Effects of using plant extracts and a probiotic on performance, intestinal morphology, and microflora population in broilers

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Primary Audience: Nutritionists, Poultry Feed Manufacturers, Researchers

SUMMARY

The effects of garlic (Allium sativum) and thyme (Thymus vulgaris) extracts and a probiotic on growth performance, intestinal tissue morphology, and microbial flora in broilers were investigated. A total of 240 Ross 308 male broilers were divided into 4 treatments, with 4 replicates of 15 birds. Treatments were 2 plant extracts (1,000 ppm) and the probiotic (1,000 ppm from 0 to 7 d, 500 ppm from 8 to 21 d, and 250 ppm from 22 to 42 d) added to drinking water, and a control group with no additives. Birds in different treatments received the same diets during the experimental period. Feed intake, BW gain, and FCR were not influenced by the treatments. The weight of the ileum in birds receiving garlic was significantly lower than that in birds receiving thyme. The villus width in the duodenum of birds receiving garlic was lower than that of birds in control group (P < 0.05). A smaller villus width in the ileum was seen in birds fed garlic and the probiotic compared with birds fed the control diet. However, villus height and crypt depth were not affected by the treatments. At 20 d, birds fed garlic had significantly higher lactobacillus and lower coliform counts compared with birds fed the probiotic. Chickens that received thyme had lower total aerobic bacterial counts in the ileum than did birds in the control group. At 40 d, birds receiving the probiotic had higher total aerobic bacteria and lactobacillus counts in the crop than did birds receiving the control diet. It was concluded that these plant extracts and the probiotic can be used as natural growth promoters for broilers. The beneficial effects of plant extracts and the probiotic should be further studied on commercial farms.

Key words: broiler, intestine, performance, plant extract, probiotic

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DESCRIPTION OF PROBLEM

Antibiotics have been used as growth-promoting substances in poultry nutrition. However, using an antibiotic as a feed additive is viewed as risky by some because of the potential resistance buildup in pathogenic bacteria [1]. Therefore, the use of most antibiotic growth promoters has

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been banned by the European Union since 2006. Consequently, the animal and poultry feed industries are under increasing consumer pressure to reduce the use of antibiotic growth promoters as feed additives and find alternatives [2]. Many studies have been performed to find alternatives to antibiotics [2–4], with probiotics and plant extracts being the most probable alternatives. Probiotics are viable microbial additives that assist in the establishment of a beneficial intestinal population antagonistic to harmful microbes [5]. The action of probiotics can be explained by their production of antimicrobial substances that protect villi and absorptive surfaces against toxins produced by the pathogens, improvement of immunity stimulation, and ability to increase volatile fatty acids [6]. Medicinal plants, such as herbs or extracts, are being investigated as natural sources of biologically important substances that may positively influence poultry health and productivity. The large number of active components in medical plants may be more effective against bacteria than are antimicrobials [7]. Garlic (Allium sativum) and thyme (Thymus vulgaris) have several major components, including allicin, ajoene, thymol, and carvacrol, that have biological activities (e.g., antimicrobial, antioxidant, and antiseptic activities) [8]. The intestinal epithelial layer acts as a barrier that protects the host against luminal pathogens [5]. A reduction of epithelial cell proliferation and mucosal atrophy of the intestine allow various pathogens to inhabit the intestinal lumen. Feed additives, such as probiotics or plant extracts, can decrease the number of intestinal pathogens [4]. Presumably, changes in intestinal morphology could be used as an index of intestinal function and to determine the nutritional value of poultry diets [9].

The objective of the current study was to determine the effect of garlic and thyme extracts and Protexin (a mixture of advantageous bacteria) [10] as a probiotic on the performance, small intestinal microflora, and tissue morphology of broiler chickens.

**MATERIALS AND METHODS**

**Birds and Management**

Two hundred forty 1-d-old Ross 308 male broilers [11] were randomly assigned to 4 treatments, each with 4 replicates consisting of 15 chickens. All birds were placed in wire cages (120 × 120 × 70 cm) in a temperature-controlled house. The initial temperature of 33°C was gradually reduced according to the age of birds (3°C every week) until reaching 21°C, and was then kept constant. Vaccinations against Newcastle disease (8 and 17 d) and Gumboro disease (14 and 21 d) [12] were performed according to recommendations of the Iran Veterinary Organization (Tehran). The lighting program was 24 h per day for the entire experiment. Water and feed were available ad libitum. Birds and feed were weighed weekly on a pen basis and BW gain (BWG), feed intake, and FCR were determined accordingly. Mortality was recorded each day. All bird care and use procedures were approved by the Gorgan University Animal Care and Use Committee.

**Treatments and Diets**

Treatments consisted of garlic (1,000 ppm), thyme (1,000 ppm), and Protexin as a probiotic (1,000 ppm from 0 to 7 d, 500 ppm from 8 to 21 d, 250 ppm from 22 to 42 d) added in the drinking water, and a control with no additives. Protexin is a mixture of advantageous bacteria containing Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Streptococcus thermophilus, Aspergillus oryzae, Bifidobacterium bifidum, Enterococcus faecium, and Candida pintolesii, with a minimum of 6 × 10⁷ cfu/g of the product. All birds were fed the same basal diet. All diets were formulated to meet or exceed the nutrient requirements of broiler chickens based on 1994 NRC guidelines [13]. The composition of the basal diet is shown in Table 1. The garlic and thyme extracts were obtained from the Giah Esans Company [14].

**Sampling**

At the end of the study (42 d), birds in each pen were weighed. One bird in each pen that was close to the mean weight of the pen was selected and killed for carcass evaluation and intestinal morphology. Birds were stunned by exposure to CO₂ gas and killed by cervical dislocation. Selected birds were defeathered, processed, and
Feed was removed 12 h before processing.

**Intestinal Morphology**

The small intestine was opened, the intestinal contents were removed and weighed, and the length of the intestine was measured immediately after killing the bird. The weight and length of the small intestine were expressed as a percentage of BW [5]. The ileum was defined as extending from Meckel’s diverticulum to a point 4 cm distal. The jejunum was defined as being midway between the end of the duodenum and Meckel’s diverticulum. For morphometric analysis, tissue samples (3 cm) were obtained from the middle part of the duodenum, jejunum, and ileum, washed with 10% formalin, and fixed in PBS (40 g of NaH₂PO₄, 65 g of Na₂HPO₄, and 1,000 mL of 10% formalin) for 24 to 48 h [5]. Tissues were dehydrated with an Automatic Tissue Processor [15]. The process consisted of dehydrating the tissues in a series of increasing concentrations of alcohol, clearing the tissue in xylene, and embedding it in paraffin. A microtome was used to make 3 cuts of 5-μm width. The cuts were stained with hematoxylin and eosin. For each treatment, 12 glass slides were prepared and the mean of 10 villi per slide was used as the average value for further analysis. Villus height (VH), villus width (VW), crypt depth (CD), and the ratios of VH:VW and VH:CD were determined at a magnification of 10× by using a light microscope.

**Microbial Enumeration**

Enumeration of the microbial population in the crop and ileal contents was performed at 20 and 40 d in 3 birds per treatment. Various dehydrated media were prepared and sterilized according to the manufacturer’s instructions before being poured into sterile Petri dishes. The media consisted of plate count agar for total bacteria culture, modified de Man, Rogosa, Sharpe agar for *Lactobacillus* culture, and violet red bile agar for coliform culture. A 10-fold serial dilution method using sterilized water was adopted to determine colony-forming units in each gram of digesta harvested from the crop and ileum by means of the pour plate method. In the crop, *Lactobacillus* counts were determined using modified de Man, Rogosa, Sharpe agar after incubating at 37°C for 48 h. In the ileum, coliform counts were determined using violet red bile agar after incubating at 37°C for 48 h. In both the crop and ileum, total bacteria were enumerated on plate count agar after incubating at 37°C for 48 h [16].

**Statistical Analysis**

A completely randomized design was used to analyze the studied traits in the different treatments. Data were analyzed using the GLM procedure of SAS statistical software [17]. Duncan’s multiple-range test was used for means comparisons between treatments [18].

**RESULTS AND DISCUSSION**

Effects of the treatments on growth performance are shown in Table 2. No significant differences were observed in feed intake, BWG, and FCR among treatments in the starter phase.
(0 to 21 d), grower phase (22 to 42 d), and entire period (0 to 42 d). Adding thyme and garlic extracts and the probiotic in water did not increase feed intake and BWG; accordingly, no improvement was observed for FCR in the experiment. The same results have been observed by others [5, 16, 19–21]. In contrast, Alcicek et al. [22] reported that adding a blend of plant extracts (36 to 48 mg/kg) to the diet resulted in an increase in growth performance. Cross et al. [23] reported that birds fed diets that included thyme oil and yarrow herb had better growth performance than birds fed diets that included other essential oil and herb supplements. The present results may be associated with the environmental conditions of the experiment. Well-nourished, healthy chickens do not respond positively to growth promoters when they are reared under clean conditions and at a moderate stocking density. Several researchers reported that when chickens were housed in a clean environment, growth promoters, such as probiotics or plant extracts, were ineffective on performance [5, 19]. Furthermore, it is necessary to investigate the digestive enzymes that exist in the pancreas and small intestine to reveal the effect of probiotics and plant extracts on digestive function. It has been reported that plant extracts increase the secretion of pancreatic enzymes in broiler chickens [16].

The weight, length, and weight:length ratio in the different segments of the small intestine are presented in Table 3. No parameters were influenced by treatments, except the weight of the ileum. The weight of the ileum in birds receiving garlic was significantly lower than that of birds receiving thyme ($P < 0.05$). No differences were observed for the weight of the ileum among the control, thyme, and probiotic treatments. Guo et al. [24] found no significant differences in intestinal morphology among a medicinal plant group, an antibiotic group, and a control group. Jin et al. [25] also found that adding *Lactobacillus* to the diet did not affect the weight of the intestine. Gunal et al. [5] found a lower small intestinal weight in birds receiving an antibiotic. They suggested that the reduction in intestinal weight was due to a thinner intestine wall, which increased nutrient absorption. In the present study, the reduction in ileum weight observed in birds receiving the garlic treatment may be associated with a reduction in muscular thickness. During a pathogenic bacteria infection, the lymphocytes that accumulate to kill the pathogens cause inflammation, which in turn increases muscular thickness. Antimicrobial compounds, such as garlic, reduce the microbial population and the toxins produced in the lumen [23]. In contrast, Tortuero [26] reported a decrease in the weight of the cecum after using a probiotic based on *L. acidophilus* in chickens.

The effect of plant extracts and the probiotic was considerable on VW in the duodenum and ileum (Table 4). Birds receiving garlic had a significantly lower VW in the duodenum than did birds in the control group ($P < 0.05$). A lower VW in the ileum was observed in birds in the garlic and probiotic groups compared with those in the control group. However, VH and CD in different segments of the small intestine were not influenced by treatments. The effects of plant extracts and the probiotic on the VH:VW and VH:CD ratios are shown in Table 5. The VH:VW ratio in the jejunum and the VH:CD ratio in different segments of the small intestine were not significantly influenced by treat-

**Table 2. The effect of treatments on the growth performance of broilers**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 to 21 d</th>
<th>22 to 42 d</th>
<th>0 to 42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feed intake, g</td>
<td>BW gain, g</td>
<td>FCR, g/g</td>
</tr>
<tr>
<td>Control</td>
<td>1,269.28</td>
<td>704.15</td>
<td>1.80</td>
</tr>
<tr>
<td>Garlic</td>
<td>1,218.12</td>
<td>675.68</td>
<td>1.81</td>
</tr>
<tr>
<td>Thyme</td>
<td>1,249.28</td>
<td>719.26</td>
<td>1.74</td>
</tr>
<tr>
<td>Probiotic</td>
<td>1,239.05</td>
<td>705.22</td>
<td>1.76</td>
</tr>
<tr>
<td>SEM</td>
<td>30.20</td>
<td>19.45</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1Garlic and thyme at 1,000 ppm; Protexin (Probiotics International Ltd., Lopen Head, UK) as the probiotic at 1,000 ppm from 0 to 7 d, 500 ppm from 8 to 21 d, and 250 ppm from 22 to 42 d.
The ratio of VH:VW in the ileum was significantly lower in birds in the control group and the thyme treatment group than in the ileum of birds in the probiotic and garlic treatment groups. Garcia et al. [9] also reported no significant differences in VH and CD between birds in the control group and a group receiving a blend of plant extracts. Generally, the VH:VW ratio is reduced in older birds. The reduction in the VH:VW ratio is associated with an increase in both mitosis and the ratio of immature to mature enterocytes. Antimicrobial agents are known to reduce the intestinal microbial load, which in turn reduces the presence of toxins. An increase in toxins is associated with changes in intestinal morphology, such as shorter villi and deeper crypts [27]. Our results are not in accordance with the findings obtained by Demir et al. [3], who showed that CD in the ileum were significantly reduced by including garlic and thyme in the diet compared with including antibiotics, oregano, and Du-sacch (an herbal natural feed additive). The same results were reported by Pelicano et al. [28] and Santos et al. [29], who observed no difference in VH in the ileum after using probiotics. Schwarz et al. [1] found no difference in CD in the jejunum between the control group and birds receiving diets containing Bacillus subtilis. However, Gunal et al. [5] showed that a probiotic treatment significantly increased the VH in the jejunum and ileum at 21 or 42 d compared with a nonsupplemented basal diet. Pelicano et al. [4] indicated that the VH and CD in all segments of the small intestine were significantly increased in diets containing a probiotic.

The effect of plant extracts and the probiotic on crop and ileal microbial colony-forming units at 20 and 40 d are presented in Tables 6 and 7, respectively. The bacteriological data were natural

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**Table 3.** The effect of treatments on the length, weight, and weight-to-length ratio of the small intestine of broilers at 42 d of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duodenum</th>
<th></th>
<th></th>
<th>Jejunum</th>
<th></th>
<th></th>
<th>Ileum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length, cm</td>
<td>Weight, g</td>
<td>Weight: length</td>
<td>Length, cm</td>
<td>Weight, g</td>
<td>Weight: length</td>
<td>Length, cm</td>
<td>Weight, g</td>
</tr>
<tr>
<td>Control</td>
<td>13.09</td>
<td>6.93</td>
<td>0.53</td>
<td>28.54</td>
<td>12.83</td>
<td>0.46</td>
<td>30.47</td>
<td>9.30</td>
</tr>
<tr>
<td>Garlic</td>
<td>12.45</td>
<td>5.78</td>
<td>0.47</td>
<td>27.05</td>
<td>10.71</td>
<td>0.40</td>
<td>30.17</td>
<td>8.00</td>
</tr>
<tr>
<td>Thyme</td>
<td>12.21</td>
<td>5.71</td>
<td>0.47</td>
<td>27.55</td>
<td>11.89</td>
<td>0.43</td>
<td>30.43</td>
<td>10.50</td>
</tr>
<tr>
<td>Probiotic</td>
<td>12.53</td>
<td>6.32</td>
<td>0.50</td>
<td>25.96</td>
<td>11.68</td>
<td>0.45</td>
<td>30.46</td>
<td>9.62</td>
</tr>
<tr>
<td>SEM</td>
<td>0.70</td>
<td>0.43</td>
<td>0.04</td>
<td>1.99</td>
<td>0.82</td>
<td>0.03</td>
<td>2.58</td>
<td>0.71</td>
</tr>
</tbody>
</table>

a,b Means within a column with no common letters are significantly different ($P < 0.05$).

Length and weight of different segments of intestine are based on kilograms of BW.

Garlic and thyme at 1,000 ppm; Protexin (Probiotics International Ltd., Lopen Head, UK) as the probiotic at 1,000 ppm from 0 to 7 d, 500 ppm from 8 to 21 d, and 250 ppm from 22 to 42 d.

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**Table 4.** The effect of treatments on villus height, villus width, and crypt depth of the small intestine of broilers at 42 d of age

| Treatment | Duodenum | | | Jejunum | | | Ileum | |
|-----------|----------|----------|----------|----------|----------|----------|----------|
|           | VH, µm | VW, µm | CD, µm | VH, µm | VW, µm | CD, µm | VH, µm | VW, µm | CD, µm |
| Control   | 1,495.37 | 313.33a | 275.00 | 1,362.50 | 237.92 | 231.94 | 872.92 | 197.22a | 181.25 |
| Garlic    | 1,300.00 | 236.11b | 304.80 | 1,329.17 | 194.44 | 209.72 | 842.36 | 150.00c | 157.64 |
| Thyme     | 1,274.07 | 284.26ab | 250.00 | 1,315.97 | 231.25 | 213.19 | 902.78 | 189.58ab | 181.25 |
| Probiotic | 1,299.53 | 273.79ab | 232.45 | 1,286.81 | 209.03 | 229.17 | 948.61 | 168.06bc | 195.14 |
| SEM       | 72.89 | 16.67 | 29.61 | 58.04 | 15.57 | 12.30 | 46.09 | 7.12 | 15.63 |

a,b Means with no common letters in columns are significantly different ($P < 0.05$).

VH = villus height; VW = villus width; CD = crypt depth.

Garlic and thyme at 1,000 ppm; Protexin (Probiotics International Ltd., Lopen Head, UK) as the probiotic at 1,000 ppm from 0 to 7 d, 500 ppm from 8 to 21 d, and 250 ppm from 22 to 42 d.
logarithm-transformed before statistical analysis. At d 20 of the experiment, the colony-forming units of total bacteria in digesta harvested from the crop were not significantly influenced by treatments. The colony-forming units of lactobacilli in the crop were significantly higher ($P < 0.05$) in birds receiving garlic than in birds receiving the probiotic. A significant reduction ($P < 0.05$) in colony-forming units of coliforms was found in digesta harvested from the ileum of birds receiving garlic and thyme compared with birds receiving the probiotic.

In the second phase of microflora enumeration (d 40), the colony-forming units of total bacteria in the crop of birds in the probiotic treatment were significantly higher ($P < 0.05$) than those for birds in the control group and the thyme treatment group. However, the colony-forming units of total bacteria in the ileum were not influenced by treatments at 40 d. Chickens in the treatments containing the probiotic and garlic showed a significant increase ($P < 0.05$) in colony-forming units of lactobacilli in the crop compared with chickens in the control group. No detectable colony-forming units of coliforms in the ileal digesta were obtained from birds in any treatments.

Microflora inhabiting the gastrointestinal tract interact with host animals, and their populations vary with the animal species, the site along the gastrointestinal tract, age, diet, and the environment. Healthy animals generally maintain a balanced microbial population, which plays an important role in the growth and health of the animals. For example, intestinal bacteria metabolize nutrients in the contents, produce short-chain fatty acids and lactic acid, and synthesize some vitamins. Some of these activities can be beneficial to the host animals [30]. It is important to consider that some feed additives originating from plant products and probiotics have a large effect on gut microflora either directly or indirectly [7]. However, birds receive little nutritional advantage from intestinal mi-

<table>
<thead>
<tr>
<th>Table 5. The effect of treatments on ratios of villus height to villus width and crypt depth of the small intestine of broilers at 42 d of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Garlic</td>
</tr>
<tr>
<td>Thyme</td>
</tr>
<tr>
<td>Probiotic</td>
</tr>
<tr>
<td>SEM</td>
</tr>
</tbody>
</table>

Means with no common letters in columns are significantly different ($P < 0.05$).

Table 6. The effect of treatments on microbial population (log10 cfu/g) of the crop and ileum of broilers at 20 d of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Crop</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total bacteria</td>
<td>Lactobacilli</td>
</tr>
<tr>
<td>Control</td>
<td>7.60</td>
<td>8.38ab</td>
</tr>
<tr>
<td>Garlic</td>
<td>7.23</td>
<td>8.98a</td>
</tr>
<tr>
<td>Thyme</td>
<td>6.93</td>
<td>8.62ab</td>
</tr>
<tr>
<td>Probiotic</td>
<td>7.39</td>
<td>8.32b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.54</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Means within a column with no common letters are significantly different ($P < 0.05$).

Garlic and thyme at 1,000 ppm; Protexin (Probiotics International Ltd., Lopen Head, UK) as the probiotic at 1,000 ppm from 0 to 7 d, 500 ppm from 8 to 21 d, and 250 ppm from 22 to 42 d.
croflora compared with other species of animals. The same results were reported by Guo et al. [24], who showed that mushroom and herb extracts were largely associated with reduced Bacteroides spp., enterococci, and Escherichia coli numbers and with increased numbers of bifidobacteria and lactobacilli compared with the control and antibiotic treatment groups. Jamroz et al. [31] reported that the number of Lactobacillus colony-forming units was significantly increased after using a blend of plant extracts. In earlier studies, significant reductions of E. coli and Clostridium have been obtained after using natural plant extracts [32]. In contrast, Jang et al. [16] observed no change in the number of colony-forming units of lactobacilli in the ileocecal digesta when using a commercial blend of essential oils in a broiler diet. Gunal et al. [5] observed that the probiotic decreased ileal and cecal negative bacterial counts at 21 or 42 d. Ceylan et al. [33] reported that the Enterococcus-containing probiotic Cylactin reduced aerobic and coliform bacteria counts. However, Ozturk and Yildirim [34] found that a probiotic containing lactobacilli had no effect on ileal and cecal gram-negative bacteria counts.

CONCLUSIONS AND APPLICATIONS

1. The plant extracts and the probiotic had no significant effects on the growth performance of broilers.
2. Birds that received the garlic treatment had lower ileum weights and lower VW in the duodenum and ileum than did birds in the control group.
3. Birds receiving the probiotic treatment had more total bacteria and lactobacilli in the crop than did birds in the control group at 42 d.
4. Because of differences in the herbal active components (terpenes), the combination of plant extracts or a probiotic in the diet could be of great interest in future studies.

REFERENCES AND NOTES


10. Protexin, Probiotics International Ltd., Lopen Head, UK.


12. Vaccines against Newcastle and Gumboro diseases, Intervet International B.V., Boxmeer, the Netherlands.


14. Garlic (Allium sativum) and thyme (Thymus vulgaris) extracts, Giah Esans Company, Gorgan, Iran.

15. DS 2000/H Automatic Tissue Processor, Dib Sabz, Tehran, Iran.


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