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Effects of Xylanase on Growth and Gut Development of Broiler Chickens Given a Wheat-based Diet

Y. Yang, P. A. Iji*, A. Kocher[1], L. L. Mikkelsen and M. Choct[2]

School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia

ABSTRACT : To study the working mechanisms for non-starch polysaccharidases to improve the growth performance of broiler chickens, a 21-day feeding trial was conducted. Two dietary treatments were included: 1) wheat diet (the control); 2) wheat+xylanase diet (xylanase, Allzyme PT, Alltech, Kentucky, USA). There were 8 replicates with 8 birds each for each treatment and the experimental diets were given to birds from hatch. Feed intake and body weight were measured on days 7 and 21. At the same ages, samples were taken for the determination of selected groups of luminal and mucosa-associated bacteria, mucosal morphology, brush-border membrane (BBM) bound enzyme activity and ileal nutrient digestibility. The xylanase supplement increased ($p<0.05$) body weight gain (BWG) and improved feed conversion ratio (FCR) at the end of the experiment but protein and starch digestibilities were not affected ($p>0.05$) by xylanase. Up to day 7, xylanase increased the counts of *C. perfringens* in the ileum and total anaerobic bacteria (TAB) in the caeca ($p<0.05$, $p=0.07$, respectively). By day 21, the counts of ileal lactobacilli ($p<0.05$) and TAB ($p=0.07$) were lower in birds given the xylanase-supplemented diet than in those on the control diet. No significant differences were observed in the counts of mucosa-associated lactobacilli and coliforms between xylanase treatment and the control at both ages. Villus height at the jejunum was not affected ($p>0.05$) by the supplement but crypt depth at the same site was reduced at day 7. Also, xylanase tended to increase the concentration of BBM protein ($p = 0.09$) and the specific activity of sucrase ($p = 0.07$) at day 21. (**Key Words :** Xylanase, Growth Performance, Nutrient Digestibility, Gut Microflora, Intestinal Mucosal Morphology, Specific Activity of BBM Bound Enzymes)

INTRODUCTION

The inclusion of non-starch polysaccharidases, such as xylanases and/or glucanases, to improve the performance of broiler chickens fed diets based on wheat and barley has become a routine practice (Ravindran, 2006). The underlying mechanisms causing the improvements have been extensively examined in the past fifteen years but still not fully understood. Besides reduction in the viscosity of intestinal contents, the growth-promoting effects of enzymes(s) also appear to be partly related to the modulation of gut microflora (Choct et al., 1996; Bedford, 2001). A few reports have indicated that non-starch polysaccharidase supplement could alter the development of gut microflora, including those attached to the mucosa (Vahjen et al., 1998; Hubener et al., 2002); however, there is limited information on the link among the enzyme(s), luminal as well as mucosal microflora of the small intestine, and gut function in broiler chickens (Danicke et al., 1999). The present work was designed to particularly determine the influence of xylanase on the development of selected groups of intestinal luminal and mucosa-associated bacteria, mucosal morphology and enzyme activity and nutrient digestibility to further explore the underlying mechanisms of growth-promoting effects of non-starch polysaccharidases in a wheat-based diet.

MATERIALS AND METHODS

Experimental design and diets

A wheat basal diet, with the composition shown in Table 1 and 2, was used. Two dietary treatments were included 1) no supplementation (None, the control) or, 2) xylanase (1,000 XU/kg diet, Allzyme PT, Alltech, Kentucky, USA). Acid-insoluble ash (AIA) was included at 0.8% in the diets at the expense of wheat, as an indigestible marker. The experiment was approved by the Animal Ethics Committee

* Corresponding Author: P. A. Iji. Tel: +61-2-6773-2082, Fax: +62-2-6773-3922, E-mail: piji@une.edu.au

[1] Alltech Biotechnology P/L 68-70 Nissan Drive, Dandenong South, Vic 3175 Australia.

[2] Australian Poultry Science CRC, PO Box U242, University of New England, Armidale NSW 2351, Australia.

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Table 1. Ingredients (g/kg) of basal diet and nutrient composition (g/kg)

Ingredients	Wheat diet
Wheat	624.0
Soybean meal	265.0
Meat meal	50.0
Limestone	9.90
Dicalcium phosphate	3.0
Lysine-HCl	2.8
Methionine	1.9
Salt	0.5
Sodium bicarbonate	3.3
Choline chloride	3.0
Sun oil	34.8
L-threonine	0.2
Vitamin and mineral premix[1]	2.0
Chemical composition	
ME (MJ/kg)	12.33
Crude protein	230.00
Crude fibre	29.00
Crude fat	55.00
Lys	13.00
Met+cys	9.00
Ca	10.00
P available	4.20
Na	1.80
Cl	2.00
Analysed composition	
Crude protein	234.00
Starch	415.00

[1] Supplied per kg of diet (mg): vitamin A (as all-trans retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K3, 2 mg; thiamin, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B12, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-calcium pantothenate, 12 mg; folic acid, 2 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg; and Mo, 1 mg.

of the University of New England. Since the main aim of the study was to examine the interaction between xylanase, gut microflora, mucosal morphology and function of birds during the early growth period, the experiment was conducted within the first three weeks after hatch; therefore, the examination of growth performance was conducted during these three weeks rather than a full growth period (a five-week time length).

Birds, housing and management

One hundred and twenty-eight (128) day-old birds were obtained from a local hatchery and randomly allocated to the two treatments, each with 8 replicates (cages) of 8 birds per replicate. The birds were kept in brooders in an environmentally controlled room. Room temperature was at 34±1°C on the first day and gradually decreased to 24°C by the end of the third week. The lighting program was 18 h light and 6 h darkness throughout the trial. Feed and water were offered *ad libitum* during the experimental period. Feed intake (FI) and body weight were measured at the end of weeks 1 and 3. Mortality was recorded as it occurred and feed per gain values were corrected for mortality.

Table 2. Simple sugar composition (g/kg) of the basal diet

	Free sugar	Insoluble NSP fraction	Soluble NSP fraction
Rhamnose	0.00	0.00	0.00
Fucose	0.42	0.67	0.00
Ribose	0.00	0.00	0.23
Arabinose	0.40	20.43	2.80
Xylose	0.00	22.21	3.02
Mannose	4.44	2.32	1.30
Galactose	7.94	12.10	1.98
Glucose	22.05	22.67	1.01
Total NSP	35.25	80.4	10.34

Sampling procedure

At the end of weeks 1 and 3, two birds per cage from 6 cages per treatment were randomly chosen and killed by cervical dislocation, and the gastrointestinal tract (GIT) was excised. The small intestine was divided into three segments: duodenum (from the gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the caecal junction). Approximately 2 cm of the proximal jejunum was removed for morphological measurements. The gut samples were flushed with ice-cold phosphate buffered saline (PBS) at pH 7.4 and immediately placed in 10% formalin solution. Another section, of about 2 cm, was taken from the proximal part of the jejunum, rinsed with PBS to remove the digesta, wrapped in aluminium foil before snap-freezing in liquid nitrogen, and then transferred into a -80°C freezer until analysis of BBM enzymes. In addition, approximately 5 cm of the proximal end of the jejunum was ligated and prepared according to the method described by Untawale et al. (1978), with modifications for the determination of selected mucosa-associated bacteria groups. The intestinal contents of the ileum and caeca were collected and fresh digesta samples were taken for bacterial analyses. The contents of the ileum of 3-week old birds were freeze-dried, and milled (0.5 mm screen) for determination of starch and protein digestibility.

Microbial analysis

Intestinal content from each segment was mixed in a 10 ml pre-reduced salt medium (Holdeman et al., 1977) and then prepared and serially diluted, according to the procedure described by Engberg et al. (2004), to examine the counts of lactobacilli (Rogosa, CM 0627, incubated anaerobically 48 h), coliforms (MacKonkey, CM 0115, incubated aerobically 24 h), *Clostridium perfringens* (CM 0543 OPSP, incubated aerobically 24 h) and TAB (Wilkins-Chalgren anaerobe agar, CM 0619, incubated anaerobically 7 days). All the media were supplied by Oxoid. Gut tissue

Table 3. Effects of xylanase on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), body weight and ileal digestibility of nutrients in broilers given a wheat-based diet[1]

	Treatment[2]		
	None	Xylanase	SED
0-7 days			
FI (g/bird)	199	195	6.4
BWG (g/bird)	159	162	7.3
FCR (g/g)	1.26	1.21	0.035
8-21 days			
FI (g/bird)	1,354	1,304	31.8
BWG (g/bird)	740[b]	797[a]	17.59
FCR (g/g)	1.83[a]	1.65[b]	0.049
Body weight (g/bird)			
Day 0	44.5	44.4	0.84
Day 7	204	206	11.9
Day 21	943[b]	1,017[a]	34.0
Digestibility (%) on day 21			
Starch	83	83	3.4
Protein	68	73	3.3

[1] Values are means of 8 replicates except for the digestibility, which is the mean of 6 replicates.

[2] Two dietary treatments: None = no supplementation; Xylanase, 1,000×U/g diet.

[a, b] Means within a row not sharing the same superscripts differ at $p<0.05$.

samples were serially diluted from 10^{-1} to 10^{-3}. From each dilution, 0.1 ml of the sample was plated onto the appropriate medium for enumeration of bacteria.

Histological and BBM bound enzyme analysis

The intestinal segments fixed with formalin solution were prepared for paraplast embedding, sectioned at 7 μm thickness, and stained with haematoxylin-eosin. About 15 villi and 15 crypts were measured from each chicken.

The mucosal homogenate and BBM were prepared as described by Shirazi-Beechey et al. (1991) with minor modifications by Iji (1998). The activities of BBM enzymes, maltase (EC. 3.2.1.20), sucrase (EC. 3.2.1.26) and alkaline phosphatase (AP, EC. 3.1.3.1) were analysed according to methods described previously in research with mammalian tissues (Dahlqvist, 1964; Forstner et al., 1968; Holdsworth, 1970). An enrichment factor was derived to ascertain the purity of the BBM vesicles, because the specific activity of membrane-bound enzymes should be higher in BBM vesicles than in the crude homogenate under ideal condition. The enrichment factor was determined by analysing and comparing the specific activities of maltase and AP in the mucosal homogenate with activities in the vesicles. At the end of week 1, the specific activities of maltase and AP were enriched by 6 and 3 times, respectively, and at the end of week 3, the specific activities of these two enzymes were enriched by 15 and 7 times, respectively. This indicated that the membrane vesicles had been purified compared to the homogenate samples.

Chemical and statistical analysis

Crude protein content (Kjeldahl N×6.25) was determined according to the methods of the Association of Official Analytical Chemists International (1994). Starch content was derived from glucose value by using a glucose oxidase and peroxidase method (GPD-Perid kit supplied by Boehringer-Mannheim Australia, Castle Hill, NSW, Australia). Acid-insoluble ash in the ileal digesta was determined according to the method described by Choct et al. (1992). Nutrient digestibility (%) was calculated according to the following equation:

$$\text{Digestibility} = \left(1 - \frac{\text{digesta nutrient (g/kg)/digesta AIA (g/kg)}}{\text{diet nutrient (g/kg)/diet AIA (g/kg)}}\right) \times 100$$

Statistical analysis of results was performed by one way ANOVA (SPSS Version 12.0, SPSS Inc). Bacterial numbers were logarithmically transformed to secure a normal distribution of the data and the results obtained were analysed separately for the jejunum, ileum and caeca. Differences between treatments were deemed to be significant only if the p-value was less than 0.05. All data were expressed as means.

RESULTS AND DISCUSSION

The production and digestibility results are shown in Table 3. The addition of xylanase did not have any positive effects on the growth performance of birds during the first 7 days. From day 8 to day 21, the BWG of birds was increased ($p<0.05$) and the FCR was improved ($p<0.05$) by xylanase. This observation is consistent with published reports that the supplementation of non-starch polysaccharidases can improve the growth performance of birds fed on a wheat and/or rye diet (Annison and Choct, 1991; Bedford and Classen, 1992; Selle et al., 2003; Wu et al., 2004a, 2004b; Qiao et al., 2005). The poor performance of birds fed on a wheat/rye-based diet was improved by exogenous enzymes through the breaking-down of the gel-forming capacity (viscosity) of non-starch polysaccharides (NSP), which is the cause of low nutrient digestibility and/or availability (Bedford and Classen, 1992). Indeed, Choct et al. (1995, 1999) observed that starch and/or protein digestibility of birds on xylanase treatment was better compared to the control. However, there were no significant ($p>0.05$) differences in the ileal protein and starch digestibility between xylanase treatment and the control at day 21 in the present trial although slight numerical increases were observed (Table 3).

Table 4 presents the results of luminal bacterial counts. The addition of xylanase increased ($p<0.05$) the numbers of *C. perfringens* in the ileum and tended ($p = 0.07$) to

Table 4. Effects of xylanase on the numbers (log CFU/g digesta) of luminal bacteria in the ileum and caeca of broilers given a wheat-based diet[1]

	Treatment[2]		
	None	Xylanase	SED
Day 7			
Ileum			
TAB[3]	8.45	8.66	0.232
Lactobacilli	8.40	8.23	0.165
Coliform	6.39	6.62	0.148
C. perfringens	5.38[b]	7.35[a]	0.490
Caeca			
TAB[3]	9.64	10.03	0.134
Lactobacilli	9.39	9.40	0.170
Coliform	8.90	9.23	0.164
C. perfringens	5.68	5.42	0.516
Day 21			
Ileum			
TAB[3]	8.96	8.37	0.202
Lactobacilli	9.06[a]	7.22[b]	0.376
Coliform	6.23	6.65	0.220
C. perfringens	7.61	7.85	0.205
Caeca			
TAB[3]	9.82	9.77	0.124
Lactobacilli	9.69	9.22	0.181
Coliform	8.56	8.60	0.086
C. perfringens	8.36	8.65	0.156

[1] Values are means of 6 replicates.
[2] Two dietary treatments: None = No supplementation; Xylanase, 1,000×U/g diet.
[3] TAB = Total anaerobic bacteria.
[a, b] Means within a row not sharing the same superscripts differ at p<0.05.

increase the number of TAB in the caeca of 7-day old birds. At day 21, xylanase reduced the counts of lactobacilli and TAB in the ileum (p<0.05 and p = 0.07, respectively). It was surprising to observe that xylanase significantly increased the populations of *C. perfringens* in the caeca at day 7. There is no published literature on findings similar to this; also we did not observe significant differences in mortality rate between xylanase treatment and the control (data not shown). The bacterial results for 3-week-old birds are in agreement with the reports of Vahjen et al. (1998) that xylanase supplementation reduced the density of certain group(s) of bacteria, such as total presumptive enterobacteria and lactobacilli, in the gut lumen in the first two or three weeks of life. Similar results were also noticed in weaned piglets given a wheat-based diet supplemented with xylanase (Vahjen et al., 2007). Less bacterial load means less competition with the host for nutrients (Gaskins, 2005). By reducing the density of luminal bacteria, xylanase may save nutrients for growth. Furthermore, the reduction in luminal lactobacilli populations may improve nutrient, in particular fat, digestibility. Although this parameter was not examined in the present experiment, Steenfeldt et al. (1998), Huberner et al. (2002) and Chiang et al. (2005) observed a higher fat digestibility and/or lipase activity in birds on

Table 5. Effects of dietary treatments on the counts (log CFU/ g wet tissue) of mucosa-associated bacteria and mucosal morphology of jejunum in broilers given a wheat-based diet[1]

	Treatment[2]		
	None	Xylanase	SED
Mucosa-associated bacteria			
Day 7			
Lactobacilli	6.08	6.82	0.285
Coliform	4.61	4.67	0.116
Day 21			
Lactobacilli	5.70	5.73	0.517
Coliform	4.80	5.13	0.248
Mucosal morphology			
Day 7			
Villus height (μm)	763	818	50.4
Crypt depth (μm)	137[a]	120[b]	3.53
Day 21			
Villus height (μm)	1,086	1,152	71.2
Crypt depth (μm)	170	162	8.8

[1] Values are means of 6 replicates.
[2] Two dietary treatments: None = No supplementation; Xylanase, 1,000×U/g diet.
[a, b] Means within a row not sharing the same superscripts differ at p<0.05.

xylanase treatment. In addition, Engberg et al. (2004) reported that the overgrowth of lactobacilli may lead to an impaired lipid digestion by catalyzing bile acid deconjugation.

The xylanase supplement did not alter the numbers of mucosa-associated lactobacilli and coliforms in the jejunum of birds at both ages (Table 5). In contrast, Vahjen et al. (1998) observed that the counts of lactobacilli associated with gut tissue were increased in birds given a xylanase-supplemented diet during the first week and remained stable thereafter. The inconsistency in results is difficult to explain but it is known that the profile of gut microflora can be affected by a variety of factors such as diet composition (Apajalahti et al., 2004), animal strain (Zoetendal et al., 2004) and digesta flow rate (Danicke et al., 1999).

The jejunal villus height was not affected by xylanase but the crypt depth was decreased (p<0.05) at 7 days of age (Table 5). At the same age, the addition of xylanase did not affect (p>0.05) the activity of the mucosal enzymes, whereas the content of BBM protein and the activity of the sucrase tended to be increased with xylanase treatment at day 21 (p = 0.09 and 0.07, respectively, Table 6). Crypt depth, the size of the proliferative compartment, can serve as an indicator of epithelial cell proliferation activity (Geyra et al., 2001) and a shallow crypt probably means a reduction in metabolic cost of the replacement of intestinal epithelium (Willing and Van Kessel, 2007). Silva and Smithard (1996) examined the effects of xylanase on crypt cell proliferation rate of birds given a rye-based diet and found that the rate was significantly reduced by xylanase (29 and 33 cells/ 2 h for xylanase treatment vs. 45 cells/2 h for the control

Table 6. The effects of xylanase on mucosal protein concentrations (mg/g tissue) and brush-border membrane (BBM) bound enzyme(s) activity (μmol product/mg protein/min) of jejunum in broilers given a wheat-based diet[1]

	Treatment[2]		
	None	Xylanase	SED
Day 7			
Total protein[3]	36.0	38.2	1.78
BBM protein[4]	0.34	0.26	0.046
Maltase	18.1	20.2	1.251
Sucrase	0.99	1.21	0.119
AP	14.2	14.2	1.60
Day 21			
Total protein[3]	38	40	2.11
BBM protein[4]	0.64	0.80	0.062
Maltase	8.28	9.00	0.587
Sucrase	0.84	1.06	0.076
AP	7.53	7.24	0.342

[1] Values are means of 6 replicates.

[2] Two dietary treatments: None, no supplementation; Xylanase, 1,000×U/g diet.

[3] Protein content in the mucosal homogenate.

[4] Protein content in the brush-border membrane (BBM) vesicles.

[a, b] Means within a row not sharing the same superscripts differ at $p<0.05$.

treatment). This result may indirectly support our observation of the lower crypt depth in birds on xylanase treatment in the present trial. However, Iji et al. (2001) did not find any significant effects of xylanase on the mucosal morphology of the small intestine of birds fed a wheat-based diet. On the other hand, Wu et al. (2004b) reported that xylanase increased ileal crypt depth but this effect was noticed only on a whole wheat diet but not on a ground wheat diet. Therefore, the exact active site of xylanase, the corresponding effect(s) and the interaction of the type of diet need to be further examined.

To our knowledge, there is no published information on the effects of xylanase on the development of BBM enzyme activity, the final digestion step of nutrients (Sklan and Noy, 2000). Sharma et al. (1997) reported that xylanase could modify mucin composition and induce major changes in the expression of goblet cell glycoconjugates of broiler chickens. However, it is not known whether these changes can either positively or negatively interact with the final digestion and/or absorption by the gut in birds given a xylanase-supplemented diet. In piglets, xylanase did not significantly affect the activities of BBM maltase and lactase (Li et al., 2004; Sileikiene et al., 2006) but increased the activity of γ-glutamyl transpeptidase in the jejunum mucosa (Li et al., 2004). In the present study, xylanase tended to increase the sucrase activity. These observations may indicate that xylanase can exert enterotrophic effects on the development of certain kind(s) of BBM enzyme(s) and thus nutrient digestion in farm animals.

In conclusion, xylanase altered the development of selected groups of intestinal bacteria with a reduction observed in the density of ileal lactobacilli at the end of the experiment. The crypt depth of jejunum was reduced but the mucosal sucrase activity tended to be increased by xylanase at different ages. These changes may help to partly explain the growth promoting effects induced by xylanase.

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