Properties of *Aspergillar* Xylanase and the Effects of Xylanase Supplementation in Wheat-based Diets on Growth Performance and the Blood Biochemical Values in Broilers

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ABSTRACT : Three experiments were conducted to study the property of xylanase and the effects of xylanase in wheat-based diets on growth performance of broilers, respectively. Experiment 1 was performed *in vitro* to evaluate the effect of different pH and temperature on xylanase activity, and to evaluate the enzymic stability under different conditions. The results indicated that the optimum temperature and pH for xylanase activity were 50°C and 4.5, respectively. The activity of enzyme solution was reduced rapidly after the treatment of water bath above 60°C for 10 min. The enzyme was relatively stable at pH 3.5 to 8.0 and deteriorated when incubated at pH below 3.5. In Experiment 2, a total of 378 d-old male Arbor Acres broilers were randomly distributed to 7 different treatments with 6 replicates (9 birds) in each treatment. The treatments were as follows: (1) corn based diet (CS), (2) wheat based diet (WS), (3) WS+ 0.05% xylanase, (4) WS+0.15% xylanase, (5) WS+0.25% xylanase, (6) WS+0.35% xylanase, (7) WS+0.45% xylanase. The results showed that the body weight and feed/gain ratio of the broilers fed wheat-based diets have been significantly improved ($p<0.05$) compared to that fed corn-based diet in the first 3 wk. With regard to the wheat-based diets, the xylanase supplementation had a tendency to improve the growth performance in first 3 wk. After 3 wk, no significant difference ($p>0.05$) was found among all these different treatments. The supplementation of xylanase and the type of diets did not affect the feed intake but increased the concentration of triglyceride in serum. In Experiment 3, a total of 360 d-old male Arbor Acres broilers were assigned to 30 groups with 12 birds in each group randomly. These groups were then randomly distributed to 5 different treatments with 6 replicates within each treatment. The broilers of each treatment were fed one of the diets as follows: (1) Corn based diet, (2) White wheat based diet (WW) (3) White wheat based diet+0.25% xylanase, (4) Red wheat based diet, (5) Red wheat based diet+0.25% xylanase. The results showed that the body weight and feed/gain ratio had been significantly improved ($p<0.05$) by xylanase supplementation in the first 2 or 3 wk. The effect of xylanase in red wheat diet is a little higher than that used in white wheat diet. From the results of the present experiments, it can be concluded that the supplementation of *Aspergillar* xylanase can improve the performance of the broilers fed the wheat-based diet. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 1 : 66-74)***

Key Words : *Aspergillar Xylanase, Performance, Broilers*

INTRODUCTION

Wheat is an important feed ingredient in poultry diets. However, it contains relatively high levels of arabinoxylans/ pentosans at concentrations of 50-80 g/kg dry matter (Annison, 1991), making these the main non-starch polysaccharide (NSP) component. Pentosans are the main constituent in the endosperm cell wall of wheat, rye and triticale (Mares and Stone, 1973; Henry, 1985), and are assumed to have antinutritional effects on broiler chickens (Annison, 1991; Choct and Annison, 1990, 1992a). Bedford et al. (1991) and Bedford and Classen (1992) demonstrated that a significant negative relationship exists between intestinal viscosity and feed conversion efficiency in chickens fed on rye and wheat based diets. Choct and Annison (1992b) confirmed that isolated wheat pentosans contributed to the negative effects on broiler performance by means of increased intestinal viscosity which leads to reduced absorption of nutrients.

Feed enzymes have come to be regarded by many nutritionists as being a necessary ingredient for formulating poultry rations (Choct, 2001). Numerous investigators have shown that the addition of commercial xylanase enzymes and others NSP degrading enzymes to rye-based broiler diets (Grootwassink et al., 1989; Bedford et al., 1991; Bedford and Classen, 1993, Dänicke et al., 1999; He et al., 2003) and wheat-based diets (Annition, 1992; Preston et al., 2001; Peng et al., 2003; Selle et al., 2003) can largely eliminate the antinutritive effects of pentosans in chickens and in pigs (Li et al., 2004).

The objectives of the present experiments were to study the properties of *Aspergillar* xylanase produced in our laboratory by solid-state fermentation, to conduct a comparative trial to evaluate the efficacy of the xylanase, and to examine the effects of xylanase supplementation in wheat-based diets on the growth performance of broilers.

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MATERIALS AND METHODS

Experiment 1

Crude xylanase preparation

i) *Microorganism* : The xylanolytic strain used in this experiment was *Aspergillus sulphureus*. It was originally isolated from soil and was identified by the Institute of Microbiology, Chinese Academy of Sciences.

ii) *Cultivation* : The strain was cultivated in a slant medium containing 0.3% $NaNO_3$, 0.05% KCl, 0.1% K_2HPO_4, 0.001% $FeSO_4$, 0.05% $MgSO_4$, 3% sucrose and 2.0% agar in distilled water at an initial pH of 7.0.

iii) *Xylanase production in solid-state fermentation* : Each 250 ml Erlenmeyer flask containing 15 g of wheat bran and 15 ml mineral salt solution with 2% K_2HPO_4 and 3% $(NH_4)_2SO_4$ was autoclaved at 1.05 kg per cm^2 for 30 min, cooled , inoculated with 5% (v/w) inoculum (3 d-old in slant medium) and incubated at 28°C. The flasks were gently tapped intermittently to mix the contents. The incubator was humidified using a tray with sterile distilled water (relative humidity 60%-65%). After 72 h cultivation, the flasks were removed and the contents were dried in an oven at 45°C for 10 h. The dried product was comminuted using a Willey mill equipped with 1 mm screen.

Enzyme extraction : The enzyme produced by *Aspergillus sulphureus* using solid-state fermentation was extracted with distilled water or 10 mM sodium citrate buffer at pH 4.5 (1 g wheat bran with 100 ml) and agitated in a constant temperature water bath (Pulsator HZ-9202S, manufactured by Taicang City) at a speed of 80 rpm/min for 30 min. After shaking, the enzyme extraction was filtrated using Whatman filter paper and further diluted using sodium citrate buffer at pH 4.5. The supernatant was stored for subsequent analysis of enzyme activity.

Xylanase assay : One unit (U) of xylanase activity is defined as the amount of enzyme that releases 1 μmol xylose from a 1% xylan (Oat spelt xylan, X0627, Sigma Chemical Co., 3050 Spruce St. Louis, MO 63103, USA) solution in one min. The enzyme assay was performed in triplicate with analytical grade reagents. Xylanolytic activity of the enzyme supernatant was determined according to the method of Miller (1959).

One ml of clear supernatant was transferred to a 20 ml test tube and placed in a 40°C water bath for 2 min. Then, 1 ml of 1% xylan substrate solution (Oat spelt xylan, Sigma, in solution of sodium citrate buffer at pH 4.5) was added, stirred and the tube was incubated for 10 min. After incubation, the released sugars were quantified by adding 2 ml 3,5-dinitro-salicylic acid solution (DNS) and placed the test tube in a boiling water bath for 5 min to stop the hydrolyzing reaction. The tube was removed, cooled and then 10 ml distilled water was added to the tube and stirred. A blank was prepared with the addition of 2 ml DNS prior to the 1% xylan using the same procedure. The absorbance was measured at 540 nm using the 752Z raster Spectrophotometer (Beijing Optical Instrument Factory, Beijing, China, 101149). The concentration was calculated based on a xylose standard curve.

Effect of different pH on enzyme activity : The enzyme was diluted using distilled water with the dilution rate of 0.5 g/100 ml and filtered using Whatman filter paper. One ml clear supernatant was then transferred to a 20 ml test tube and further diluted with 19 ml of a sodium citrate buffer (0.5 M) with different pH (3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0 and 8.0, respectively). The pH was ensured to be constant. The Oat spelt xylan substrate was dissolved to form a 1% solution in corresponding buffer to ensure the pH was equal to that of the enzyme dilution. The enzyme was then assayed using the corresponding substrate. The total amount of sugars released by the enzyme was determined at different pH (3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0 and 8.0, respectively) in a 40°C water bath for 10 min to evaluate the effect of pH on the hydrolysis reaction.

Effect of different temperatures on enzyme activity : The enzyme was diluted with sodium citrate buffer at pH 4.5 with the dilution rate of 0.5 g/100 ml and filtered using Whatman filter paper. It was then further diluted 20 times using the sodium citrate buffer. The Oat spelt xylan substrate was dissolved to form a 1% solution in corresponding buffer and then the diluted enzyme solution was used to determine the xylanase activity. The total amount of sugars released by the enzyme at different temperatures (30, 40, 50, 55, 60, 65 and 70°C, respectively) during a 10 min incubation in a water bath was determined to examine the effect of temperature on the hydrolysis reaction.

Thermostability of the enzyme : The dried enzyme product was diluted using sodium citrate buffer, pH 4.5 (0.5 g/100 ml). After shaking, the enzyme extraction was filtered using filter paper and further diluted (20 times) using the same buffer. From the clear enzyme supernatant, 1 ml was transferred to a 20 ml test tube and placed in water bath of different temperatures (30, 40, 50, 60, 65, 70 and 80°C) for 10 min. After the appropriate incubation time, the tubes were removed, cooled and the survival enzyme activity was determined according to the assay described previously to evaluate the thermostability when the enzyme is in solution.

Stability under different pH : The enzyme product was diluted using distilled water with the dilution rate of 0.5 g /100 ml and filtered using Whatman filter paper. The clear enzyme supernatant was further diluted using sodium citrate buffers of different pH (2.0, 2.5, 3.0, 4.0, 4.5, 5.0, 6.0, 7.0 and 8.0) and then incubated in a 40°C water bath for 10 min. After incubation, the pH was adjusted immediately to 4.5 and then the survival enzyme activity was determined in conduction of pH 4.5, 40°C water bath for 10 min to

Table 1. Composition and nutritional levels of basal diets (%)

Ingredients	0-3 wk		3-6 wk	
Corn	-	53	-	56.64
Wheat	63.9	-	68.3	-
Soybean meal	26.27	37.15	22.5	34.2
Soybean oil	3	3	4.52	4.5
Fish meal	2.58	2.58	-	-
Limestone	1.25	1.37	1.35	1.5
Di-calcium phosphate	1.15	1.25	1.4	1.45
Salt	0.3	0.3	0.3	0.3
Premix[1]	1	1	1	1
Methionine[2]	0.2	0.22	0.2	0.22
Lysine[2]	0.35	0.13	0.43	0.19
Total	100	100	100	100
Nutritional levels				
ME, MJ/kg	12.3	12.3	12.6	12.6
Crude protein[3]	23.4	23.5	20.1	20.3
Calcium[3]	1.07	1.1	0.96	1.04
Available phosphorus[4]	0.45	0.45	0.37	0.37
Lysine[4]	1.23	1.23	1.06	1.06
Methionine[4]	0.56	0.56	0.4	0.4

[1] Premix provided the following per kg of complete diet: Vitamin A, 14,400 IU; Vitamin D_3, 2,000 IU; Vitamin E, 20 IU; Vitamin K_3, 1.5 mg; Vitamin B_1, 2.25 mg; Vitamin B_2, 9 mg; D-pantothenic acid, 10 mg; Niacin, 40 mg; Vitamin B_6, 4 mg; Biotin, 0.15 mg; Vitamin B_{12}, 0.03 mg; Choline chloride, 100 mg; Mn, 60 mg ; Zn, 75 mg ; Cu, 9 mg; Fe, 80 mg; Se, 0.3 mg; I, 0.35 mg.

[2] Amino acids were provided by Ajinomoto, Japan.

[3] Each value represents the mean of chemical analysis conducted in duplicate (as fed).

[4] Calculated values (as fed).

examine the enzyme stability after the treatment of solutions at different pH.

Experiment 2

Enzyme and activities : The enzyme used in this study was derived from the fermentation of *Aspergillar*, with xylanase activity of 2,000 unit/g. One unit of xylanase activity was defined as the amount of enzyme that liberated 1 μmol of xylose from 1% xylan at pH 4.5 and 40°C in 1 min.

Animals and treatments : A total of 378 d-old male Arbor Acres broilers were randomly assigned to 42 groups with 9 birds in each group. The groups were then randomly distributed to 7 treatments with 6 replicates within each treatment. The treatments were as follows: (1) corn based diet, (2) wheat-based diet (WS), (3) WS+0.05% xylanase, (4) WS+0.15% xylanase, (5) WS+0.25% xylanase, (6) WS+0.35% xylanase, and (7) WS+0.45% xylanase. The trial was conducted for 42 d.

Experimental diets : All diets were formulated to meet or exceed the minimum NRC (1994) requirements for broilers.

The composition and nutritional levels of the basal diets are shown in Table 1.

Bird management : The broilers were raised in three-layer cages, three broilers per cage. Each replicate consisted of 3 cages in one column. Temperature was maintained at 34°C-36°C using infrared lamps for the first two wk. The lighting schedule was as follows: 24 h light from 0 to 3 wk, and 20 h light from 3 to 6 wk. Mash-type feed and water were available *ad libitum*. All broilers were weighed individually at 21 d and 42 d. Feed intake and feed conversion were also recorded and calculated on a per replicate basis.

Serum biochemical values : At 21 d of age, one bird from each replicate was selected at random and an 8 ml sample of blood from the wing vein was taken and immediately centrifuged for 15 min (3,000 rpm). The serum samples were stored at -20°C until analyzed using the automatic Chemistry Analyzer (TECHNICON RA-1000^{TM}, Miles Inc., Diagnostics Division Headquarters, 511 Benedict Avenue, Tarrytown, New York 10591-5097 USA).

Xylanase activity in digesta supernatant : After the blood samples were taken, birds were sacrificed by cervical dislocation. Then, the abdomen was opened and the intestinal tract was exposed. The intestinal contents of the small intestine (entrance of bile ducts to ileo-ceco-colic junction) were collected. Digesta contents were placed directly in a centrifuge tube (10 ml) and immediately centrifugated at 3,000 g for 10 min. The supernatant was withdrawn after centrifugation and stored on ice until the xylanase activity was determined

To estimate xylanase activity in a complex sample (digesta supernatant), an agar diffusion method was used. The agar medium was made with 20 mmol sodium acetate buffer (pH 4.5) containing 10 mmol calcium chloride, 10 g/kg agar and 2 g/kg xylan (Oat spelt xylan, Sigma). After boiling and then cooling to 60°C, 10 ml medium was poured into petri dishes (9 cm diameter). Circular wells (8 mm diameter) were punched with a cork borer. Fifteen μl blank (buffer), samples and standards were added to each of the wells. The standards contained different concentrations of the enzyme as estimated by the previous method. Incubation at 40°C for 24 h was followed by flooding the surface of agar with congo red solution (1.0 g/kg). Then, the staining solution was discarded and the application wells were indicated by unstained circular area while the remainder was stained red. The lysis diameters were linear in relation to the log of the enzyme concentration.

Statistical analysis : Data were analyzed using a one-way ANOVA (SPSS 8.0). The differences among treatment means were compared using Duncan's multiple range test. The effect of enzyme in wheat-based diet was analyzed using the contrast procedure in one-way ANOVA.

Experiment 3

Birds and treatments : A total of 360 d-old male Arbor

Table 2. Composition and nutritional levels of basal diet (%)

Ingredients	0-3 wk		
Corn	-	-	56.15
White wheat	66	-	-
Red wheat	-	63	
Soybean meal	25.5	28.61	36.6
Soybean oil	2.5	2.5	1.5
Fish meal	1.5	1.5	1.5
Limestone	1.3	1.3	1.0
Di-calcium phosphate	1.5	1.5	1.85
Salt	0.3	0.3	0.3
Premix[1]	1	1	1
Methionine[2]	0.1	0.09	0.1
Lysine[2]	0.3	0.2	0
Total	100	100	100
Nutritional levels			
ME, MJ/kg	12.3	12.3	12.3
Crude protein[3]	22.4	22.5	22.5
Calcium[3]	1.07	1.07	1.1
Available phosphorus[4]	0.45	0.45	0.45
Lysine[4]	1.23	1.23	1.23
Methionine[4]	0.56	0.56	0.56

[1] Premix provided the foling per kg of complete diet: Vitamin A, 14,400 IU; Vitamin D_3, 2,000 IU; Vitamin E, 20 IU; Vitamin K_3, 1.5 mg; Vitamin B_1, 2.25 mg; Vitamin B_2, 9 mg; D-pantothenic acid, 10 mg; Niacin, 40 mg; Vitamin B_6, 4 mg; Biotin, 0.15 mg; Vitamin B_{12}, 0.03 mg; Choline chloride, 100 mg; Mn, 60 mg; Zn, 75 mg; Cu, 9 mg; Fe, 80 mg; Se, 0.3 mg; I, 0.35 mg.

[2] Amino acids were provided by Ajinomoto, Japan.

[3] Each value represents the mean of chemical analysis conducted in duplicate (as fed).

[4] Calculated values (as fed).

Acres broilers were randomly assigned to 30 groups with 12 birds in each group. These groups were then randomly distributed to 5 different treatments with 6 replicates within each treatment. The broilers were fed one of following diets: (1) Corn based diet. (2) White wheat based diet (WW), (3) White wheat based+0.25% xylanase, (4) Red wheat based diet. (5) Red wheat based diet+0.25% xylanase.

Experimental diets : The diets were formulated to meet or exceed the minimum NRC (1994) requirements for broilers. The composition and nutritional levels of the basal diets are shown in Table 2. The xylanase enzyme used in this experiment is the same as that used in Experiment 2.

Birds management : Broilers were raised in three-layer cages, 4 broilers per cage. Each replicate included 3 cages in one column. The temperature was kept within 34°C-36°C using infrared lamps for the first 2 wk. The light was provided 24 h daily from 0 to 3 wk. Mash-formed feed and water were available *ad libitum*.

All broilers were weighed individually at 14 d and 21 d. Both feed intake and feed conversion were determined on a per replicate basis on d 14 and d 21.

Statistical analysis : Data were analyzed using a one-way ANOVA (SPSS 8.0) to find the differences among treatment. The effects of enzyme and wheat type were analyzed using the procedure of 2-factors ANOVA.

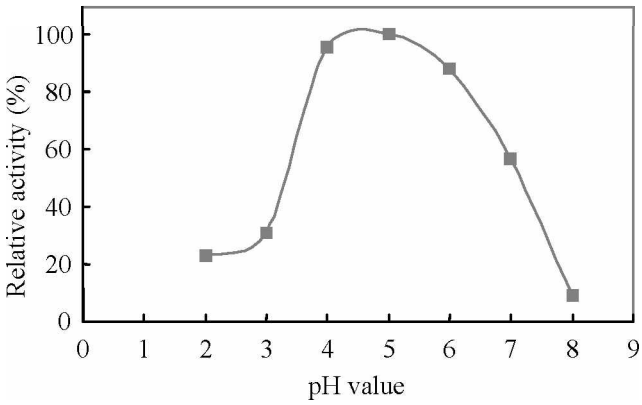

Figure 1. Effect of different pH values on enzyme activity.

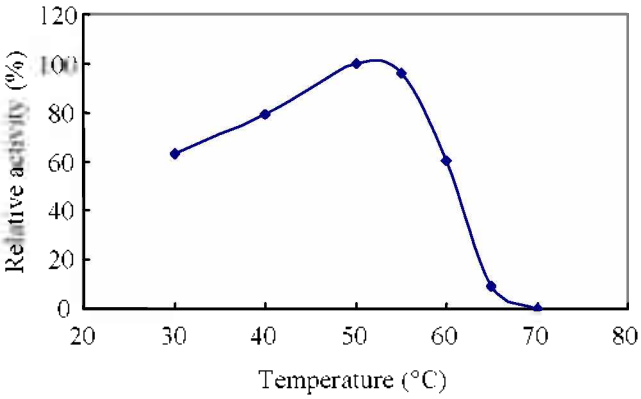

Figure 2. Effect of different temperature on enzyme activity.

RESULTS

Experiment 1

Effect of different pH on enzyme activity : The highest activity of xylanase occurred at pH 4.5 and at 40°C (Figure 1). The enzyme activity was maintained greater than 80% of its highest activity from pH 4.0 to 6.0. At pH 3.0, the activity was only 35% of the highest measured activity. The pH in the gastrointestinal tract of the broilers ranged from 2.8 to 7.2. Therefore, in most sections of the gut, this enzyme will retain xylanolytic activity.

Effect of the temperature on the activity : The influence of the temperature on xylanase activity is shown in Figure 2. The optimum temperature for xylanase activity was 50°C. The enzyme activity was about more than 60% of the highest activity when the temperature ranged from 30°C to 60°C. When the temperature increased to 60°C, the xylanase activity rapidly declined. At 70°C, the enzyme lost activity completely. The temperature of the gut in chicks is approximately 40°C. Therefore, our enzyme product can exert xylanolytic activity in the range of temperatures of a broiler's gut.

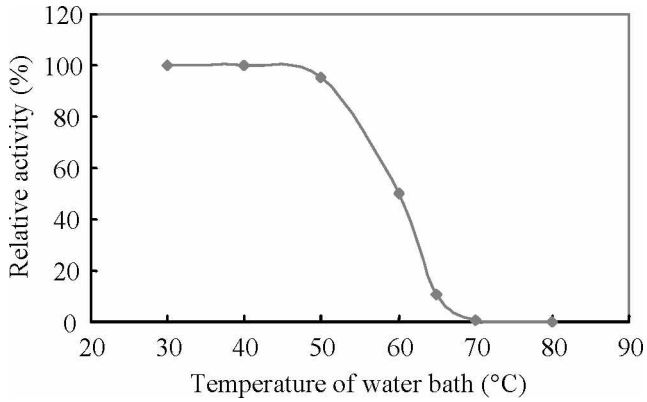

Figure 3. Enzyme thermorstability in solution.

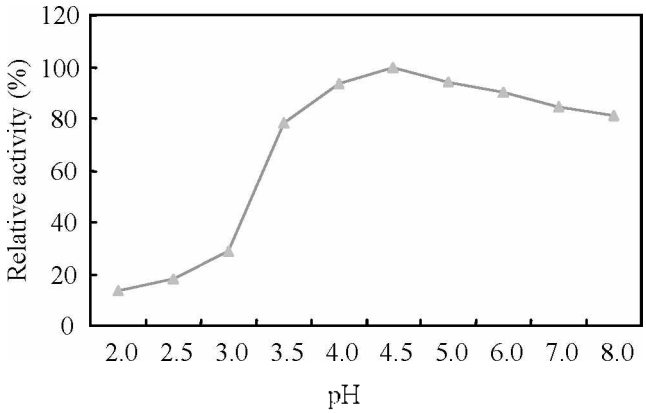

Figure 4. Enzyme activity after treatment using different Ph solution.

Thermostability of the enzyme : The thermostability of the enzyme when in solution is shown in Figure 3. Little enzyme activity was lost when the enzyme solution was incubated be 50°C. However, when the temperature was increased to 60°C and 65°C, the activity was reduced approximately 50% and 90%, respectively.

Stability under different pH : The enzymic stability at different pH is shown in Figure 4. After incubation in solutions of pH from 3.5 to 8.0 for 10 min, the enzyme retained more than 80% of its highest activity. When the pH was below 3.0, the activity was reduced about 70%. Results indicate that the enzyme was not impaired by the pH changed from 3.5 to 8.0. However, the enzyme activity was damaged by the low pH solutions (<pH 3.0) and the activity was not recovered.

Experiment 2

Body weight : The results of the growth performance are shown in Table 3. The body weight of the birds fed the corn-based diet was significantly lower ($p<0.05$) than those fed wheat-based diets in the first 3 wk. After 3 wk, no significant differences ($p>0.05$) were found among the treatments for body weight. Within those birds fed wheat-based diets, numerical improvements in body weight were found, but they were not significant.

The supplementation of xylanase and the dietary grain source did not alter feed intake. The feed/gain ratio of the broilers fed wheat-based diets was significantly improved ($p<0.05$) compared with that of those fed corn-based diets in first 3 wk. After 3 wk, no differences ($p>0.05$) were found among the treatments. No difference due to the supplementation of xylanase was detected in the feed/gain ratio of broilers consuming the wheat-based diets.

Serum biochemical values : Effects of the enzyme on serum biochemical values in broiler are shown in Table 4. The total protein and albumin in the serum were not affected by the enzyme supplementation. There were significant differences ($p<0.01$) in uric acid and triglyceride values in serum among different treatments. Enzyme supplementation increased the concentrations of triglycerides in serum. The concentrations of triglycerides of birds consuming WS+0.25% xylanase were highest among all treatments.

Xylanase activity in digesta : The xylanase activity of

Table 3. Effects of different treatment on the growth performance of broilers

Items	CS[1]	WS[2]						SEM	P value	
		+0%	+0.05%	+0.15%	+0.25%	+0.35%	+0.45%		ANOVA	Contrast[3]
Body weight, g										
1 d	42	41	42	41	41	41	41	0.244	0.811	0.165
21 d	758[a]	784[b]	794[b]	805[b]	795[b]	801[b]	806[b]	8.262	0.003	0.086
42 d	1,873	1,847	1,895	1,898	1,885	1,876	1,861	33.77	0.935	0.332
Average daily feed intake, g										
1-21 d	48	48	48	48	48	48	48	0.035	0.507	0.669
22-42 d	106	106	108	107	107	105	109	3.067	0.967	0.414
1-42 d	76	76	76	76	77	77	78	1.185	0.943	0.382
Feed/gain ratio										
1-21 d	1.42[a]	1.38[b]	1.36[b]	1.34[b]	1.36[b]	1.35[b]	1.34[b]	0.015	0.003	0.070
22-42 d	1.89	2.07	1.93	1.95	1.99	1.99	2.04	0.070	0.610	0.247
1-42 d	1.74	1.80	1.72	1.73	1.75	1.76	1.76	0.036	0.762	0.134

[a,b] Means within a row lacking a common superscript differ ($p<0.05$).
[1] corn-based diet, [2] wheat-based diet with different addition of xylanase.
[3] ws+0% vs. ws−0.05%, ws−0.15%, ws+0.25%, ws+0.35%, ws−0.45%.

Table 4. Effects of enzyme on serum biochemical values in broiler and the xylanase activity of digesta supernatant

Item	WS[1]						CS[2]	SEM	P value	Contrast[3]
	0	0.05%	0.15%	0.25%	0.35%	0.45%				
Uric acid (mg/100 ml)	6.983^{ab}	3.950^{a}	5.883$^{a\,b}$	13.517^{c}	8.350^{abc}	11.683^{bc}	5.020^{a}	1.895	0.008	0.414
Triglyceride (g/100 ml)	62.333^{ab}	55.667^{a}	83.000^{abc}	144.667^{d}	128.667^{cd}	116.833^{bcd}	71.600^{ab}	17.908	0.005	0.031
Total protein (g/100 ml)	2.345	1.853	2.298	3.03	2.511	3.094	2.344	0.3489	0.176	0.578
Albumin (g/100 ml)	0.9	0.767	0.967	1.183	1.033	1.133	0.92	0.1422	0.418	0.453
Enzyme activity of digesta supernatant										
Diameters (mm)	-	14.7	16.2	17.8	18.8	20.3	-	-	-	-
Estimative activity (U/ml)	-	0.1442	0.23	0.3864	0.5276	0.82	-	-	-	-

[a, b, c, d] Means within a row lacking a common superscript differ ($p<0.05$).
[1] wheat-based diet with different addition of xylanase. [2] corn-based diet.
[3] ws+0% vs. ws−0.05%, ws−0.15%, ws+0.25%, ws+0.35%, ws−0.45%.

Table 5. Effects of different treatment on the growth Performance of Broilers*

Treatment	0-2 wk			0-3 wk		
	Body weigh, g	ADFI, g	F/G	Body weigh, g	ADFI, g	F/G
Corn	398	35	1.398	728	46	1.403
RW	392	36	1.435	689	46	1.483
RW+E	416	36	1.370	728	46	1.402
WW	400	35	1.382	722	46	1.410
WW+E	414	35	1.320	730	46	1.400
P value						
One-way ANOVA	0.211	0.391	0.150	0.077	0.574	0.071
Main effects	0.115	0.212	0.042	0.020	0.624	0.023
Enzyme	0.042	0.711	0.047	0.023	0.495	0.029
Wheat	0.739	0.088	0.100	0.072	0.495	0.069
Enzyme+wheat	0.582	0.486	0.956	0.110	0.819	0.082

WW: white wheat, RW: red wheat, E: enzyme.

intestinal digesta from different treatment is shown in Table 3. It also can be clearly seen in the agar plate for xylanase activity estimation (Figure 5). The increase of xylanase activity in digesta was linear with respect to the amount of enzyme supplementation in the diets. These results indicate that the enzyme can exert its effect in these segments of the gut and that the small intestine had suitable environment for the enzyme activity.

Experiment 3

Body weight : The results of the growth performance are shown in Table 5. The effect of xylanase used in red wheat diet is better than that used in white wheat diet ($p>0.05$). The supplementation of xylanase in red-wheat based diet increased the body weight by 6.1% in two wk ($p<0.05$). The type of wheat had no significant effect on the growth performance. At 3 wk of age, the xylanase in red-wheat based diet increased the body weight by 5.66% ($p<0.05$).

Feed intake and feed/gain ratio : The feed intake and feed/gain ratio of different treatments is shown in Table 5. No significant difference for average feed intake among treatments was found. The effects on growth performance of xylanase in red-wheat based diet was greater than that used in white-wheat based diet ($p>0.05$). The supplementation of xylanase in red-wheat based diet improved the feed/gain ratio by 4.74% in two wk ($p<0.05$). The type of wheat had no significant effect on the feed/gain ratio. At the end of 3 wk, the supplementation of xylanase in red-wheat based diet improved the feed/gain ratio by 5.78% ($p<0.05$) significantly. However, there was no significant interaction between wheat and xylanase ($p>0.05$).

DISCUSSION

Wheat arabinoxylans are similar to those of rye consisting of a (1-4)-β-xylan chain with α-arabinose substituted at the O_2- and O_3-positions (Fincher and Stone, 1986). It is generally conceded that the detrimental effect of such kinds of NSP is associated with the viscous nature of these polysaccharides, their physiology and morphological effects on the digestive tract, and the interaction with the microflora of the gut (Choct, 2001). The viscous polysaccharides, such as pentosans and β-glucans, may directly form a complex with digestive enzymes and reduce their activity (Edwards et al., 1988). The NSP supplementation to diets can increase the endogenous secretion of water, proteins, electrolytes and lipids (Low, 1989). This will increase the metabolic cost. The study of Angkanaporn et al. (1994) indicates that the effect of wheat arabionxylans on the apparent protein digestibility is mainly due to increased endogenous secretions of amino acids at low levels of inclusion, while at high levels a direct

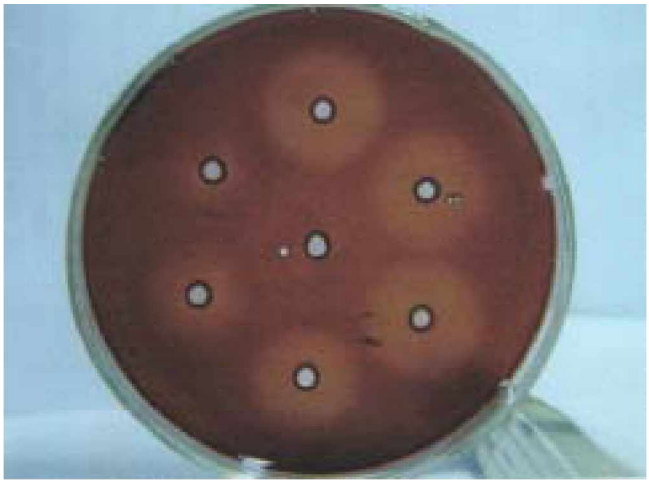

Figure 5. Xylanase activity of digesta supernatant in small intestine. Numbers represented the treatments: 1 wheat based diet; 2 wheat based diet+0.05% xylanase; 3 wheat based diet+0.15% xylanase; 4 wheat based diet+0.25% xylanase; 5 wheat based diet +0.35% xylanase; 6 wheat based diet+0.45% xylanase; 7 corn based diet.

inhibition of protein breakdown and/or absorption occurs. Other experiments have shown that viscous polysaccharides can increase the retention time of digesta (Gohl and Gohl, 1977), which may result in increased microbial activity within the intestine and change the microbial ecosystem. Choct et al. (1996) demonstrated that the addition of soluble NSP in broiler diets significantly elevated the fermentation activity in the small intestine. However, *in vivo* depolymerization of soluble NSP using the enzyme almost totally overcame this problem. An increased microbial load can increase animal requirements for maintenance of the integrity of the small intestine (Abrams et al., 1963; Lesher et al., 1964). The negative effect of excessive microbial growth has been implicated from the demonstration that the nutritive value of rye is greatly improved by dietary antibiotics (MacAuliffe and McGinnis, 1971; Misir and Marquardt, 1978a,b). Most of these detrimental effects of NSP, such as wheat arabinoxylans and β-glucans, were related to the viscosity of digesta.

Wheat pentosans appear to elicit antinutritional activity predominantly through increasing the viscosity of digesta (Choct and Annison, 1992a). Some experiments have indicated that the enzyme treatment reduced the viscosity of digesta and thus improved the broilers performance (Annition, 1992; Preston et al., 2001). Bedford et al. (1991) confirmed that the viscosity of both fore and hind gut contents was significantly reduced with pentosanase supplementation and that the weight gain and feed conversion efficiency of the broilers were correlated with fore but not hind gut viscosity. They also found that the viscosity of gut samples was best described by the concentration of carbohydrate complexes with an average molecular weight greater than 500,000 Da and that the pentosanase treatment reduced lumenal concentration of these complexes and improved the growth performance. Together, these experimental results supported the hypotheses that viscosity can contribute to the anti-nutritive effect in broiler diets.

The antinutritional activity of wheat arabinoxylans has been demonstrated through increasing digesta viscosity and by interference of the diffusion and movement of the solutes and the interaction between the solutes and the enzyme in the gut. This viscous polysaccharide may also have direct or indirect effects on physiology, morphology and the microflora of the gut. Therefore, the addition of the xylanase enzyme may restore the normal environment of the gut through reducing the viscosity of the digesta, leading to improved broiler performance.

The diet may also influence the effects of the xylanase. There were no significant difference in chicken performance when iso-nitrogenous and iso-energetic diets base on increasing levels of wheat (cv. Holme) and triticale (cv. Lasko) were compared with a maize diet (Pettersson, 1987). In Experiment 2, the xylanase activity of digesta supernatant was linear in relation to the amount of enzyme supplementation in diets (shown in Table 3 and Figure 5). This indicates that the xylanase exerted an effect in the gut. We found no significant difference in chicken performance among the treatments of corn-based diets and wheat-based diets at the end of the trial. In those birds fed a wheat-based diet in Experiment 2, the xylanase supplementation had no significant effect on the body weight and the feed/gain ratio. But it tended to improve the performance of chicks from 0 to 3 wk of age. In Experiment 3, however, the effect of xylanase used in red-wheat based diet is better than that used in white wheat based diet ($p<0.05$). The body weight and feed/gain ratio of broilers had been significantly improved ($p<0.05$) by xylanase supplementation in the first 2 or 3 wk. These results indicate that broilers can tolerate small increases in digesta viscosity without a reduction in growth performance. The response to xylanase supplementation in a wheat-based diet indicated that the anti-nutritional effect of the endosperm cell wall pentosans was different in different type of wheat.

Not only can the diet impact the effects of xylanase enzyme but also the age of the broilers. As the bird ages, the digestive system and gut microflora becomes more established, allowing the bird to adapt to the environment. In addition, digesta viscosity decreases as birds get older (Dusel et al., 1998; Steenfeldt et al., 1998) and older birds can better cope with low-ME wheats (Rogel et al., 1987). In the present trial, the results indicated that the effect of the enzyme was reduced in older chicks. Veldman and Vahl (1994) also found the similar phenomena. When they compared the effect of diets containing 15%, 30%, 45% or

50% of wheat with the diet containing 50% wheat plus enzyme. they found significant differences of feed intake and feed/gain ratio in the first 14 d. However. the difference decreased from 14 to 42 d and the digesta viscosity decreased. These results indicate that the age of the birds may influence the effects of the enzyme to some extent.

In general. xylanases are specific for the internal β-1.4 linkages of polymeric xylan and are designated as endoxylanases. Therefore. the supplementation of xylanase can degrade the wheat pentosans and reduce the viscosity of the digesta. This action can elevate the nutrient value of pentsoan-rich ingredients and contribute to improving the performance of broilers fed a pentsoan-rich diet. The concentration of pentosans in wheat was lower than that in rye (50-80 vs. 80-100 g/kg dry matter). Therefore. the effects of the xylanase supplementation in the rye-based diet would be more evident than those in the wheat-based diet. The enzyme can exert a greater effect when the ration has enough corresponding substrate.

IMPLICATIONS

From the results of the present experiments. it can be concluded that the *Aspergillar* xylanase keep its activity in broliers' gut and can exert its effect in these segments of the gut. The supplementation of *Aspergillar* xylanase can improve the performance of the broilers fed the wheat-based diet.

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