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Supplementation of β-Mannanase in Diet with Energy Adjustmenton Affect Performance, Intestinal Morphology and Tight Junction Proteins mRNA Expression in Broiler Chickens

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Abstract: The objective of this study was to assess the effects of commercial β-mannanase enzyme supplementation in low-energy corn-soybean meal diets on performance, intestinal morphology and mRNA expression of intestinal tight junction proteins in 1-21 days broiler chickens. The 1800, 1 day old broiler chicks were divided into 5 treatment groups, 6 pens in each treatment. The study was performed in a randomised complete block design. The 5 treatments were Positive diet Group (PG) with basal energy level, Negative diet Group (NG) with lower energy of 502.08 kJ kg^{-1} and other groups supplemented with 150, 300 and 450 mg kg^{-1} β-mannanase based on NG. Decrease of energy in NG resulted in loss of the Average Daily Gain (ADG) ($p<0.05$) and Feed Conversion Ratio (FCR) ($p<0.05$). Based on the NG, supplementation with β-mannanase in diet significantly improved the Average Daily Gain (ADG) ($p<0.05$) and Feed Conversion Ratio (FCR) ($p<0.05$) of birds. ADG of groups with enzyme and lower energy reached to the level of PG. Supplementation with 300 g ton^{-1} β-mannanase increased the villus height of the small intestine ($p<0.05$) and 450 g ton^{-1} β-mannanase significantly decreased the crypt depth ($p<0.01$). The addition of β-mannanase at 150, 300 and 450 g ton^{-1} to the diets increased the ratio of crypt depth to villus height of the duodenum and jejunum in birds ($p<0.01$). The ratio of crypt depth to villus height of the ileum was also increased with 300 and 450 g ton^{-1} β-mannanase supplementation compared to the low-energy diet group ($p<0.01$). Chicks fed diet with the β-mannanase showed an increased mRNA expression of ZO-1 in the duodenum compared with the low energy diet ($p<0.05$). Supplementation with 300 and 450 g ton^{-1} β-mannanase to the diets enhanced the mRNA expression of Occludin and ZO-1 in the jejunum ($p<0.05$) and 300 g ton^{-1} β-mannanase increased enhanced the mRNA expression of Occludin and ZO-1 in the ileum ($p<0.05$). Thus, the addition of β-mannanase to low energy diets improved the performance, gut morphology and mRNA expression of intestinal tight junction proteins.

Key words: β-mannanase, performance, intestinal morphology, intestinal tight junction protein, broiler

INTRODUCTION

In China, the corn-soybean meal diet is the typical and common feed source of broiler diets. Several factors influence the nutrient bioavailability of corn-soybean meal diets such as diet design, individual animal and anti-nutritional factors. However, among these factors, the most important are anti-nutritional factors. Mannan is a strong anti-nutritive factor for monogastric animals (Mehri *et al*., 2010). The β-mannan content of the soybean meal is approximately 1.3-1.6% (Jackson *et al*., 1999). It occurs in the form of glucomannans and galactomannans in plant cell walls. Daskiran *et al*. (2004) and Ray *et al*. (1982) reported that β-mannan significantly reduced growth and increased the feed:Gain ratio in broilers. In addition, Patel and McGinnis (1985) demonstrated that β-mannan significantly decreased egg production, egg weight and feed intake in laying hens. In addition, several studies have shown that β-mannan reduced insulin secretion (Sambrook and Rainbird, 1985) and glucose absorption in swine (Rainbird *et al*., 1984). The addition of the enzyme in feed can also reverse the negative effects. Several studies have demonstrated that enzyme supplementation of poultry rations improved the efficiency of converting feedstuffs into broiler tissue (Annison and Choct, 1991; Campbell and Bedford, 1992; Bedford and Morgan, 1996; Marquardt *et al*., 1996; Choct, 2001). Hemicell is a fermentation product of *Bacillus lentus*; its active ingredient is β-mannanase which can degrade β-mannan in feed (Zou *et al*., 2006). The addition of β-mannanase in feed can improve soybean meal utilisation and diminish negative effects

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due to the enzymatic degradation of β-mannan (Li *et al*., 2010). Broiler experiments have shown that supplementation with β-mannanase in corn-soybean meal diets can improve the growth performance and feed efficiency by approximately 3% (McNaughton *et al*., 1998). Moreover, Pettey *et al*. (1999) and Odetallah *et al*. (2002) reported that β-mannanase also improved the feed efficiency of weaning pigs and turkeys, respectively.

However, only a few studies have investigated the effect of β-mannanase on the intestinal tight junction proteins of broilers fed with a corn-soybean meal diet. Thus, the objectives of the experiments were to evaluate the effects of β-mannanase on growth performance, intestinal relative weight and length as well as the intestinal morphology and intestinal tight junction proteins of broilers.

MATERIALS AND METHODS

Animals and group formation: In this experiment, 1800, 1 day old male yellow-feathered broiler chicks were randomly assigned to 5 dietary treatments groups with 6 replicate pens of 60 chicks each, consisting of a control diet formulated to be normal in energy and four low-energy diets supplemented with 0, 150, 300 and 450 g ton^{-1} β-mannanase. The basal diet was based on corn and soybean meal (Table 1) which were formulated to meet the specification of the Nutrient Requirements of Poultry for broiler chickens. In addition, the low-energy diets were formulated to be 502.08 kJ kg^{-1} lower in ME than the basal diet for diets that were more sensitive to enzymatic effects. The β-mannanase (Hemicell) was provided by ChemGen Co., Ltd. (Shanghai, China) and the enzymatic activity was >160 MU kg^{-1}. The temperature was maintained at 35±1°C for up to 7 days of age and then gradually decreased to 25±1°C by 21 days of age. Feed and water were provided *ad libitum* and artificial lighting was continuous during the experiment.

All birds were vaccinated according to the company's management guide. The care and management of the broiler chicks followed the recommended guidelines. The experiment was approved by South China Agricultural University (Guangzhou, China).

Performance variables: Broiler chicken performance was assessed through the Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR). The feed conversion ratio was calculated based on the G:F for each replicate-pen. For each treatment group of animals, 6 replications (pens) with 60 animals were analyzed (n = 6). Data were collected during days 1 and 21, before and after breeding experiment.

Table 1: Diets composition and nutrient levels (air dry basis, %)

	Starter (0-3 weeks)	
Ingredients	Normal-energy diet	Low-energy diet
Corn	54.04	57.25
Soybean meal (43% CP)	38.40	37.80
Mixed oil	3.26	0.64
Dicalcium phosphate	1.92	1.90
Limestone	0.90	0.91
Methionine (98%)	0.25	0.25
Salt	0.24	0.24
Lysine (70%)	0.15	0.17
Sodium bicarbonate	0.12	0.12
Choline chloride (50%)	0.10	0.10
Threonine	0.02	0.02
Premix*	0.60	0.60
Total	100.00	100.00
Nutrient levels**		
ME (MJ kg^{-1})	12.13	11.63
Crude protein	21.00	21.01
Crude fat	5.47	3.01
Crude fiber	28.60	28.80
Calcium	0.90	0.90
Total phosphorus	0.71	0.71
Non-phytate P	0.45	0.45
Lysine	1.27	1.27
DLys-P	1.15	1.15
D (M+C)-P	0.83	0.83
DThr-P	0.71	0.71
DTrp-P	0.22	0.22
DArg-P	1.28	1.27
Sodium	0.15	0.15
Electrolyte balance (mEq kg^{-1})	248.00	247.00

*Added per kg of diet; DL-Methionine:1200 mg; retinylpalmitate, 5.5 mg; cholecalciferol: 0.05 mg; DL-α-tocopheryl acetate: 40 mg; menadione: 3 mg; thiamin: 3 mg; riboflavin: 4.5 mg; pyridoxine: 7 mg; cyanocobalamin: 0.03 mg; nicotinic acid: 50 mg; Ca-pantothenate: 8 mg; folic acid: 1.5 mg; choline chloride: 600 mg; Mn: 80 mg as $MnSO_4.H_2O$; Fe: 80 mg as $FeSO_4.H_2O$; Zn: 50 mg as $ZnSO_4.H_2O$; Cu: 10 mg as $CuSO_4.5H_2O$; Co: 0.4 mg as $CoSO_4$; iodine: 0.35 mg as KI; Se: 0.25 mg as $NaSeO_3$

Measurement for intestinal relative indexes: At the end of the experiment (21 days of age), two birds from each pen was randomly selected (closest to the mean pen body weight), weighed and sacrificed by cervical dislocation. The digestive tract was carefully removed accordingly: Duodenum (from the gizzard to the pancreas and bile duct was referred to as the duodenum), jejunum (from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to ileocaecal junction). After removing the intestinal contents, the duodenum, jejunum and ileum were weighed empty. And then their relative lengths and weights were measured and divided by Body Weight (BW).

Small intestinal morphology: Ring fragments of approximately three centimetres in length were removed from the duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after Meckel's diverticulum) for intestinal morphological measurements. The samples were processed as previously described (Viveros *et al*., 2011). The intestinal samples

were flushed twice with ice-cold saline to remove the intestinal contents and fixed in 10% formalin. Next, the samples were dehydrated in 70% ethanol for 24 h and embedded in paraffin. Tissue sections (5 μm) were obtained and stained with haematoxylin-eosin and a combination of the Periodic Acid-Schiff Method (PAS staining). The specimens were then examined using light microscopy (BA310 motic, Xiamen, China) and the images were analysed using the JD-801 morphological image analysis system. The variables (villus height, crypt depth, ratio of villus height: Crypt depth) were measured. Villus height (μm) was measured from the tip of the villus to the villus crypt junction and the crypt depth (μm) was defined as the depth of the invagination between adjacent villi.

Different mRNA expression of occludin and ZO-1: The duodenum, jejunum and ileum samples were dissected longitudinally to expose the mucosa and washed two times in ice-cold saline to remove the digesta. Next, 1.5 cm of the centrifugal pipe was sheared, quickly frozen in liquid nitrogen and stored at -80°C until RNA extraction. Total RNA was extracted from the small intestine (100 mg) using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The yield and quality of the RNA were measured using the Nanodrop ND-2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and the OD260: OD280 ratios were from 1.80-2.00. Approximately, 2 μg RNA was reverse-transcribed into cDNA in a reaction using oligo (dT) 18 primers (Sangon, Shanghai, China), M-MLV Reverse Transcriptase (Promega, Madison, USA), M-MLV buffer and RNase inhibitor (TaKaRa, Shiga, Japan).

To determine the mRNA abundance of the genes, quantitative real-time RT-PCRs was performed using the Stratagene MxPro 3005 P apparatus (Agilent Technologies, Santa Clara, CA, USA) and SYBR® Green Real-time PCR Master Mix (TOYOBO, Tokyo, Japan). In this study, β-actin was used as the reference gene. The relative mRNA expression was calculated using the $2^{-\Delta C}$T Method (ΔCt = ΔCt of the target gene -ΔCt of the house keeping gene) as previously described (Livak and Schmittgen, 2001).

The gene-specific primers for Zonula Occluden protein-1 (ZO-1), Occludin and β-actin were the following: Forward 5'-GCG CCT CCC TAT GAG GAG CA-3', reverse 5'-CAA ATC GGG GTT GTG CCG GA-3' for ZO-1 (160 bp); forward 5'-TCG TGC TGT GCA TCG CCA TC-3', reverse 5'- CGC TGG TTC ACC CCT CCG TA-3' for occludin (178 bp); Forward 5'-CCC CAG CCA TGT ATG TAG CC-3', reverse 5'-TCTG TCA GGA TCT TCA TGA GG TAG-3' for β-actin (154 bp).

Statistical analysis: Data were subjected to one-way ANOVA using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, 2010) and presented as mean±SME. The difference among the means was separated using Duncan's multiple range tests. Statistical significance was established at a probability of $p<0.05$.

RESULTS

Growth performance: The effect of β-mannanase in low energy corn-soybean meal diets on the growth performance of broilers is presented in Table 2. The ADG of broilers fed with low-energy diets was significantly lower ($p<0.05$) compared to the normal-energy group. The ADG of the broilers fed with 150, 300 and 450 g ton^{-1} β-mannanase were higher ($p<0.05$) than the low-energy group and were similar to the normal-energy group which improved by 6.04, 6.11 and 8.13%, respectively. However, significant differences were not found among the groups with the addition of β-mannanase ($p>0.05$). However, the ADFI of broilers fed with 450 g ton^{-1} β-mannanase was higher ($p<0.05$) than the low-energy group and there was no significant difference among the various groups ($p>0.05$). The FCR of the broilers fed with the low-energy diets was poorer ($p<0.001$) than the normal-energy group. Supplementation with 150, 300 and 450 g ton^{-1} β-mannanase significantly improved ($p<0.001$) the FCR by 4.62, 4.62 and 5.13% compared with the low-energy group, respectively. However, there was no significant difference among the animals treated with β-mannanase ($p>0.05$).

Intestinal relative weight and length: The effects of β-mannanase on the relative weight and length in different sections of the small intestine of broilers are summarised in Table 3. The relative weight of the duodenum, jejunum and small intestine of broilers were not significantly different among the various groups ($p>0.05$). Moreover,

Table 2: The growth performance of broilers (n = 6)

			Low-energy+β-mannanase (g ton^{-1})				
Items (0-3 weeks)	Normal-energy group	Low-energy group	150	300	450	SEM	p-values
ADFI (g)	28.02^{b}	28.05^{b}	28.43^{ab}	28.35^{ab}	28.85^{a}	0.21	0.680
ADG (g)	15.07^{a}	14.40^{b}	15.27^{a}	15.28^{a}	15.57^{a}	0.15	0.001
FCR	1.86^{b}	1.95^{a}	1.86^{b}	1.86^{b}	1.85^{b}	0.01	<0.001

^{a,b}Superscripts for means belong to one-way ANOVA analyses and means within a row with no common superscript differ significantly ($p<0.05$)

Table 3: The relative small intestine indexes of broilers (n = 12)

Items (0-3 weeks)	Normal-energy group	Low-energy group	Low-energy + β-mannanase (g ton^{-1}) 150	300	450	SEM	p-values
Relative weights (g kg^{-1} body weight)							
Duodenum	11.40	12.04	10.90	11.92	12.15	0.61	0.589
Jejunum	17.31	17.44	16.83	18.40	17.12	0.81	0.726
Ileum	13.11^{b}	14.28^{ab}	15.93^{a}	15.06^{a}	15.88^{a}	0.56	0.005
Small intestine	42.57	43.32	43.59	45.77	45.15	1.60	0.615
Relative lengths (cm kg^{-1} body weight)							
Duodenum	59.04^{ab}	61.04^{a}	54.80^{b}	59.92^{a}	57.29^{ab}	1.55	0.061
Jejunum	122.05	124.85	123.60	126.48	126.48	3.67	0.915
Ileum	124.43^{b}	133.26^{ab}	137.24^{a}	135.30^{a}	134.70^{ab}	3.44	0.125
Small intestine	303.84	314.24	316.39	320.62	319.86	6.30	0.404

Table 4: The small intestinal morphology of broilers (n = 12)

Items (0-3 weeks)	Normal-energy group	Low-energy group	Low-energy + β-mannanase (g ton^{-1}) 150	300	450	SEM	p-values
Duodenum							
Villus height (μm)	1114.33^{ab}	1072.39^{b}	1209.55^{ab}	1229.08^{a}	1064.75^{b}	43.16	0.046
Crypt depth (μm)	190.75^{a}	186.35^{a}	155.34^{b}	145.00^{b}	127.97^{c}	5.65	<0.001
Ratio	5.85^{b}	5.83^{b}	7.63^{a}	8.49^{a}	8.15^{a}	0.35	<0.001
Jejunum							
Villus height	1073.20^{a}	870.78^{b}	1063.23^{ab}	1084.53^{a}	894.02^{ab}	66.12	0.09
Crypt depth	136.04^{b}	151.17^{a}	126.99^{b}	132.12^{b}	107.33^{c}	3.96	<0.001
Ratio	7.91^{a}	5.74^{b}	8.35^{a}	8.32^{a}	8.31^{a}	0.51	0.008
Ileum							
Villus height	617.90^{b}	610.27^{b}	744.81^{ab}	819.05^{a}	676.50^{b}	43.02	0.023
Crypt depth	84.90^{bc}	92.12^{ab}	100.05^{a}	84.06^{bc}	81.60^{c}	3.08	0.004
Ratio	7.30^{bc}	6.61^{c}	7.45^{bc}	9.74^{a}	8.31^{b}	0.44	0.002

$^{a-c}$Superscripts for means belong to one-way ANOVA analyses and means within a row with no common superscript differ significantly ($p<0.05$)

there were no significant differences in the relative length of the jejunum and small intestine of broilersamong various groups ($p>0.05$). The addition of β-mannanase had increased the relative weight and length of the ileum; however there was no significant difference among the low-energy diet groups. Interestingly, relative to the low energy diet group, the addition of 150 g ton^{-1} β-mannanase reduced ($p<0.05$) the relative length of the duodenum.

Intestinal morphology: The morphological and histological measurements of the small intestinal of broilers are shown in Table 4. The villus height for 300 g ton^{-1} β-mannanase-treated broilers were greater ($p<0.05$) than the low-energy group in the duodenum, jejunum and ileum and met or exceeded that of the normal-energy group. Compared with the low-energy group, 450 g ton^{-1} β-mannanase supplementation significantly reduced the duodenum, jejunum and ileum crypt depth ($p<0.01$); in addition, the crypt depth of the duodenum and jejunum were reduced ($p<0.05$) with 150 and 300 g ton^{-1} β-mannanase supplementation. However there was no significant difference between the two groups ($p>0.05$). Supplementation with 150, 300 or 450 g ton^{-1} β-mannanase increased ($p<0.01$) the ratio of crypt depth to villus height in the duodenum by 30.87, 45.63 and 39.79% and in the jejunum by 45.47, 44.95 and 44.77% compared with the low-energy group, respectively. However, there were no significant differences among the β-mannanase supplementation groups ($p>0.05$). The ratio of crypt depth to villus height in the ileum was increased ($p<0.01$) by 300 and 450 g ton^{-1} β-mannanase supplementation compared with the low-energy diet group. Moreover, there was a significant difference between the 300 and 450 g ton^{-1} β-mannanase-treated groups ($p<0.05$).

mRNA expression of Occludin and ZO-1: The effect of β-mannanase in the diet of broilers on the mRNA levels for Occludin and ZO-1 are presented in Fig. 1 and 2. Supplementation with β-mannanase significantly enhanced the mRNA levels of ZO-1 in the duodenum ($p<0.05$) relative to the low-energy diet group. Furthermore, there were no significant differences among the animals treated with different β-mannanase concentrations ($p>0.05$). However, a significant difference was not found in the Occludin mRNA levels of the duodenum when treated with β-mannanase. Supplementation of 300 and 450 g ton^{-1} β-mannanase to the diets significantly enhancedthe mRNA levels of Occludin and ZO-1 in the jejunum compared with the low-energy diet group ($p<0.05$). However there was a significant difference between 300 and 450 g ton^{-1} β-mannanase on the mRNA levels of occludin in the

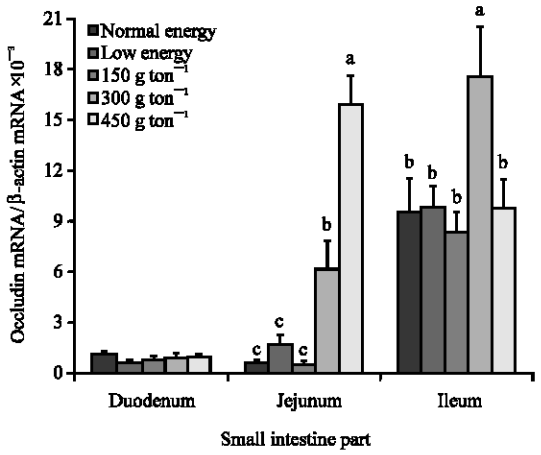

Fig. 1: Occludin mRNA expression in small intestine of broilers (n = 12); [a-c] Superscripts for means belong to one-way ANOVA analyses and means within a row with no common superscript differ significantly ($p<0.05$)

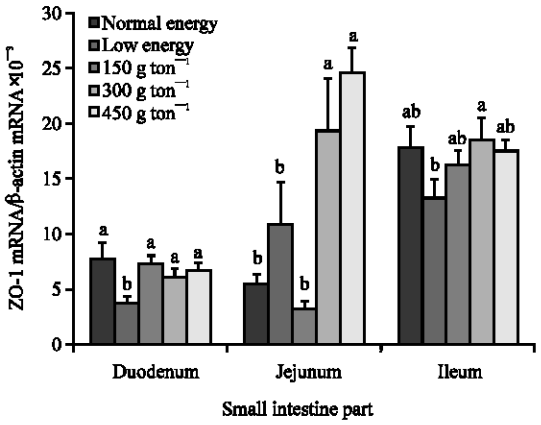

Fig. 2: ZO-1 mRNA expression in small intestine of broilers (n = 12); [a, b]Superscripts for means belong to one-way ANOVA analyses and means within a row with no common superscript differ significantly ($p<0.05$)

jejunum ($p<0.05$). The Occludin and ZO-1 mRNA levels in the ileum of broilers fed with the 300 g ton^{-1} β-mannanase diet were significantly higher than the low-energy diet group ($p<0.05$).

DISCUSSION

In this study, the results suggested that supplementation of β-mannanase at different levels improved the ADG, ADFI and FCR of broilers compared with the low-energy diet group. Numerous studies have demonstrated that the addition of β-mannanase in diets improved the ADG and FCR of broilers fed with a corn-soybean meal diet (McNaughton *et al*., 1998; Jackson *et al*., 2004; Zou *et al*., 2006; Li *et al*., 2010). Wu *et al*. (2005) demonstrated that supplementation with β-mannanase significantly improved the overall average feed conversion of hens fed with a low-energy diet. In particular, supplementation with 450 g ton^{-1} β-mannanase significantly increased ($p<0.05$) the ADG, ADFI and FCR of broilers relative to the low-energy diet group. the results also suggested that the addition of higher levels of β-mannanase may be beneficial which did not achieve the maximum response. Previous reports have found that inclusion at 80 MU ton^{-1} improved broiler gains and feed conversion and increases to 110 MU ton^{-1} resulted in no significant additional response (Jackson *et al*., 2004). In contrast, the results suggested that broilers fed with a low energy diet supplemented with β-mannanase had the same performance as broilers fed with the normal-energy diet which indicated an energy advantage with the addition of β-mannanase in the corn-soybean meal diet. This result was consistent with reports that broilers fed with low-energy diets supplemented with β-mannanase performed slightly better than broilers fed with high energy diets without enzyme (McNaughton *et al*., 1998). Li *et al*. (2010) demonstrated that broilers fed with low-energy diets supplemented with β-mannanase enhanced energy utilisation by >0.5 MJ kg^{-1} (119 kcal kg^{-1}). Pettey *et al*. (2002) also suggested that β-mannanase may provide an energy equivalent of 100 kcal kg^{-1} in a corn-soybean meal diet for weaning pigs. In the present study, the addition of β-mannanase improved the growth performance of broilers which may have resulted from an increase in feed intake.

The present results showed thatthe addition of enzyme to corn-soybean meal diets did not result in statistically different ($p>0.05$) effects on the relative weight (duodenum + jejunum + small intestine) and length (jejunum + small intestine). Wu *et al*. (2004) reported that there were no significant differences ($p>0.05$) on the relative weight or length of the different sections of the intestine of broilers with xylanase supplementation. Interestingly, compared with the low-energy diet group, 150 g ton^{-1} β-mannanase supplementation reduced ($p<0.05$) the relative length of the duodenum which may have resulted from physiological adjustment. In the study, supplementation with β-mannanase increased the relative weight and length of the ileum which may have increased the intestinal absorption area and thus improved the digestibility of nutrients and performance of broilers. This result was consistent with those obtained by Yusrizal and

Chen (2003) who reported that longer gut lengths exhibited better nutrient absorption which resulted in a heavier body weight.

Gut morphology is an index of intestinal health (Tufarelli *et al*., 2010). For example, changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of toxins (Yason *et al*., 1987; Anonymous, 1999). In the study, the addition of β-mannanase to the corn-soybean meal diet increased the villus height and ratio of crypt depth to villus height and decreased the crypt depth of the small intestine compared with the low energy diet group. Yaghobfar *et al*. (2007) reported that supplementing enzymes to broilers diets improved the villus height and crypt depth of the duodenum and jejumum. This result was consistent with the reported changes in gut morphology induced by exogenous enzyme supplementation (Bedford *et al*., 1991). Increased villi height has been shown to improve nutrient absorption, resulting in increased performance (Coates *et al*., 1955; Izat *et al*., 1989). It was concluded that an increased villus height and decreased crypt depth increased the surface area for nutrient absorption and thus improved nutrient digestibility thereby improving the feed conversion and performance in the present study. The higher ratio of crypt depth to villus height was an indication of a decreased turnover rate of the intestinal mucosa which resulted in a lower maintenance requirement and thereby leading to a higher growth rate or growth efficiency in rabbits (Tufarelli *et al*., 2010). Thus, an improvement in performance may be attributed to changes in the small intestine morphology of broilers and thus to an increased area for the absorption of nutrients (Onderci *et al*., 2006).

Tight junctions played a very important role in the intestinal mucosal barrier against macromolecular transmission (Ballard *et al*., 1995). Accumulating evidence has shown that Occludin and ZO-1 are the most important and critical proteins responsible for the structural and functional organisation of tight junctions (Fanning *et al*., 1998). The mRNA levels for Occludin and ZO-1 are considered an indicator of intestinal permeability and damage. The decrease in the expression of Occludin and ZO-1 resulted in an increase in intestinal permeability. Several results have suggested that the increased intestinal permeability resulted in local inflammation and thus compromised barrier function (Bruewer *et al*., 2003; Moeser *et al*., 2007; Farhadi *et al*., 2003).

The consequences of increasing intestinal permeability could enable the indiscriminate entry of extracellular antigens and pathogenic micro-organisms (Zhang and Guo, 2009). However, very few studies have been performed to determine the effects of β-mannanase supplementation on the expression of Occludin and ZO-1. The results of this study indicated that the mRNA levels of Occludin and ZO-1 broilers fed with 300 or 450 g ton^{-1} β-mannanase diet was significantly higher than the other groups ($p<0.05$) which resulted in a decrease in intestinal permeability.

CONCLUSION

Supplementation of β-mannanase in corn-soybean meal diets improved performance, intestinal morphology and the expression of intestinal tight junction proteins of broilers compared with the low-energy diet group. Furthermore, the results showed that broilers fed with low-energy diets supplemented with β-mannanase achieved the same performance as the normal energy diet group which might be due to the enzymatic degradation of β-mannanase that resulted in changes in intestinal morphology thus, improving the digestibility and utilisation of nutrients.

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