The aim of this study was to evaluate the effect of dietary *Euphorbia hirta* and an acidifier mixture supplementation on gut morphology and some blood parameters of broiler chickens. A total of 240 day old male broiler chicks were randomly assigned to one of the four dietary treatment groups including: (1) basal diet (control), (2) basal diet + 7.5 g/kg *E. hirta* (*Eh* 7.5), (3) basal diet + 1.5 g/kg acidifier (OA) and (4) basal diet + 7.5 g/kg *E. hirta* and 1.5 g/kg acidifier (*Eh* OA). The *Eh* 7.5, OA and *Eh* OA supplementation significantly improved overall feed conversion ratio compared to the control group. The addition of *Eh* 7.5, OA and their combination increased the villus height compared to the control birds. Crypt depth was markedly decreased by OA treatment. The highest ratio of villi to crypt was observed in OA fed broilers. Blood serum biochemical parameters did not influence by the dietary treatments. In conclusion, the results indicated that addition of *Eh* 7.5 and OA to the broiler diet enhanced maintenance and function of the small intestine and broiler performance.

**KEY WORDS** broiler, *Euphorbia hirta*, feed additive, gut morphology, herbal plants.

**INTRODUCTION**

High growth performance and efficient feed conversion could be achieved in poultry industry by application of specific feed additives. Poultry diets contain a wide variety of additives. Common feed additives used in poultry diets include antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes. Antibiotic feed additives as growth and health promoters supplemented to poultry diets to stabilize the gut microflora improve performance and prevent some specific intestinal diseases (Truscott and Al-Sheikhly, 1997; Miles *et al.* 1984; Waldroup *et al.* 1995; Hashemi and Davoodi, 2011). Antibiotic use in animals, however, is a potential problem for human medicine because antibiotic resistant bacteria can pass through the food chain to people. As a result of increasing concerns over the transfer of resistance between different bacteria and between human and animals (Ratcliff, 2000), the European union (EU) in 2006 banned antibiotic growth promoters used as additives in animal feed (Hashemi and Davoodi, 2010). Hence, large investments have been made by researchers and multinational companies in order to investigate alternative products to maintain growth and performance in poultry and at the same time, take consideration into the demands of consumers that the new antibiotic-replacers must be safe, acceptable and healthy. Consequently, an
intensive search for alternatives such as probiotics, prebiotics, symbiotics, enzymes, toxin binders, organic acids, organic minerals, oligosaccharides and other feed additives has started in the last decade (Fulton et al. 2002; Griggs and Jacob, 2005; Owens et al. 2008).

Phytopgenic feed additive has gained increasing interest, especially for their application in poultry diets (Windisch et al. 2008; Hashemi and Davoodi, 2010). Some positive changes in digestive enzymes, gut morphology and immune system were noticed in birds given phytogen supplemented feed (Windisch et al. 2008). Small intestine is a critical digestive organ involved in nutrient absorption, the development of this organ is essential to poultry health and performance (Kawalilak et al. 2011). Bi and Chiou (1996) found that broiler chicks developed larger intestinal villi resulting in faster growth rates.

It is demonstrated that improvement of gut morphology is paralleled by increased absorptive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems (Awad et al. 2008). Euphorbia hirta is a small herb common to the tropical countries and number of reports has shown that E. hirta possess antibacterial activity (Hashemi et al. 2008b), in vitro antioxidant (Sharma et al. 2007), analgesic, antipyretic, anti-inflammatory properties and anti-depressant for blood pressure (Williams et al. 1997). The positive effects of E. hirta supplementation on broiler performance and gut microflora have been demonstrated (Hashemi et al. 2009a). However, the exact growth-promoting mechanisms of phytobiotics in broiler chickens are poorly understood.

On the other hand, the beneficial effects of organic acids on the productive traits of pigs have been demonstrated in many studies, but in poultry production, organic acids have not gained as much attention as in pig production (Radecki and Yokoyama, 1991; Læghout, 2000). Supposed benefits of acidifiers feed additives would be associated with the increase of intestinal nutrient assimilation (Pelicanò et al. 2005; Roser, 2006; Paul et al. 2007; Kum et al. 2010) but comprehensive information on the effects of organic acids on the gut histology in poultry still not available. Therefore, the objective of this study was to investigate the effect of dietary supplementation with E. hirta and acidifiers on gut morphology and some blood parameters of broiler chickens.

**Materials and Methods**

**Preparing chicken rations**

*Euphorbia hirta* was selected on the basis of the preliminary evaluation tests (Hashemi et al. 2008a; Hashemi et al. 2008b). The whole plant was washed and dried at 50 °C.

The dried plants were ground using a Wiley mill (Thomas® Wiley Cutting Mill Model 4) through a 1 mm screen and then the powder was added to the proper chicken ration. Ingredients and nutrient compositions of the diet are shown in Tables 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Ingredients and nutrient composition of basal diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (g/100 grasses)</td>
<td>Starter (1-21 d)</td>
</tr>
<tr>
<td>Corn</td>
<td>49.47</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>34.91</td>
</tr>
<tr>
<td>Palm oil</td>
<td>6</td>
</tr>
<tr>
<td>Fish meal</td>
<td>6</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.3</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>1.4</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.9</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.15</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline-HCl (70%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>0.50</td>
</tr>
<tr>
<td>Carrier</td>
<td>0.9</td>
</tr>
<tr>
<td>Calculated analyses (g/kg)</td>
<td></td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>3103</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>22.8</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.60</td>
</tr>
<tr>
<td>Methionine + cysteine (%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Arginine (%)</td>
<td>1.55</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Available phosphorus (%)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1 Supplied per kg of diet: vitamin A: 1500 IU; Cholecalciferol: 200 IU; vitamin E: 10 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; Pyridoxine: 3.0 mg; Iron: 80 mg; Zinc: 40 mg; Manganese: 60 mg; Iodine: 0.18 mg; Copper: 8 mg and Selenium: 0.15 mg.

2 The diets of treatments contained 0, 1.5 g/kg acidifier; 7.5 g/kg *E. hirta* or 9 g/kg acidifier and *E. hirta* combination and carrier (sand powder: 9, 7.5, 1.5, or 0 g/kg), respectively.

3 Based on NRC (1994) feed composition table.

**Experimental design**

A total of 240 day old male broiler chicks (Cobb 500) were obtained from a local hatchery, wing banded and randomly allocated to one of the four dietary treatment groups:

1. Basal diet (control, NRC recommendation).
2. Basal diet + 7.5 g/kg *E. hirta* (Eh 7.5).
3. Basal diet + 1.5 g/kg acidifier (OA) and (4) basal diet + 7.5 g/kg *E. hirta* and 1.5 g/kg acidifier (*EhOA*).

Each dietary treatment was replicated 4 times with 15 birds per replicate. The acidifier (Orgacids™) consisted of formic, phosphoric, lactic, tartaric, citric and malic acids (Sunzen Corporation Sdn Bhd. Malaysia). The area of each pen measuring was 1.5 m². Feed and water were provided *ad libitum* and lighting was continuous. The chicks were vaccinated against Newcastle disease (animal health, fort dodge, Iowa, USA) on d 7 eye drop and nasal route on d 21. No antibiotic and anticoccidials were used during the experiment.
Performance parameters
The chicks were weighed individually at the end of each week and feed consumption was recorded weekly. Four hours prior to bird weighing, the diets were removed and feed consumption was determined. Feed conversion ratio (FCR) was calculated weekly. Mortality of broilers in each replicate was recorded daily.

Measurement of blood biochemical parameters
On d 21 and 42, two birds from each pen were randomly selected for blood biochemical parameters. Blood samples were collected from the wing vein within 45 s after the capture of each bird using a sterilized syringe with a 23 gauge needle to obtain serum. The blood samples were then centrifuged at 2000 × g at 4 °C for 20 min within 1 h of collection to separate the serum. Serum stored at -20 °C until further analysis. Serum cholesterol, triglyceride and electrolytes (Na, K and Cl) levels were measured by specific commercial kits (Roche Diagnostica, Basel, Switzerland) using an autoanalyzer (Hitachi 902, Hitachi Ltd., Tokyo, Japan).

Morphometric analysis of the gut
On day 42, eight birds per treatment were killed by cervical dislocation. The gastrointestinal morphometric variables including villus height, crypt depth, villus surface area, lamina propria and muscularis mucosa thickness from the duodenum were evaluated. A 2 cm segment of the midpoint of the duodenum was dissected and fixed in 10% buffered formalin. Each segment was embedded in paraffin. A 5 μm section of each sample was placed onto a glass slide and stained with hematoxylin and eosin (Sakamoto et al. 2000; Solis de los Santos et al. 2005). Slides were viewed with an upright microscope (BX51; Olympus, Tokyo, Japan) equipped with a microscope digital camera (U-TV1X; Olympus, Tokyo, Japan). Villus length, width and surface, crypt depth, lamina propria and muscularis mucosa thickness were acquired and measured using image analysis software (Olympus Soft Imaging Solutions, version 3.2, Germany). The villus height was measured from the top of the villus to the top of the lamina propria. The surface area was calculated using the formula (Sakamoto et al. 2000):

\[ \text{Surface area} = (2\pi) \times \left( \frac{\text{VW}}{2} \right) \times (\text{VL}) \]

Where:

- VW: villus width.
- VL: villus length.

The lamina propria thickness was measured in the space between the base of the villus and the peak of the muscularis mucosa. Crypt depth was measured from the base upward to the region of transition between the crypt and villus (Aptekmann et al. 2001).

Statistical analysis
A completely randomized design (CRD) with 4 treatments and 4 replicates and 15 birds per replicate was employed. Statistical analyses were performed using the procedure in the SAS statistical package (SAS, 2005). The significance of differences between means was tested using the Duncan multiple range test of the GLM procedure. The mortality rate was analyzed by the chi-square test. Statistical significance was considered as P < 0.05.

RESULTS AND DISCUSSION
The effects of Euphorbia hirta, acidifier and their combination on broiler chickens performance and mortality rate are shown in Table 2. Dietary treatments affected body weight gain, feed intake and FCR from day 22 to 42. An increase in body weight gain was observed in Eh 7.5 and OA groups compared to EhOA and the control groups. There were no significant differences in body weight gain between EhOA and control groups. Overall, all treatment groups showed better FCR than the control group. The control group had the poorest FCR. There were no significant differences in mortality rate between treatment groups.

Histological examinations of the small intestine from birds fed the dietary treatments are shown in Table 3 and Figure 1. The addition of Eh 7.5, OA and EhOA significantly increased the villus height compared to the control birds. Crypt depth was markedly decreased by OA treatment. No significant differences in crypt depth were observed between Eh 7.5, EhOA and control groups. The villus height to crypt depth ratio in the duodenum was influenced by any dietary treatments. The ratio of villus to the crypt was the highest in the OA treatment. Treated birds had a higher villus surface area and lamina propria thickness than that of the control birds. There were no significant differences between treatment diets on muscularis mucosa thickness.

The impact of Euphorbia hirta, acidifier and their combination on clinical blood chemistry values of broiler chickens are presented in Table 4. Blood serum cholesterol, triglyceride and electrolytes (Na, K and Cl) levels were not influenced by dietary treatments on d 21 and 42.
Dietary supplementation with herbal plants, and acidifiers improved the health status of the gastrointestinal tract (Garcia et al. 2007; Windisch et al. 2008; Ao et al. 2009; Yang et al. 2009). To our knowledge, little is known about the effect of new antibiotic growth promoter replacements such as acidifiers and herbal plants on broiler chickens performance. The present findings indicate that birds fed Eh 7.5, EHOA and OA had significantly better FCR compared to the no added control program. An increase in broiler performance due to the use of single acids such as formic acid (Vogt et al. 1979; Vogt et al. 1981) and fumaric acid (Kirchgessner et al. 1991) have been documented. Patten and Waldroup, (1998) showed that supplementation of fumaric acid significantly improved body weights of broilers. Improvement in live body weight, body weight gain and feed conversion ratio by organic acid supplementation has been reported (Abdel-Fattah et al. 2008; Vieira et al. 2008; Luckstadt et al. 2004; Canibe et al. 2001; Skinner et al. 1991). On the other hand, growth promoting effects of E. hirta could be associated with the antibacterial properties of this plant (Vijaya et al. 1995; Ogbulie et al. 2007) and their phytochemical compounds such as flavanoids, tannin, saponin and alkaloids (Cowan, 1999; Draughon, 2004; Hashemi et al. 2008a).

Table 2: The effect of Euphorbia hirta, mix of acidifier and their combination on performance and mortality rate of broiler chickens (Mean±SEM)

<table>
<thead>
<tr>
<th>Weight gain (g)</th>
<th>Control</th>
<th>Eh 7.5</th>
<th>OA</th>
<th>EHOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-21 d</td>
<td>870±8.29</td>
<td>878±7.50</td>
<td>885±7.40</td>
<td>873±7.16</td>
</tr>
<tr>
<td>22-42 d</td>
<td>1407±26.18(^a)</td>
<td>1516±14.26(^a)</td>
<td>1520±17.66(^a)</td>
<td>1457±18.71(^a)</td>
</tr>
<tr>
<td>1-42 d</td>
<td>2276±28.46(^a)</td>
<td>2393±16.31(^a)</td>
<td>2402±18.24(^a)</td>
<td>2332±22.14(^a)</td>
</tr>
</tbody>
</table>

Table 3: Feed intake (g/bird)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1-21 d</th>
<th>22-42 d</th>
<th>1-42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1183±5.85</td>
<td>1091±3.84</td>
<td>4059±86.84(^b)</td>
</tr>
<tr>
<td>Eh 7.5</td>
<td>1205±6.54</td>
<td>1143±5.28</td>
<td>3965±40.18(^b)</td>
</tr>
<tr>
<td>OA</td>
<td>1195±7.56</td>
<td>1117±5.28</td>
<td>3431±32.87 (^b)</td>
</tr>
</tbody>
</table>

Table 4: Blood biochemical parameter

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cholesterol (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
<th>Na (mmol/L)</th>
<th>K (mmol/L)</th>
<th>Cl (mmol/L)</th>
<th>SEB(^2) (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.53±0.13</td>
<td>0.85±0.06</td>
<td>142.81±0.45</td>
<td>3.78±0.20</td>
<td>105.01±0.74</td>
<td>42.58±0.74</td>
</tr>
<tr>
<td>Eh 7.5</td>
<td>2.63±0.10</td>
<td>0.91±0.10</td>
<td>140.27±2.27</td>
<td>3.96±0.21</td>
<td>100.71±1.95</td>
<td>43.52±0.43</td>
</tr>
<tr>
<td>OA</td>
<td>2.60±0.12</td>
<td>0.81±0.05</td>
<td>141.35±1.21</td>
<td>4.15±0.31</td>
<td>102.40±1.16</td>
<td>43.30±0.17</td>
</tr>
<tr>
<td>EHOA</td>
<td>2.68±0.08</td>
<td>0.68±0.10</td>
<td>143.25±0.74</td>
<td>4.48±0.13</td>
<td>103.05±1.57</td>
<td>42.68±0.45</td>
</tr>
</tbody>
</table>

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

Table 5: Effect of Euphorbia hirta (Eh 7.5), acidifier (OA) and their combination (EHOA) on performance and mortality rate of broiler chickens on d 42

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed intake (g/bird)</th>
<th>FCR (feed/gain)</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.35±0.01(^a)</td>
<td>1.24±0.01(^a)</td>
<td>1.25±0.09(^a)</td>
</tr>
<tr>
<td>Eh 7.5</td>
<td>2.04±0.04(^c)</td>
<td>1.91±0.01(^c)</td>
<td>2.02±0.02(^c)</td>
</tr>
<tr>
<td>OA</td>
<td>1.79±0.09(^a)</td>
<td>1.66±0.03(^b)</td>
<td>1.63±0.01(^b)</td>
</tr>
</tbody>
</table>

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

Mean ± SEM representing 8 birds per group and the average of 10 measurements per parameter, per bird.
Furthermore, the mode of action for improved FCR in birds given \textit{Eh} 7.5, \textit{EhOA} and OA supplementation could be closely associated to improve in gut health and intestinal morphology. Acidifiers and their salts added to poultry and monogasteric animal diets could potentially help to improve growth performance by improving digestive processes through several mechanisms such as: reduction of pH and buffering capacity of diets, promoting the beneficial bacterial growth, inhibiting growth of pathogenic microbes for example, \textit{E. coli}, \textit{clostridia} and \textit{salmonella spp}. Organic acids may also stimulate pancreatic secretions, which increase the digestibility, absorption and retention of protein and amino acids (Papatsiros et al. 2012).

On the other hand, a number of phytobiotics are capable of modifying the gut microflora substantially, which, in turn, can bring about a cascade of changes in the animal’s responses to nutrients. The exact modes of action by which plant bioactive substances and phytochemicals exert their positive effects are not well understood. Some bioactive substances from plants, like most antimicrobial agents, exert their effects by modulating the cellular membrane of microbes and also the effects of phytobiotics are often indirectly mediated by metabolites generated by gut microflora that use the bioactive compounds for their own metabolism (Hashemi and Davoodi, 2011). Several bioactive compounds from mushrooms and plants have been identified as compounds that differentially stimulate favourable bacteria such as \textit{Lactobacilli} and \textit{Bifidobacteria} without promoting the growth of pathogenic species (Jamroz et al. 2003; Guo et al. 2004).

The small intestine is a critical digestive organ involved in nutrient absorption and development of this organ is essential to broiler health and performance (Kawalilak et al. 2011). Bi and Chiou (1996) found that broiler chicks developed larger intestinal villi resulting in faster growth rates. Villus condition has become a common measurement in supporting the effects of nutrition on gastrointestinal physiology. However, relationships between live performance improvements and villus height or crypt depth measurements many times have documented to show significant correlations.

The present study showed duodenal villus height, villus surface area and lamina propria thickness increased in birds fed with \textit{Eh} 7.5, OA and \textit{EhOA} compared with the control
group. The increases in villus height and villus surface area are capable of greater absorption of available nutrients (Awad et al. 2008).

The villus height to crypt depth ratio in the duodenum was the highest in the OA. The villus to crypt ratio is an indicator of the likely digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption (Montagne et al. 2003). On the other hand, a decrease in villus to crypt ratio or lower crypt to villus ratio is indicative of a higher rate of enterocyte-cell migration from the crypt to the villus (Adibmoradi et al. 2006; Silva et al. 2009).

The smallest depth of the crypts was observed in OA birds. Decreasing crypt depth by OA diet might be explained by the fact that the crypt can be regarded as the villus factory and a large crypt indicates rapid tissue turnover and a high demand for new tissue (Choct, 2009). In addition, in previous studies, acidifiers exhibit strong antibacterial activity against E. coli and Salmonella (Skrivanova and Marounek, 2007; Hashemi et al. 2009b; Hashemi, 2010). It has been suggested that decreasing colonization of pathogens and production of toxic metabolites, would reduce damage of enterocytes and the need for cell renewal in the gut (Hughes, 2003). Furthermore, this decrease may possibly be related to the mucous reduction as the crypt is in the intestinal layer. As the crypts present, basal cells capable of dividing several times by mitosis and differentiate amongst the number of intestine epithelium. Smaller crypt depth has probably interfered in the normal functioning of the mucosa, its regeneration, and nutrient absorption (Hermes et al. 2008).

It has been reported that organic acids stimulate the proliferation of normal crypt cells, enhancing healthy tissue turnover and maintenance (Scheppach et al. 1995). This trophic effect was demonstrated by Frankel et al. (1994), who found an increase in villus height and surface area in the colon and jejunum of rats fed diets supplemented with butyric acid. Le Blay et al. (2000) and Fukunaga et al. (2003) also reported that organic acids can accelerate gut epithelial cell proliferation, thus increase intestinal tissue weight and changing mucosal morphology. The short chain fatty acids are believed to increase plasma glucagon-like peptide 2 (GLP-2) and ileal pro-glucagon mRNA, glucose transporter (GLUT2) expression and protein expression, which are potential signals mediating gut epithelial cell proliferation (Tappenden and McBurney, 1998). Paul et al. (2007) reported that the organic acid supplementation increased duodenal villus height.

Similar results were observed by Garcia et al. (2007) who found improved villus height with formic acid and also greater crypt depth but the villus surface area was not influenced. The increased villus height in the small intestines could be associated with higher absorptive intestinal surface (Loddi et al. 2004) which facilitates the nutrient absorption and hence, has a direct impact on growth performance. Garcia et al. (2007) showed that diet supplementation with herbal plants and plant derived products causes a higher villus in chickens. Herbal plants decrease the total pathogen bacteria in the intestinal wall and cause a reduction in production of toxic compounds and damage to intestinal epithelial cells, inhibit the destruction of villus and decreases reconstruction of the lumen. This function could lead to a conversion in intestinal morphology (Garcia et al. 2007; Hashemi, 2010). The results in our study are in agreement with other researches (Yakhkeshi et al. 2011; Garcia et al. 2007). Furthermore, previous study revealed that acidifiers exhibit strong antibacterial activity against E. coli and Salmonella and E. hirta had a positive effect on improvement of the microflora balance and the decrease of E. coli and Salmonella population and stimulating of the Lactobacillus spp. Proliferation (Hashemi et al. 2009a; Hashemi, 2010). It has been suggested that reduced microbial activity in digesta or microbial activity at the level of the brush border would reduce both the damage to enterocytes and the need for cell renewal in the gut (Hughes, 2003). Cook and Bird (1973) reported a shorter villus and a deeper crypt when the counts of pathogenic bacteria increase in the GIT, which result in fewer absorptive and more secretory cells (Schneeman, 1982).

CONCLUSION

Changes in intestinal morphology as described above can lead to privileged nutrient absorption, decreased secretion in the gut, reduced disease resistance and impaired overall performance (Nabuurs et al. 1993). In view of the concern for increased drug resistance bacteria and antibiotic residual effects following use of subtherapeutic antibiotic growth promoters as feed supplements, the non therapeutic antibiotic replacements such as enzymes, probiotics, prebiotics, herbs and their derivatives, essential oils, and acidifiers are the potential candidates as feed additives in broiler production.

REFERENCES


