log_{10} CFU/g)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total aerobic count</th>
<th>Lactic acid bacteria</th>
<th>Colibacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.83a</td>
<td>9.50a</td>
<td>8.08b</td>
</tr>
<tr>
<td>B. laterosporus</td>
<td>8.64d</td>
<td>9.71b</td>
<td>7.16c</td>
</tr>
<tr>
<td>E. coli Nissle 1917</td>
<td>7.79c</td>
<td>10.32a</td>
<td>7.32c</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>8.62e</td>
<td>10.67c</td>
<td>7.67d</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>8.35f</td>
<td>10.28d</td>
<td>8.52e</td>
</tr>
<tr>
<td>SEM</td>
<td>0.10</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>P. value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

**Means within same column with different superscripts are significantly different (P<0.05).**

According to results of this experiment it could be concluded that Gram negative probiotic performed similar to Gram positive probiotics on biochemical parameters in serum and intestinal microflora of broiler chickens. Therefore, usage of these probiotic bacteria as antibiotic alternative in poultry nutrition can be recommended.

REFERENCES


MALFA/WHO. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina, October 1–4


