Effect of the Three Herbal Extracts on Growth Performance, Immune System, Blood Factors and Intestinal Selected Bacterial Population in Broiler Chickens

S. Rahimi1∗, Z. Teymouri Zadeh1, M. A. Karimi Torshizi1, R. Omidbaigi2, and H. Rokni3

ABSTRACT

A research study was conducted to evaluate the effects of three herbal extracts and an antibiotic – virginiamycin- on growth performance, immune system, blood factors and selected intestinal bacterial populations in broiler chickens. A total of four hundred and eighty 1-day old male broiler chicks were assigned to the basal diet (control), basal diet supplemented with 15 ppm of virginiamycin, basal diets with a 0.1% dose of either thyme (Thymus vulgaris), coneflower (Echinacea purpurea), garlic (Allium sativum) or a blend of the three extracts in the drinking water. The highest and the lowest body weight and weight gain were related to virginiamycin and coneflower, (P< 0.05) respectively. The lowest and the highest feed conversion rates were respectively related to virginiamycin and coneflower (P< 0.05). Relative weight of bursa Fabricius in the garlic group showed a significantly more increase as compared with other groups, while the relative weight of spleen was unaffected by treatments. Cutaneous basophils hypersensitivity response (to phytohemaglutinin injection) and antibody response to Sheep Red Blood Cells (SRBC) was higher in coneflower group (P< 0.05). Antibody responses to Newcastle Disease vaccine (LaSota) was unaffected by treatments but coneflower improved antibody levels (P> 0.05). Garlic (Allium sativum) significantly reduced the serum levels of cholesterol, LDL, and triglyceride as well as significantly increasing the level of HDL. Thyme (Thymus vulgaris) improved hematocrit percentage and hemoglobin concentration, but not significantly. The colony forming units of Escherichia coli in digesta of ileo-cecum in the blend group showed a significantly lower number compared with control. However, there was no difference observed in E. coli counts between blend group and others, except for control. The lactic acid bacteria counts in the thyme group increased as compared to other groups, except for coneflower (P< 0.05).

Keywords: Blood factors, Coneflower, Garlic, Immune system, Performance, Thyme.

INTRODUCTION

A number of feed additives including antibiotics have been widely employed in the poultry industry for several decades. A manipulation of gut function and microbial habitat of domestic animal with feed additives has been recognized as an important tool for improving growth performance and feed efficiency (Collington et al., 1990). Approximately 80% of domestic animals have been fed synthetic compounds for the purpose of either medication or growth promotion (Lee et al., 2001). Recently, the concerns about possible antibiotic residues and antibiotic resistance have aroused great caution in the usage of antibiotics in the animal industry.

The banning of the use of antibiotics as feed additives has accelerated and led to investigations of alternative feed additives in...
animal production. As one of the alternatives, herbal extracts are already being used as feed supplements to improve growth performance under intensive management systems (William and Losa, 2001). Plant extracts and spices as single compounds or as mixed preparations can play a role in supporting both performance and health status of the animal (Janssen, 1989; Horton et al., 1991; Bakhiet and Adam, 1995; Skrabka Blotnicka et al., 1997; Gill, 2000; Manzanilla et al., 2001). Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelmintic actions. Isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract. The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism (Horton et al., 1991; Baratta et al., 1998; Jamroz et al., 2003). The pharmacological action of active plant substances or herbal extracts in humans is well known, but in animal nutrition the number of precise experiments is relatively low.

Volatile oil from thyme (Thymus vulgaris) was assessed for antibacterial and antiviral activity as inhibitors of microbial growth (Dorman and Deans, 2000). Garlic (Allium sativum) is widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases. Garlic is thought to have various pharmacologic properties. For example, it has been found to lower serum and liver cholesterol (Qureshi et al., 1983), inhibit bacterial growth (Cavallito and Bailey, 1994), inhibit platelet growth and reduce oxidative stress (Horie et al., 1992). Tollba and Hassan (2003) found that garlic as a natural feed additive, improved broiler growth and Feed Conversion Ratio (FCR), and decreased mortality rate. Echinacea plant extract is widely used as a self prescribed agent against such upper respiratory tract infections as common cold (O’Hara et al., 1998). According to many in vitro studies, it is thought to stimulate macrophage activity and hence the immune system (Stimpel et al., 1984; Fry et al., 1998). In recent years, much effort has been made to identify the potential components in Echinacea plant extract that could account for its in vitro immunostimulatory effects (Bauer and Wagner, 1991). Some of these bioactives include polysaccharides, cichoric acid and alkylamides. Despite the in vitro immunomodulatory potential of these compounds in Echinacea plant extract, the principle active(s), its optimal dose level and its action in vitro, have not been well known. In older animals the effectiveness of plant extract supplementation was relatively low, but higher digestibility of nutrients and reduction of E. coli and Clostridium sp. in intestinal content were stated (Jamroz et al., 2003).

The objective of this study was to compare the effects of three commercial herbal extracts as alternative to antibiotic on growth performance, immune system, blood factors and intestinal microflora in broiler chickens.

**MATERIALS AND METHODS**

**Experimental Animals and Design**

A total of 480 one-day old chicks (Ross male-308) were purchased from a local hatchery. On arrival, chicks were weighed and randomly housed in wood shavings covered floor pens (20 chicks per pen). Continuous lighting was provided throughout the experiment. The ambient temperature was gradually decreased from 30°C on day 7 to 25°C on day 21 and was then kept constant. There were 6 treatments, each consisting of 4 replicates. The replicate was a pen with 20 male chicks so that each treatment was comprised of 80 animals. Chicks were assigned to the basal diet (control) and basal diet supplemented with 15 ppm virginiamycin, 0.1% aqueous extract of: thyme (Thymus vulgaris), coneflower (Echinacea purpurea),...
garlic (*Allium sativum*) and blend of extracts with the same dose in drinking water. The ingredients and composition of the basal diet (starter from 7 to 21, grower from 21 to 35 and finisher from 36 to 42 days of age) are presented in Table 1. All birds employed in the experiment were fed according to applicable recommendations of the National Research Council (NRC, 1994).

Thyme (*Thymus vulgaris*) extract, coneflower (*Echinacea purpurea*) extract, garlic (*Allium sativum*) were commercially purchased supplements (Zardband Pharmaceutical Co., Tehran).

### Performance Data

Body weight, feed intake and feed conversion were assessed on days 21 and 41 or during the age ranges of 1-21 and 22-41 days. Mortality rate was reported. For performance data, pen means served as the experimental unit for statistical analysis. For data on relative weight, individual birds were considered as the experimental unit.

### Immune System

**Toe-Web Hypersensitivity**

At 42 days of age, cutaneous basophils hypersensitivity response (to phytohemaglutinin injection) was determined through the methodology of Corrier and Deloach (1990). Briefly, the right foot was cleansed with 70% ethanol and the thickness of the toe web between the third and fourth digits measured, using a micrometer. One hundred microliters of a 100 μg mL⁻¹ solution of phytohemaglutinin (PHA) (Pars-Azmoon Co., Tehran) in sterile 0.85% saline was injected intradermally.

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**Table 1. Composition of the basal (control) diet.**

<table>
<thead>
<tr>
<th>Ingredients (% diet)</th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize, yellow</td>
<td>58.4</td>
<td>85.3</td>
<td>61.5</td>
</tr>
<tr>
<td>Soybean meal (48%)</td>
<td>27.9</td>
<td>23.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Wheat</td>
<td>4.6</td>
<td>9.4</td>
<td>13.9</td>
</tr>
<tr>
<td>Fish meal</td>
<td>4.6</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Bagasse</td>
<td>1.8</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.9</td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>Mineral premix (0.25%) †</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix (0.25%) †</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>DL-Metionine</td>
<td>0.094</td>
<td>0.095</td>
<td>0.093</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.094</td>
<td>0.096</td>
<td>0.093</td>
</tr>
<tr>
<td>Oil</td>
<td>0.9</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
</tr>
</tbody>
</table>

**Calculated chemical composition (%):**

<table>
<thead>
<tr>
<th>ME (Kcal kg⁻¹)</th>
<th>2850</th>
<th>2950</th>
<th>3050</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>21.5</td>
<td>19.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Calcium</td>
<td>1</td>
<td>0.95</td>
<td>0.9</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>0.5</td>
<td>0.46</td>
<td>0.43</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.18</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.2</td>
<td>1.15</td>
<td>1</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.53</td>
<td>0.48</td>
<td>0.43</td>
</tr>
<tr>
<td>Methionine+cystine</td>
<td>0.95</td>
<td>0.85</td>
<td>0.75</td>
</tr>
</tbody>
</table>

† The 10 g of premix consisted of 500,000 IU vitamin A (500,000 IU g⁻¹); 6.0 mg of vitamin D3 (100,000 IU g⁻¹); 60.0 mg of vitamin E (500 IU g⁻¹); 6.6 mg of vitamin K3 (purity, 22.7%); 100.0 mg of vitamin B12 (purity, 0.1%); 2,000.0 mg of biotin (purity, 0.01%); 1,100.0 mg of choline chloride (purity, 50%); 1.1 mg of folic acid (purity, 90%); 65.2 mg of nicotinic acid (purity, 100%); 16.3 mg of d-pantothenate (purity, 92%); 4.5 mg of vitamin B6 (purity, 100%); 12.5 mg of riboflavin (purity, 80%); 2.5 mg of vitamin B1 (purity, 100%); 32.00 mg of CuSO₄·5H₂O; 333.20 mg of FeSO₄·H₂O; 166.80 mg of MnO; 1.0 mg of Na₂SeO₃·6H₂O; 220.00 mg of ZnSO₄·7H₂O; 4.80 mg of CoSO₄·7H₂O; 0.56 mg of KI; 100.00 mg of ethoxyquin, and 5,742.94 mg of corn meal as carrier.

‡ The values were calculated from NRC (1994).
After 24 hours, the toe webs were cleansed and measured again. Cutaneous basophil hypersensitivity response was determined as the difference in skin thickness pre and post (24 hours) injection.

Sheep Red Blood Cells

At 27 and 36 days of age SRBC suspension (5% in sterile PBS) was injected in breast muscle of 12 birds per treatment. Total antibody (Ab) titers to SRBC were determined by agglutination according to Van der Zijpp and Leenstra (1980) in serum from all 72 birds. Therefore, 7 days after each sensitization (35 and 42 days) antibody titers against SRBC were measured and expressed as the log$_2$ of the reciprocal of the highest serum dilution giving complete agglutination.

Lymphoid Organ Weights.

At 42 days of age relative weights of immune organs (spleen and bursa of Fabricius) as two immune indexes were determined.

Newcastle Disease Vaccine Response

Newcastle disease vaccine (LaSota) was administrated with drinking water at 23 days of age. Blood samples were collected on 7 and 14 days after vaccination and antibody response was determined by using HI method.

Blood Factors

Total Cholesterol (TC), LDL cholesterol, Triglycerides (TG), HDL cholesterol, hematocrit percentage and hemoglobin concentration were measured at 42 days of age. Concentrations of these parameters except for LDL cholesterol were determined by enzymatic kits (Pars-Azmoon Co., Tehran). To measure LDL cholesterol, Friedwald (Soltani et al., 2007) formula was used.

Selected Bacterial Population in the Intestinal Contents

For a determination of some selected micro-organisms in intestinal digesta from 48 birds (8 birds per treatment and pen) the contents of the distal part of the small intestine (10 cm anterior to the junction with caecum and rectum) and whole caeca of two birds per replicate pen were separately collected, cooled and used for microbial assays. The populations of Escherichia coli and lactic acid bacteria (LAB) were then estimated as CFU g$^{-1}$. Sterilized PBS (99 mL) was added (1:100) to 1 g of fresh material, and then subsequent dilutions prepared. E. coli was cultured on MacConkey agar (Merck, Germany) at 37°C for 24 hours, and the presence of E. coli then determined. LAB was enumerated on MRS (Merck, Germany) agar after incubation under anaerobic condition for 72 hours at 37°C.

Statistical Analysis

A complete randomized model was employed to analyze data for weight gain, feed intake, and efficiency, characteristics of carcass, immune system, blood factors, as well as intestinal microflora. The data were analyzed using the GLM procedure of the statistical software (SAS Institute Inc, 1989). Mean differences among treatments were evaluated through Duncan multiple range test at $P<0.05$. The original data for microbial counts were transformed to log$_{10}$ CFU g$^{-1}$ of intestinal content for statistical analysis.
RESULTS

Performance

In the present study, feed intake and feed conversion ratio were significantly influenced by the addition of herbal extracts to diets and at the 42 days of age (Table 2). The coneflower group presented a significantly lower feed intake (4,125.6 g vs. 4,376.3 g) and body weight (2,269.00 g vs. 2,595.40 g) and while a higher FCR (1.86 vs. 1.71) as compared with the virginiamycin group. At 21 days of age all the herbal extracts except coneflower improved the FCR as compared with the control group, although this difference was not significant. Also at the end (1-42 days) of the experimental period, among herbal extracts, thyme presented a lower FCR as compared with the other treatments except for virginiamycin (P< 0.05). Relative weights of the carcass, abdominal fat pad, and digestive organs except small intestine weight were not affected by treatments. Virginiamycin treatment presented a lower small intestinal weight than that in the other treatments (Table 2). The effects of three herbal extracts on relative weight (% body weight) of some internal organs of broilers are shown in Table 3.

Immune System

Relative weight of bursa Fabricius in the garlic group showed a significant increase as compared with that in other groups, but relative weight of spleen was unaffected by the different treatments. Cutaneous basophil hypersensitivity response (to phytohemaglutinin injection) and antibody responses to SRBC were recorded as higher in coneflower group (P< 0.05). Antibody response to Newcastle disease vaccine (LaSota) was unaffected by the treatments but the antibody levels were improved in the coneflower group (P> 0.05) (Table 4).
Table 3. The effect of three herbal extracts and antibiotic on relative weight (% body weight) of some internal organs of broilers at 42 days of age.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Coneflower</th>
<th>Garlic</th>
<th>Blend of Extracts</th>
<th>Thyme</th>
<th>Antibiotic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative weight (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass yield</td>
<td>70.45</td>
<td>72.15</td>
<td>69.78</td>
<td>69.53</td>
<td>69.93</td>
<td>71.90</td>
<td>0.313</td>
</tr>
<tr>
<td>Fat pad</td>
<td>2.09</td>
<td>2.05</td>
<td>1.92</td>
<td>2.04</td>
<td>2.19</td>
<td>2.05</td>
<td>0.066</td>
</tr>
<tr>
<td>Liver</td>
<td>2.26</td>
<td>2.27</td>
<td>2.35</td>
<td>2.15</td>
<td>2.43</td>
<td>2.39</td>
<td>0.034</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.255</td>
<td>0.260</td>
<td>0.260</td>
<td>0.230</td>
<td>0.230</td>
<td>0.242</td>
<td>0.005</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>0.376</td>
<td>0.415</td>
<td>0.395</td>
<td>0.390</td>
<td>0.327</td>
<td>0.355</td>
<td>0.01</td>
</tr>
<tr>
<td>Gizzard</td>
<td>1.48</td>
<td>1.43</td>
<td>1.41</td>
<td>1.88</td>
<td>1.35</td>
<td>1.39</td>
<td>0.063</td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.059</td>
</tr>
<tr>
<td>Heart</td>
<td>0.504</td>
<td>0.531</td>
<td>0.536</td>
<td>0.490</td>
<td>0.541</td>
<td>0.491</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Values in the same row with no common superscript differ significantly.

Blood Factors

Garlic (<i>Allium sativum</i>) significantly reduced the serum levels of TC, LDL, and TG and significantly increased the level of HDL. There was no significant difference observed for triglyceride levels among extracts in all of which a significant reduction from the control and antibiotic group was observed. Cholesterol level was recorded as the same in groups thyme (<i>Thymus vulgaris</i>) and coneflower (<i>Echinacea purpurea</i>). Thyme (<i>Thymus vulgaris</i>) improved hematocrit percentage and hemoglobin concentration, but not significantly (Table 5).

Selected Bacterial Population

The colony forming units of <i>Escherichia coli</i> in digesta of ileo-cecum in the blend group showed a significantly lower number compared with control group. However, there was no difference observed in <i>E. coli</i> counts between blend group and others, except for control. The LAB counts in the thyme group increased as compared with the other groups, except for coneflower (P< 0.05) (Table 6).

Table 4. The effect of three herbal extracts and antibiotic on immune system of broilers.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Coneflower</th>
<th>Garlic</th>
<th>Blend of extracts</th>
<th>Thyme</th>
<th>Antibiotic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody response to SRBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27day</td>
<td>3.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.75&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.75&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.189</td>
</tr>
<tr>
<td>42 days</td>
<td>6.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.198</td>
</tr>
<tr>
<td>Antibody response to Newcastle vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 d</td>
<td>2.82</td>
<td>3.20</td>
<td>2.92</td>
<td>2.97</td>
<td>3.02</td>
<td>3.17</td>
<td>0.088</td>
</tr>
<tr>
<td>42 d</td>
<td>2.07</td>
<td>2.57</td>
<td>2.60</td>
<td>2.40</td>
<td>2.40</td>
<td>2.12</td>
<td>0.088</td>
</tr>
<tr>
<td>Bursa Fabricious relative weight (%)</td>
<td>0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.007</td>
</tr>
<tr>
<td>Spleen relative weight (%)</td>
<td>0.113</td>
<td>0.099</td>
<td>0.133</td>
<td>0.129</td>
<td>0.125</td>
<td>0.113</td>
<td>0.006</td>
</tr>
<tr>
<td>Hypersensitivity cutaneous basophiles response (mm)</td>
<td>0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.043</td>
</tr>
</tbody>
</table>

* Values in the same row with no common superscript differ significantly.
Table 5. The effect of three herbal extracts and antibiotic on blood factors of broilers.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Coneflower</th>
<th>Garlic</th>
<th>Blend of Extracts</th>
<th>Thyme</th>
<th>Antibiotic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerid</td>
<td>44.92</td>
<td>35.50</td>
<td>30.98</td>
<td>32.27</td>
<td>32.15</td>
<td>50.73</td>
<td>1.84</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>93.92</td>
<td>86.75</td>
<td>76.13</td>
<td>81.17</td>
<td>85.77</td>
<td>102.78</td>
<td>2.19</td>
</tr>
<tr>
<td>LDL</td>
<td>47.03</td>
<td>32.01</td>
<td>16.85</td>
<td>24.95</td>
<td>28.11</td>
<td>39.37</td>
<td>2.31</td>
</tr>
<tr>
<td>HDL</td>
<td>45.65</td>
<td>47.64</td>
<td>53.08</td>
<td>49.76</td>
<td>51.22</td>
<td>45.60</td>
<td>1.20</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>27.28</td>
<td>28.49</td>
<td>27.50</td>
<td>29.19</td>
<td>30.40</td>
<td>28.74</td>
<td>0.447</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>7.69</td>
<td>8.88</td>
<td>7.88</td>
<td>8.46</td>
<td>9.45</td>
<td>7.81</td>
<td>0.387</td>
</tr>
</tbody>
</table>

* Values in the same row with no common superscript differ significantly.

DISCUSSION

Over the entire period (1-42 days) coneflower group had significantly lower body weight and weight gain when compared with the other groups especially virginiamycin. However, during the period of 21-42 days, daily weight gain presented no significant difference between thyme (*Thymus vulgaris*) and virginiamycin. Thus, when comparing coneflower with virginiamycin in relation to body weight and weight gain, it appears that coneflower decreases feed intake, leading to lower body weight and weight gain. Among herbal extracts employed, coneflower (*E. purpurea*) did not improve FCR. Findings in this study are in agreement with those of Dora *et al.* (2008) who reported that use of 2.4% *Echinacea* cob or 10 mg kg$^{-1}$ feed flavomycin showed a significantly lower feed intake (2,713 g vs. 2,995 g) and body weight (1,719 g vs. 1,895 g) as compared to flavomycin and control group. Roth-Maier *et al.* (2005), who fed cobs prepared from *E. purpurea* arial parts to healthy broiler and layer chickens, found no beneficial effect of the treatment on feed intake and growth performance of the animals. They concluded that *E. purpurea* cobs cannot be considered as a suitable alternative for antibiotics as a growth promoter in the animals’ feed.

Results of the experiment indicated that thyme extract has improved FCR in comparison with other groups except for virginiamycin (P< 0.05). This finding is in agreement with results of Lee *et al.* (2003) and Ocak *et al.* (2008) who reported that use of 2% thyme supplement could significantly improve the growth of broilers. Results of studies by some other investigators are also in agreement with the present findings (Cross *et al.*, 2002; 2007; Bampidis *et al.*, 2005; Demir *et al.*, 2003; Lee *et al.*, 2003). There was no significant difference in relative weight of carcass, fat pad, or digestive organs among treatments except for the small intestine. These finding are in agreement with the results of Ocak *et al.* (2008) who found no differences in carcass and organs’ weight of broilers fed a diet containing 2% thyme powder. Similar results were observed by Hernandez (2004)

Table 6. The effect of three herbal extracts and antibiotic on *E.coli* and LAB numbers in ileal contents of broilers.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Coneflower</th>
<th>Garlic</th>
<th>Blend of extracts</th>
<th>Thyme</th>
<th>Antibiotic</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (log CFU g$^{-1}$)</td>
<td>7.10</td>
<td>4.82</td>
<td>5.20</td>
<td>4.32</td>
<td>4.40</td>
<td>4.42</td>
<td>0.248</td>
</tr>
<tr>
<td>Lactic acid bacteria (log CFU g$^{-1}$)</td>
<td>4.52</td>
<td>6.20</td>
<td>5.47</td>
<td>5.82</td>
<td>5.95</td>
<td>4.92</td>
<td>0.149</td>
</tr>
</tbody>
</table>

* Values in the same row with no common superscript differ significantly.
who found no difference in weight of organs of broiler chickens fed diets containing an extract from thyme and oregano.

In contrast, it has been proposed that immunostimulation may have adverse effects on growth performance, because more nutrients will be repartitioned to synthesize antibodies and develop the immune organs, thereby decreasing the nutrients available for growth (Hevener et al., 1999; Takahashi et al., 2000). Coneflower increased production of such acute phase proteins as interleukin (IL)-1 and IL-6, or tumor necrosis factor (TNF-α) - like substances. Cachectins are produced by such extra vascular effector cells as macrophages in response to invasive stimuli. Among various multiple effects, cachectins induce a state of anorexia, as occurs during injecting antigens. Consequently cachectins induce a reduction of growth and feed utilization in chickens, and an accelerated muscle protein degradation (Klasing and Johnston, 1991); whereas a negative relationship between BW and Ab titers is found in commercial broilers (Martin et al., 1990). Intraperitoneal stimulation of such chickens with IL-1, SRBC resulted in reduced growth and feed intake, probably due to the cachectin activities of IL-1, IL-6, TNF-α (Klasing et al., 1987). These cytokines are the earliest mediators secreted by the host in response to antigens and other injurious stimuli (Van Miert, 1995). Acute phase proteins act in concert with a decrease in feed intake (partially as a result of loss of appetite) (Grimble, 1994), increase resting expenditure, gluconeogenesis, glucose oxidation, and synthesis of fatty acids as well as acute phase proteins by the liver. Also, metabolism of circulating insulin, glucagon, and corticosterone are affected (Klassing, 1988). Amino acids and glutamine released from muscle, and glucose derived from increased hepatic gluconeogenesis may provide endogenous sources of nutrients for immune system. Diversion of nutrient pool of the host as a consequence of cachectin release may also lead to retarded growth (Grimble, 1994).

Negative correlations between BW and antibody response might be based on pleiotropic effects for genes associated with immunoresponsiveness (Martin et al., 1990). In the present experiment, growth of birds was significantly retarded by coneflower.

In poultry production, it's very important to improve immunity so as to prevent infectious diseases. A variety of such factors as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics can induce immunodeficiency. Utilization of immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious disease (Liu, 1999). The study of the immune system showed that coneflower (Echinacea purpurea) was most effective in immune system improvement. Increasing response to phytohemaglutinin injection (hypersensitivity on toe-web) and SRBC in coneflower group was expected, because this herb increased stimulation of non-specific immune system. Rehman et al. (1999) showed production of IgG in rats increasing after being challenged with antigens. Schranner et al. (1989) studied the effect on the humoral immune response of a complex drug containing E. angustifolia homoeopathic mother tincture and E. angustifolia mother tincture alone on normal and on immunodeficient chickens. The administration of the complex drug enhanced humoral immune parameters in both group of chickens. Herbs that are rich in such flavonoids as thyme (Thymus vulgaris) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Manach et al., 1996; Cook and Samman, 1996). It is thought that immune enhancement of Echinacea is provided by certain polysaccharides, flavonoids, and isobutylamides (Bruneton, 1995).

Garlic lowers total and LDL-cholesterol as well as triacylglycerol concentrations without affecting HDL-cholesterol concentrations (Warshafsky et al., 1993; Kleijnen et al., 1989). Terpenoids (from essential oil components of medicinal
plants) also elicit a significant reduction in total and LDL-cholesterol concentration (Elson and Yu, 1994; Zheng et al., 1992; Pearce et al., 1992).

This study showed that the lowest E. coli counts were related to thyme group, but there was no significant difference observed among coneflower, garlic and virginiamycin supplemental groups. In earlier studies (Jamroz et al., 2005) significant reduction of E. coli number has been obtained following an application of natural plant extract. It has been documented that garlic extracts exert a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Rees et al., 1993). Inhibition observed in E. coli was more than 10 times greater than that seen in Lactobacillus casei for the same garlic dose (Skyrme, 1997). Exactly why this differential inhibition should occur is not clear, but it may be due to differing composition of bacterial membranes and their permeability to allicin (Miron et al., 2000). Garlic extract and allicin have been shown to exert bacteriostatic effects on some vancomycin-resistant enterococci. Decreasing number of such viable Gram-positive bacteria, as Lactobacilli and Bifidobacteria, may increase the presence of Gram-negative species. Antibiotics decrease the number of viable Gram-positive bacteria in gastrointestinal tract of chickens (Ferket et al., 2002). Polysaccharides from coneflower showed bacteriostatics against E. coli (Schulte et al., 1967).

Jang (2006) reported that birds fed diets containing antibiotics showed a significant (P< 0.05) reduction in CFU of E. coli when compared to those fed the basal diet (control). There was a similar CFU number of E. coli among birds fed the basal diets fortified with antibiotics and two levels of essential oil. Canan Bolukbasi and Kuddusi Erhan (2006) showed that control group and the 1% thyme group had the highest average concentration of E. coli in feces. The group fed 0.1% and 0.5% thyme had a significantly lower E. coli count than the control and the 1% thyme group. Average E. coli counts significantly differed (P< 0.05) from each other, with the 0.1% thyme group bearing the lowest concentration. Thymol (from thyme essential oil) has been shown to reduce the number of coliforms within the digesta of chickens (Cross et al., 2004). Furthermore, it has been suggested that supplementation with oligosaccharides may have a prebiotic effect through an increase in production of lactic acid, thus increasing the proliferation of beneficial bacteria and reducing the presence of Gram-negative bacteria (Savage et al., 1996). Polysaccharides in coneflowers include: Echinacein, echinacosid, echinalon. Carvacrol is the thyme essential oil component that has a stimulating effect on Lactobacillus proliferation (Tschirch, 2000). Jamroz et al. (2005) reported that plant extract supplement also significantly increases the Lactobacillus numbers.

In conclusion, use of herbal extracts specially garlic improved FCR comparable to virginiamycin in broilers. This effect could be attributed to improvement of digestive enzymes secretion. According to the obtained results in this study, it can be concluded that coneflower is not a suitable feed additive alternative for antibiotic as a growth promotor in broilers. Among the herbal extracts employed, coneflower and garlic were the most effective in immune function enhancement. Also these extracts were able to reduce the serum lipids. Improvement of selected intestinal bacterial populations through these extracts was remarkable. However, more trials are needed to clarify the effect of different medicinal plants on performance of broilers with regard to varied management conditions, including different stress factors, herbal extracts and their optimal dietary inclusion levels, active substances of extracts, dietary ingredients and nutrient content.

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اجرای آزمایش ارتباط پیشنهادی عصاره گیاهی (آویشن باغی، سرخارگل و سیر) و آنتی-پروکانوئید و چربی‌های کلسترولی

این مطالعه به منظور ارزیابی اثرات عصاره گیاهی (آویشن باغی، سرخارگل و سیر) و آنتی-پروکانوئید و چربی‌های کلسترولی بر عملکرد رشد، سیستم ایمنی، فاکتورهای خونی و جمعیت باکتریایی انتخابات شده در چندین دستگاه گوشتی انجام گردید. 

۶۰ طبقه چرخه گوشتی از گروه کنترل در باعث گروه کنترل (۱۵ ppm) و جریه پا به گروه سیر (۱/۰ درصد عصاره آویشن، سرخارگل، سیر) و مخلوط از عصاره‌ها با دور مشابه تقسیم شدند. بر اساس مبنا و کمترین وزن بدین ترتیب مربوط به تیمارهای وریچینامپسین و سرخارگل پیدا (۰/۵) (P < ۰/۵). هم‌چنین کمترین وزن بیشترین ضریب تبدیل غذایی ترکیب مربوط به تیمارهای سیر پرداخته و سرخارگل پیدا (۰/۵) (P < ۰/۵). وزن نسبی بیوس نیتریژن در گروه سیر افزایش معنی‌داری داشت را در مقایسه با دیگر گروهها نشان داد. اما وزن نسبی طالب به وسیله تیمارهای تحت تأثیر قرار نگرفت. پاسخ به افزایش حساسیت آنزیم‌ها (تریلیو) و عیار آنتی‌بایدن بر روی گلیبول قرمزگسیخت در گروه سرخارگل بالاتر بود (۰/۵) (P < ۰/۵). این آنتی‌بایدن بر روی گلیبول قرمزگسیخت در سرخارگل بالاتر بود (۰/۵) (P < ۰/۵). 
