Iranian Journal of Applied Animal Science

Effect of Feed Additives on Intestinal Histomorphology of Broilers Fed Wheat-Based Diet

Research Article

V. Khaksar[1*], A. Golian[1] and A. Raji[2]

[1] Excellence Centre for Animal Science and Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Department of Basic Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Received on: 12 Sep 2012
Revised on: 25 Dec 2012
Accepted on: 31 Dec 2012
Online Published on: Dec 2013

*Correspondence E-mail: khaksar_80@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

A 2 × 3 factorial experiment was conducted to investigate the effect of enzyme Endofeed W (EEW) at 0 and 0.05%, thyme essential oil (TEO), probiotic Primalac® (PP) and non-feed additive, on intestinal histomorphology of broilers fed wheat-based diet. Each of the 6 dietary treatments was replicated 4 times with 12 chicks each from 0 to 42 days of age. There was no interaction between enzyme and growth promoters on any measured parameters. Data showed that supplementation of wheat-based diet with EEW enhanced ($P<0.05$) intestinal histomorphology including jejunal villus height (VH), villus width (VW), villus surface area (VS), crypt depth (CD), mucosa layer thickness (MCL), muscular layer thickness (MSL) and number of goblet cells (GC) and ileal VH, MCL and GC at 21 and 42 days of age, as compared to broilers fed control diet. The TEO in diet did ameliorate ($P<0.05$) some jejunal and ileal morphological parameters, but it was less effective as compared to EEW or PP supplementation. The PP improved ($P<0.05$) the intestinal histomorphology in the present study in all measured parameters except the jejunal and ileal VH:CD. It was concluded that dietary EEW and growth promoters (TEO or PP) supplementation improved ($P<0.05$) gut health in broilers fed wheat-based diet, but the effect of probiotic Primalac® was more pronounced as compared to natural thyme essential oil.

KEY WORDS essential oil, exogenous enzyme, intestinal histomorphology, probiotic, wheat.

INTRODUCTION

Compared to maize, wheat contains considerably higher levels of anti-nutritional factors consisting mainly of water soluble nonstarch polysaccharides (NSP). The NSP fraction increases digesta viscosity and protects lipids, starch, and protein, thereby decreasing nutrient digestibility, modifying intestinal microflora in the digestive system and reducing physiological and morphological changes (Basmacioglu *et al.* 2010). The use of enzymes or antibiotic growth promoters as dietary supplements, may positively affect poultry health and productivity fed diets containing grains such as wheat, rye, barley and oats (Basmacioglu *et al.* 2010).

Exogenous enzymes have been used extensively to remove anti-nutritional factors from feeds, to increase the digestibility of existing nutrients, and to supplement the activity of the endogenous enzymes of poultry (Wang *et al.* 2005).

The routine use of antibiotics in poultry feed is now less common, as new antibiotic-resistant strains of pathogens emerge. Thus, other growth promoters that have no side effects in poultry meat or human health are used as alternatives to antibiotics in poultry feed. Thyme essential oil could be used as a growth promoter due to its mode of action, including: improving feed flavor and intake, stimulating the secretion of digestive enzymes, increasing gastric and intestinal motility, gut development and antimicrobial activity (Jamroz *et al.* 2006; Tipu *et al.* 2006). Carvacrol, an active component of thyme essential oil, has been noted to have a positive effect on intestinal morphology and secretion of mucin in the intestine (Jamroz *et al.* 2006). Probiotics are sources of live (viable) naturally occurring microorganisms that improve microbial balance in the animal gastrointestinal tract and, therefore, are beneficial (Isolauri *et al.* 2001). Mode of action of probiotics include inhibition of pathogen growth in the gastrointestinal tract, increase in digestive enzyme activity (Rolf, 2000) and gut development. With regard to this last action, Gunal *et al.* (2006) reported that the increments of villus height (VH), crypt depth (CD) and VH:CD in jejunum and ileum of probiotic-fed broilers, were greater compared to the control groups.

Therefore, the aim of this study was to determine the effect of enzyme Endofeed W, thyme essential oil and probiotic Primalac® on intestinal histomorphology of broilers fed wheat-based diet.

MATERIALS AND METHODS

Birds, diets and management

A total of two hundred eighty, eight day-old Ross-308 male broiler chicks with a similar weight and wing-banded, were randomly distributed to 24 pens. The six dietary treatments were arranged factorially with two levels of enzyme Endofeed W (0.0 and 0.05%) and two types of growth promoters (unsupplemented or supplemented with thyme essential oil or probiotic Primalac®). Each diet was fed to four replicates of twelve birds each. All starter, grower and finisher diets were formulated (Table 1) to meet the nutrient requirements according to Ross-308 rearing guideline (Aviagen, 2007).

Enzyme Endofeed W produced from Aspergillus niger fermentation product, contains the arabinoxylanase and β-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). Stability tests under dry warehouse conditions show no changes in enzyme activity during a 36 month period. Enzyme activity is guaranteed for a storage period of 24 months. Zataria, an important genus of the family Lamiaceae (previously called Labiatae), is widely available in Iran, Afghanistan and Pakistan, and from Zataria multiflora (thyme), an aromatic member of genus Zataria, an essential oil is extracted and used in this study. The thyme essential oil was first dissolved in the vegetable oil component of the ration and homogenized by mixer and then the mixture was blended with wheat bran. Wheat bran with essential oil was added to pre-mixture, and finally, the pre-mixture was gently mixed with the basal diet. This experimental diet was prepared weekly and stored in airtight containers. Thyme essential oil was added at the rate of 0.1% of diet.

Table 1 Composition of starter, grower, and finisher wheat-based diets

Ingredients, %	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Wheat	57.49	59.94	61.47
Soybean meal, 44% CP	34.05	30.70	29.00
Wheat bran	0.15	0.15	0.15
Vegetable oil	4.00	5.60	6.14
Limestone	1.45	1.25	1.20
Dicalcium phosphate	1.35	1.10	1.00
Salt (NaCl)	0.37	0.36	0.34
DL-methionine	0.19	0.15	0.11
L-lysine	0.33	0.20	0.08
Threonine	0.12	0.05	0.01
Vitamin mix[1]	0.25	0.25	0.25
Mineral mix[2]	0.25	0.25	0.25
Calculated nutrient contents			
ME, kcal/kg	2850	2970	3020
Crude Protein, %	22.14	20.74	19.82
Calcium, %	1.00	0.85	0.80
Available Phosphorus, %	0.47	0.42	0.40
Lysine, %	1.35	1.17	1.03
Methionine, %	0.48	0.42	0.39
Threonine, %	0.89	0.78	0.70

[1] Vitamin mix provided the following per kilogram of diet: vitamin A (trans-retinyl acetate): 10000 IU; vitamin D_3 (cholecalciferol): 3500 IU; vitamin E (DL-α-tocopheryl acetate): 60 mg; vitamin K (menadione): 3 mg; Thiamine: 3 mg; Riboflavin: 6 mg; Pyridoxine: 5 mg; vitamin B_{12} (cyanocobalamin): 0.01 mg; Niacin: 45 mg; Pantothenic acid (D-calcium pantothenate): 11 mg; Folic acid: 1 mg; Biotin: 0.15 mg; Choline chloride: 500 mg and Ethoxyquin (antioxidant): 150 mg.

[2] Mineral mix provided the following per kilogram of diet: Fe: 60 mg; Mn: 100 mg; Zn: 60 mg; Cu: 10 mg; I: 1 mg; Co: 0.2 mg and Se: 0.15 mg.

Primalac® is a commercial microbial culture including a minimum presence of 1.0×10^8 CFU of friendly bacteria Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium thermophilus and Enterococcus faecium per gram (Primalac®, Star Labs, St. Joseph, Clarksdale, MO). Primalac® has been shown to withstand extreme temperature and humidity and remains stable up to a year after its container is opened. Its unique formulation ensures that a large portion of what is ingested actually gets into the bowel or gastrointestinal tract and is not destroyed in the crop or stomach. Therefore, its desired effect is achieved. The probiotic Primalac® was added to the starter (0-10 d), grower (11-24 d) and finisher (25-42 d) diets at the rate of 0.09%, 0.045% and 0.0225%, respectively. Continuous lighting was provided during the first wk of age and afterwards lighting schedule of 23 h L:1 h D was used. The initial temperature of 33 ˚C was gradually reduced according to the age of birds until reached 21 ˚C at day 28 and kept constant thereafter. Feed and water were offered ad libitum.

Extraction of thyme essential oil and GC/MS analysis

Fresh plants were collected at the flowering stage and processed immediately after harvest. Essential oil was distilled from the ground plant material using Clevenger distillation apparatus (Herbal Exir Co., Mashhad, Iran). The samples were distilled for two hours and the oils obtained, dried with anhydrous sodium sulphate, and stored in dark sealed glass vials at +4 ˚C until required. The main active compounds of the thyme essential oil were determined by GC/MS and contained thymol and its isomer, carvacrol at the rate of 21.9 and 31.9% of oil, respectively. The concentrations of two predominant components of thyme essential oil, thymol and carvacrol have been reported to range from as low as 3% to as high as 60% of total essential oil (Lawrence and Reynolds, 1984).

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 histomorphometric 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. Then they were excised and washed by 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), 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 (Sakamoto *et al.* 2000; Aptekmann *et al.* 2001) included: villus height (VH) measured from the villus-crypt junction; villus width (VW) measured at midvillus height; villus surface area (VS) measured using the formula: $(2\pi) \times (VW/2) \times (VH)$; crypt depth (CD) 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. Experimental procedures followed the principles of the Animal Care Committee of the Ferdowsi University of Mashhad.

Statistical analysis

All data were checked for normality before analysis. The data were subjected to ANOVA using SAS (SAS, 2000). Differences between treatment means were evaluated by Tukey's multiple range tests. A value of P<0.05 was considered significant.

RESULTS AND DISCUSSION

The effects of feeding wheat-based diet containing enzyme Endofeed W (EEW) or growth promoters (thyme essential oil (TEO) or probiotic Primalac® (PP) on jejunal and ileal histomorphology of broilers at 21 and 42 days of age are shown in Tables 2-5. There was no interaction between enzyme and growth promoters on any measured parameters. Supplementation of wheat-based diet with EEW enhanced (P<0.05) intestinal histomorphology including jejunal villus height (VH), villus width (VW), villus surface area (VS), crypt depth (CD), mucosa layer thickness (MCL), muscular layer thickness (MSL) and number of goblet cells (GC) and ileal VH, MCL and GC at 21 and 42 days of age, as compared to those in broilers fed control diet. TEO ameliorated (P<0.05) some jejunal and ileal morphological parameters, but it did not perform as comprehensive as EEW or PP.

The intestinal histomorphology in broilers has been associated with intestinal functions and birds growth. However, little information is available on how enzymes or phytogenic compounds may affect gastrointestinal histomorphology and functionality. Similar observations were reported by Jamroz *et al.* (2006), who observed qualitative increase in the number of GC and in mucin secretion at the surface of the jejunum villi when feeding broilers a mixture of 5, 3, and 2 mg/kg of carvacrol, cinnamaldehyde, and capsicum oleoresin, respectively.

Birds fed a blend of essential oils from oregano, anise, and citrus peel had longer ileal VH (P<0.05) and more GC, than birds fed unsupplemented (Reisinger *et al.* 2011). The number of GC per villus increases as the villi grows (Tucker and Taylor-Pickard, 2004), and GC secretes throughout the GI tract that forms an adherent gel on the mucosal surface and may play an important role in epithelial cell repair (Ikeda *et al.* 2002; Sklan, 2004). However, hydroalchoholic plant extracts from sage, thyme, and rosemary leaves did not exert any influence on intestinal VH, VS and CD (Garcia *et al.* 2007).

It has been observed that probiotics affect gastrointestinal tract histology (Awad *et al.* 2006) and the regulation of mucus synthesis and secretion (Deplancke and Gaskins, 2001).

Probiotics may also enhance the integrity of the tight junctions between the intestinal epithelial cells during infections or inflammatory conditions (Montalto *et al.* 2004). As expected, PP improved (P<0.05) the intestinal histomorphology in the present study in all measured parameters except the jejunal and ileal VH:CD.

The villi play a crucial role in the digestion and absorption processes of the small intestine, as is the first to make contact with nutrients in the lumen (Gartner and Hiatt, 2001).

Table 2 Effect of feeding wheat-based diet containing enzyme or growth promoters[1] on jejunal[2] histomorphology of broilers at 21 day of age

Main effects	Morphological parameters							
	VH[3] (µm)	VW[3] (µm)	VS[3] (µm²)	CD[3] (µm)	VH:CD[3]	MCL[3] (µm)	MSL[3] (µm)	GC[3]
Enzyme Endofeed W (EEW), %								
0	953[b]	128[b]	383,029[b]	111[b]	8.59	1064[b]	311[b]	230[b]
0.05	1201[a]	172[a]	648,636[a]	149[a]	8.06	1350[a]	428[a]	269[a]
SEM	46.50	6.41	24,717	5.22	0.16	51.20	21.17	15.31
Growth promoters (GP)								
Unsupplemented	944[b]	132[b]	391,269[b]	110[b]	8.58[ab]	1054[b]	320[b]	222[b]
TEO[1]	1117[ab]	141[b]	494,540[ab]	118[b]	9.47[a]	1235[ab]	379[ab]	271[a]
PP[1]	1276[a]	184[a]	737,221[a]	161[a]	7.92[b]	1437[a]	452[a]	282[a]
SEM	51.50	7.11	24,312	5.29	0.18	61.20	22.22	15.30
	P-value							
EEW	0.019	0.009	0.021	0.022	0.390	0.022	0.008	0.041
GP	0.009	0.012	0.034	0.008	0.041	0.001	0.037	0.010
EEW × GP	0.876	0.350	0.422	0.521	0.390	0.442	0.121	0.311

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] TEO: thyme essential oil and PP: probiotic PrimaLac®.
[2] 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).
[3] 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.

Table 3 Effect of feeding wheat-based diet containing enzyme or growth promoters[1] on jejunal[2] histomorphology of broilers at 42 day of age

Main effects	Morphological parameters							
	VH[3] (µm)	VW[3] (µm)	VS[3] (µm²)	CD[3] (µm)	VH:CD[3]	MCL[3] (µm)	MSL[3] (µm)	GC[3]
Enzyme endofeed W (EEW), %								
0	1144[b]	193[b]	693,286.9[b]	157[b]	7.29	1301[b]	382[b]	310[b]
0.05	1359[a]	222[a]	947,331.7[a]	191[a]	7.12	1550[a]	507[a]	358[a]
SEM	67.41	6.53	24,800	5.60	0.21	62.94	20.01	17.82
Growth promoters (GP)								
Unsupplemented	1187[b]	201[b]	749,163.2[b]	131[b]	9.06[a]	1318[b]	359[b]	298[b]
TEO[1]	1399[a]	212[b]	931,286.3[a]	142[b]	9.85[a]	1541[a]	433[ab]	351[a]
PP[1]	1418[a]	244[a]	1,086,415[a]	177[a]	8.01[b]	1595[a]	491[a]	370[a]
SEM	67.20	6.97	24,899	5.99	0.24	64.50	21.08	16.99
	P-value							
EEW	0.023	0.011	0.019	0.014	0.721	0.017	0.024	0.030
GP	0.044	0.039	0.015	0.022	0.030	0.020	0.022	0.041
EEW × GP	0.365	0.311	0.289	0.318	0.450	0.711	0.392	0.279

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] TEO: thyme essential oil and PP: probiotic PrimaLac®.
[2] 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).
[3] 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.

Longer villi increase the absorptive surface of intestine, while smaller crypt indicates a decrease of enterocyte replacement and tissue turnover, and lower demand for tissue development as well. It can be stated that, increments in VH and CD are directly correlated with enhanced epithelial cell turnover (Fan *et al.* 1997).

Possibly, in the present study the increments reported with Primalac® treatment are associated with increased intestinal nutrient absorption and accelerated enterocyte turnover rates. The crypts of the villus contain several specialized cells such as absorptive cells, GC, and regenerative cells that are responsible for the production of mucus and the replacement of old cells.

As it happened in the present investigation, the increased crypt depth may also be due to a higher number of GC particularly concentrated in the crypt, which can result in increased mucus secretion (Langhout *et al.* 1999).

In line with this study, several researches provided data suggesting that Primalac® increases metabolic efficiency via changes in intestinal physiology and metabolism. Feeding Primalac® increased (P<0.05) intestinal VH, CD, MSL and number of GC compared to birds fed control diet (Chichlowski *et al.* 2007). Rahimi *et al.* (2009) reported that there was a significant increase in GC count, area, mean size and villi density due to feeding of Primalac®.

Table 4 Effect of feeding wheat-based diet containing enzyme or growth promoters[1] on ileal[2] histomorphology of broilers at 21 day of age

Main effects	Morphological parameters							
	VH[3] (μm)	VW[3](μm)	VS[3] (μm[2])	CD[3] (μm)	VH:CD[3]	MCL[3] (μm)	MSL[3] (μm)	GC[3]
Enzyme Endofeed W (EEW), %								
0	704[b]	101	223,266.6	94	7.49[b]	798[b]	347	181[b]
0.05	911[a]	111	317,519.9	107	8.51[a]	1018[a]	380	210[a]
SEM	48.32	5.45	23,393	4.13	0.17	44.13	22.60	16.11
Growth promoters (GP)								
Unsupplemented	712[b]	107[b]	239,217.8[b]	100[b]	7.12[b]	812[b]	341[b]	205[b]
TEO[1]	951[a]	115[b]	343,406.1[ab]	103[b]	9.23[a]	1054[a]	402[ab]	249[a]
PP[1]	947[a]	147[a]	437,116.3[a]	134[a]	7.07[b]	1081[a]	457[a]	258[a]
SEM	53.52	6.74	24,402	5.07	0.21	53.71	21.38	17.12
	P-value							
EEW	0.034	0.482	0.466	0.343	0.017	0.039	0.421	0.019
GP	0.012	0.020	0.027	0.013	0.024	0.011	0.031	0.021
EEW × GP	0.217	0.419	0.617	0.712	0.387	0.528	0.444	0.421

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] TEO: thyme essential oil and PP: probiotic PrimaLac®.
[2] 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).
[3] 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.

Table 5 Effect of feeding wheat-based diet containing enzyme or growth promoters[1] on ileal[2] histomorphology of broilers at 42 day of age

Main effects	Morphological parameters							
	VH[3] (μm)	VW[3] (μm)	VS[3] (μm[2])	CD[3] (μm)	VH:CD[3]	MCL[3] (μm)	MSL[3] (μm)	GC[3]
Enzyme Endofeed W (EEW), %								
0	963[b]	144	435,430.1	128	7.52	1091[b]	400	207[b]
0.05	1185[a]	159	591,623.1	143	8.29	1328[a]	441	250[a]
SEM	54.63	6.46	24,259	4.87	0.17	50.91	23.00	15.14
Growth promoters (GP)								
Unsupplemented	971[b]	138[b]	420,753.7[b]	130[b]	7.47[b]	1101[b]	382[b]	247[b]
TEO[1]	1164[a]	140[b]	511,694.4[ab]	135[b]	8.62[a]	1299[a]	417[b]	302[a]
PP[1]	1182[a]	170[a]	630,951.6[a]	172[a]	6.87[b]	1354[a]	510[a]	313[a]
SEM	55.28	7.17	24,603	5.52	0.22	59.44	22.50	16.55
	P-value							
EEW	0.031	0.612	0.317	0.427	0.622	0.009	0.790	0.033
GP	0.030	0.017	0.033	0.040	0.040	0.032	0.011	0.008
EEW × GP	0.411	0.521	0.512	0.616	0.518	0.567	0.620	0.619

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
[1] TEO: thyme essential oil and PP: probiotic PrimaLac®.
[2] 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).
[3] 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.

The supplementation with Primalac® led to (P<0.05) an increase in VH and VW, but a decrease in CD in all parts of the intestine (Markovicv *et al.* 2009).

Gunal *et al.* (2006) reported that increments of VH, CD, and VH:CD in jejunum and ileum of probiotic-fed broilers were greater compared to birds control group.

Awad *et al.* (2008) showed that the addition of symbiotic (prebiotic and probiotic) increased the VH and VH:CD in ileum compared to control. However, the ileal CD was decreased by synbiotic supplementation compared to control birds.

CONCLUSION

In conclusion, the present data suggest that one mechanism for the improvement of growth of broilers fed wheat-based diet supplemented with EEW and growth promoters (TEO or PP), can be seen jn the increase in health and functionality of the intestine. In this aspect, Primalac® is more efficient as a growth promoter than natural thyme essential oil. Supplementation of wheat-based diet with enzyme Endofeed W enhances (P<0.05) intestinal histomorphology including jejunal VH, VW, VS, CD, MCL, MSL and GC and ileal VH, MCL and GC, at 21 and 42 days. Natural thyme essential oil enhanced (P<0.05) some jejunal and ileal morphological parameters, but it did not perform as comprehensive as EEW or PP.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Ferdowsi University of Mashhad for financial support of this project (No. 494P9-12-88) and the staff at the Animal Science Department for technical assistance.

REFERENCES

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. *Anatom. Histol. Embryol.* **30,** 277-280.

Aviagen W. (2007). Ross 308: Broiler Nutrition Specification. Aviagen Inc., Huntsville, AL.

Awad W.A., Bohm J., Razzazi-Fazeli E., Ghareeb K. and Zentek J. (2006). Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. *Poult. Sci*. **85,** 974-979.

Awad W.A., 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.

Bancroft J.D. and Gamble M.N. (2002). Theory and Practice of Histological Techniques. Pp. 175- 181 in 5th Ed. Churchill Livingstone, London, Edinburgh, New York, Philadelphia, St Louis, Sydney and Toronto.

Basmacioglu H., Baysal S., Misirlioglu Z., Polat M., Yilmaz H. and Turan N. (2010). Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat-soybean meal diets. *Br. Poult. Sci.* **51,** 67-80.

Chichlowski M., Croom W.J., Edens F.W., McBride B.W., Qiu R., Chiang C.C., Daniel L.R., Havenstein G.B. and Koci M.D. (2007). Microarchitecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct-fed microbial, Primalac®, and salinomycin. *Poult. Sci.* **86,** 1121-1132.

Deplancke B. and Gaskins H.R. (2001). Microbial modulation of innate defence: goblet and the intestinal mucus layer. *Am. J. Clin. Nutr*. **72,** 1131-1141.

Fan Y., Croom J., Christensen V., Black B., Bird A., Daniel L., McBride B. and Eisen E. (1997). Jejunal glucose uptake and oxygen consumption in turkey poults selected for rapid growth. *Poult. Sci.* **76,** 1738-1745.

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.

Gartner L.P. and Hiatt J.L. (2001). Color Textbook of Histology. 2nd Ed. W.B. Saunders, Baltimore, MD.

Gunal M., Yayli G., Kaye O., Karahan N. and Sulak O. (2006). The effect of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *Int. J. Poult. Sci.* **5,** 149-155.

Ikeda H., Yang C.L., Tong J., Nishimaki H., Masuda K., Takeo T., Kasai K. and Itoh G. (2002). Rat small intestinal goblet cell kinetics in the process of restitution of surface epithelium subjected to ischemia-reperfusion injury. *Dig. Dis. Sci*. **47,** 590-600.

Isolauri E., Sutas Y., Kankaanpaa P., Arvilommi H. and Salminen S. (2001). Probiotics: effects on immunity. *Am. J. Clin. Nutr*. **73,** 444-450.

Jamroz D., Wertelecki T., Houszka M. and Kamel C. (2006). Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *J. Anim. Physiol. Anim. Nutr*. **90,** 255-268.

Langhout D.J., Schutte J.B., Van Leeuwen P., Wiebenga J. and Tamminga S. (1999). Effect of dietary high and low methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *Br. Poult. Sci*. **40,** 340-347.

Lawrence B.M. and Reynolds R.J. (1984). Progress in essential oils. *Perfu. Flavor*. **9,** 23-31.

Markovicv R., Sefer D., Krsticv M. and Petrujkic B. (2009). Effect of different growth promoters on broiler performance and gut morphology. *Arch. Med. Vet.* **41,** 163-169.

Montalto M., Maggiano N., Ricci R., Gasbarrini A. and Gasbarrini G. (2004). Lactobacillus acidophilus protects tight junctions from aspirin damage in HT-29 cells. *Digestion*. **69,** 225-228.

Rahimi S., Grimes J.L., Fletcher O., Oviedo E. and Sheldon B.W. (2009). Effect of a direct-fed microbial (Primalac®) on structure and ultrastructure of small intestine in turkey poults. *Poult. Sci.* **88,** 491-503.

Reisinger N., Steiner T., Nitsch S., Schatzmayr G. and Applegate T.J. (2011). Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. *J. Appl. Poult. Res.* **20,** 272-283.

Rolf R.E. (2000). The role of probiotic cultures in the control of gastrointestinal health. *J. Nutr*. **130,** 396-402.

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. Surgical. Res.* **94,** 99-106.

SAS Institute. (2000). SAS®/STAT Software, Release 8.2. SAS Institute, Inc., Cary, NC.

Sklan D. (2004). Early gut development. Pp. 9-32 in The Interaction between Feed, Gut Health and Hmmunity. Interfacing Immunity, Gut Health and Performance. L.A. Tucker and J.A. Taylor-Pickard Eds. Nottingham University Press. UK.

Tipu M.A., Akhtar M.S., Anjum M.I. and Raja M.L. (2006). New dimension of medicinal plants as animal feed. *Pakistan Vet. J.*

26, 144-148.

Tucker L.A. and Taylor-Pickard J.A. (2004). Interfacing Immunity, Gut Health and Performance. 1st Ed. Nottingham Univ. Press, UK.

Wang Z.R., Qiao S.Y., Lu W.Q. and Li D.F. (2005). Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poult. Sci.* **84,** 875-881.