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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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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.

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1. Introduction

Efforts to improve nutrient digestibility by the pigs can have effects on profitability of the pork industry (National Pork Board,

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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 cornsoybean 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 μ m. 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 μ mol 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 \pm 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.

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

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 B₁₂ 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.

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 = $100 - [(ND/NF) \times (CrF/CrD) \times 100]$, 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 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,

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