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Short communication

Dietary inclusion of xylanase improves growth performance, apparent total tract nutrient digestibility, apparent ileal digestibility of nutrients and amino acids and alters gut microbiota in growing pigs

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ABSTRACT

The study investigated the effects of dietary inclusion of xylanase on growth performance, apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N), gross energy (GE), apparent ileal digestibility (AID) of nutrients and amino acids, and faecal and ileal microflora counts in twelve cannulated growing pigs fed corn-soybean meal based diet. Pigs with a body weight of 24.32 ± 1.77 kg were surgically equipped with T-cannulas and randomly allotted to one of four dietary treatments (basal diet supplemented with 0, 450, 900, and 1800 of xylanase U/kg) for a 21-days trial period. Dietary inclusion of xylanase significantly improved daily gain and tended to improve gain: feed ratio (P = 0.048; 0.089, respectively). It also improved AID of DM, N, and GE (P = 0.042; 0.084; and 0.093, respectively). The AID of histidine and glutamic acid were significantly improved by xylanase supplementation (P = 0.015 and 0.021) while the AID of lysine, methionine and threonine tended to be improved (P = 0.097; 0.082; and 0.086, respectively). Xylanse supplementation also increased counts of faecal and ileal *Lactobacillus* (P = 0.036 and 0.048, respectively) and reduced counts of faecal and ileal *E. coli* (P = 0.007 and 0.071, respectively). In conclusion, dietary inclusion of xylanase improved growth performance, AID of nutrients and amino acids and had beneficial effects on faecal and ileal microflora, increasing *Lactobacillus* and reducing *E. coli* counts in pigs.

1. Introduction

Corn-soybean meal (SBM) based diets are used globally for pigs. However, corn and SBM contain 10% and 20% non-starch polysaccharides (NSP) respectively and can adversely affect the nutritional value of such diets, as monogastrics do not produce the xylanase (CVB, 1998; Li et al., 2010; Moeser et al., 2002; van Kempen et al., 2006). Exogenous xylanase has been used to improve the digestibility of energy by breaking down arabinoxylans that are poorly digested in pigs (Barrera et al., 2004) and to enhance nutrient

Abbreviations: BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; ATTD, apparent total tract digestibility AID apparent ileal digestibility; DM, dry matter; N, nitrogen; GE, gross energy; G:F, gain feed ratio; NSP, non-starch polysaccharides; SBM, soybean meal

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Table 1
Ingredient composition of basal experimental diet (g/kg, as-fed basis).

Items	Content
Ingredients	
Corn	553.8
Soybean meal	334.4
Molasses	25.0
Animal Fat	53.3
Dicalcium phosphate	19.3
Limestone	7.8
L-Lysine HCL	1.7
Trace mineral premix[a]	1.1
Vitamin premix[b]	1.2
Salt	2.1
Choline chloride	0.3
Calculated composition	
ME (MJ/kg)	14.06
NE (MJ/kg)	9.84
Crude protein (%)	20.00
Lysine (%)	1.30
Calcium (%)	0.90
Phosphorus (%)	0.80
Analyzed composition	
GE (MJ/kg)	17.22
Crude Protein (%)	19.65
Calcium (%)	0.87
Phosphorus (%)	0.76
Lysine	1.52
Digestible lysine	1.46
Methionine	0.62

[a] Provided per kg of complete diet: 12.5 mg Mn, 179 mg Zn, 140 mg Cu, 0.5 mg I and 0.4 mg Se.
[b] Provided per kg of complete diet: 20,000 IU of vitamin A; 4000 IU of vitamin D3; 80 IU of vitamin E; 16 mg of vitamin K3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B12; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.

values of corn-based diets for pigs (Li et al., 2010; Passos et al., 2015). However, pigs receiving dietary xylanase supplementation do not always show consistent improvement in growth performance or nutrient digestibility (Atakora et al., 2011; Barrera et al., 2004; Leek et al., 2007; Widyaratne et al., 2009). The effects of exogenous xylanase supplementation on nutrient digestibility and performance can be influenced by the type and dose of xylanase used, diet composition and the physiological status of the animal (Fang et al., 2007). The present study was conducted to investigate the effects of a 1, 4-beta xylanase on the performance, apparent nutrient digestibility and the some aspects of the faecal and ileal microflora of growing pigs fed a corn based SBM diet.

2. Materials and methods

2.1. Ethical statement

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University, Cheonan, South Korea.

2.2. Experimental design, diets, animals, and housing

Twelve [(Landrace × Yorkshire) × Duroc] growing pigs with average body weight (BW) of 24.32 ± 1.77 kg were surgically equipped with simple T-cannulas approximately 15 cm prior to the ileocecal junction according to procedures described by Sauer et al. (1983). Pigs were allocated to one of four dietary treatments: basal diet supplemented with 0, 450, 900 or 1800 of xylanase U/kg (*endo*-1,4-β-xylanase, derived from *Bacillus subtilis*, Nutrase-Xyla®, Nutrex Nv™, Belgium). There were three replicates per treatment for a 21-day trial period. Diets (Table 1) in mash form were formulated to meet or exceed the requirements suggested by the NRC (2012). The pigs were fasted for 16–20 h prior to surgeries. After the surgery, pigs were transferred to individual pens (1.2 × 0.6 m) in a temperature-controlled room (28 °C) and allowed to recover for 10 days prior to the initiation of the trial as previously described by Li et al. (1994). Daily feed allowance was 0.05 × $BW^{0.9}$ based on the recommendation of Armstrong and Mitchell (1955). The daily feed was provided as two meals at 12 h interval (08.00 and 20.00 h), and water was provided *ad libitum*.

2.3. Sampling and measurements

The BW was determined at the start and end of the experimental period, and feed consumption was recorded throughout the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain: feed ratio (G:F). Chromic oxide (2 g/kg) was added to the diet as an indigestible marker to allow digestibility determinations (Fenton and Fenton 1979). Fresh faecal samples were collected directly via rectum massaging of pigs at the end of the experiment. A plastic bag (225 mL) was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed as filled with digesta or at least every 30 min and immediately stored at −20 °C to prevent bacterial degradation of AA in the digesta (Upadhaya et al., 2016). Before chemical analysis, faecal samples and ileal digesta were thawed and dried at 70 °C for 72 h. They were subsequently ground to pass through a 1 mm screen. The procedures used for the determination of dry matter (DM), nitrogen (N), and gross energy (GE) digestibility were in accordance with the methods established by AOAC (2005). Nitrogen was determined by a Kjectec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as nitrogen × 6.25. Gross energy (GE) was determined by using a Parr 6100 Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, Illinois, USA). Dietary DM (method 930.15), CP (method 968.06), calcium (method 984.01), phosphorus (method 965.17) were analyzed according to the procedures described by AOAC (2005). The individual amino acid composition was measured using an amino acid analyzer (Hitachi Amino Acid Analyzer, model No. L8800; Hitachi High Technologies America Inc., Pleasanton, CA, USA) analysis using ninhydrin for postcolumn derivatization and norleucine as internal standard. The apparent ileal digestibility (AID) of individual amino acids was measured and calculated according to the method described by Stein et al. (2007). The apparent total tract digestibility (ATTD) of nutrients, was calculated using the following formula:

$$ATTD = 1 - \{(Nf \times Cd)/(Nd \times Cf)\},$$

where Nf is the nutrient concentration in feces (% DM), Nd the nutrient concentration in the diet (% DM), Cd the chromium concentration in the diet (% DM), and Cf the chromium concentration in feces (% DM). The digestibility values for nutrients and amino acids were calculated and expressed as coefficient of digestibility.

To analyze microflora counts, one gram of composite ileal and excreta faecal sample from each pig was diluted with sterile saline (10^{-7}–10^{-3}) and homogenized. Viable counts of bacteria in the faecal samples were determined by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates or MRS agar plates (Difco, USA) to isolate

E. coli or *Lactobacillus*, respectively. The number of colonies of *E. coli* and *Lactobacillus* was counted immediately after incubation at 37 °C for 38 h. The microbial populations were log transformed before statistical analysis.

2.4. Statistical analysis

Data were analyzed using the general linear model procedure of SAS/STAT® (Statistical Analysis System, version 9.2). The pig was the experimental unit for all analyses. Mean values and standard errors of the mean (SEM) are reported. Analysis of variance (ANOVA) was used followed by Turkey's Test when significant differences were obtained. A probability value of $P \leq 0.05$ was considered to be statistically significant and trends were noted under conditions of $0.05 < P < 0.10$.

3. Results and discussion

We expected that dietary supplementation of xylanase might hydrolyze NSP present in the diet or supplement activity of endogenous enzymes and improve performance and nutrient digestibility (Tables 2 and 3). Supplementing the diet with xylanase significantly improved final weight, growth rate and tended to improve feed efficiency, but had no effect on feed intake (Table 2). Our findings are in line with Fang et al. (2007), who reported improvements in both growth performance and apparent nutrient digestibility for grower pigs fed xylanase supplemented corn based SBM diets. In the present study, growth rate and feed efficiency were improved 17–20% and 17–23% by xlyanase respectively suggesting the enzyme investigated has considerable potential to improve the performance of younger pigs offered SBM based diets. In contrast, Passos et al. (2015) reported quite large effects of xylanase

Table 2
Effects of xylanase supplementation of a corn-SBM based diet on the performance of grower pigs over 21 days.

Items[a]	Dietary xylanase inclusion, U/kg				SEM[b]	P-value
	0	450	900	1800		
Body weight, kg						
Initial	24.06	24.56	24.06	24.56	0.46	0.768
Final	35.73	37.03[a]	36.26	37.33	0.44	0.021
ADG, g	508	594	581	608	21.23	0.048
ADFI, g	897	892	875	870	13.84	0.501
G:F	0.572	0.669	0.666	0.704	0.029	0.089

[a] ADG, average daily gain; ADFI, average daily feed intake; G:F, gain:feed ratio.
[b] Standard error of means.

Table 3
Effects of xylanase supplementation of a corn-SBM based diet on apparent ileal (AID) and total tract digestibility (ATTD) of nutrients and AID of amino acids in growing pigs.

Particulars	Dietary xylanase inclusion, U/kg				SEM[a]	P-value
	0	450	900	1800		
AID of Essential amino acids						
Arginine	0.73	0.74	0.75	0.76	1.29	0.585
Histidine	0.78	0.80	0.79	0.81	0.55	0.015
Isoleucine	0.79	0.80	0.80	0.79	0.90	0.727
Leucine	0.71	0.72	0.72	0.73	1.22	0.941
Lysine	0.75	0.77	0.78	0.78	1.00	0.097
Methionine	0.81	0.83	0.83	0.83	0.66	0.082
Phenylalanine	0.76	0.76	0.76	0.76	1.28	0.984
Threonine	0.78	0.79	0.80	0.80	0.94	0.086
Valine	0.86	0.87	0.87	0.87	1.02	0.875
Total	0.77	0.78	0.79	0.79	0.73	0.167
AID of Non-essential amino acids						
Alanine	0.81	0.82	0.83	0.82	1.07	0.828
Asparatic acid	0.72	0.74	0.74	0.75	1.02	0.171
Cystine	0.70	0.72	0.74	0.72	3.07	0.853
Glutamic acid	0.77	0.79	0.79	0.79	0.59	0.090
Glycine	0.77	0.79	0.79	0.79	1.23	0.908
Proline	0.76	0.76	0.77	0.77	3.04	0.987
Serine	0.77	0.77	0.77	0.76	0.73	0.960
Tyrosine	0.76	0.76	0.76	0.76	1.12	0.957
Total	0.76	0.76	0.77	0.78	2.52	0.978
Total amino acids	0.76	0.77	0.77	0.77	2.10	0.948
AID of nutrients						
Dry matter	0.77	0.79	0.79	0.80	0.66	0.042
Nitrogen	0.76	0.78	0.78	0.79	0.87	0.084
Gross energy	0.77	0.78	0.79	0.79	0.67	0.093
ATTD of nutrients						
Dry matter	0.75	0.77	0.77	0.80	0. 89	0.171
Nitrogen	0.76	0.77	0.78	0.79	0. 56	0.167
Gross energy	0.76	0.77	0.79	0.80	0.57	0.425

[a] Standard error of means.

supplementation on AID of nutrients but not on growth performance. The difference between experiments might reflect differences in the basal diet, xylanase dose and/or the age of the pigs involved. In the experiment reported by Passos et al. (2015), the study lasted only 10 days and the pigs were housed in metabolism cages. In our study xylanase, supplementation improved the AID and ATTD of DM, N and GE by 2.4–3.6% and 3.9–6.6% respectively. Only the improvement in the AID of DM reached significance (Table 3).

There is a general agreement that measurement of pig's ileal digestibility rather than faecal digestibility represents a more accurate estimate of amino acids availability since amino acids are only absorbed proximal to the distal ileum (Barrera et al., 2004; Mosenthin et al., 2000; Upadhaya et al., 2016). In our study, xylanase supplementation singnificantly improved the AID of histidine and tended to improve the AID of lysine, methionine, threonine, glutamic acid (Table 3). These findings are in line with Yin et al. (2001). Our results are also consistent with those of Ao et al. (2010) and Upadhaya et al. (2016). Both reported improved AID of amino acids in grower pigs fed corn-SBM based diets supplemented with NSP degrading enzymes. According to Barrera et al. (2004) the contents of AID of amino acids in the diet are increased as the dose of xylanase supplementation is increased. Yin et al. (2000) also reported only small improvements in the AID of some of the indispensable amino acids in pigs fed diets based on wheat or wheat by-products supplemented with xylanase. In addition, the improvement in N digestibility in our experiment might partially explain the improvement in the performance and ileal amino acid digestibility of pigs. The xylanase used in our study is designed to be highly thermo and pH stable and to target both the soluble and insoluble parts of the arabinoxylan fraction of the feed. It likely improves nutrient digestibility by effectively increasing the digestion of NSPs and releasing entrapped nutrients.

It has been suggested that NSP degrading enzyme supplementation might influence intestinal microbiota by reducing undigested substrates and releasing oligosaccharides from cell wall NSP (Kiarie et al., 2013). In our study, the xylanase supplementation increased faecal and ileal *Lactobacillus* counts (Table 4), suggesting that xylanase hydrolysis products containing arabinose and xylose may have positive effects on lactic acid bacteria activity. Our results are in agreement with Kiarie et al. (2007) who reported higher *Lactobacillus* counts in weaner pigs fed diets supplemented with xylanase. Yi et al. (2013) also reported xylanase supplementation improved faecal and ileal microbial balance in weaner pigs. These observations support the recent interest in using xylanase for their beneficial effect on gut microbiota balance.

In conclusion, our results confirmed the hypothesis that supplementation of a corn- SBM improved the performance and nutrient digestibility of growing pigs. Xylanase supplementation also increased and reduced ileal and faecal *Lactobacillus* and *E. coli* counts

Table 4
Effects of xylanase supplementation of a corn-SBM based diet on ileal and faecal *Lactobacillus* and *E. coli* counts in grower pigs.

Particulars, log_{10}cfu/g	Dietary xylanase inclusion, U/kg				SEM[a]	P-value
	0	450	900	1800		
Faecal						
Lactobacillus	7.52	7.64	7.66	7.75	0.04	0.036
E. coli	5.76	5.66	5.67	5.50	0.03	0.007
Ileal						
Lactobacillus	7.83	7.89	7.90	7.95	0.02	0.048
E. coli	2.15	2.08	1.94	1.81	0.01	0.071

[a] Standard error of means.

respectively. The quite large improvements in growth performance elicited by the xylanase used in the study clearly warrant further investigation in studies involving more animals.

Conflict of interest

The authors declare no conflicts of interest.

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