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Effects of Xylanase on Performance, Blood Parameters, Intestinal Morphology, Microflora and Digestive Enzyme Activities of Broilers Fed Wheat-based Diets*

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ABSTRACT : The study was conducted to investigate the effects of different levels of xylanase on performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers. The wheat-based diets were supplemented with 0, 500, 1,000, 5,000 U/kg xylanase. Xylanase supplementation significantly ($p<0.05$) improved the feed:gain ratio of broilers from 1 to 21 d and 1 to 42 d. Supplementing 500 U/kg and 1,000 U/kg xylanase improved ($p<0.05$) the villus height and the ratio of villus height to crypt depth in the small intestine. Excess supplementation of xylanase (5,000 U/kg) increased the villus height in the ileum ($p<0.01$) and the ratio of villus height to crypt depth in the duodenum and ileum ($p<0.05$). The microflora in the ileum and caecum, digestive enzyme activities in the small intestine and the concentrations of serum glucose, uric acid, insulin and IGF-1 were not affected by the supplementation of xylanase. Excess level of xylanase (5,000 U/kg) had a tendency to induce the multiplication of *E. coli* and total aerobes. The results suggested that supplementing 500 U/kg and 1,000 U/kg xylanase was beneficial for broilers and excess xylanase supplementation resulted in no further improvement or negative effects. (**Key Words** : Xylanase, Broilers, Blood Parameters, Intestinal Morphology, Digestive Enzyme Activities)

INTRODUCTION

Although wheat is becoming an important source of energy in poultry diets, its high level of xylans, the principal water-soluble non-starch polysaccharides (NSP), limits its use. The presence of xylans increases the viscosity of the digesta, impeding the digestion and absorption of nutrients and causing poor performance (Almirall et al., 1995; Choct et al., 1995). There is considerable evidence that negative effects of NSP in poultry diets are related to the gut microflora of broilers, as supplementation of antibiotics to diets increases their nutritive value (Annison and Choct, 1991). Diet composition may produce microscopic alterations in the intestinal mucosa (Yamauchi, 2002) and it is possible that the change in morphology of the gastrointestinal tract (GIT) may be associated with dietary NSP levels. NSP also alters digestive functions and, in particular, digestive enzyme activities. It has been demonstrated in rats that there may be an attempt to compensate for the inefficiency of digestion and absorption with hyperplasia and hypertrophy of digestive organs and an increased secretion of digestive juice, although nutrient digestibility does not improve (Ikegami et al., 1990).

Adding NSP-degrading enzymes is a routine practice to improve the performance of broilers fed diets based on rye, wheat, barley or oats (Bedford, 2000a; Acamovic, 2001; Cowieson, 2005). However, although the efficacy of exogenous enzymes has been well established, the underlying mechanism is not clearly understood. It has been proposed that the NSP-degrading enzymes reduce digesta viscosity in the small intestine, and result in improvements in nutrient absorption. *In vitro* study showed that the endosperm cell wall of barley was completely degraded by the NSP-degrading enzyme and supplementation with NSP-degrading enzymes increased the digestibilities of dry matter, crude protein, nitrogen-free extract, crude fat and crude fiber of barley by 18.1%, 20.3%, 16.4%, 26.9% and

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30.0%, respectively (Li et al., 2004). Some studies have demonstrated that enzyme treatment can influence the intestinal morphology of birds fed barley-based diets (Brenes et al., 1993) and there are interactions between enzymes and the host animal, its microflora, and also dietary ingredients (Bedford, 2002).

Furthermore, the growth performance of poultry is also closely associated with the regulation of metabolism and function of the growth-related endocrine system. There is a high correlation between the relative growth rate of broiler cockerels and the concentrations of some hormones such as IGF-I and tri-iodothyronine (T_3) (Buys et al., 1999). Nutritional status is an important factor in the regulation of blood hormones and intermediary metabolism in broiler chickens (Buyse et al., 2002; Swennen et al., 2005). However, relatively few studies have been conducted to evaluate the effects of different levels of xylanase on intestinal development and hormone levels of broilers fed wheat-based diets. Besides, some researchers showed that excess supplementation of enzyme complex had no effect on performance or even inhibited endogenous enzyme secretion and destroyed small intestine structure (Iji et al., 2001; Ai et al., 2004). However, few reports are available on the effects of a high level of xylanase as a single source of enzyme on the performance and intestinal parameters of broilers. Therefore, the objective of the present study was to investigate the effects of different levels of xylanase on performance, blood hormones, intestinal morphology, microflora and digestive enzyme activities in broilers fed wheat-based diets.

MATERIAL AND METHODS

Enzyme preparation

The enzyme preparation used in this study was a microbial xylanase containing 10,000 U/g xylanase activity. One unit of xylanase is defined as the amount of enzyme that liberates 1 μmol of reducing sugars from 5 mg/ml at xylan solution per min at pH 5.5 and 37°C.

Birds and diets

A total of 240 one-day-old Arbor Acres, female broiler chicks were obtained from a commercial hatchery. The chicks were weighed and allocated to 4 dietary treatments in a completely randomized design. Each treatment was replicated 5 times with 12 chicks each. The birds had free access to feed and water. The experimental period was divided into 2 phases: growing phase (1 to 21 d) and finishing phase (22 to 42 d). The compositions of the diets are listed in Table 1. The 4 dietary treatments were wheat-based diets adequate in all nutrients for both experimental phases and supplemented with 0, 500, 1,000, and 5,000 U/kg xylanase. Diets of the finishing phase were antibiotic-free. All experimental diets were given in mash form. Diets were formulated to the nutrient requirements recommended by NRC (1994) for broilers of each matching age.

All chicks were provided 24-h light for the first three days, followed by 18 h of light and 6 h of dark for the growing phase and 16 h of light and 8 h of dark for the finishing phase. Room temperature was maintained at 33°C for the first three days and then gradually reduced 2 to 3°C

Table 1. Composition and nutrient levels of the experimental diets

Ingredient (%)	Experiment phase		Calculated analysis (%)[3]	Experiment phase	
	Growing phase	Finishing phase		Growing phase	Finishing phase
Wheat	40.00	40.00	ME, kcal/kg	2,960	3,050
Corn	19.00	24.50	Crude Protein	22.40	19.80
Soybean meal	30.00	22.00	Ca	0.92	0.86
Cottonseed meal	3.00	5.00	Total P	0.61	0.56
Soy oil	4.00	4.60	Nonphytate P	0.45	0.40
Limestone	1.24	1.33	Lysine	1.35	1.20
Dicalcium phosphate	1.50	1.30	Methionine	0.55	0.50
Salt	0.30	0.30	Methionine+cystine	0.90	0.82
Lysine	0.47	0.47	Threonine	0.80	0.73
Methionine	0.22	0.20			
Threonine	0.02	0.05			
Mineral premix[1]	0.01	0.10			
Vitamin premix[2]	0.04	0.05			
Choline-Cl	0.10	0.10			

[1] The mineral premix provided per kilogram of diets of 1-21 d and 22-42 d: iron, 100, 60 mg; zinc, 100, 80 mg; copper, 8, 8 mg; manganese, 120, 60 mg; iodine, 0.7, 0.6 mg; and selenium, 0.3, 0.3 mg, respectively.

[2] The vitamin premix provided per kilogram of diets of 1-21 d and 22-42 d: vitamin A, 8,000, 6,000 IU; vitamin D_3, 1,000, 500 IU; vitamin E, 20, 30 IU; menadione, 0.5, 0.5 mg; thiamine, 2.0, 2.0 mg; flavin, 8.0, 5.0 mg; niacin, 35, 30 mg; pyridoxine, 3.5, 3.0 mg; vitamin B_{12}, 0.01, 0.01 mg; pantothenic acid, 10.0, 10.0 mg; folic acid, 0.55, 0.55 mg; biotin, 0.18, 0.15 mg; choline-Cl, 1, 1 g, respectively.

per week to a final temperature of 22°C. House conditions and animal management followed standard recommendations (Institute of Laboratory Animal Resources Commission on Life Science, 1996).

On d 21 and d 42, birds were weighed by the pen. Feed consumption was recorded weekly and mortality was recorded daily.

Sample collection

On d 42, one bird from each pen with body weight closest to the mean was selected and killed by cervical dislocation. The digesta from the duodenum, jejunum and ileum 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.

On d 21 and d 42, one bird from each pen was selected randomly, and blood was obtained after morning feeding by heart puncture for the determination of blood glucose, uric acid, insulin and IGF-I. Blood samples were allowed to clot at 4°C and centrifuged at 1,520 ×g for 20 min before harvesting serum. Serum samples were stored at -20°C until assayed. For broilers at 21 d, approximately 5 cm lengths of the duodenum, jejunum and ileum were removed for measurements of intestine histology.

Bacteriological analysis

On 42 d, five broilers from each treatment were randomly selected and killed by cervical dislocation. The following procedures were conducted according to the method described by Zhang et al. (2003). About 1 g of ileal or cecal contents was placed into a bottle containing 50 ml of sterilized physiological salt solution (NaCl, 9 g/L) together with a few glass beads to aid dispersion. The sample was homogenized and the suspension was then serially diluted to 10^{-8} in 9 ml of sterilized physiological salt solution for viable counts of total aerobes, *Lactobacillus*, and *E. coli*. Aliquot volumes (0.2 ml) of appropriate dilutions were spread on the appropriate selective agar plates and incubated at 37°C. Nutrient agar was used as the medium for counting total aerobes with dilutions of 10^{-2} to 10^{-6}. Aliquots of these dilutions were also placed onto MacConkey agar for *E. coli* counts. Nutrient agar and MacConkey agar were incubated aerobically for 1 d. For *Lactobacillus*, Rogasa medium was used with dilutions of 10^{-4} to 10^{-8}, and the plates were incubated in 5% CO_2 for 48 h. All dilutions were plated in duplicate. After incubation, colonies were counted according to their morphology. Counts from duplicate plates were averaged. Numbers of colony-forming units were expressed as log colony-forming units per gram of the digesta content.

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, 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 were calculated.

Chemical analysis

The activities of amylase and protease of the small intestinal digesta were determined using standard kits (Jiancheng Bioengineerign Institute, Nanjing, China). The concentrations of blood glucose and uric acid were measured by kits (Shanghai Fuxing Changzheng Medical Science, Ltd. Co., Shanghai, China), and insulin and IGF-I were measured by RIA using standard kits (Tianjin Jiuding Biological Technology Ltd. Co., Tianjin, China). All blood parameters were measured according to the manufacturer's instructions. All measurements for each variable were run in the same assay in order to avoid inter-assay variability.

Statistical analysis

Data were analyzed using the General Linear Model procedure of SAS (SAS Institute, 1996) to determine the treatment effects. Means with a significant F ratio were separated by the least significant difference test. Differences were considered significant at $p<0.05$.

RESULTS

Birds were in good health throughout the experimental period. Mortality was less than 0.2% and was not related to dietary treatment. At the start of the experimental period, there were no differences ($p>0.05$) in initial BW among the treatments.

The effects of exogenous xylanase supplementation on the performance of broilers fed wheat-corn-based diets are shown in Table 2. Different levels of xylanase tended to increase ($p>0.05$) body weight gain (BWG) during the finishing phase. Enzyme had no significant effects on average daily feed intake (ADFI) of broilers in both phases. Feed:gain ratio was significantly improved by enzyme supplementation in broilers from 1-21 d and 1-42 d ($p<0.05$). There were no significant differences in the performance of broilers among levels of enzyme supplementation in both phases.

Table 2. Effects of different levels of xylanase supplementation on the performance of broilers

Parameters	Dietary enzyme levels (U/kg)				SEM	p
	0	500	1,000	5,000		
1-21 d						
BWG[1] (g)	543	557	557	549	7	0.556
ADFI[2] (g)	930	866	876	866	14	0.164
Feed:gain ratio	1.712[a]	1.556[b]	1.573[b]	1.577[b]	0.046	0.013
22-42 d						
BWG (g)	1,382	1,495	1,540	1,462	34	0.155
ADFI (g)	3,043	2,958	3,211	2,821	67	0.162
Feed:gain ratio	2.210	1.979	2.039	1.933	0.100	0.065
1-42 d						
BWG (g)	1,925	2,052	2,098	2,011	36	0.137
ADFI (g)	3,973	3,824	3,998	3,687	69	0.156
Feed:gain ratio	2.068[a]	1.862[b]	1.913[ab]	1.835[b]	0.075	0.030

[1] BWG means body weight gain. [2] ADFI means average daily feed intake.
Means in the same row with different superscripts differ significantly ($p<0.05$).

Xylanase supplementation had no influence on intestinal microflora of broilers at 42 d (Table 3). However, supplementing 500 U/kg and 1,000 U/kg xylanase tended to reduce ($p = 0.06$) the counts of *E. coli* in the ileum compared with the control.

Supplementing 500 U/kg and 1,000 U/kg xylanase increased ($p<0.05$) the villus height in the duodenum, jejunum and ileum (Table 4). There was no significant difference between the two levels except in the jejunum where 1,000 U/kg increased the villus height ($p<0.01$). In the duodenum and jejunum, there was no significant difference in villus height between 5,000 U/kg xylanase and the control, but the height was decreased in the 5,000 U/kg group compared with 500 U/kg and 1,000 U/kg groups ($p<0.05$). In the ileum, 5,000 U/kg increased the villus height compared with the control ($p<0.01$). The ratio of villus height to crypt depth was increased by 1,000 U/kg xylanase supplementation in the duodenum, jejunum and ileum ($p<0.01$). There was a significant difference in the ratio between 500 U/kg xylanase supplementation and the control ($p<0.05$) in the ileum. 5,000 U/kg treatment increased the height in the duodenum and ileum compared with the control ($p<0.05$). However, xylanase supplementation had no effects on crypt depth, epithelial thickness and intestinal muscle thickness in the three segments.

No significant differences were observed among dietary treatments on amylase and protease activities of the small intestinal digesta (Table 5) and on blood parameters (Table 6).

DISCUSSION

It is well documented that supplementing exogenous enzymes to wheat-based diets for broilers can improve performance (Peng, 2003; Wang et al., 2005). The xylanase preparation improved weight gain and feed:gain ratio throughout the experiment. The feed to gain ratio was decreased by 9.11%, 8.11%, 7.88% during 1-21 d and 9.96%, 7.50%, 11.27% during 1-42 d with 500 U/kg, 1,000 U/kg, and 5,000 U/kg xylanase supplementation, respectively. However, the effects on feed to gain ratio were not significant among the three different levels of xylanase supplementation (Table 2). The data were in general agreement with those of Wang et al. (2005) and Gao et al. (2008). The improved performance may be due to lowered

Table 3. Effects of different levels of xylanase on the intestinal microflora composition of broilers at 42 d (log cfu/g)

Parameters		Dietary enzyme levels (U/kg)				SEM	p-value
		0	500	1,000	5,000		
Ileum	*E. coli*	5.84	5.16	5.56	6.32	0.525	0.062
	Total aerobes	6.51	5.60	6.15	7.54	0.563	0.090
	Lactobacillus	7.43	8.04	8.07	7.59	0.568	0.319
Caecum	*E. coli*	6.67	7.17	6.95	7.51	0.446	0.108
	Total aerobes	7.19	7.37	7.02	7.64	0.317	0.094
	Lactobacillus	8.03	8.40	7.73	8.18	0.431	0.173

Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Effects of different levels of xylanase on the small intestine histology of broilers at 21 d (μm)

Parameters	Dietary enzyme levels (U/kg)				SEM	p-value
	0	500	1,000	5,000		
Duodenum						
Villus height	876.06^{Cb}	987.78^{ABa}	1020.60^{Aa}	905.44^{BCb}	32.56	0.001
Crypt depth	69.65	67.76	56.81	61.32	4.78	0.058
Villus height: crypt depth	12.62^{Bc}	14.70^{Bbc}	18.02^{Aa}	15.10^{ABb}	1.05	0.001
Epithelial thickness	55.42	39.57	38.13	46.34	6.55	0.067
Muscle thickness	148.64	143.99	137.83	139.05	24.37	0.968
Jejunum						
Villus height	775.89^{BCb}	673.54^{Cc}	975.05^{Aa}	795.40^{Bb}	37.28	0.000
Crypt depth	50.54	43.94	38.57	48.20	4.51	0.079
Villus height: crypt depth	15.98^{Bb}	15.40^{Bb}	25.53^{Aa}	16.72^{Bb}	1.80	0.000
Epithelial thickness	37.13	26.05	25.55	34.21	4.60	0.052
Muscle thickness	113.00	114.58	110.64	139.53	20.27	0.469
Ileum						
Villus height	354.82^{Bc}	521.90^{Aab}	556.89^{Aa}	493.82^{Ab}	26.97	0.000
Crypt depth	51.95	49.91	37.15	44.50	5.90	0.095
Villus height: crypt depth	7.55^{Bb}	10.79^{ABa}	13.74^{Aa}	11.84^{ABa}	1.454	0.000
Epithelial thickness	33.86	29.50	21.27	30.42	4.93	0.986
Muscle thickness	111.39	105.73	104.17	105.53	20.82	0.112

Means in the same row with superscripts of different small and capital letters differ significantly at $p<0.05$ and $p<0.01$, respectively.

viscosity and/or disruption of cell wall. However, the feed:gain ratio over 22-42 d was not improved by xylanase addition. This may be explained by effects of enzyme supplementation being dependent on the bird's age and older birds having a greater capacity to endure the effects of high viscosity because of enhanced fermentation capacity of the microflora in their intestines (Vukic-Vranjes and Wenk, 1995; Choct et al., 1996).

Composition of the diet affects the gastrointestinal microflora in broilers. The presence of viscous polysaccharides has been shown to increase the intestinal microbial activity associated with poor broiler performance (Choct et al., 1996; Hubner et al., 2002). Enzyme supplementation can significantly influence microbial populations in the intestine. NSP-degrading enzymes such as xylanase are hypothesized to work in two steps, described as an ileal phase and a cecal phase (Bedford, 2000b). 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, 2000b). Engberg et al. (2004) found that xylanase addition to wheat-based broiler diets stimulated growth of lactic acid bacteria in the ileum, which was confirmed by higher lactic acid concentrations. However, in the present study, there was no influence of xylanase on the counts of *Lactobacillus*, *E. coli* and total aerobes in the ileum and caecum (Table 3). These results are consistent with those of Gao et al. (2008) who reported no significant change of lactobacillus and coliform bacteria counts in caecum contents of 21-day-old birds. In our study, we observed that in the ileum there was a tendency for decreased ($p = 0.06$) counts of *E. coli* when 500 U/kg and 1,000 U/kg xylanase were added. The ceca contain the largest number of bacteria in the chicken GIT so the regulation of microflora composition by diet ingredients might be more complicated than in the ileum.

In the present study, compared with the control 500 U/kg and 1,000 U/kg xylanase supplementation increased villus height of the duodenum, jejunum and ileum. The ratio of villus height to crypt depth of the three segments was increased when 1,000 U/kg xylanase was added but only the ratio in the ileum was increased by 500 U/kg xylanase (Table 4). A significant positive correlation between xylan level in wheat and the relative weights of the duodenum, jejunum and ileum has been reported by Steenfeldt (2001). Iji (1999) found that guar gum and xanthin gum significantly increased crypt depth of both the jejunum and ileum, suggesting that NSP may promote GIT cell turnover. The length of the villus is related to the absorption capacity of the enterocytes. Presence of short villi decreases the surface area for nutrient absorption. The epithelial cells of the villi originate in the crypt and a large crypt indicates fast tissue turnover and a high demand for new tissues (Parsaie, 2007). Any additional tissue turnover will increase nutrient requirements for maintenance and will therefore lower the feed efficiency of the animal. Shortening of the villi and

Table 5. Effects of different levels of xylanase on amylase and protease activities in the small intestinal digesta of broilers at 42 d (μ/mg prot.)

Parameters		Dietary enzyme levels (U/kg)				SEM	p-value
		0	500	1,000	5,000		
Amylase	Duodenum	62.31	66.99	65.21	61.53	5.25	0.708
	Jejunum	147.43	142.94	139.75	137.79	25.49	0.983
	Ileum	38.59	46.77	37.90	36.88	10.64	0.779
Protease	Duodenum	19,339.75	13,142.60	8,158.72	11,932.43	4,769.95	0.209
	Jejunum	32,550.80	34,209.22	28,802.13	30,078.37	12,017.25	0.968
	Ileum	8,114.83	16,632.17	15,102.99	12,412.01	5,911.18	0.529

deepening crypts can also lead to increased secretion in the GIT, diarrhea, reduced disease resistance and lower overall performance (Parsaie, 2007). Wang et al. (2005) reported a linear decrease in ileal relative length and relative weight on d 21 and d 42 as the level of enzyme supplementation (primarily xylanase and β-glucanase) increased in a broiler wheat-based diet. These studies indicated that NSP-degrading enzymes may counter the negative effects of NSP on intestinal morphology.

In this study, no influence was found on digestive enzyme activities in the small intestine when xylanase was added (Table 5). Engberg et al. (2004) found that whole wheat feeding resulted in lower amylase activity in the pancreatic tissue, whereas xylanase supplementation increased chymotrypsin and lipase activities of broilers. Qian et al. (2004) reported that the feeding of 0.2% β-glucosidase significantly increased intestinal amylase activity, while it had little effect on lipase and trypsin activities of broiler chicks fed corn-soybean meal. Exogenous enzymes release nutrients trapped by the fiber in plant cell walls, causing an increase of substrate in the GIT. The increased activities of digestive enzymes support the hypothesis that birds modulate specific enzymes according to substrate levels, rather than constantly maintaining high enzyme activities (Karasov and Hume, 1997). Almirall et al. (1995) reported that barley reduced amylase and lipase activities in small intestine contents, and that β-glucanase addition increased these activities in broiler chicks, along with a reduction in the intestinal viscosity. However, no changes were observed in adult birds, except for lipase, suggesting that adult birds appear to be able to cope with the intestinal viscosity and digestive enzyme activities are less affected by the diet in adult birds than in young birds (Almirall et al., 1995). In our study, the effects of xylanase on digestive enzyme activities were determined on broilers of 42 d. Thus, the absence of effects may also be associated with the age of broilers.

No effects were observed on the concentration of blood glucose. Gao (2001) reported that xylanase supplementation did not affect plasma glucose concentration, but significantly increased the level of glucose in digesta which indicated that, although the digestion of starch was improved by xylanase, the absorption of glucose was not affected. It is possible that birds modulate glucose absorption to an appropriate level for the needs of metabolism, rather than maintaining a constantly high level of blood glucose. So the results of the present study may be a consequence of the interaction between absorption and metabolism.

The concentration of blood uric acid can accurately reflect the state of protein metabolism and balance of amino acids, and the concentration is low when urea synthesis is reduced by improvement of dietary amino acid profile (Borg et al., 1987). However, the concentration of blood uric acid was not influenced by xylanase addition (Table 6). The possible reason was that amino acid profile in the experimental diets was suitable, and thus an improvement in the utilization of amino acids was not observed.

The growth of birds is modulated by the concentration of hormones such as thyroid hormones, GH, insulin and IGF-I. A close relationship between the somatotropic and thyrotropic axis in regulation of growth and development of broiler chickens has been found to play an important role in poultry growth (Cogburn et al., 1995). Gao et al. (2008) reported that xylanase supplementation to wheat-based diets increased the concentration of blood IGF-I and insulin of 21-day-old broilers, which indicated that enhanced digestion and absorption of nutrients, caused by the the enzyme supplementation, could have an effect on hormone concentrations. In our study, the concentration of insulin and IGF-I was not affected by xylanase supplementation (Table 6). Wang (2004) found that the concentrations of blood thyroxine (T_4), T_3, thyroid stimulating hormone (TSH) and GH were not affected by the supplementation of xylanase to wheat-based diets. The mechanism of exogenous enzyme on hormone regulation is complicated and requires the further study.

In addition, in the present study the excess addition of 5,000 U/kg xylanase had no negative effects on performance, blood hormones, intestinal morphology, microflora and digestive enzyme activities in broilers fed wheat-based diets. The enzyme used in their study was a

Table 6. Effects of different levels of xylanase on blood parameters of broilers

Parameters	Dietary enzyme levels (U/kg)				SEM	p-value
	0	500	1,000	5,000		
21 d						
Glucose (mmol/L)	15.61	15.59	16.61	15.13	1.56	0.812
Uric acid (mmol/L)	0.40	0.42	0.48	0.34	0.09	0.506
Insulin (μIU/ml)	24.79	22.64	20.15	22.79	3.16	0.551
IGF-I (μIU/ml)	43.74	41.36	39.73	41.54	2.69	0.539
42 d						
Glucose (mmol/L)	14.74	15.03	14.06	14.99	1.08	0.792
Uric acid (mmol/L)	0.14	0.18	0.21	0.18	0.07	0.800
Insulin (μIU/ml)	16.49	17.88	16.42	17.25	2.55	0.931
IGF-I (μIU/ml)	41.60	41.89	41.23	38.51	2.16	0.401

mixture of digestive (protease and amylase) and non-digestive (xylanase, β-glucanase, pectinase, cellulase and cellobiose) enzymes. Thus, we suggest that the mechanisms of digestive and non-digestive enzymes may not be similar and more studies should be conducted to investigate these mechanisms.

In conclusion, supplementation of xylanase improved the feed:gain ratio of broilers fed wheat-based diets. Supplementing 500 U/kg and 1,000 U/kg xylanase was beneficial to the morphology of the small intestine. Blood hormones, intestinal microflora and digestive enzyme activities of the intestinal digesta were not affected by xylanase supplementation. Furthermore, excess supplementation of xylanase did not result in further improvement or negative effects on those parameters tested in broilers.

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