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Effects of Supplementing Different Levels of a Commercial Enzyme Complex on Performance, Nutrient Availability, Enzyme Activity and Gut Morphology of Broilers

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ABSTRACT : A trial was conducted to study the influence of different levels of a commercial enzyme complex on performance, nutrient availability, blood parameters, digestive tract measurements, amylase and trypsin activity of the digestive tract and gut morphology in broilers fed the typical diets in north China. There were four treatments: the control diet and the other three enzyme complex supplemented diets which were 180 mg/kg, 360 mg/kg and 720 mg/kg enzyme complex supplemented to the control diet, respectively. The birds fed the diets supplemented with 180 mg/kg and 360 mg/kg enzyme complex had better performance and nutrient availability, the activities of amylase and trypsin in the digestive tract in the two treatments were improved, the villus height and surface area of villus in the small intestine increased and the crypt depth and epithelial thickness of small intestine decreased. Relative weights of pancreas and relative weights and lengths of small intestine decreased. However, the addition of 720 mg/kg enzyme complex had no effects on these parameters and increased crypt depth and epithelial thickness of the small intestine. The data suggested that suitable supplementation of enzyme complex was beneficial for the birds, while excess enzyme complex inhibited secretion of endogenous enzyme and destroyed the structure of the small intestine. (**Key Words :** Enzyme Complex, Broilers, Performance, Nutrients Utilization, Enzyme Activity, Guts Morphology)

INTRODUCTION

The typical formulation of broiler diets in north China is quite variable and is mainly dependent upon the cost of ingredients; as a result, regional differences in diet formulation, feed quality and broiler performance can be quite dramatic. With increasing feed cost and rapid development of the enzyme industry, use of exogenous enzymes as a cost-effective means of improving feed efficiency, poultry performance and environmental quality is already relatively commonplace. There are diverse benefits to be gained from the use of enzymes in poultry diets. Some of the benefits influencing the performance of poultry are increased feed value of the dietary raw materials, reduction in the variation of nutrient quality of the diet, increased nutrient digestibility, reduction in water content of the excreta (Sarmiento-Franco et al., 2003), reduced viscosity of intestinal contents and the weight of digestive organs and accelerated rate of passage of digesta through the gastrointestinal tract (Lazaro et al., 2004). The efficacy of exogenous enzymes depends on many factors such as the chemical characteristics of the ingredient and diet being evaluated, the microbial population in the gut (and, consequently, the age of the bird), the characteristics and amounts of the enzymes used (Sarmiento-Franco et al., 2003), feeding regimes and feed processing methods, and dietary nutrition levels. Many commercial enzyme additives are mixtures of protease, cellulase and amylase. In this case, protease may attack other enzymes and decrease their efficiency. To be fully functional in the digestive tract, exogenous enzymes should be resistant to attack of protease in the small intestine and able to exhibit catalytic activity in the pH range 6 to 8 (Wang and Hsu, 2006). Although the practical efficacy of supplementing feed enzymes has been well established, the precise mechanism(s) involved and their site(s) of action have been less extensively explored

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Table 1. Ingredients and chemical composition of the basal diet

Ingredients	g/kg	Chemical composition[3]	g/kg
Yellow corn	612.90	Crude protein	185.00
Soybean meal	138.00	Calcium	9.40
Cotton seed meal	40.00	Total phosphorus	6.30
Corn oil	35.00	Available phosphorus	4.30
Rapeseed meal	30.00	Lysine	1.00
Corn gluten meal	25.00	Methionine	3.80
Meat and bone meal	30.00	Met+cys	7.00
Ground limestone	8.00	Threonine	7.30
DDGS[1]	70.00	AME (MJ/kg)	12.96
Methionine	0.80		
Lysine-HCl	4.00		
Threonine	0.30		
Premix[2]	6.00		

[1] Distillers dried grains and solubles produced from corn.

[2] The premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 800 IU; vitamin E, 30 IU; menadione,1.0 mg; thiamine, 2.0 mg; riboflavin, 5.0 mg; niacin, 50.0 mg; pyridoxine, 5.0 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 10.0 mg; folic acid, 0.60 mg; biotin, 0.30 mg; iron, 60 mg; zinc, 80 mg; copper, 10 mg; manganese, 60 mg; iodine, 0.6 mg; and selenium, 0.3 mg.

[3] The value of crude protein and apparent metabolizable energy (AME) were analyzed.

(Silva and Smithard, 2002). Some experiments showed that excess addition of enzyme complex inhibited the secretion of endogenous enzymes (Inborr, 1990; Mahagna et al., 1995) and decreased nutrient digestibility (Ni, 2000). An understanding of the mechanisms of enzyme supplementation will enable optimized supplementation with the required enzyme activity. However, there is no systematic information on the influence of different levels of enzyme supplementation in broiler diets. Therefore, the present study was conducted to investigate the effects of different levels of an exogenous enzyme complex supplemented to typical broiler diets in north China on performance, nutrient availability, blood parameters, amylase and trypsin activity, relative weight or length of digestive tract and gut morphology. Furthermore, we investigated the correlation of exogenous enzyme supplementation with endogenous enzyme secretion.

MATERIALS AND METHODS

Enzyme complex

The enzyme complex used in the present experiment was supplied by Guangdong VTR Bio-tech Co. Ltd., (Zhuhai, China) and contained mainly neutral protease (3,000 U/g), acid protease (3,000 U/g), endoamylase (500,000 U/g) and xylanase (65,000 U/g). It also contained lower amounts of exoamylase, β-glucanase, pectinase, cellulase and cellobiose.

Experiment design and diets

There were four treatments and six replicates per treatment. The basal diet (Table 1) was formulated to meet the requirements recommended by National Research Council (1994) for broilers. Three experimental diets were formulated by supplementing the basal diet (T_0) with 180 mg/kg (T_1), 360 mg/kg (T_2) and 720 mg/kg (T_3) of the enzyme complex, respectively. All diets were fed in a mash form.

General procedures

Three hundred and eight-four 21-d-old Avian broilers were obtained from a commercial broiler group and randomly assigned to 24 pens in two-tier cages. All birds received fluorescent lighting throughout the trial.

Body weight and feed intake were obtained for each pen at two-weekly intervals. Birds were allowed to consume feed and water *ad libitum* during the 28-d trial period. Mortality was recorded daily and feed to gain ratio was corrected for mortality.

Sample collection

During the last week (d 42 to 46), total collection of excreta was carried out for the determination of apparent digestibility of crude protein (APD) and gross energy (AED). Feed intake and excreta were measured by pen over 5 consecutive days. Excreta were pooled within each pen, mixed well using a blender and two representative samples were taken. The samples were freeze-dried. Dried samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at -4°C until chemical analysis.

On d 49, three birds from each pen with body weights closest to the mean were selected and weighed. Two birds were used for digestive tract measurements and the third for examination of blood parameters and gut morphology. The birds were killed by cervical dislocation and the digestive tract, from the pancreas to ileum, was carefully and swiftly excised. The weight of pancreas was recorded; then the intestinal contents were removed and the weights and lengths of the empty duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and

Table 2. Effects of enzyme levels on performance and apparent utilization of nutrients of broilers

Parameters	T_0	T_1	T_2	T_3	SEM	p value
Body weight (g)						
21 d	669.57	673.46	670.25	674.11	3.70	0.7718
35 d	1,344.13^{a}	1,427.88^{b}	1,427.22^{b}	1,324.70^{a}	0.027	0.0196
49 d	2,184.75^{a}	2,288.69^{ab}	2,350.81^{b}	2,171.02^{a}	0.041	0.0166
Average daily gain (g)						
22-35 d	48.18^{a}	53.86^{b}	54.22^{b}	46.47^{a}	1.90	0.0140
36-49 d	59.88^{a}	66.16^{b}	67.06^{b}	59.15^{a}	1.50	0.0016
22-49 d	54.11^{a}	57.69^{ab}	60.02^{b}	53.46^{a}	1.50	0.0141
Average daily feed intake (g)						
22-35 d	112.51	115.46	114.16	109.81	2.30	0.3707
36-49 d	148.75	149.25	150.33	146.28	3.10	0.8226
22-49 d	129.28	132.87	132.76	127.45	2.30	0.2972
Feed to gain ratio						
22-35 d	2.35^{b}	2.15^{a}	2.11^{a}	2.39^{b}	0.062	0.0101
36-49 d	2.49^{b}	2.26^{a}	2.24^{a}	2.48^{b}	0.031	<0.0001
22-49 d	2.42^{b}	2.30^{a}	2.20^{a}	2.40^{b}	0.033	0.0005
DMR (%)	64.64^{a}	74.10^{Bb}	71.02^{b}	62.90^{Aa}	1.90	0.0015
AED (%)	64.27^{a}	75.10^{b}	73.95^{b}	61.86^{a}	3.30	0.0186
APD (%)	38.18^{Aa}	57.58^{Bb}	50.12^{b}	36.20^{Aa}	3.40	0.0007
Livability (%)	85.40^{a}	91.67^{ab}	95.83^{b}	92.70^{ab}	3.10	0.1416
Excreta moisture (%)	82.11^{b}	78.43^{a}	79.64^{a}	80.10^{a}	0.55	0.0013

T_0, T_1, T_2 and T_3 represent basal diets supplemented with 0, 180 mg/kg, 360 mg/kg, and 720 mg/kg enzyme complex, respectively.
Means in the same row with superscripts of different lower case and capital letters differ significantly at $p<0.05$ and $p<0.01$, respectively.
SEM = Standard error of means.

ileum (from Meckel's diverticulum to ileocaecal junction) were recorded. After weighing, a pancreas sample of about 1 g was taken from the proximal, medial and distal portions of the pancreas and cut into pieces. Pancreas samples (according to W/V = 1/10 (g/ml)) and digesta of duodenum, jejunum and ileum (according to W/V = 1/5) were added into ice-cold deionized water. The mixture was homogenized and centrifuged (13,000 g for 5 min) and the supernatant was transferred into a 2 ml eppendorf tube immediately and frozen at -20°C until analyzed.

Before the third bird was killed, blood samples were obtained for subsequent determination of blood sugar and urea nitrogen in plasma. Approximately 5 cm lengths of duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after Meckel's diverticulum) were removed for measurements of gut morphology.

Intestinal morphology

Examinations of intestinal morphology were carried out according to the method of Iji et al. (2001). Intestine samples from each section were fixed in 10% buffered formalin until analyzed. Each segment was embedded in paraffin. A 7-μm section of each sample was placed onto a glass slide and stained with alcian blue/haematoxylin and eosin for examination with a light microscope. Villus height , crypt depth , the width of the apical and basal part of the villus and the thickness of epithelium and muscle were measured at 100×magnification using computer software (Sigma Scan, Jandel Scientific, San Rafael, CA, USA), and then the ratio of villus height to crypt depth and villus surface area was calculated.

Chemical analysis

Dry matter (DM) content was determined using standard procedures (AOAC, 1990). DM retention (DMR) was calculated as described by Marquardt et al. (1979). Gross energy was determined using an adiabatic bomb calorimeter (GR-3500 Autobomb, Changsha, China). APD and AED were determined as described by Rotter et al. (1989). The activities of amylase and trypsin of the pancreas and small intestinal digesta were determined using reagent boxes (Jiancheng Bioengineering Institute, Nanjing,China). One amylase unit was the amount of enzyme that hydrolyzed 5 mg starch in 15 min at 37°C. Trypsin activity was expressed as units per milligram of protein, which was determined by the method of Lowry et al. (1951).

Statistical analysis

For performance and nutrient availability measurements, each pen of birds was considered as an experimental unit for statistical analysis. For digestive tract measurements and gut morphology, the individual bird was considered as an experimental unit. All data were statistically analyzed using the GLM Procedure of SAS (SAS Institute, 1996). The differences between treatment means were considered to be significant at $p<0.05$.

Table 3. Effects of enzyme levels on relative weight, length and amylase and trypsin activity of digestive organs and blood parameter of broilers

Parameters	T_0	T_1	T_2	T_3	SEM	p value
Blood sugar (mmol/L)	12.37^{a}	13.43^{b}	13.47^{b}	12.14^{a}	0.298	0.0063
Blood Urea nitrogen (mmol/L)	0.33^{b}	0.22^{ab}	0.18^{a}	0.32^{b}	0.039	0.0298
Relative weight (g/kg live weight)						
Pancreas	2.66^{Bb}	2.09^{Aa}	2.08^{Aa}	2.45^{b}	0.102	0.0012
Duodenum	8.67^{Cc}	6.26^{ab}	5.37^{Aa}	7.41^{bc}	0.63	0.0081
Jejunum	13.16^{b}	9.87^{a}	9.89^{a}	11.96^{ab}	0.94	0.0550
Ileum	7.68^{b}	5.72^{a}	5.22^{a}	6.44^{ab}	0.60	0.0472
Relative length (cm/kg live weight)						
Duodenum	15.22^{Bb}	12.58^{a}	11.43^{Aa}	13.37^{ab}	0.71	0.0095
Jejunum	30.84^{Cc}	24.74^{ab}	23.76^{Ab}	28.54^{bc}	1.54	0.0134
Ileum	20.28^{b}	18.42^{ab}	16.15^{Aa}	20.52^{Bb}	0.93	0.0117
Relative activity of amylase (U/g chyme)						
Pancreas ($\times 10^5$ U/g)	15.11^{A}	21.24^{B}	23.62^{B}	12.73^{A}	1.335	<0.0001
Duodenum	$6,687.00^{ab}$	$12,791.00^{Cc}$	$10,553.50^{bc}$	$5,098.50^{Aa}$	1,665.00	0.0147
Jejunum	$1,380.00^{a}$	$3,016.00^{ab}$	$4,253.00^{b}$	$1,071.50^{a}$	750.00	0.0240
Ileum	656.50^{a}	$2,019.00^{b}$	$1,699.00^{ab}$	843.50^{a}	415.00	0.0894
Specific activity of trypsin (U/mg protein)						
Pancreas ($\times 10^5$ U/g)	472.79^{Aa}	931.35^{Bb}	815.51^{b}	506.01^{Aa}	99.00	0.0077
Duodenum	$19,355.76^{Aa}$	$38,541.33^{Bb}$	$34,695.81^{b}$	$21,991.05^{Aa}$	3,393.30	0.0013
Jejunum	$37,095.50^{a}$	$41,823.54^{ab}$	$49,515.36^{Bb}$	$35,275.50^{Aa}$	3,086.50	0.0179
Ileum	$43,113.30^{Aa}$	$59,313.32^{Bb}$	$57,026.12^{b}$	$43,566.94^{Aa}$	3,335.40	0.0027

T_0, T_1, T_2 and T_3 represent basal diets supplemented with 0, 180 mg/kg, 360 mg/kg, and 720 mg/kg enzyme complex, respectively.
Means in the same row with superscripts of different small and capital letters differ significantly at $p<0.05$ and $p<0.01$, respectively.
SEM = Standard error of means.

Table 4. Effect of enzyme levels on the small intestine morphology of broilers

Parameters	T_0	T_1	T_2	T_3	SEM	p value
Duodenum						
Villus height (μm)	$1,332.80^{a}$	$1,635.20^{b}$	1918.00^{Cc}	$1,173.20^{Aa}$	84.15	<0.0001
Crypt depth (μm)	282.80^{B}	154.00^{A}	245.00^{B}	369.60^{C}	18.80	<0.0001
Ratio	4.71^{A}	10.82^{C}	8.39^{B}	3.29^{A}	0.50	<0.0001
Surface area (mm^2)	3.78^{a}	6.78^{Bb}	5.78^{b}	3.13^{Aa}	0.586	0.0002
Epithelial thickness (μm)	76.30^{B}	45.85^{A}	52.50^{A}	53.20^{A}	3.62	<0.0001
Muscle thickness (μm)	145.60^{Aa}	128.80^{Aa}	156.80^{a}	189.00^{Bb}	9.89	<0.0011
Jejunum						
Villus height (μm)	$1,020.60^{A}$	$1,697.50^{Cc}$	$1,499.40^{Cb}$	$1,079.68^{A}$	64.68	<0.0001
Crypt depth (μm)	274.40^{ab}	220.50^{a}	226.10^{a}	288.75^{b}	18.49	0.0271
Ratio	3.93^{A}	7.73^{B}	7.08^{B}	3.92^{A}	0.45	<0.0001
Surface area (mm^2)	3.11^{Aa}	4.68^{Cc}	4.09^{bc}	3.43^{ab}	0.323	0.0068
Epithelial thickness (μm)	49.00^{ab}	58.28^{b}	44.80^{a}	57.75^{b}	3.56	0.0255
Muscle thickness (μm)	194.60	182.00	203.00	222.25	17.05	0.4120
Ileum						
Villus height (μm)	809.20^{a}	938.00^{b}	$1,040.20^{Bb}$	715.40^{Aa}	38.17	<0.0001
Crypt depth (μm)	238.00^{Bb}	168.00^{Aa}	176.40^{Aa}	193.20^{a}	14.35	0.007
Ratio	3.63^{A}	5.87^{B}	6.12^{B}	3.73^{A}	0.39	<0.0001
Surface area (mm^2)	2.04^{ab}	2.49^{bc}	2.64^{c}	1.97^{a}	0.169	0.0169
Epithelial thickness (μm)	45.50^{a}	51.80^{ab}	53.20^{ab}	56.70^{b}	3.26	0.1233
Muscle thickness (μm)	193.20	170.80	157.50	172.20	15.05	0.4215

T_0, T_1, T_2 and T_3 represent basal diets supplemented with 0, 180 mg/kg, 360 mg/kg, and 720 mg/kg enzyme complex, respectively.
Means in the same row with superscripts of different small and capital letters differ significantly at $p<0.05$ and $p<0.01$, respectively.
SEM = Standard error of means.

RESULTS

Bird performance and nutrient availability

Effects of enzyme complex levels on the performance, nutrient availability, livability and fecal moisture of broilers are presented in Table 2. Average weight gain, feed:gain ratio(FCR), DMR, AED, and APD were significantly improved for the birds of T_1 and T_2 compared to those of T_0

and T_3 ($p<0.05$).There was no difference in feed intake among the four treatments ($p>0.05$). All enzyme treatments improved livability and reduced excreta moisture of birds significantly ($p<0.05$).There were no significant differences in the above parameters between T_0 and T_3 ($p>0.05$).

Weight and length of digestive tract, activity of amylase and trypsin, and blood parameters

The content of blood sugar and the relative activity of amylase and trypsin of the pancreas and small intestine were higher, and blood urea nitrogen content, the relative weight of pancreas and the relative length of jejunum were lower for birds fed T_1 and T_2 than for those fed T_0 and T_3 ($p<0.05$) (Table 3). Comparing with the control group (T_0), the addition of 720 mg/kg enzyme complex (T_3) had no significant effects on blood parameters, relative weight and length of the digestive tract and the relative activities of amylase and trypsin ($p>0.05$).

Gut morphology

Effects of enzyme complex levels on gut morphology are shown in Table 4. The height and surface area of small intestine and the villus height: crypt depth ratio were increased and the crypt depth of small intestine was decreased by treatment T_1 and T_2 ($p<0.05$). Supplementing 720 mg/kg enzyme complex (T_3) increased the crypt depth of duodenum, duodenum muscle thickness and ileum epithelial thickness ($p<0.05$).There were no significant differences in other gut morphology between treatments T_0 and T_3 ($p>0.05$).

Figure 1 to 4 showed light micrographs of sections of the duodenum from the four treatments. T_1 and T_2 treatments increased height of the villus while the villi were thinner than other groups. However, addition of 720 mg/kg enzyme complex (T_3) resulted in shortened, widened and atrophied villi. The changes in length and width of villi in the jejunum and ileum were consistent with changes in the duodenum.

DISCUSSION

The addition of 180 mg/kg and 360 mg/kg enzyme

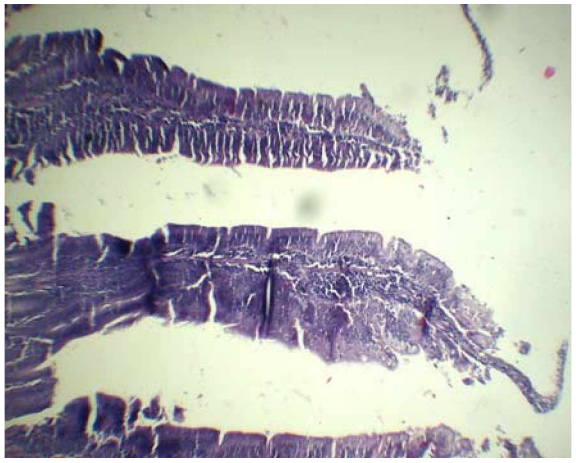

Figure 1. (duodenum 0 mg/kg) (×100)

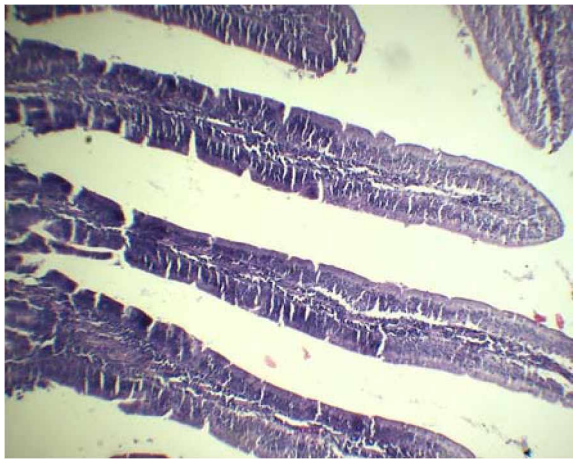

Figure 2. (duodenum 180 mg/kg) (×100)

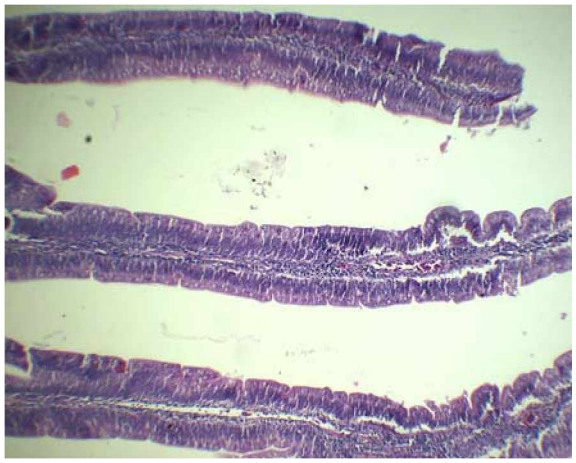

Figure 3. (duodenum 360 mg/kg) (×100)

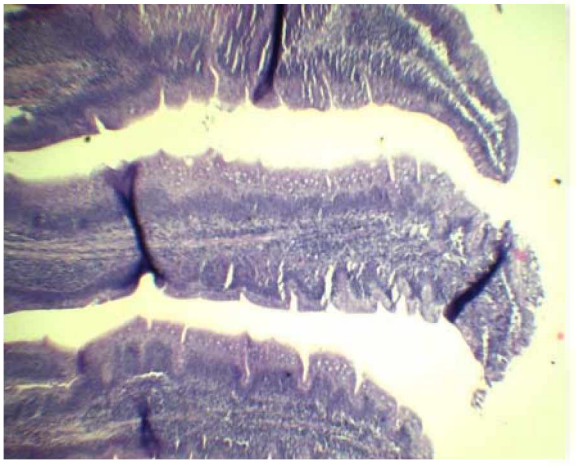

Figure 4. (duodenum 720 mg/kg) (×100)

significantly increased weight gain and reduced feed to gain ratio. These results agree with previous research (Pack et al., 1998; Gracia et al., 2003), which reported that supplementing corn-based diets with enzyme produced significant positive responses in growth performance, although the enzyme used in those studies contained predominantly amylase. Early studies also showed beneficial effects of amylase and protease preparations on growth and feed efficiency of chicks when added to diets (Fry et al., 1958; Burnett, 1966). Zanella et al. (1999) found that supplementation of a corn-soybean meal (SBM) diet with a mixture of amylase, protease, and xylanase, similar to our study, did not affect intestinal viscosity and the viscosity was low, so the improvement of performance may not be correlated to intestinal viscosity. Café et al. (2002) reported that supplementation of corn-SBM diets with an enzyme complex containing amylase, protease, and xylanase improved body weight gain of male broilers at 16, 35, and 49 d while FCR was worse than the control treatment; this may have been due to the nutritional level which was higher than other studies. Bi Yu and Chung (2004) reported that by lowering the activity of α-amylase the weight gain of birds at 21 d was lower; when adequate activity of α-amylase was supplemented along with non-starch polysaccharide-degrading enzymes, further numerical growth responses were obtained. Mahagna et al. (1995), however, did not find any beneficial effect of enzyme complexes on performance or digestibility of nutrients in broilers from 1 to 14 d of age fed sorghum-SBM diets; this may be correlated to supplementation levels of the enzyme. The activities of enzymes supplemented in our study are much higher than in the study of Bi Yu and Chung (2004). The present study showed that T_2 treatment did not produce better performance than T_1, and adding 720 mg/kg enzyme complex (T_3) caused no improvements in performance. Similar results were found with wheat-based diets (Iji et al., 2001), on which supplementation of enzyme complex at a low dose was shown to be beneficial in improving bird performance while a high dose had no effect or tended to decrease performance.

Digestibility of nutrients in these ingredients was affected by exogenous enzyme supplementation. T_1 and T_2 treatments significantly improved DMR, AED and APD. Previous studies have shown that enzyme complex containing amylase, protease, and xylanase, similar to our study, improved the digestibility of starch, DM and energy (Zanella et al., 1999; Bi Yu and Chung, 2004; Scheideler et al., 2005). Bi Yu and Chung (2004) concluded that exogenous enzyme addition appeared to be beneficial for reduced-energy diets. Kocher et al. (2003) reported that combined addition of pectinase, protease, and amylase significantly improved AME_n when added to a corn-SBM diet low in energy and protein, whereas addition of the same enzyme combination to a corn-SBM diet with increased energy and protein content resulted in a significant reduction of AME_n. Addition of 720 mg/kg enzyme complex (T_3) tended to decrease nutrient digestibility. Similar results were found in wheat-based diets (Ni, 2000) for broilers, on which 0.1% enzyme complex increased availability of CP, ME and DM, while 0.23% enzyme complex decreased the availability of CP and DM. This may also be explained similarly to a previous report (Almirall et al., 1995) that opportune exogenous enzyme could increase the activity of endogenous enzymes and interrelating hormones which regulate the endocrine system of the animal body and promote nutrient availability and performance. However, excess exogenous enzymes may inhibit the activity of endogenous digestive enzymes and the secretion of interrelating hormones.

The enzyme treatments significantly reduced excreta moisture of broilers, which is due to enzyme action which releases NSP in diets, disrupts the cell wall matrix and decreases viscosity of digesta. The addition of 360 mg/kg enzyme complex significantly increased livability of broilers which is consistent with published reports (Pack and Bedford, 1997). This may be due to the fact that enzyme complex accelerates the growth of immunity organs of the animal body. Treatments T_1 and T_2 significantly increased blood sugar and decreased urea nitrogen, while T_3 had no effects on blood parameters. This is consistent with previous reports (Borg et al., 1987; Friesen et al., 1992). Friesen et al. (1992) found that enzyme complex increased the digestion rate of starch, while the product of starch digestion was absorbed into blood in the form of glucose and so increased blood sugar content. Borg et al. (1987) reported that blood urea nitrogen could accurately reflect the state of protein metabolism and balance of amino acids, and urea nitrogen was low when the balance of amino acids was good.

Birds fed T_1 and T_2 had lower relative weight of pancreas and lower relative weights and lengths of duodenum, jejunum and ileum than other groups, while supplementing with 720 mg/kg enzyme complex (T_3) had no effect on the measurements of digestive tract. Onderci et al. (2006) reported that supplementing an amylase-producing culture in broiler diets reduced the relative weight of pancreas. This was in agreement with Wang et al. (2005), who found that enzyme inclusion in wheat-based diets decreased the size of the digestive organs and the gastrointestinal tract to some extent. The relative weight of pancreas has been shown to decrease when an exogenous amylase was supplemented, which indicates that secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine, which may in

turn be due to the reduction of viscosity of the digesta (Brenes et al., 2002). Brenes et al. (1993) reported that 1,000 mg/kg Avizyme SX in barley diets significantly reduced the relative weights of pancreas, duodenum, jejunum and ileum. Cowieson et al. (2003) reported that addition of enzyme in 5 kinds of pea-meal diet caused significant reductions or tended to decrease the relative weights and lengths of small intestine. The reduction in relative weight of digestive tract in treatments T_1 and T_2 was also of direct economic benefit, as the dressing yield of broilers should increase proportionally.

Supplementing with 180 mg/kg enzyme complex (T_1) had greater effects on digestive enzyme activity of the pancreas, duodenum and ileum than T_0 and T_3 treatments, and T_2 group had higher enzyme activity in the jejunum. This was correlated to performance of the broilers since high enzyme activity accelerates the digestion and absorption of nutrients in the feed. Gracia et al. (2003) concluded that reduction in pancreas weight might have been related to less secretion of endogenous amylase due to the presence of exogenous amylase in the intestine. However, in our study, activities of pancreatic amylase and trypsin increased though the relative organ weight decreased. These results were consistent with a previous report (Inborr, 1990) which concluded that supplementation of higher exogenous protease in diets offered to pigs could disrupt the protein of diets by simultaneously inhibiting secretion of endogenous protease and decomposition of exogenous protease. Mahagna et al. (1995) found that secretion of amylase and protease by the pancreas was reduced when chicks were fed diets supplemented with amylase and protease, which may be due to the fact that excess enzyme was supplemented. Zhengyu Jiang et al. (2008) also showed that the oral administration of different levels of exogenous amylase for broilers affected activities of intestinal enzymes and the production of pancreatic digestive enzymes in a dose-dependent manner. The enzyme activity in the small intestine is composed of exogenous and endogenous enzymes and the enzyme activity of the pancreas is only endogenous. Furthermore, we can conclude that an effective enzyme complex (180 mg/kg and 360 mg/kg) can significantly increase secretion of endogenous enzyme by the pancreas and accelerate the action of exogenous enzyme in the small intestine. However, excess enzyme complex inhibits secretion of endogenous enzymes of the pancreas and impedes the action of exogenous enzyme in the small intestine.

Treatments T_1 and T_2 increased villus height, surface area and ratio of villus height to crypt depth of the duodenum, jejunum and ileum. Therefore, improvement in performance of T_1 and T_2 treatments may not only be due to the release of simple sugars and protein but also to increased area for absorption of nutrients in small intestine. However, 720 mg/kg enzyme treatment had no effect on these parameters. These results are consistent with the report of Iji et al. (2001) that addition of a high dose of enzyme to wheat-based diets had no effect on villus height, crypt depth or villus surface area in the duodenum, jejunum and ileum of broilers. Reductions of villus height and surface area can reduce the absorption of nutrients. Therefore, the improvement of performance may be also correlated to the small intestine morphology (Onderci et al., 2006).

The enzyme treatments significantly reduced crypt depth of the duodenum, jejunum and ileum and the epithelial thickness of duodenum and jejunum except on the 720 mg/kg treatment. The reduction of epithelial thickness of the small intestine improves absorption by the pillar generative cell. Photomicrographs (Figures 1 to 4) showed that the villus shape in the duodenum of T_1 and T_2 groups was longer and thinner than in T_0 and T_3 groups, and similar effects were observed in the jejunum and ileum. Jaroni et al. (1999) found that the shortening, thickening and atrophy of the villus in the jejunum of laying hens fed on diets based on wheat middling were reversed with xylanase addition. The enzyme complex in the present study contained xylanase, β-glucanase, cellulase and pectinase. The improvement of small intestine morphology adapted to the increased nutrients in the small intestine.

In conclusion, supplementation of 180 mg/kg and 360 mg/kg enzyme complex improved the performance and nutrient availability, decreased the relative weights and lengths of pancreas, duodenum, jejunum and ileum, improved blood parameters, and increased amylase and trypsin activities of the digestive tract. These supplements were also associated with increased villus height, surface area and decreased crypt depth and epithelial thickness. However, the addition of 720 mg/kg enzyme complex had no beneficial effects on the above parameters. Therefore, excess supplementation of enzyme complex is not beneficial for broilers. There was no significant difference between treatments of 180 mg/kg and 360 mg/kg enzyme complex, so the addition of 180 mg/kg enzyme complex would be recommended to perform most cost effectively under the conditions of this experiment.

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