

# Intestinal Histomorphology Changes and Serum Biochemistry Responses of Broiler Chickens Fed Herbal Plant (Euphorbia hirta) and Mix of Acidifier Research Article S.R. Hashemi<sup>1</sup>, I. Zulkifli<sup>2</sup>, H. Davoodi<sup>3\*</sup>, M. Hair Bejo<sup>4</sup> and T.C. Loh<sup>2</sup> <sup>1</sup> Department of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran

<sup>2</sup> Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia
 <sup>3</sup> Department of Microbiology and Immunology, Golestan University of Medical Science, Gorgan, Iran

<sup>4</sup> Department of Veterinary Pathology and Microbiology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Received on: 23 Sep 2012 Revised on: 29 Nov 2012 Accepted on: 12 Feb 2013 Online Published on: Mar 2014

\*Correspondence E-mail: homdavoodi@yahoo.com © 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran Online version is available on: www.ijas.ir

# 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).

Phytogenic 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 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 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 antidepressant 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 growthpromoting 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	Table 1 Ingredients and nutrient composition of basal d
---	---

Ingredient <sup>1</sup> (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 <sup>2</sup>	0.9	0.9				
Calculated analyses <sup>3</sup> (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				

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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<sup>TM</sup>) consisted of formic, phosphoric, lactic, tartaric, citric and malic acids (Sunzen Corporation Sdn Bhd. Malaysia). The area of each pen measuring was 1.5 m<sup>2</sup>. Feed and water were provided *ad libitium* 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  $2000 \times \text{g}$  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 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):

Surface area=  $(2\Pi) \times (VW/2) \times (VL)$ 

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 *Eh*OA and the control groups. There were no significant differences in body weight gain between *Eh*OA and control groups. The total feed consumption (d 1-42) of OA birds was higher than those of *Eh* 7.5 and *Eh*OA 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 *Eh*OA and control groups. The total feed intake in *Eh* 7.5 and *Eh*OA 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 *Eh*OA 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, *Eh*OA 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.

Weight gain (g)	Control	Eh 7.5	OA	EhOA
1-21 d	870±8.29	878±7.50	885±7.40	873±7.16
22-42 d	$1407 \pm 26.18^{b}$	$1516 \pm 14.26^{a}$	1520±17.66 <sup>a</sup>	$1457 \pm 18.71^{b}$
1-42 d	2276±28.46°	2393±16.31 <sup>a</sup>	2402±18.24ª	2332±22.14 <sup>b</sup>
		Feed intake (g/bird)		
1-21d	1183±5.85	1091±3.84	1134±5.28	1117±10.38
22-42 d	$2876 \pm 84.07^{b}$	$2904 \pm 28.54^{ab}$	3049±52.22ª	2835±54.66 <sup>ab</sup>
1-42 d	$4059 \pm 86.84^{ab}$	$3986 \pm 40.18^{b}$	4431±42.87 <sup>a</sup>	3952±72.04 <sup>b</sup>
		FCR (feed/gain)		
1-21d	$1.35 \pm 0.01^{a}$	$1.24\pm0.01^{\circ}$	1.25±0.009°	1.29±0.01 <sup>b</sup>
22-42 d	$2.04\pm0.04^{a}$	$1.91\pm0.01^{\circ}$	$2.02{\pm}0.02^{ab}$	1.96±0.01 <sup>bc</sup>
1-42 d	$1.79{\pm}0.09^{a}$	$1.66 \pm 0.03^{b}$	$1.63 \pm 0.01^{b}$	$1.66 \pm 0.03^{b}$
		Mortality rate (%)	_	
1-42 d	4	3	2	3

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

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

EH7.5: 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.

	Intestinal parameters					
Treatments	Villus length (µm)	Crypt depth (µm)	Villus/crypt ration	Villus surface area (µm <sup>2</sup> )	Lamina propria thickness (µm)	Muscularis mucosa thickness (µm)
Control	$866 \pm 14.40^{b}$	$185{\pm}8.97^{a}$	4.89±0.24 <sup>c</sup>	331988±19717 <sup>b</sup>	230±8.84°	12.87±0.74
Eh 7.5	972±26.20 <sup>a</sup>	$170{\pm}6.16^{a}$	$5.86 \pm 0.26^{b}$	515729±31174 <sup>a</sup>	334±13.35 <sup>a</sup>	15.72±0.69
OA	953±38.54 <sup>a</sup>	144±9.26 <sup>b</sup>	6.78±0.31 <sup>a</sup>	503402±36503 <sup>a</sup>	298±12.42 <sup>ab</sup>	13.10±0.92
EhOA	1000±32.93ª	186±9.25 <sup>a</sup>	$5.56 \pm 0.30^{bc}$	644815±10320 <sup>a</sup>	275±18.04 <sup>b</sup>	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; EhOA: 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 of	combination (EhOA) on clinical blood chemistry values of male broiler chickens

<b>T</b> ( )	Blood biochemical parameter					
Treatments	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	SEB <sup>2</sup> (mmol/L)
			21 <sup>th</sup> day of exper	iment		
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
Eh 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	$0.81 \pm 0.05$	$141.35 \pm 1.21$	4.15±0.31	$102.40{\pm}1.16$	43.10±0.47
EhOA	$2.68 \pm 0.08$	0.68±0.10	143.25±0.74	4.48±0.13	$103.05 \pm 1.57$	42.68±0.45
42 <sup>th</sup> day of experiment						
Control	2.93±0.15	0.97±0.07	$148.57 \pm 3.11$	3.05±0.21	$108.40 \pm 0.90$	43.22±3.06
Eh 7.5	3.29±0.10	1.11±0.20	$146.68 \pm 0.82$	3.21±0.33	107.92±0.79	41.97±0.55
OA	3.32±0.17	1.00±0.08	$147.03 \pm 0.69$	3.20±0.29	109.17±0.69	42.06±0.89
EhOA	3.30±0.17	0.75±0.06	$144.35 \pm 1.19$	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].

EH7.5: 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 (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).

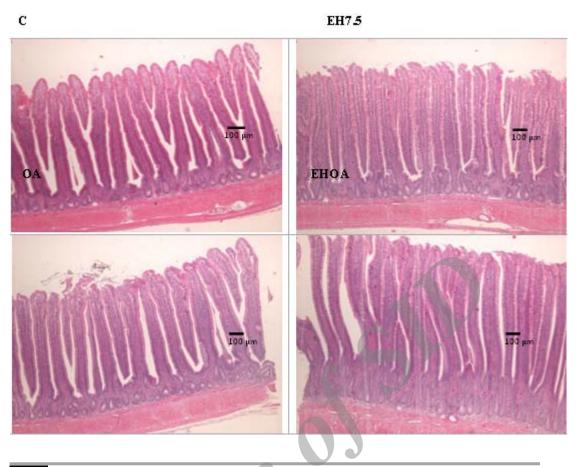


Figure 1 Effect of *Euphorbia hirta* (EH7.5), acidifier (OA) and their combination (EHOA) on intestinal histology of broiler chickens A. Histological microscopy of the duodenum, shown with H and E staining and 40 × magnification

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 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, *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 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 *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.

# REFERENCES

- Abdel Fattah S.A., El Sanhoury M.H., El Mednay N.M. and Abdel Azeem F. (2008). Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *Int. J. Poult. Sci.* 7, 215-222.
- Adibmoradi M., Navidshad B., Seifdavati J. and Royan M. (2006). Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. *Poult. Sci.* 43, 378-383.
- Ao T.A. Cantor H., Pescatore A.J., Ford M.J., Pierce J.L. and Dawson K.A. (2009). Effect of enzyme supplementation and

acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poult. Sci.* **88**, 111-117.

- Aptekmann K.P., Baraldi Arton S.M., Stefanini M.A. and Orsi M.A. (2001). Morphometric analysis of the intestine of domestic quails (*Coturnix coturnix japonica*) treated with different levels of dietary calcium. *Anat. Histol. Embryol.* **30**, 277-280.
- Awad W., Ghareeb K. and Böhm J. (2008). Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. *Int. J. Mol. Sci.* 9, 2205-2216.
- Bi Y.U. and Chiou P.W.S. (1996). Effects of crude fibre level in the diet on the intestinal morphology of growing rabbits. *Lab. Anim.* **30**, 143-148.
- Canibe N., Engberg R.M. and Jensen B.B. (2001). An Overview of the Effect of Organic Acids on Gut Flora and Gut Health. Danish Institute of Agricultural Science. Research Center Foulum, Denmark.
- Choct M. (2009). Managing gut health through nutrition. Br. Poult. Sci. 7, 9-15.
- Cowan M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12**, 564-582.
- Cook R.H and Bird F.H. (1973). Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. *Poult. Sci.* 52, 2276-2280.
- Draughon F.A. (2004). Use of botanicals as biopreservatives in foods. Food. Technol. 58, 20-28.
- Fulton R.M., Nersessian B.N. and Reed W.M. (2002). Prevention of *Salmonella enteritidis* infection in commercial ducklings by oral chicken egg derived antibody alone or in combination with probiotics. *Poult. Sci.* **81**, 34-40.
- Frankel W.L., Zhang W., Singh A., Klurfeld D.M., Don S., Sakata T. and Rombeau J.L. (1994). Mediation of the trophic effects of short chain fatty acids on the rat jejunum and colon. *Gastroenterology*. **106**, 385-390.
- Fukunaga T.M., Sasaki Y., Araki T., Okamoto T., Yasuoka T., Tsujikawa T., Fujiyama Y. and Bamba T. (2003). Effects of the soluble fiber pectin on intestinal cell proliferation, fecal short chain fatty acid production and microbial population. *Digestion.* 67, 42-49.
- Garcia V., Catala Gregori P., Hernandez F., Megias M.D. and Madrid J. (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology and meat yield of broilers. *J. Appl. Poult. Res.* **16**, 555-562.
- Griggs J.P. and Jacob J.P. (2005). Alternatives to antibiotics for organic poultry production. J. Appl. Poult. Res. 14, 750-756.
- Guo F.C., Williams B.A., Kwakkel R.P., Li H.S., Li X.P., Luo J.Y., Li W.K. and Verstegen M.W.A. (2004). Effects of mush-room and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poult. Sci.* 83, 175-182.
- Hashemi S.R., Zulkifli I., Hair Bejo M., Farida A. and Somchit M.N. (2008a). Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. *Int. J. Pharmacol.* 4, 352-360.
- Hashemi S.R., Zulkifli I., Zunita Z. and Somchit M.N. (2008b). The effect of selected sterilization methods on antibacterial ac-

tivity of aqueous extract of herbal plants. J. Biol. Sci. 8, 1072-1076.

- Hashemi S.R., Zulkifli I., Hair Bejo M., Karami M. and Soleimani A.F. (2009a). The effects of *Euphorbia hirta* and acidifier supplementation on growth performance and antioxidant activity in broiler chickens. Pp. 215-217 in Proc. 21<sup>st</sup> Vet. Assoc. Malaysia (VAM) Cong., Port Dickson, Malaysia.
- Hashemi S.R., Zulkifli I., Zunita Z., Hair Bejo M., Loh T.C., Somchit M.N., Kok P.C. and Davoodi H. (2009b). Effects of dietary supplementation with *Euphorbia hirta* and acidifier on performance and *Salmonella* colonization in broiler chickens. Pp. 69-70 in Proc. 30<sup>th</sup> Malaysia Soc. Anim. Prod. Annu. Conf., Kota Kinabalu, Malaysia.
- Hashemi S.R. (2010). Selected herbal plants as growth and health promoters in broiler chickens. Ph D Thesis. Univ., Putra Malaysia (UPM), Malaysia.
- Hashemi S.R and Davoodi H. (2010) Phytogenics as new class of feed additive in poultry industry. J. Anim. Vet. Adv. 9, 2955-2304.
- Hashemi S.R and Davoodi H. (2011) Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Vet. Res. Commun.* 35, 169-180.
- Hermes C., Azevedo J.F., Araujo E.J.A. and Santana D.M.G. (2008). Intestinal ascending colon morphometrics in rats submitted to severe protein malnutrition. *Int. J. Morphol.* 26, 5-11.
- Hughes R.J. (2003). Energy Metabolism of Chickens Physiological Limitations. A report for the Rural Industries Research and Development Corporation, RIRDC Publication.
- Jamroz D., Orda J., Kamel C., Wiliczkiewicz A., Wertelecki T. and Skorupinska J. (2003). The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics and gut microbial status in broiler chickens. J. Anim. Feed Sci. 12, 583-596.
- Kawalilak L.T., Ulmer Franco A.M. and Fasenko G.M. (2011). Impaired intestinal villi growth in broiler chicks with unhealed navels. *Poult. Sci.* 89, 82-87.
- Kirchgessner M., Roth F.X. and Steinruck U. (1991). Nutritive wirkung von fumarsäure bei anderung der proteinqualität und des proteingehaltes im futter auf die mastleistung von broilern. *Arch. Geflügelkd.* 55, 224-232.
- Kum S., Eren U., Onol A.G. and Sandikci M. (2010). Effects of dietary organic acid supplementation on the intestinal mucosa in broilers. *Rev. Med. Vet.* **10**, 463-468.
- Langhout P. (2000). New additives for broiler chickens. *World's Poult.* **16**, 22-27.
- Le Blay G., Blottiere H.M., Ferrier L., Le Foll E., Bonnet C., Galmiche J.P. and Cherbut C. (2000). Short-chain fatty acids induce cytoskeletal and extracellular protein modification associated with modulation of proliferation on primary culture of rat intestinal smooth muscle cells. *Dig. Dis. Sci.* **45**, 1623-1630.
- Loddi M.M., Maraes V.M.B., Nakaghi I.S.O., Tucci F., Hannas M.I. and Ariki J.A. (2004). Mannan oligosaccharide and organic acids on performance and intestinal morphometric characteristics of broiler chickens. Pp. 45 in Proc. 20<sup>th</sup> Annu. Symp. Comp. Geom., Brooklyn, NY.

- Luckstadt C., Nizamettin S., Hasan A. and Aylin A. (2004). Acidifier a modern alternative for antibiotic free feeding in livestock production, with special focus on broiler production. *Vet. Zootech.* 27, 91-95.
- Miles R.D. Janky D.M. and Harms R.H. (1984). Virginiamycin and broiler performance. *Poult. Sci.* 63, 1218-1221.
- Montagne L., Pluske J.R. and Hampson D.J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Technol.* **108**, 95-117.
- Nabuurs M.J., Hoogendoorn A., Van Der Molen E.J. and Van Osta A.L.M. (1993). Villus height and cryp depth in weaned pigs reared under various circumstances in the Netherland. *Res. Vet. Sci.* 55, 78-84.
- NRC. (1994). Nutrient Requirements of Poultry, 9<sup>th</sup> Rev. Ed. National Academy Press, Washington, DC.
- Ogbulie J.N., Ogueke C.C., Okoli I.C. and Anyanwu1 B.N. (2007). Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. *African J. Biotechnol.* **6**, 1544-1548.
- Owens B., Tucker L., Collins M.A. and Mc Cracken K.J. (2008). Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. *Br. Poult. Sci.* 49, 202-212.
- Papatsiros V.G., Christodoulopoulos G. and Filippopoulos L.C. (2012). The use of organic acids in monogastric animals (swine and rabbits). J. Cell Anim. Biol. 6, 154-159.
- Patte J.D. and Waldroup P.W. (1998). Use of organic acids in broiler diets. *Poult. Sci.* 67, 1178-1182.
- Paul S.K., Halder G., Mondal M.K. and Samanta G. (2007). Effect of organic acid salt on the performance and gut health of broiler chicken. *Poult. Sci.* 44, 389-395.
- Pelicano E.R.L., Souza P.A., Souza H.B.A., Figueiredo D.F., Boiagom M., Carvalho S.R. and Bordon V.F. (2005). Intestinal mucosa development in broiler chicken fed natural growth promoters. *Brazilian J. Poult. Sci.* 4, 221-229.
- Radecki S.V. and Yokoyama M.T. (1991) Intestinal bacteria and their influence on swine nutrition. Pp. 439-447 in Swine Nutrition. E.R. Miller, E.U. Duane and A.J. Lewis, Eds. Boston: Butter Worth Heinemann.
- Ratcliff J. (2000). Antibiotic bans A European perspective. Pp. 135-152 in Proc. 47<sup>th</sup> Maryland Nutr. Conf., Feed Manufac. Univ. Maryland, College Park.
- Roser U. (2006) Effects of organic acids in liquid and solid forms on the survival rate of *Salmonella* in pelleted compound feed after recontamination. *Immunology*. **82**, 12-19.
- Sakamoto K., Hirose H., Onizuka A., Hayashi M., Futamura N., Kawamura Y. and Ezaki T. (2000). Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. J. Surg. Res. 94, 99-106.
- SAS. (2005). SAS<sup>®</sup>/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC.
- Scheppach W., Bartram H.P. and Richter F. (1995). Role of shortchain fatty acids in the prevention of colorectal cancer. *European J. Cancer.* **31**, 1077-1080.
- Schneeman B.D. (1982). Pancreatic and digestive function. Pp. 73-83 in Dietary Fibre in Health and Disease. G.V. Vahuoung

and D. Kritchevsky, Eds. Plenum Press, New York.

Sharma N.K., Dey S. and Prasad R. (2007). In vitro antioxidant potential evaluation of Euphorbia hirta. Pharmacologyonline. 1, 91-98.

- Silva M.A., Pessotti B.M.S., Zanini S.F., Colnago G.L., Rodrigues M.R.A., Nunes L.C., Zanini M.S. and Martins I.V.F. (2009). Intestinal mucosa structure of broiler chickens infected experimentally with *Eimeria tenella* and treated with essential oil of oregano. *Cienc. Rural.* 5, 1471-1477.
- Skinner J.T., Izat A.L. and Waldroup P.W. (1991). Research note: fumaric acid enhances performance of broiler chickens. *Poult. Sci.***70**, 1444-1447.
- Skrivanova E. and Marounek M. (2007). Influence of pH on antimicrobial activity of organic acids against rabbit enteropathogenic strain of *Escherichia coli*. Folia Microb. 52, 70-72.
- Solis de los Santos F., Tellez G., Farnell M.B., Balog J.M., Anthony N.B., Pavlidis H.O. and Donoghue A.M. (2005). Hypobaric hypoxia in ascites resistant and susceptible broiler genetic lines influences gut morphology. *Poult. Sci.* 84, 1495-1498.
- Tappenden K.A. and McBurney M.I. (1998). Systemic short-chain fatty acids rapidly alter gastrointestinal structure, function and expression of early response genes. *Dig. Dis. Sci.* 43, 1526-1536.
- Truscott R.B. and Al-Sheikhly F. (1997). The production and treatment of necrotic enteritis in broilers. *Am. J. Vet. Res.* **38**, 857-861.
- Vieira S.L., Oyarzabal O.A., Freitas D.M., Berres J., Pena J.E.M., Torres C.A. and Coneglian J.L.B. (2008). Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic acids. J. Appl. Poult. Res. 17, 128-133.
- Vijaya K., Ananthan S. and Nalini R. (1995). Antibacterial effect of theaflavin, polyphenon 60 (*Camellia siensis*) and *Euphorbia hirta* on *Shigella spp*. A cell culture study. J. Ethnopharmacol. 49, 115-118.
- Vogt H., Matthes S. and Harnisch S. (1981). The effect of organic acids in the rations on the performance of broilers and laying hens. Arch. Geflugelkd. 45, 221-232.
- Vogt H., Matthes S., Harnisch S. and Ristic M. (1979). Fumaric acid in broiler rations. *Arch. Geflugelkd.* **43**, 54-60.
- Waldroup A., Kaniawati S. and Mauromoustakos A. (1995). Performance characteristics and microbiological aspects of broiler fed diets supplemented with organic acids. *J. Food Protec.* 58, 482-489.
- Williams L.A.D., Williams M.G., Sajabi A., Barton E.N. and Fleischhacker R. (1997). Angiotensin converting enzyme inhibiting and anti dipsogenic activities of *Euphorbia hirta* extracts. *Phyto. Res.* 11, 401-405.
- Windisch W., Schedle K., Plitzner C. and Kroismayr A. (2008). Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86, 140-148.
- Yakhkeshi S., Rahimi S. and Gharib Naseri K. (2011). The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. *J. Med. Plants.* **10**, 80-95.
- Yang Y., Iji P.A. and Choct M. (2009). Dietary modulation of gut

microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poult. Sci. J.* **65**, 97-114.