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Original article

Effect of dietary supplementation of xylanase on apparent ileal digestibility of nutrients, viscosity of digesta, and intestinal morphology of growing pigs fed corn and soybean meal based diet

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ABSTRACT

This study was to determine apparent ileal digestibility of acid detergent fiber (ADF), neutral detergent fiber (NDF), dry matter (DM), energy, organic matter (OM), crude ash, digesta viscosity, and gut morphology in nursery pigs fed diets containing xylanase (Lohmann Animal Nutrition GmbH, Cuxhaven, Germany). The diet (61% corn, 35% soybean meal, 1% poultry fat, and 3% minerals and vitamins) was mixed with 3 levels of xylanase (0, 700, and 1400 LXU/kg). Thirty-six barrows (17.6 ± 3.3 kg) received one of 3 treatment diets based on a randomized complete block design with the initial body weight (BW) as a block. Pigs were individually housed and received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs ($0.09 \times BW^{0.75}$ kg). Pigs were fed diets for 10 d, and chromium oxide (0.3%) was added to the diets from d 6 as an indigestible external marker. Pigs were euthanized at the end of d 10 for the collection of digesta and tissues. Jejunal digesta were centrifuged to measure viscosity using a viscometer (Brookfield Engineering Laboratories, Stoughton, MA). Diets and freeze-dried ileal digesta were used to measure ADF, NDF, and chromium to calculate apparent ileal digestibility of ADF and NDF. Villus height and crypt depth of jejunum were measured using a microscope (Fisher Scientific, Hampton, NH). Data were analyzed using polynomial contrasts in the MIXED procedure of SAS version 9.3 (SAS Inc., Cary, NC, USA). Morphological measurements and ileal ADF digestibility were not affected by increasing xylanase. However, increasing xylanase supplementation from 0 to 1400 LXU/kg enhanced ileal digestibility of NDF ($P < 0.042$, linear) from 27.9 to 40.3%, DM ($P < 0.006$, linear) from 55.4 to 64.6%, OM ($P < 0.006$, linear) from 59.2 to 67.7%, and energy ($P < 0.003$, linear) from 58.8 to 68.0%. Viscosity of jejunal digesta decreased ($P < 0.023$) in a non-linear manner from 2.9 to 2.5 centipoises (cP). In conclusion, the usage of xylanase in corn and soybean meal based pig diets linearly enhanced digestibility of nutrients and affected viscosity of digesta in a non-linear manner.

1. Introduction

Efforts to improve nutrient digestibility by the pigs can have effects on profitability of the pork industry (National Pork Board, 2012). Studies indicate that non-starch polysaccharides (NSP) in corn and soybean meal negatively affect nutrient digestibility (Moeser et al., 2002; van Kempen et al., 2006). Whole corn grain contains 27–32 g of xylose/kg (Knudsen, 1997) as arabinoxylans in pericarp and aleurone (Landis et al., 2001). Soybean meal contains 18–19 g of xylose/kg (Knudsen, 1997; Irish and Balnave, 1993) as xyloglucan in the structural polysaccharides (Karr-Lilienthal et al., 2005).

Feed enzymes supplementation to corn (Cozannet et al., 2012; Li et al., 2010), soybean meal (Cozannet et al., 2012), and complete feed (Ji et al., 2008; Jo et al., 2012; Kim et al., 2003; Pettey et al., 2002) fed to pigs were previously reported. Endo-1,4-β-xylanase (xylanase) catalyzes endohydrolysis of 1,4-β-D-xylosidic linkages

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in xylans (International Union of Biochemistry and Molecular Biology, 1992) releasing oligosacharides from corn and wheat fiber (Katapodis and Christakopoulos, 2008; Katapodis et al., 2003). Xylanase has been evaluated to improve nutrient digestibility in pigs (Moehn et al., 2007; Nortey et al., 2007; Woyengo et al., 2008). The mechanism proposed to explain the effect of fiber degrading enzymes involves degradation of polysaccharides in the cell wall (Adeola and Cowieson, 2011; Masey et al., 2012; Meng et al., 2005; Tervila-Wilo et al., 1996) and reduction of digesta viscosity (Garcia et al., 2008; Mathlouthi et al., 2002). However, viscosity might not be the most important factor affecting nutrient digestibility in pigs (Bartelt et al., 2002). Type of fiber and intestinal fermentation should be considered (Hooda et al., 2010; Jensen, 1996).

The hypothesis of this study is that supplementation of xylanase in corn-soybean meal based diets reduces digesta viscosity and thus enhances digestibility of nutrients. The objective of this study is to measure viscosity of jejunum digesta, intestinal morphology, and ileal digestibility of dry matter (DM), energy, acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude ash of a corn-soybean meal based diet supplemented with xylanase fed to pigs.

2. Materials and method

The experimental protocol was approved by North Carolina State University Animal Care and Use Committee.

2.1. Experimental diets and pigs

The experiment was conducted at the Swine Educational Unit at the North Carolina State University (Raleigh, NC). Pigs were used to evaluate digestibility of DM, energy, protein, ADF, NDF, and crude ash of a diet (Table 1) supplemented with feed enzyme. Corn was ground to 400 μm. Xylanase (Carboflex, Lohmann Animal Nutrition GmbH, Cuxhaven, Germany) was supplemented at 0 (C), 100 (T1), and 200 mg/kg of diet (T3) to provide 0, 700, and 1400 LXU of xylanase/kg of diet respectively. LXU is the amount of enzyme which releases 1 μmol of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50 °C (EURL, 2013).

Thirty six barrows (17.6 ± 3.3 kg) were placed in metabolic cages (0.6 m wide, 1.8 m long) equipped with stainless-steel feeder attached to the front of the pen, nipple water drinker next to the feeder, and slatted flooring. There were 12 cages available for the study and 3 groups of 12 pigs were allotted in the metabolism room. Pigs received one of the 3 treatment diets based on a randomized complete block design with initial body weight as block. The experimental period consisted of 10 days. Ileum content of ADF and NDF, ileal digestibility of ADF and NDF, villus height/crypt depth in jejunum and digesta viscosity were measured.

Table 1
Ingredient composition of experimental diets (as-fed basis).

Item	Xylanase, LXU[a]/kg		
	0	700	1400
Ingredients, %			
Yellow corn, ground	61.12	61.11	61.10
Soybean meal	35.30	35.30	35.30
Limestone	1.10	1.10	1.10
Monocalcium phosphorus	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Trace mineral premix[b]	0.15	0.15	0.15
Vitamin premix[c]	0.03	0.03	0.03
Xylanase	0.00	0.01	0.02
Calculated composition			
ME, MJ/kg	14.02	14.02	14.02
SID[d] Lys, %	1.01	1.01	1.01
SID Met + Cys, %	0.59	0.59	0.59
SID Thr, %	0.63	0.63	0.63
SID Trp, %	0.21	0.21	0.21
Ca, %	0.89	0.89	0.89
P total, %	0.61	0.61	0.61
P available, %	0.33	0.33	0.33
Analyzed composition			
DM, %	91.77	91.83	91.66
GE, MJ/kg	16.86	16.89	16.67
CP, %	20.36	20.73	21.01
Fat, %	2.55	2.91	2.39
Ca, %	0.76	0.71	0.78
P, %	0.56	0.58	0.56
Ash, %	4.64	4.82	4.81
Xylanase, LXU/kg	<200	674	1231

GE = gross energy; CP = crude protein; Ca = calcium; P = phosphorus.
[a] LXU is the amount of enzyme which releases 1 μmol of reducing sugars equivalents (as xylose or glucose) from birch xylan or barley glucan per minute at pH 5.5 and 50 °C.
[b] Trace mineral premix supplied per kg of feed: 16.5 mg/kg of Cu as copper sulfate, 165.3 mg/kg of Fe as ferrous sulfate, 39.60 mg/kg of Mn as manganous oxide, 165.30 mg/kg of Zn as zinc sulfate, 0.30 mg/kg of I as ethylenediamine dihydroiodine and 0.30 mg/kg of Se as sodium selenite.
[c] Vitamin premix supplied per kg of feed: 6171 IU of vitamin A as vitamin A acetate, 880 IU of vitamin D as cholecalciferol, 35 IU of vitamin E as tocopheryl acetate, 0.02 mg/kg of vitamin B_{12} as cyanocobalamin, 0.18 mg/kg of biotin, 2.91 mg/kg of vitamin K as menadione sodium bidulfite, 4.40 mg/kg of riboflavin, 17.64 mg/kg of pantothenic acid as calcium pantothenate, 26.45 mg/kg of niacin as nicotidamide, 1.32 mg/kg of folate as folic acid.
[d] Standardized ileal digestibility.

2.2. Experimental procedures, chemical analyses, and digesta viscosity

Pigs received experimental diets twice daily (0700 and 1700 h) at a fixed amount based on BW of pigs ($0.09 \times BW^{0.75}$ kg). Dietary treatments were fed to pigs for 10 days. Chromium oxide was added to experimental diets (0.3%) from day 6 as an indigestible external marker for calculation of ileal digestibility. Pigs were euthanized via captive-bolt stunning and exsanguination at day 10 for sample collection 8 h after the last meal. Immediately after the euthanasia, an ileal portion (a portion of 20 cm prior to ileo-cecal connection) of small intestine was used to obtain digesta in ileum. Digesta from ileum was stored in sterile container and kept frozen at −20 °C. Jejunum tissue sample (3 cm) was collected and stored in formaline for further histological analysis. Intestine (20 cm) from distal portion of jejunum was also used to obtain digesta to measure viscosity. Jejunal contents were emptied into 50 mL tubes, samples were kept on ice and viscosity was measured immediately after the collection.

Frozen Ileal digesta were freeze-dried (24D × 48, Virtis, Gardiner, NY) for storage and chemical analysis. Diets and freeze dried digesta were analyzed for moisture (Method 934.01, AOAC, 2006), ADF (Method 973.18, AOAC, 2006), NDF (Van Soest et al., 1991), ash (Method 942.05, AOAC, 2006), chromium (Williams et al., 1962), and energy using a calorimeter (6200, Parr Instrument Company, Moline, IL). Apparent ileal digestibility (AID, %) of ADF and NDF were calculated using the chromium concentration in the diets and digesta by using AID = 100 − [(ND/NF) × (CrF/CrD) × 100], where ND is the nutrient concentration present in the ileal digesta, NF is the nutrient concentration in the diet, CrF is the chromium concentration in the feed, and CrD is the chromium concentration in the ileal digesta.

Viscosity was done using a viscometer (Brookfield Digital Viscometer, Model DV-II Version 2.0, Brookfield Engineering Laboratories Inc., Stoughton, MA). The tubes were centrifuged at 3000 rpm for 5 min and then 2 mL of the supernatant was centrifuged at 12,500 rpm for 5 min. Viscometer was set at 25 °C, 0.5 mL of digesta supernatant was placed in the viscometer. Viscosity measurement was the average between 45.0 s^{-1} and 22.5 s^{-1} shear rates.

2.3. Histology

Jejunum morphology were analyzed according to Fan et al. (2001) to obtain villus height, crypt depth and the relation villus height to crypt depth. Jejunum samples (2 sections per pig) were fixed in formaline and sent to North Carolina State University histology laboratory for hematoxylin and eosin staining and sectioning according to standard histological technique. The sections were dehydratated and embedded in paraffin. Staining was done using hematoxylin and eosin dyes (Junqueira and Carneiro, 2005).

Villus height, crypt depth, and relation villus height and crypt depth were measured in the microscope (Micromaster, Fisher Scientific International Inc., Pittsburgh, PA). For each section, 15 measurements of adjacent villus height and crypt depth were obtained. The measurements were done with ImageJ software (NIH, 2013) and transferred to Microsoft Excel software. The relation villus height to crypt depth of each measurement was calculated. The averages of the 30 measurements per pig were calculated and reported as one number per pig.

2.4. Statistical analysis

Data were analyzed using polynomial contrasts in the Mixed procedure of SAS version 9.3 (SAS Inc., Cary, NC, USA). The experiment was a randomized complete block design using initial BW and group of pigs allotted in the metabolism as blocking factor. The experimental unit was the individual pig. Initial BW and group of pigs were considered random effect. Statistical differences were considered significant with $P < 0.05$. Probabilities less than 0.10 and equal or greater than 0.05 were considered as a tendency.

3. Results

The average BW of pigs utilized on this study was 17.6 kg and average daily feed intake (ADFI) was 757 g/d (Table 2). The weight gain of the pigs during the metabolism study was 352 g/d and the feed conversation ratio (G:F) was 0.47 in average. Increasing the level of xylanase in the diet (0–1400 LXU/kg) did not affect ($P > 0.10$) growth performance of pigs individually house in the metabolism cages. Pigs received a limited amount of feed based on their BW and this study was not designed to measure growth performance.

Increasing xylanase in the diet from 0 to 1400 LXU/kg did not affect histological measurements (Table 3) including villus height, crypt depth, and relation villus height to crypt depth. Increasing xylanase resulted in a quadratic change ($P = 0.023$) in viscosity of jejunal digesta from 2.94 to 2.52 centipoises (cP) when xylanase increased from 0 to 700 LXU/kg and from 2.52 to 3.20 cP when xylanase increased from 700 to 1400 LXU/kg respectively.

Increasing xylanase (0–1,400 LXU/kg) yielded greater AID of DM (linear increase from 55.43 to 64.58%, $P = 0.006$), organic matter (OM) (linear increase from 59.19 to 67.70%, $P = 0.006$), and energy (linear increase from 58.78 to 68.04%, $P = 0.003$). Similarly, AID of crude ash increased by 16% (quadratic increase from 18.71 to 34.34%, $P = 0.045$) and AID of NDF by 12% (linear increase from 27.91 to 40.32%, $P = 0.042$). However AID of ADF was not affected by supplementation of xylanase ($P > 0.10$).

4. Discussion

The digesta viscosity obtained on this study ranged from 2.52 to 3.20 cP. The viscosity can be affected by the type of ingredient in the diet (Willamil et al., 2012). Digesta viscosity in the ileum was reported to be 2.8 cP for a corn-soybean meal based diet (Willamil et al., 2012), 1.7 cP in corn-soybean meal-DDGS based diet (Agyekum et al., 2012), 4.6 cP in a wheat based diet (Mavromichalis et al., 2000), and 7.0 cP in a rye-wheat based diet (Bartelt et al., 2002). Corn was the major ingredient in the diet of the present study, and it has lower content of soluble NSP than wheat, rye, barley, and oats (Knudsen, 1997) yielding low viscous solutions (Mathlouthi et al., 2002).

This study indicated that by increasing the use of xylanase yields a quadratic change in the viscosity of the digesta in pigs fed corn-soybean meal based diets. Corn grain NSP contains arabinoxylans (Landis et al., 2001), and contains 30 g total xylose/kg of corn (Knudsen, 1997). Soybean meal contains 18–19 g xylose/kg of soybean meal (Irish and Balnave, 1993; Knudsen, 1997) as xyloglucan (Karr-Lilienthal et al., 2005), therefore the main substrate for xylanse in a corn-soybean meal-based diet will be the arabinoxylans in the corn. The effect of xylanase on corn fiber was previously demonstrated by *in vitro* studies (Grabber et al., 1998; Hu et al., 2008; Saha, 2001). The limitations regarding the xylanase activity on corn fiber (Rose and Inglett, 2011) involve the arabinose side-chains in the xylan back-bone of the arabinoxylan (Doner et al., 2001; Rose et al., 2010). However, arabinofuranosyl groups attached to xylan can be partially released under acidic pH conditions in the stomach (Zhang et al., 2003). In addition, the corn fiber utilization on xylanase production increases the number of side activity enzymes (β xylosidase and α-L-arabinofuranosidase) that enhance the release of arabinose and xylose from arabinoxylans (Saha, 2001). Arabinoxylans can form viscous solutions (Izydorczyk and Biliaderis, 1982, 1992) and increase viscosity of digesta (Choct and Annison, 1992). Xylanase can break arabinoxylans (Grabber et al., 1998; Pedersen et al., 2012) and reduce viscosity of *in vitro* solutions (Mathlouthi et al., 2002) and also digesta viscosity (Adeola and Bedford, 2004; Yin et al., 2001).

Increasing supplementation of xylanase yields a quadratic response on digesta viscosity. Corn contains a greater proportion of xylose in the insoluble NSPs (Knudsen, 1997) and some xylanases

Table 2
Initial BW, ADFI, average daily gain (ADG), and G:F of pigs housed individually in metabolism cages with daily feed allowance of 0.09 × $BW^{0.75}$ kg of corn-soybean meal based diets supplemented with xylanase (n = 12).

Item	Xylanase, LXU/kg			SEM	P-value	
	0	700	1,400		Linear	Quadratic
Initial BW, kg	17.4	17.4	17.7	1.0	0.145	0.370
ADFI, g/d	753	756	763	28	0.249	0.799
ADG, g/d	340	353	364	57	0.408	0.964
G:F	0.458	0.471	0.482	0.093	0.498	0.952

BW = body weight; ADFI = average daily feed intake; G:F = feed conversion ratio.

Table 3
Jejunum villus height, crypt depth, villus height/crypt depth, and viscosity of jejunal digesta in pigs fed corn-soybean meal based diets supplemented with xylanase (n = 12).

Item	Xylanase, LXU/kg			SEM	P-value	
	0	700	1400		Linear	Quadratic
Villus height, μm	431	407	403	18	0.263	0.652
Crypt depth, μm	233	212	226	14	0.642	0.171
Villus height/crypt depth	1.89	1.97	1.80	0.14	0.553	0.295
Viscosity, cP[a]	2.94	2.52	3.20	0.12	0.314	0.023

[a] cP = centipoise (1 cP = 1/100 dyne s/cm^2).

Table 4
Apparent ileal digestibility (%) of DM, OM, energy, crude ash, NDF, and ADF in pigs fed corn-soybean meal based diets supplemented with xylanase.

Item	Xylanase, LXU/kg			SEM	*P*-value	
	0	700	1400		Linear	Quadratic
DM	55.43	66.80	64.58	2.46	0.006	0.020
OM	59.19	69.80	67.70	3.60	0.006	0.021
Energy	58.78	69.00	68.04	2.80	0.003	0.038
Crude ash	18.71	34.34	29.40	4.04	0.062	0.045
NDF	27.91	43.63	40.32	4.80	0.042	0.070
ADF	1.54	13.20	8.77	7.90	0.430	0.312

DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.

have affinity to insoluble xylan (Connerton et al., 1999; Sun et al., 1998). There is evidence that xylanases can degrade insoluble NSP into soluble NSP increasing digesta viscosity (Choct et al., 2004). Therefore, one can speculate that at greater dosages of xylanase (as treatment T2 in the study reported herein), the insoluble NSP become more soluble, and thus increase digesta viscosity. Therefore, the NDF result of this study supports the degradation of corn NSP. However, this needs further investigation with limited biological and practical meaning at this moment.

This study observed that by increasing the dietary supplementation level of xylanase, there will be a linear increase in the ileal digestibility of DM, OM, energy, and NDF (Table 4). The mode of action of xylanase on enhancing nutrient digestibility may involve the degradation of the cell wall NSPs, thus enabling endogenous digestive enzymes to access nutrients trapped (Adeola and Cowieson, 2011; Masey et al., 2012; Tervila-Wilo et al., 1996). The greater NDF digestibility can be explained by the method utilized to analyze NDF. Xylanase release oligosaccharides (xylobiose to xylopentose) from arabinoxylans (He et al., 2010; Rajagopalan et al., 2013). The filter bags utilized in the NDF analysis procedure have pore sizes of 25 μm (F57, ANKOM, Macedon, NY) and the smaller particles of xylotriose to xylopentose released by xylanase might not be retained by the filter bags. There are nutritional benefits of NSP degradation (Choct and Annison, 1992). The use of xylanase in corn-soybean meal based diets improved ileal digestibility of energy by 2% (Nian et al., 2011) and also an enzyme blend containing xylanse, protease, and amylase improved protein digestibility (Zanella et al., 1999). Improvement in NDF, DM, gross energy (GE), and starch digestibility were observed utilizing *in vitro* and *in vivo* digestibility methods in pigs when an enzyme blend composed of xylanase, protease, and amylase was added to the diet (Li et al., 2010). The present study indicated that as dietary level of xylanase increased, digestibility of DM, OM, energy, NDF, and crude ash increased by 9.2, 8.5, 9.3, 12.4, and 10.7%, respectively.

Dietary level of NSP can affect intestinal morphology (Montagne et al., 2003). Diets with high content of NSP from wheat and barley affected villus height and the relation villus height to crypt depth in the ileum of pigs compared to diet formulated with corn and soybean meal (Willamil et al., 2012). The use of feed enzyme can also mitigate the negative effect of NSP from wheat and barley on intestinal morphology, however it does not affect intestinal morphology in corn-soybean meal based diet (Willamil et al., 2012). Similarly, there was no significant effect of dietary xylanase supplementation of corn-soybean meal diet on intestinal morphology measured in the study reported herein.

5. Conclusion

The ileal nutrient digestibility of a corn-soybean meal based diet improved when dietary xylanase supplementation level increased from 0 to 1400 LXU/kg. There was a quadratic change in viscosity of jejunum digesta, but no effect on intestinal morphology. The results confirm our hypothesis that xylanase can be supplemented to swine diets in order to improve nutrient digestibility.

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