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Effect of Antibiotic Alternatives on Ileal Microflora and Intestinal Histomorphology of Broiler Chickens Fed Wheat Based Diet

Research Article

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ABSTRACT

An experiment was conducted to study the effects of enzyme and phytogenic product (thymol+carvacrol) on the ileal microbial population and jejuna and ileal histomorphology of broilers fed wheat based diet. The experiment was conducted as a 2 × 3 factorial arrangement with 2 levels of enzyme endofeed W (0 and 0.05%) and 3 levels of next enhance 150® (0, 100 and 200 mg/kg). Each of the 6 dietary treatments was fed to 5 replicate pens (12 birds/pen) from 0 to 24 d of age. Adding enzyme and phytogenic product into wheat based diet increased (P<0.05) *Lactobacillus* and decreased (P<0.05) *E. coli* population while it had no effect on *Bifidobacterium* when compared to the control group. For jejunal histomorphology at d 24, addition of enzyme into wheat based diet increased (P<0.05) villus height (VH), villus surface (VS), crypt depth (CD), mucosa layer thickness (MCL) and goblet cell (GC) although villus width (VW) decreased (P<0.05) when compared to the control group. Pytogenic product supplementation significantly increased VH, VS, villus height to crypt depth (VH:CD) and GC but it was not significant for CD, MCL and mascular layer (MSL). Results of jejunal measurement showed that enzyme supplementation increased (P<0.05) VH, CD, MCL and GC although decreased (P<0.05) VW in contrast with control group: enzyme increased ileal VH, VS and GC at 24 days of age, but VW, CD, MCL and MSL was not affected by that. Effect of thymol + carvacrol on ileal morphology at d 24 followed the similar pattern of enzyme addition. At d 42, ileal measurement showed that enzyme supplement significantly enhanced VH and CD and thymol + carvacrol increased VH and VS. Thus, the addition of enzyme and phytogenic product can modify microbial status of intestinal by increasing beneficial microbial population, decreasing pathogens and improve mucosa structure of jejunum and ileum by increasing villus height, villus surface area and goblet cell.

KEY WORDS broiler, carvacrol, enzyme, microflora, morphology, thymol.

INTRODUCTION

The use of wheat in poultry diets is limited, because its high content in non starch polysaccharides (NSP) results in increased intestinal viscosity, reduced litter quality and poor productive performance (Basmacioglu *et al*. 2010). Most of the adverse effects of wheat feeding have been attributed to the content of arabinoxylan (Annison, 1991). Negative effect of arabinoxylans have been found to be growth depression, reduced nutrient digestibility, physiological and morphological changes in the digestive system, increased digesta viscosity and modified intestinal microflora (Basmacioglu *et al.* 2010). The dietary NSPs increase gut populations of pathogenic bacteria which may influence goblet cell dynamics by releasing bioactive compounds or by indirect activation of immune system. Negative effects of NSP can be reduced or even eliminated by supplementation of feeds with xylanase and / or ß-glucanase enzyme

(Veldman and Vahl, 1994) and / or antibiotic alternatives (Basmacioglu *et al*. 2010). The use of NSP degrading enzymes in poultry diets is therefore a common practice. The addition of appropriate exogenous enzymes (xylanases and ß-glucanases) to broiler diets based on wheat or barley, or both, has been shown to reduce intestinal viscosity and improve nutrient digestibility and performance (Meng *et al.* 2005).

The bioefficacy of exogenous enzymes can be improved when enzymes are added in combination with antibiotic growth promoters (AGP), particularly to overcome the detrimental effects of NSP hydrolysis products derived from the increased bacterial activity in the small intestine (Parsaie *et al.* 2007). However, the use of AGP is banned in the European Union member countries, and for this reason, one possible alternative to AGP might be found in the use of certain herbs such as thyme and oregano. Essential oils are usually used for their antimicrobial effect (Lee *et al.* 2004).

The antimicrobial properties of essential oils have encouraged their use as a natural replacement for antibiotic growth promoters in animal feeds in addition to the positive effects of essential oils against the colonization and proliferation of pathogenic bacteria (Lee *et al.* 2004). The main constituents of the essential oils are terpenes, which are responsible for the bulk of the antimicrobial activity (Charai *et al.* 1996). The oils of *Thymus vulgaris L.* (thyme), *O. vulgare subsp. hirtum* (oregano) have *in vitro* antimicrobial properties (Dorman and Deans, 2000).

The aim of the present experiment was to study the influence of feeding phytogenic product (thymol+carvacrol) and enzyme supplementation both individually and in combination on the composition of intestinal microflora and intestinal histomorphology of broiler chicken fed wheat based diet.

MATERIALS AND METHODS

Dietary treatments

The experiment consisted of a 3 × 2 factorial arrangement of the treatments with 3 concentrations of next enhance 150® (0, 100 or 200 mg/kg of diet) and 2 concentrations of supplemental enzyme endofeed W (0 or 0.05% of diet). Next enhance 150® (Novus International, Inc., Missouri, USA) was a commercial product consists of 50% thymol and 50% carvacrol. Enzyme endofeed W produced from *Aspergillus niger* fermentation product contains the arabinoxylanase and ß-glucanase activity of 2250 and 700 units per gram, respectively as reported by the manufacturer with barley malt sprouts dehydrated as carrier and standardizer (endofeed W, GNC Bioferm Inc., Saskatoon, Saskatchewan, Canada). Thymol + carvacrol were added to 500 g of soybean meal, homogenized and then was blended with premix. Finally, the premix was added to the basal diet. The composition of the starter, grower and finisher basal diets are shown in Table 1 and were calculated to meet the nutrient requirements of Ross 308 (Aviagen, 2007). All experimental diets were free from antibiotics and were provided in mash form. Experimental diets were offered to the birds from d 1 of age. Birds had free access to feed and water.

Table 1 Ingredients and composition of the basal diets

Ingredient (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Wheat	57.50	59.95	61.47
Soybean meal	34.12	30.77	29.08
Soybean oil	4.00	5.60	6.14
Wheat bran	0.07	0.07	0.07
Limestone	1.45	1.25	1.20
Dicalcium phosphate	1.35	1.10	1.00
DL-met	0.19	0.15	0.11
L-lys HCL	0.33	0.02	0.08
L-thr	0.12	0.05	0.01
Salt	0.37	0.36	0.34
Vitamin-mineral permix[1]	0.50	0.50	0.50
Composition, calculated (%, unless otherwise noted)			
ME (kcal/kg)	2850	2970	3020
CP	22.14	20.75	20.00
Ca	1.00	0.85	0.80
Available phosphorus	0.47	0.42	0.40
Lys	1.35	1.17	1.03
Met	0.48	0.42	0.39
Met + cys	1.01	0.90	0.81
Thr	0.89	0.78	0.70

[1] Supplied the following per kg of diet: vitamin A (trans retinyl acetate): 9000 IU; vitamin D_3 (cholecalciferol): 2000 IU; vitamin E (all-ractocopherol acetate): 18 IU; vitamin K (bisulfate menadione complex): 2 mg; Riboflavin: 6.6 mg; Pantothenic acid (D-calcium pantothenate): 10 mg; Pyridoxine (pyridoxine·HCl): 3 mg; Folic acid: 1 mg; Thiamine (thiamine mononitrate): 1.8 mg; vitamin B12 (cyanocobalamin): 15 µg; D-biotin: 0.1 mg; Niacin: 30 mg; Choline (choline chloride): 500 mg; Ethoxyquin: 0.1 mg; Se (Na_2SeO_3): 0.2 mg; I (KI): 1 mg; Cu: 10 mg; Fe: 50 mg; Zn: 85 mg and Mn: 100 mg.

Birds and experimental facilities

A total of 360 male Ross 308 chicks, were used in this experiment. Chicks were randomly allocated to groups of 12 birds to each of 30 floor pens, with 5 pens per treatment. Each pen was equipped with a feeding trough and 4 nipple drinkers and the floor was covered with clean litter. The initial temperature of 32 ˚C was gradually reduced according to the age of the birds until reaching 20 ˚C at the end of the experiment at 42 days of age**.** The lighting program was 24 h from 1 to 7 d of age and 23 h from 8 to 42 d of age. Experimental procedures followed the protocols of animal care committee of the Ferdowsi University of Mashhad, Iran.

Data collection

At 21 and 42 days of age, two birds per replicate were randomly selected, euthanized by cervical dislocation, and then the mid part of jejunum and ileum were excised for histo-

morphometric analysis. Briefly, the small intestine was divided into three segments: the duodenum (from gizzard to pancreo-biliary ducts), the jejunum (from pancreo-biliary ducts to Meckel's diverticulum) and the ileum (from Meckel's diverticulum to ileo-caecal junction). Samples of jejunum and ileum (0.5 cm×0.5 cm segments) were obtained at its midpoint and immersed in a 10% buffered formalin solution for 72 h.

The sample were then excised and washed with physiological saline. The samples were treated in tissue processor apparatus and embedded in paraffin wax (Bancroft and Gamble, 2002). Transverse sections were cut (6 µm) using a rotary microtome (LEICA RM 2145), were placed on a glass slide and stained with hematoxylin and eosin, and analyzed under a light microscope to determine morphometric indices.

Morphological parameters were measured using the Image Pro Plus v 4.5 software package. The measured morphometric variables (Aptekmann *et al.* 2001) included: villus height (VH) was measured from the villus-crypt junction; villus width (VW) was measured at midvillus height; villus surface area (VS) was measured using the formula: (2π) (VW/2) (VH); crypt depth (CD) was measured from the villus-crypt junction until the end of gland; VH: CD ratio; mucosa layer thickness (MCL); muscular layer thickness (MSL) and number of goblet cells (GC). The mean from 10 villus per sample was used as the average value for further analysis.

These birds were also considered for assessing the ileal microbial population. The ileum was assigned from Meckel's diverticulum to ileo-caecal junction. The ileums were excised and contents were collected by gently fingers into tubes.

Digesta were pooled with a replicate, put on ice until they were transported to the laboratory for enumeration of microbial population. One gram of ileal contents homogenized in 9 mL sterile water. Each sample was serially diluted. Using these diluted subsamples, *Lactobacillus* was enumerated on De Man-Rogosa-Sharpe (MRS) agar and *E. coli* was counted on Mac Conkey (MC) agar after incubated at 37 ˚C in an anaerobic chamber for 48 h and in an aerobic chamber for 24 h, respectively (Guban *et al.* 2006). The population of *Bifidobacterium* in ileal samples was determined using the standard laboratory method (Ibrahim and Salameh, 2001). Briefly, ileal samples (10 g) were diluted with 90 mL sterilized 0.1% peptone water and homogenized using stomacher 400 lab system 4 (Seward, Norfolk, UK) for 2 min, and 100 mL of appropriate dilution was surface plated onto modified BIM 24 agar (Ibrahim and Salameh, 2001). The level was determined at the serial dilution of 10^{-5}. Plates were incubated at 37 ˚C for at least 3 days.

Statistical analysis

Statistical analysis of results was performed using the GLM procedure of the SAS software (SAS Institute, 2001). Treatment means were compared using Tukey's multiple range test. Data were analyzed considering the pen of birds as an experimental unit.

RESULTS AND DISCUSSION

Ileal microflora

The effects of phytogenic product and enzyme on ileal microflora of broiler fed wheat based diet are shown in Table 2. Adding enzyme and phytogenic product into wheat based diet increased (P<0.05) *Lactobacillus* and decreased (P<0.05) *E. coli* population while it had no effect on *Bifidobacterium* when compared to the control group. There was no interaction effect between enzyme and photogenic product in ileal microflora.

Table 2 Ileal microbial population (log CFU/g of digesta) in broilers fed wheat based diet and thymol + carvacrol with or without enzyme at 42 days of age

Treatments	*Lactobacillus*	*Bifidobacterium*	*E. coli*
Enzyme, %			
0	8.38[b]	8.11	6.72[a]
0.05	8.62[a]	8.06	6.17[b]
SEM	0.031	0.025	0.177
Thymol + carvacrol (NE), mg/kg			
0	8.15[b]	8.21	7.09[a]
100	8.45[a]	8.23	6.57[b]
200	8.51[a]	8.21	6.32[b]
SEM	0.032	0.033	0.194
P-value			
Enzyme	0.031	0.121	0.034
NE	0.013	0.245	0.021
Enzyme × NE	0.106	0.119	0.569

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means and NE: net energy.

Jejunal and ileal histomorphology

The effects of phytogenic product and enzyme on gut morphology of broiler fed wheat based diet are shown in Table 3-6. For jejunal histomorphology at d 24, addition of enzyme into wheat based diet increased (P<0.05) VH, VS, CD, MCL and GC although VW decreased (P<0.05) when compared to the control group. Pytogenic product supplementation significantly increased VH, VS, VH: CD and GC but it was not significant for CD, MCL and MSL.

Results of jejunal measurement showed that enzyme supplementation increased (P<0.05) VH, CD, MCL and GC although decreased (P<0.05) VW in contrast with control group. Ileal histomorphology at 24 days of age: enzyme increased ileal VH, VS and GC but VW, CD, MCL and MSL was not affected by that. Effect of pytogenic product on ileal morphology at d 24 followed the similar pattern of

Table 3 Effect of thymol + carvacrol supplementation in broiler fed wheat based diet with or without enzyme on jejunal histomorphology of broilers at 21 days of age

	Morphological parameters							
Main effect	VH (µm)	VW (µm)	VS (µm^2)	CD (µm)	VH:CD	MCL (µm)	MSL (µm)	GC
				Enzyme, %				
0	1082^b	143^b	485840^b	147^b	7.36	1364^b	4.76	233^b
0.05	1211^a	156^a	530007^a	169^a	7.16	1391^a	4.83	298^a
SEM	11.78	3.44	15067	1.61	0.150	11.32	5.42	3.65
				Thymol + carvacrol (NE), mg/kg				
0	1056^b	145	480797^c	143	7.38^b	1405	433	249^b
100	1234^a	152	588964^b	141	8.75^a	1421	441	318^a
200	1231^a	159	614589^a	152	8.10^{ab}	1419	430	322^a
SEM	15.64	3.32	18453	2.04	0.166	11.55	6.76	3.09
				P-value				
Enzyme	0.022	0.021	0.025	0.017	0.201	0.008	0.992	0.038
NE	0.001	0.462	0.033	0.265	0.048	0.124	0.081	0.041
Enzyme × NE	0.767	0.314	0.353	0.744	0.638	0.615	0.167	0.239

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
VH: villus height; VW: villus width; VS: villus surface area; CD: crypt depth; VH: CD: villus height to crypt depth ratio; MCL: mucosa layer; MSL: muscular layer and GC: number of goblet cells.
SEM: standard error of the means and NE: net energy.

Table 4 Effect of thymol + carvacrol supplementation in broiler fed wheat based diet with or without enzyme on jejunal histomorphology of broilers at 42 days of age

	Morphological parameters							
Main effect	VH (µm)	VW (µm)	VS (µm^2)	CD (µm)	VH:CD	MCL (µm)	MSL (µm)	GC
				Enzyme, %				
0	1320^b	247^a	1023766	231^b	5.34	1342^b	477	338^b
0.05	1398^a	233^b	1022805	254^a	5.50	1464^a	485	365^a
SEM	10.44	3.21	20212	1.44	0.143	12.53	4.82	3.05
				Thymol + carvacrol (NE), mg/kg				
0	1301^b	232	947752^b	216	6.02	1439^b	469	341^b
100	1354^a	229	973607^a	219	6.15	1466^a	472	369^a
200	1362^a	227	970806^a	221	6.16	1465^a	480	378^a
SEM	13.30	6.71	24755	1.77	0.156	17.86	4.70	4.48
				P-value				
Enzyme	0.015	0.010	0.341	0.001	0.541	0.037	0.732	0.005
NE	0.001	0.260	0.011	0.446	0.211	0.012	0.411	0.003
Enzyme × NE	0.169	0.537	0.421	0.204	0.207	0.452	0.119	0.456

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
VH: villus height; VW: villus width; VS: villus surface area; CD: crypt depth; VH: CD: villus height to crypt depth ratio; MCL: mucosa layer; MSL: muscular layer and GC: number of goblet cells.
SEM: standard error of the means and NE: net energy.

enzyme addition. At d 42, ileal measurement showed that enzyme supplement significantly enhanced VH and CD and pytogenic product increased VH and VS. Wheat is an important ingredient in broiler diets because of its high starch and protein content, and is often the only cereal in grower and finisher diets but it contains considerably higher levels of anti-nutritional factors consisting mainly of water soluble non-starch polysaccharides compared to maize such as arabinoxylans. Arabinoxylans after ingestion become soluble, resulting in increased viscosity. The viscos nature of intestinal digesta seems to be responsible for negative effect exhibited by wheat pentosans (Jaroni *et al.* 1999). Enzyme can improve performance of broilers by alleviating the viscosity of digesta. NSP-degrading enzymes are hypothesized to work in 2 steps, described as an ileal phase and a cecal phase (Bedford, 2000).

During the ileal phase, enzymes remove fermentable substrates. During the cecal phase, degradation products of sugars, such as xylose and xylo-oligomers, are fermented by cecal bacteria, thus stimulating the production of VFA and the growth of specific beneficial bacteria (Bedford, 2000). The presence of viscous polysaccharides increases the microbial activity in the small intestine associated with poor broiler growth performance (Langhout *et al.* 1999). Exogenic enzymes such as xylanases are currently added to commercial wheat-based compound feed for broilers in order to improve growth and feed conversion ratio. The degradation of arabinoxylans, the major NSP fraction in wheat, results in a reduction of intestinal viscosity and consequently improves performance of broiler. In the present study, wheat diet without enzyme had negative effect on ileal microbial numerous and adding enzyme to the wheat

Table 5 Effect of thymol + carvacrol supplementation in broiler fed wheat based diet with or without enzyme on ileal histomorphology of broilers at 21 days of age

Morphological parameters								
Main effect	VH (µm)	VW (µm)	VS (µm^2)	CD (µm)	VH:CD^2	MCL (µm)	MSL (µm)	GC
Enzyme, %								
0	967^b	136	412948^b	113^b	8.56	1102	376	243^b
0.05	999^a	139	436024^a	121^a	8.26	1107	387	265^a
SEM	10.23	4.021	12653	1.433	0.423	5.563	7.143	5.235
Thymol + carvacrol (NE), mg/kg								
0	870^b	139	379720^b	103	8.44	1121	356	221^b
100	952^a	141	421488^a	109	8.73	1132	357	258^a
200	985^a	140	433006^a	114	8.64	1164	359	265^a
SEM	13.54	5.351	16352	3.453	0.732	9.431	7.762	7.344
P-value								
Enzyme	0.043	0.110	0.004	0.024	0.265	0.657	0.768	0.015
NE	0.032	0.235	0.021	0.436	0.172	0.435	0.426	0.003
Enzyme × NE	0.376	0.352	0.533	0.455	0.635	0.287	0.134	0.135

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
VH: villus height; VW: villus width; VS: villus surface area; CD: crypt depth; VH: CD: villus height to crypt depth ratio; MCL: mucosa layer; MSL: muscular layer and GC: number of goblet cells.
SEM: standard error of the means and NE: net energy.

Table 6 Effect of thymol + carvacrol supplementation in broiler fed wheat based diet with or without enzyme on ileal histomorphology of broilers at 42 days of age

Morphological parameters								
Main effect	VH (µm)	VW (µm)	VS (µm^2)	CD (µm)	VH:CD	MCL (µm)	MSL (µm)	GC
Enzyme, %								
0	1173^b	197	725594	159^b	7.38	1147	502	263
0.05	1213^a	190	723676	185^a	6.56	1153	513	268
SEM	12.01	4.25	17911	1.40	0.106	11.74	5.80	5.44
Thymol + carvacrol (NE), mg/kg								
0	1156^b	170	617073^c	176	6.57	1169	494	258
100	1195^a	169	634139^a	187	6.28	1187	514	252
200	1201^a	167	629780^b	178	6.80	1198	519	264
SEM	20.45	6.447	21936	2.763	0.130	16.17	7.10	6.67
P-value								
Enzyme	0.027	0.047	0.085	0.019	0.301	0.736	0.722	0.822
NE	0.036	0.437	0.033	0.365	0.123	0.635	0.832	0.651
Enzyme × NE	0.095	0.324	0.936	0.463	0.273	0.533	0.242	0.397

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
VH: villus height; VW: villus width; VS: villus surface area; CD: crypt depth; VH: CD: villus height to crypt depth ratio; MCL: mucosa layer; MSL: muscular layer and GC: number of goblet cells.
SEM: standard error of the means and NE: net energy.

based diet alleviated the negative effect of wheat in terms of decreasing *E.coli* population. It has been noted that the efficacy of xylanase was dependent on the amount of wheat used in diet (Steenfeldt *et al.* 1998), amount of NSPs in wheat (Sharifi *et al.* 2003), viscosity of wheat (Adeola and Bedford, 2004) variety of wheat or source of wheat (Crouch *et al.* 1997), concentrations of xylanase inhibitors in wheat, type of lipid used in diet (Preston *et al.* 2001) and age of bird In this study, enzyme and phytogenic product supplementation decreased *E. coli* and increased *lactobacillus* population in ileum. Several studies have reported effects on intestinal microflora when herbs and essential oils have been included in broiler diets. Similar to our results, dietary supplementation of XTRACT, an encapsulated product containing capsaicin, carvacrol and cinnamaldehyde, reduced the numbers of *E. coli* and *C. perfringens* in broiler rectal contents (Jamroz *et al.* 2003). *Clostridium perfringens* has been reduced in number when the blended essential oil supplement, was added in poultry diets (Losa and Kohler, 2001). Tucker (2002) reported that growth of *E. coli* and *C. perfringens* reduced in broilers, when blends of essential oils were fed in industry trials, while numbers of *Lactobacillus* spp. increased. Thus, essential oils may act differently compared with synthetic antimicrobials, which tend to depress bacterial numbers across species. A recent study involving live birds showed that blends of the primary components of the essential oils could be used to control *Clostridium perfringens*, the bacterium that causes necrotic enteritis in broilers (Mitsch *et al.* 2004). Ground thyme has been shown to inhibit the growth of *S. typhi-*

murium (Aktug and Karapinar, 1986) and *E. coli* (Marino *et al*. 1999) when added to media. Cinnamon extract inhibits *Helicobacter pylori* at the concentration range of common antibiotics, its antimicrobial properties are mainly related to its cinnamaldehyde content, followed by eugenol and carvacrol contents (Taback *et al.* 1999). Cinnamon oil and it constituents (cinnamaldehyde and eugenol) have antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Enterococus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Salmonella sp.* and *Parahemolyticus* (Chang *et al.* 2001).

Also they have inhibitory properties against *Aspergillus flavus* (Montes Belmont and Carvajal, 1998). The exact anti-microbial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property (Cornner, 1993) and chemical structure (Farag *et al*. 1989) can play a role. It was suggested that terpenoids and phenylpropanoids can penetrate the membranes of the bacteria and reach the inner part of the cell because of their lipophilicity (Helander *et al.* 1998). Moreover, structural properties, such as the presence of the functional groups (Farag *et al.* 1989) and aromaticity (Bowles and Miller, 1993) are also responsible for the antibacterial activity of essential oils.

Jejunal and ileal histomorphology

The supplementation of diet with enzyme led to an increase in VH, VS and CD, as well as a decrease in VW in jejunum and ileum at 24 days of age ($P<0.05$), This result is similar to result reported by Jaroni *et al.* (1999). They showed shorter and thicker villi in birds fed with wheat. Non-sturch polysaccharides in wheat based diets caused to increase viscosity of intestine digesta which stimulate anaerobic microflora growth. Microorganisms migrate to small intestine where most nutrient absorption takes place (Campbell and Bedford, 1992), high bacterial concentration can irritate the gut lining and caused to thickening and atrophy of villis (Visek, 1978). Enzyme supplementation can reduce microbial population (Choct *et al.* 1995) and atrophy of villis (Brenes *et al.* 1993). There is substantial evidence that dietary enzyme modifies the morphology and structure of the intestinal mucous as defined by Mazhari *et al.* (2011). Enterocytes undergo a continual cycle of proliferation in the intestinal crypt, cell maturation and migration up the villi with desquamation at the tip of the villi. The depth of the crypts is correlated to cell replacement rate (Savage *et al.* 1997). Accelerated replacement of enterocytes requires energy and proteins, which can deprive growth and the development of other tissues and organ systems. Thus, the increased crypt depth will be correlated to a significant increase in absorption of nutrients. Longer villi increases the absorptive surface of intestines, while smaller crypts indicate lower tissue turnover as well as lower demand for tissue development (Markovic *et al.* 2009). The increased crypt surfaces may also be due to a higher number of goblet cells particularly concentrated in the crypt, which can result in increased mucus secretion (Langhout *et al.* 1999).

In general, measurements of villus height and crypt depth give an indication of the likely maturity and functional capacity of enterocytes (Hampson, 1976). A higher ratio indicates shallower crypts, in relation to villus height or longer villi in relation to the crypt. Longer villi would partly result in increased surface area for digestion and absorption as shown in this study.

CONCLUSION

Thus, the addition of enzyme and phytogenic product can modify microbial status of intestinal by increasing beneficial microbial population, decreasing pathogens and improve mucosa structure of jejunum and ileum by increasing villus height, villus surface area and goblet cell.

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