

Intestinal Histomorphology Changes and Serum Biochemistry Responses of Broiler Chickens Fed Herbal Plant (*Euphorbia hirta*) and Mix of Acidifier

Research Article

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ABSTRACT

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 compare 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 influenced 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).

Phytogetic feed additive has recently 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 phytoegen 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 digestive 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; Langhout, 2000). Supposed benefits of acidifiers feed additives would be associated with the increase of intestinal nutrient assimilation (Pelicano *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 1 Ingredients and nutrient composition of basal diets

Ingredient ¹ (g/100 grasses)	Starter (1-21 d)	Finisher (22-42 d)
Corn	49.47	58.29
Soybean meal	34.91	25.5
Palm oil	6	6
Fish meal	6	6.2
NaCl	0.3	0.3
Di-calcium phosphate	1.4	1.2
Limestone	0.9	0.9
DL-methionine	0.15	0.15
Lysine	0.2	-
Choline-HCl (70%)	0.08	0.06
Vitamin-mineral premix	0.50	0.50
Carrier ²	0.9	0.9
Calculated analyses ³ (g/kg)		
ME (kcal/kg)	3103	3205
Crude protein (%)	22.8	20.05
Lysine (%)	1.60	1.23
Methionine + cysteine (%)	0.90	0.70
Arginine (%)	1.55	1.31
Calcium (%)	0.97	0.92
Available phosphorus (%)	0.5	0.46

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 $2000 \times g$ 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 $5\ \mu\text{m}$ 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 on 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):

$$\text{Surface area} = (2\pi) \times (\text{VW}/2) \times (\text{VL})$$

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 up-

ward 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. The total feed consumption (d 1-42) of OA birds was higher than those of *Eh 7.5* and *EhOA* but not significantly different from the control group.

On d 42, *Eh 7.5* and OA birds had a greater body weight gain than the *EhOA* and control groups. The total feed intake in *Eh 7.5* and *EhOA* treatments was significantly lower than those of OA but not significantly different from the control group. 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 compare 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.

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

Weight gain (g)	Control	<i>Eh</i> 7.5	OA	<i>Eh</i> OA
1-21 d	870±8.29	878±7.50	885±7.40	873±7.16
22-42 d	1407±26.18 ^b	1516±14.26 ^a	1520±17.66 ^a	1457±18.71 ^b
1-42 d	2276±28.46 ^c	2393±16.31 ^a	2402±18.24 ^a	2332±22.14 ^b
Feed intake (g/bird)				
1-21d	1183±5.85	1091±3.84	1134±5.28	1117±10.38
22-42 d	2876±84.07 ^b	2904±28.54 ^{ab}	3049±52.22 ^a	2835±54.66 ^{ab}
1-42 d	4059±86.84 ^{ab}	3986±40.18 ^b	4431±42.87 ^a	3952±72.04 ^b
FCR (feed/gain)				
1-21d	1.35±0.01 ^a	1.24±0.01 ^c	1.25±0.009 ^c	1.29±0.01 ^b
22-42 d	2.04±0.04 ^a	1.91±0.01 ^c	2.02±0.02 ^{ab}	1.96±0.01 ^{bc}
1-42 d	1.79±0.09 ^a	1.66±0.03 ^b	1.63±0.01 ^b	1.66±0.03 ^b
Mortality rate (%)				
1-42 d	4	3	2	3

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

*Eh*7.5: basal diet supplemented with 7.5 g/kg *E. hirta*; OA: basal diet supplemented with 1.5 g/kg acidifier; *Eh*OA: basal diet + 7.5 g/kg *E. hirta* and 1.5 g/kg acidifier; FCR: feed conversion ratio.

Table 3 Effect of *Euphorbia hirta* (*Eh* 7.5), acidifier (OA) and their combination (*Eh*OA) on intestinal morphology of broiler chickens on d 42

Treatments	Intestinal parameters					
	Villus length (μm)	Crypt depth (μm)	Villus/crypt ration	Villus surface area (μm ²)	Lamina propria thickness (μm)	Muscularis mucosa thickness (μm)
Control	866±14.40 ^b	185±8.97 ^a	4.89±0.24 ^c	331988±19717 ^b	230±8.84 ^c	12.87±0.74
<i>Eh</i> 7.5	972±26.20 ^a	170±6.16 ^a	5.86±0.26 ^b	515729±31174 ^a	334±13.35 ^a	15.72±0.69
OA	953±38.54 ^a	144±9.26 ^b	6.78±0.31 ^a	503402±36503 ^a	298±12.42 ^{ab}	13.10±0.92
<i>Eh</i> OA	1000±32.93 ^a	186±9.25 ^a	5.56±0.30 ^{bc}	644815±10320 ^a	275±18.04 ^b	15.94±0.64

The means within the same column 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.

Eh 7.5: basal diet supplemented with 7.5 g/kg *E. hirta*; OA: basal diet supplemented with 1.5 g/kg acidifier; *Eh*OA: basal diet + 7.5 g/kg *E. hirta* and 1.5 g/kg acidifier.

Table 4 Effect of *Euphorbia hirta* (*Eh* 7.5), acidifier (OA) and their combination (*Eh*OA) on clinical blood chemistry values of male broiler chickens

Treatments	Blood biochemical parameter					
	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	SEB ² (mmol/L)
21 th day of experiment						
Control	2.53±0.13	0.85±0.60	142.81±0.45	3.78±0.20	105.01±0.74	42.58±0.74
<i>Eh</i> 7.5	2.63±0.10	0.91±0.10	140.27±2.27	3.96±0.21	100.71±1.95	43.52±0.43
OA	2.60±0.12	0.81±0.05	141.35±1.21	4.15±0.31	102.40±1.16	43.10±0.47
<i>Eh</i> OA	2.68±0.08	0.68±0.10	143.25±0.74	4.48±0.13	103.05±1.57	42.68±0.45
42 th day of experiment						
Control	2.93±0.15	0.97±0.07	148.57±3.11	3.05±0.21	108.40±0.90	43.22±3.06
<i>Eh</i> 7.5	3.29±0.10	1.11±0.20	146.68±0.82	3.21±0.33	107.92±0.79	41.97±0.55
OA	3.32±0.17	1.00±0.08	147.03±0.69	3.20±0.29	109.17±0.69	42.06±0.89
<i>Eh</i> OA	3.30±0.17	0.75±0.06	144.35±1.19	3.58±0.16	107.81±1.03	40.42±0.70

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

Serum electrolyte balance calculated by [(Na mmol/L+K mmol/L) - Cl mmol/L].

*Eh*7.5: basal diet supplemented with 7.5 g/kg *E. hirta*; OA: basal diet supplemented with 1.5 g/kg acidifier; *Eh*OA: basal diet + 7.5 g/kg *E. hirta* and 1.5 g/kg acidifier.

Values are expressed as Mean ± SEM.

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, *Eh*OA 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 (Kircheggner *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).

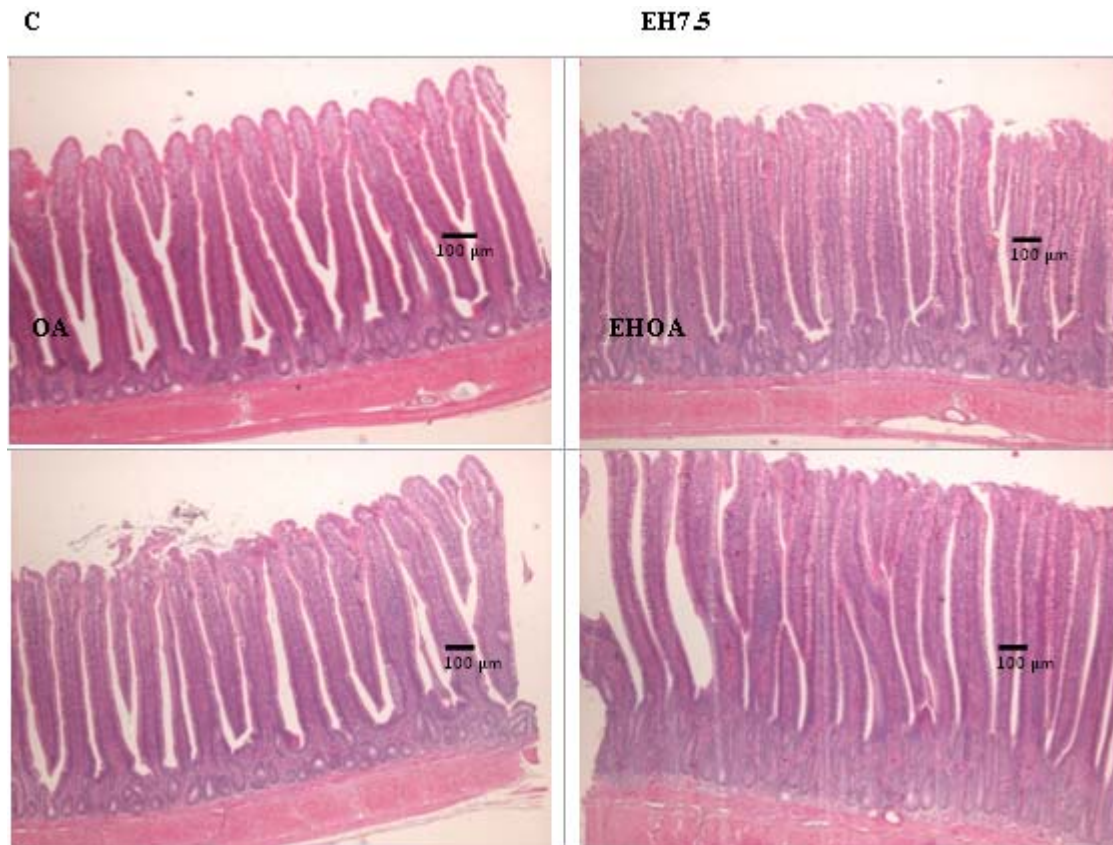


Figure 1 Effect of *Euphorbia hirta* (EH7.5), acidifier (OA) and their combination (EHOA) on intestinal histology of broiler chickens

A. Histological microscopy of the duodenum, shown with H and E staining and 40 × magnification.
B. Figure scale bar; 100μm

Furthermore, the mode of action for improved FCR in birds given *Eh* 7.5, *Eh*OA and OA supplementation could be closely associated to improve in gut health and intestinal morphology. Acidifiers and their salts added to poultry and monogastric 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, *E. coli*, *clostridia* and *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 indi-

rectly 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 *Lactobacilli* and *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 *Eh* 7.5, OA and *Eh*OA 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.

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