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Non Ruminant Nutrition

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Effect of dietary supplementation of xylanase in a wheat-based diet containing canola meal on growth performance, nutrient digestibility, organ weight, and short-chain fatty acid concentration in digesta when fed to weaned pigs

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Abstract

This study was designed to determine the effect of dietary supplementation of xylanase on growth performance, nutrient digestibility, organ weight, digesta pH, and concentration of short-chain fatty acids (**SCFA**) of weaned pigs fed wheat–canola meal (**CM**) diets over a 35-d period. A total of 144 piglets (72 barrows and 72 gilts) weaned at 18 ± 2 d of age, with initial body weight (**BW**) of 6.2 ± 0.7 kg, received one of eight dietary treatments based on randomized complete block design. BW and feed intake were recorded weekly to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed efficiency (G:F). Treatments consisted of a control wheat–soybean meal-based diet and wheat-regular (**RCM**), dehulled (**DCM**), or coarse CM (**CCM**) without and with 16,000 BXU/kg xylanase (Econase XT). All diets contained 500 FTU/kg of phytase (Quantum Blue 5G) and titanium dioxide (0.3%). Apparent total tract digestibility (**ATTD**) of neutral detergent fiber (**NDF**), crude protein (**CP**), phosphorous (P), calcium (Ca), dry matter, and diet were determined. On day 35, one pig per pen was euthanized to evaluate the main factors of protein, xylanase supplementation, and sex on organ weight, ileal and colon digesta pH, and colon digesta concentrations of SCFA. The main factors did not affect growth performance. Xylanase supplementation improved nutrient digestibilities in all diets and increased ileal and colonic digesta pH without affecting the growth performance of weaned pigs fed wheat and CM-based diets. A protein–xylanase effect ($P < 0.05$) resulted in increasing the ATTD of NDF from 28% to 32% and from 29% to 37% for RCM and DCM, respectively. The ATTD of CP was greater ($P < 0.05$) with xylanase supplementation (75% vs. 70%). Xylanase supplementation increased ATTD of P and Ca. A three-way interaction ($P < 0.05$) for protein–xylanase–gender for colon pH, acetic, and propionic acid in the colon digesta of pigs indicated that, in addition to the protein source, piglet sex could have influenced how xylanase works. Xylanase supplementation increased ($P < 0.05$) the weight of the liver and spleen and tended ($P < 0.10$) to increase the size of the kidney. In conclusion, dietary supplementation of xylanase increased nutrient digestibility and digesta pH but did not influence the growth performance of weaned pigs fed wheat and CM-based diets over a 35-d period.

Key words: canola meal, dehulling, digestibility, pig, xylanase

Abbreviations

AA	amino acid
ADFI	average daily feed intake
ADG	average daily gain
ATTD	apparent total tract digestibility
BW	body weight
CCM	coarse canola meal
CM	canola meal
CP	crude protein
DCM	dehulled canola meal
DM	dry matter
NDF	neutral detergent fiber
NE	net energy
NSP	non-starch polysaccharides
pCaSR	porcine Ca-sensing receptors
RCM	regular canola meal
SBM	soybean meal
SCFA	short-chain fatty acids
SID	standardized ileal digestible
STTD	standardized total tract digestibility
VFA	volatile fatty acid

Introduction

Canola meal (**CM**) is the second most abundant protein supplement after soybean meal (**SBM**; Canola Council of Canada, 2015). The Canadian canola seed production has augmented from 12.7 million metric tons (**MMT**) in 2010 to 21.3 MMT in 2017, increasing the availability of CM in the global market (Statistics Canada, 2018). However, the global rapeseed–canola industry has failed to process the meal to increase protein and decrease fiber content. Nevertheless, the need for protein alternatives in diet formulation has increased the interest for other options for processing in canola. Research in France on dehulling has been undertaken; however, significant fat losses have been observed (Carré et al., 2016). Dehulling also has been studied in Norway (Hansen et al., 2017) and Canada (Mejicanos et al., 2017), among others. It has been observed that dehulling of canola increased the standardized total tract digestibility (**STTD**) of phosphorous (P; Mejicanos et al., 2018) and increased the standardized ileal digestible (**SID**) amino acid (**AA**) content of the dehulled canola meal (**DCM**; Mejicanos and Nyachoti, 2018).

Slominski and Campbell (1990) indicated that non-cellulose polysaccharides in CM (13% to 16% of the meal) contain significant amounts of arabinose and xylose (33% and 13%, respectively); furthermore, in the laying hen experiment that was part of the study, the polysaccharide components showed low digestibility (<3%); however, non-starch polysaccharides (**NSP**) digestibility was increased to 37% using a multi-activity complex of fiber-degrading enzymes. Similar results have confirmed the efficacy of supplementing xylanase alone (Bedford and Morgan, 1995) or in combination with other enzymes and organic acids (Disetlhe et al., 2018) to improve the growth performance in broilers fed CM-containing diets. Furthermore, the use of xylanase has improved nutrient digestibility, and growth performance of pigs fed diets containing rapeseed meal in corn-based diets (Fang et al., 2007).

DCM produces fractions with distinctive levels of NSP, with the dehulled meal containing lower fiber and higher crude protein (**CP**; Mejicanos et al., 2017), which could affect the action of fiber-degrading enzymes such as xylanase. Ndou et al. (2015) and Zhang et al. (2018) indicated that cereal-based diets with higher NSP content promote a more diverse nutritional niche, resulting in higher microbiota diversity in the cecum. However, it is unclear if supplementing xylanase in a wheat-based diet containing regular CM or its fractions will affect growth performance, nutrient digestibility, or the concentration of short-chain fatty acids (**SCFA**) of pigs over a 35-d period. Therefore, the objective of this study was to determine the effect of xylanase supplementation in a wheat-based diet containing CM, and the influence on nutrient digestibility, organ weight, pH in ileal and colon digesta, SCFA concentration in colonic digesta, and growth performance, when fed to weaned pigs.

Materials and Methods

Animal care

The animal use protocol utilized in the present study was reviewed and approved by the Animal Care Committee of the University of Manitoba. Pigs were cared for following the guidelines of the Canadian Council on Animal Care (2009). The study was conducted at the T. K. Cheung Centre for Animal Science Research.

Animals and housing

The current experiment used a total of 144 pigs (TN70 [Large White × Landrace] × Tempo; Topigs Norsvin, Winnipeg, MB, Canada] obtained from Glenlea Swine Research Unit, University of Manitoba. Piglets were weaned at 18 ± 2 d of age, with an initial body weight (BW) of 6.2 ± 0.7 kg (mean ± SD). On day 1, piglets were weighed and randomly assigned within but not across sex to one of the eight diets in a randomized complete block. Pens with plastic-covered expanded metal floors were used (space allowed was 0.8 m^2 per piglet). Room temperature was initially set at 30 ± 1 °C and was gradually decreased by 1 °C every week. A 16-h light (0600 to 2200 hours) and an 8-h dark cycle was provided. Water and feed were provided ad libitum using nipple drinkers and stainless-steel feeding troughs throughout the 5-wk study. Body-weight and feed disappearance were monitored weekly. At the end of the study, one pig per pen was euthanized for organ weighing, ileal, and colonic digesta collection.

Dehulling of canola meal and ingredients

The **DCM** and coarse canola meal (**CCM**) fractions were produced at the Canadian International Grains Institute, Winnipeg, MB, Canada using a plansifter Model MPAR-8HK (Bühler AG, Uzwil, Switzerland), according to the methodology suggested by Mejicanos et al. (2017), with some modifications, namely using only sieve size of 355 µm, to produce two CM fractions. The DCM (particle size < 355 µm) had higher CP and P contents than the CCM (particle size > 355 µm) fraction; however, it had lower NDF, ADF, lignin, and polyphenols, whereas, the opposite was observed in the coarse fraction. The wheat used in the experiment was a Hard Canada Western Red Spring (CWRS). All other ingredients were obtained from the local market. The analyzed chemical composition of the wheat, SBM, regular CM, and the fractions (DCM and CCM) used in the current study are presented in Table 1.

Diets

The study included eight diets consisting of a control wheat/SBM basal diet as well as the combination of three types of CM, regular canola meal (**RCM**), DCM, and CCM, without and with 100 g/ton of xylanase equivalent to 16,000 BXU/kg (Econase XT 25P; AB Vista, Marlborough, Wiltshire, UK; 160,000 BXU/g). One

Table 1. Analyzed chemical composition of wheat, SBM, and CM and its fractions used in the present experiment (as-is basis)

			CM[1]		
Item, %	Wheat[3]	SBM[3]	RCM	DCM	CCM
DM	88.26	88.87	90.78	90.92	91.35
Ash	1.67	6.61	7.20	7.50	6.90
CP (N × 6.25)	11.86	47.60	39.52	41.85	37.62
Gross energy, kcal/kg	3,952	4,308	4,380	4,416	4,388
Ether extract	1.97	0.99	3.49	4.89	6.06
Total fat	2.63	1.62	4.90	6.10	7.36
Starch	61.31	7.07	3.35	2.59	1.05
Sucrose	3.23	8.37	7.68	7.35	8.74
Fiber fractions					
NDF	7.81	10.95	26.20	17.80	30.30
ADF	3.59	6.86	20.21	13.85	19.00
Total dietary fiber	—	—	33.01	24.57	37.25
NSP	—	—	20.54	16.97	20.99
Lignin and polyphenols	—	—	10.17	6.01	11.38
Total P	0.39	0.71	1.10	1.27	1.08
Non-phytate P[2]	0.11	0.33	0.39	0.59	0.31
Ca	0.06	0.33	0.67	0.60	0.67
Total glucosinolate, μmol/g	—	—	9.2	9.6	9.1
Indispensable AA					
Arg	0.60	3.45	2.10	2.49	2.03
His	0.34	1.28	1.16	1.33	1.15
Ile	0.47	2.14	1.25	1.41	1.23
Leu	0.91	3.62	2.51	2.92	2.48
Lys	0.39	2.96	2.04	2.29	1.96
Met	0.22	0.66	0.47	0.48	0.48
Phe	0.64	2.40	1.44	1.63	1.39
Thr	0.40	1.86	1.59	1.80	1.58
Trp	0.17	0.66	0.42	0.42	0.42
Val	0.58	2.23	1.55	1.74	1.58
Dispensable AA					
Ala	0.47	2.06	1.73	2.03	1.72
Asp	0.71	5.41	2.78	3.17	2.72
Cys	0.33	0.70	0.50	0.78	0.67
Glu	3.88	8.54	6.57	7.61	6.39
Gly	0.57	1.99	1.80	2.09	1.78
Pro	1.36	2.53	2.42	2.61	2.25
Ser	0.60	2.36	1.80	2.05	1.76
Tyr	0.36	1.59	0.99	1.10	0.97

[1]RCM, DCM (particle size < 355 μm), and CCM (particle size > 355 μm).
[2]Non-phytate P was calculated as the difference between total P and phytate-bound P.
[3]AA, total P, and Ca content data from NRC (2012).

BXU is defined as the amount of enzyme that produced reducing carbohydrates having a reducing power corresponding to 1 nmol xylose from birch xylan in 1 s under assay conditions (Baily and Poulanen, 1989). All diets contained 500 FTU/kg of an *Escherichia coli*-derived phytase (Quantum Blue 5G, AB Vista, Marlborough, Wiltshire, UK; 5,000 FTU/g). One FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic P per minute from 0.0051 mol/liters sodium phytate at 37 °C and pH 5.50 (AOAC, 2000). Diets were formulated to meet or exceed NRC (2012) nutrient requirements for weaned pigs and contained 0.3% titanium dioxide (TiO_2) as an indigestible marker to determine the apparent total tract digestibility (**ATTD**) of nutrients. A two-phase feeding program was used (phase I, 1 to 21 and phase II, 22 to 35 d post-weaning). The trial was terminated at day 35 post-weaning to allow pigs to consume plant-based diets for 2 wk, allowing a shift in the gut microbiome to digest fiber; furthermore, the effects of populations change in the gut due to the diets could be observed (Frese et al., 2015).

Diets were balanced according to SID AA, standardized total tract digestible P, and net energy (**NE**). The composition and nutrient contents of phase I diets are summarized in Tables 2 and 3, respectively. In the formulation of phase II diets (days 22 to 35), CM or SBM were the sole protein supplement. However, synthetic AA were added to balance the diets and meet the essential nutrient requirements of pigs (NRC, 2012). The composition and nutrient contents of phase II diets are summarized in Tables 4 and 5, respectively. The diets were offered in a mash form and fed ad libitum for 35 d.

Experimental and analytical procedures

Pigs were assigned to the eight experimental diets in a randomized complete block design to give six single-sex replicates of three pigs of each sex per experimental unit (three replicates per sex) in two separate rooms (blocks). In preparation for the ATTD sampling, pens were thoroughly washed and rinsed using warm water. Freshly voided fecal samples were

Table 2. Composition of phase I diets fed to weaned pigs (%, as-fed basis)

	Diets without xylanase				Diets with xylanase			
Ingredient	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Wheat	47.31	42.40	45.36	44.28	47.31	42.40	45.36	44.28
SBM	15.00	—	—	—	15.00	—	—	—
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dry whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
RCM	—	20.00	—	—	—	20.00	—	—
DCM	—	—	17.00	—	—	—	17.00	—
CCM	—	—	—	17.40	—	—	—	17.40
Iodized salt	0.50	0.50	0.50	0.50	0.5	0.50	0.50	0.50
Canola oil	3.50	3.50	3.50	4.00	3.50	3.50	3.50	4.00
Calcium carbonate	0.78	0.63	0.79	0.62	0.78	0.63	0.79	0.62
Dicalcium phosphate	0.25	0.13	—	0.24	0.25	0.13	—	0.24
Lysine-HCl	0.53	0.66	0.67	0.72	0.53	0.66	0.67	0.72
DL-Methionine	0.17	0.20	0.19	0.20	0.17	0.20	0.19	0.20
L-Threonine	0.16	0.17	0.18	0.21	0.16	0.17	0.18	0.21
L-tryptophan	—	0.01	0.01	0.03	—	0.01	0.01	0.03
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Enzyme premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Marker	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]Supplied per kg of diet: vitamins: vitamin A, 8,250 IU; vitamin D3, 835 IU; vitamin E, 40 IU; vitamin K 4 mg; thiamin (B1), 2.0 mg; Riboflavin, 12 mg; pantothenate, 15 mg; choline 500 mg; niacin, 22.5 mg; vitamin B6, 4.5 mg; vitamin B12 25 µg; biotin, 200 µg; folic acid, 2 mg.
[2]Minerals: Cu, 25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.
[3]Enzymes: phytase, 500 FTU/kg; xylanase, 16,000 BXU/kg, and wheat used as a carrier.

Table 3. Calculated and analyzed nutrient content of phase I diets fed to weaned pigs (%, as-fed basis)[1]

	Diets without xylanase				Diets with xylanase			
Item	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Calculated nutrient content								
CP	19.61	19.92	19.49	18.86	19.61	19.92	19.49	18.86
NE, kcal/kg	2,483	2,471	2,507	2,463	2,483	2,471	2,507	2,463
Ca, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Available P, %	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Methionine, % (SID)	0.45	0.47	0.45	0.47	0.45	0.47	0.45	0.47
Methionine + cysteine, % (SID)	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Lysine, % (SID)	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Threonine, % (SID)	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Tryptophan, % (SID)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Phytase, FTU/kg	500	500	500	500	500	500	500	500
Xylanase, BXU/kg	—	—	—	—	16,000	16,000	16,000	16,000
Analyzed nutrient content								
DM	90.38	90.88	90.82	91.10	90.60	91.02	91.34	90.93
Ash	4.18	4.54	4.76	4.37	3.93	4.67	4.45	4.49
CP, % (N × 6.25)	20.49	20.06	19.85	19.28	20.41	19.94	20.10	18.96
Ether extract	5.79	5.47	6.35	6.33	5.31	5.89	5.96	6.10
Total fat	6.74	6.43	7.35	7.33	6.27	6.85	6.96	7.10
Starch	35.19	33.61	32.32	32.82	37.54	32.07	34.06	33.95
Sugar	21.9	22.91	23.13	23.37	23.70	24.06	22.51	22.57
NDF	6.80	10.51	8.02	10.17	6.84	10.24	7.90	10.15
Ca	0.84	0.88	0.78	0.87	0.83	0.79	0.76	0.78
Total P	0.75	0.65	0.69	0.75	0.66	0.71	0.71	0.71
Phytase, FTU/kg	528	630	567	999	1,010	934	737	680
Xylanase, BXU/kg	<2,000	<2,000	<2,000	<2,000	22,800	21,900	21,000	23,300

[1]All diets were formulated to contain the following quantities of the ileal digestible indispensable AA, %: Met, 0.39; Met + Cys 0.74; Lys, 1.35; Thr, 0.79; Trp, 0.22.

Table 4. Composition of phase II experimental diets fed to weaned pigs (%, as-fed basis)

	Diets without xylanase				Diets with xylanase			
Ingredient	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Wheat	74.70	70.16	71.97	74.62	74.70	70.16	71.97	74.62
SBM	15.46	—	—	—	15.46	—	—	—
RCM	—	20.06	—	—	—	20.06	—	—
DCM	—	—	18.31	—	—	—	18.31	—
CCM	—	—	—	15.00	—	—	—	15.00
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Canola oil	4.40	4.40	4.40	4.68	4.40	4.40	4.40	4.68
Calcium carbonate	1.20	1.08	1.24	1.07	1.20	1.08	1.24	1.07
Dicalcium phosphate	0.68	0.52	0.34	0.66	0.68	0.52	0.34	0.66
Lysine-HCl	0.79	0.94	0.92	1.03	0.79	0.94	0.92	1.03
DL-Methionine	0.19	0.22	0.20	0.24	0.19	0.22	0.20	0.24
L-Threonine	0.27	0.29	0.29	0.35	0.27	0.29	0.29	0.35
L-Tryptophan	0.01	0.03	0.03	0.05	0.01	0.03	0.03	0.05
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Enzyme premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Marker	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]Supplied per kg of diet: vitamins: vitamin A, 8,250 IU; vitamin D3, 825 IU; vitamin E, 40 IU; vitamin K 4 mg; thiamin (B1), 2.0 mg; Riboflavin, 10 mg; pantothenate, 15 mg; choline, 500 mg; niacin, 22.5 mg; vitamin B6, 4.5 mg; vitamin B12, 25 µg; biotin, 200 µg; folic acid, 2 mg.
[2]Minerals: Cu, 25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.
[3]Enzymes: phytase, 500 FTU/kg; xylanase, 16,000 BXU/kg; and wheat used as a carrier.

Table 5. Calculated and analyzed nutrient content of phase II diets fed to weaned pigs (%, as-fed basis)[1]

	Diets without xylanase				Diets with xylanase			
Item	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM
Calculated nutrient composition								
CP, %	17.23	17.41	17.34	15.78	17.23	17.41	17.34	15.78
NE, kcal/kg	2,426	2,415	2,451	2,412	2,426	2,415	2,451	2,412
Ca, %	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Phosphorus, STTD	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Methionine, % (SID)	0.40	0.42	0.39	0.42	0.40	0.42	0.39	0.42
Methionine + cysteine, % (SID)	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Lysine, % (SID)	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
Threonine, % (SID)	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Tryptophan, % (SID)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase, FTU/kg	500	500	500	500	500	500	500	500
Xylanase, BXU/kg	—	—	—	—	16,000	16,000	16,000	16,000
Analyzed nutrient content								
DM	89.16	89.20	89.29	89.60	88.91	89.34	89.45	89.36
Ash	3.25	3.49	3.57	3.40	3.25	3.48	3.53	3.03
CP, % (N × 6.25)	17.83	17.16	17.91	16.16	17.38	17.98	18.00	16.05
Ether extract	5.50	5.12	5.58	5.81	5.23	5.50	5.81	5.83
Total fat	6.61	6.20	6.68	6.94	6.34	6.57	6.90	6.95
Starch	49.85	45.93	46.77	47.05	49.40	45.51	46.27	48.80
Sugar	4.98	5.29	5.31	5.16	5.29	5.89	5.51	5.46
NDF	9.35	12.30	10.16	11.89	9.05	12.31	10.59	11.59
Ca	0.66	0.63	0.59	0.61	0.75	0.77	0.82	0.81
Total P	0.56	0.58	0.60	0.58	0.52	0.59	0.67	0.64
Phytase, FTU/kg	696	744	956	1,160	794	1,020	846	991
Xylanase, BXU/kg	<2,000	<2,000	<2,000	<2,000	18,200	20,100	18,600	20,900

[1]All diets were formulated to contain the following quantities of the ileal digestible indispensable AA, %: Met, 0.36; Met + Cys, 0.68; Lys, 1.23; Thr, 0.73; Trp, 0.20.

collected by hand grab in plastic bags on day 30 of the study. Samples were carried on ice and stored at –20 °C in the freezer until analyses. Fecal samples were dried in a forced-air oven at 60 °C for 5 d. Dried samples were pooled for each pen and ground using a heavy-duty blender (model CB15, Waring Commercial, Torrington, CT), then a subsample was obtained after thoroughly mixing and sieving for homogeneity (sieve size 40, 420 µm) the ground feces for chemical analysis. Diets and CM

samples were finely ground before analysis using a Foss sample preparation Cyclotec 1093 mill (Foss Allé 1, DK-3400 Hilleroed, Denmark). Experimental diets and CM were subjected to CP (N × 6.25) analysis using an N analyzer, model TruSpec N (LECO Corp., St. Joseph, MI, USA). Samples for AA analysis were prepared according to the Association of Official Analytical Chemists (**AOAC**) procedures 994.12, alternatives 3 and 1 (sulfur AA), and then determined using an AA analyzer (S4300, Sykam GmbH, Eresing, Germany). Standard AOAC (2005) procedures were used for dry matter (**DM**) (method 930.15), fat (method 2003.06), and ash determination (method 942.05). Phytate-P was determined using the method described byHaug and Lantzsch (1983). Sucrose and glucosinolates were determined by gas-liquid chromatography as described by Slominski et al. (1994) and Slominski and Campbell (1987). Dietary fiber was determined by a combination of neutral detergent fiber (**NDF**) and detergent-soluble NSP measurements and was calculated as the sum of NDF and detergent soluble NSP (Slominski et al., 1994). NDF was determined using an Ankom fiber analyzer (Ankom Technology, Macedon, NY) and according to AOAC (2005) method 2002.04. NDF in fecal samples was determined using Ankom filter bags F58 due to significant pulverization of the sample during grinding (initially regular filter bags F57 were used). Total NSP were determined by gas-liquid chromatography (component neutral sugars) using an SP-2340 column and Varian CP3380 gas chromatograph (Varian, Inc., Palo Alto, CA) and colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) and the procedure described by Englyst and Cummings (1984, 1988) with some modifications (Slominski and Campbell, 1990). The content of NSP was measured in both the meals and the NDF residues. Neutral detergent soluble NSP was calculated as total sample NSP minus NSP present in the NDF residue, and total dietary fiber was determined by the summation of NDF and NDF-soluble NSP. The contents of CP (N × 6.25) and ash in NDF residue were also measured. The value for lignin and associated polyphenols was calculated by difference [NDF – (NSP + protein + ash)] (Slominski et al., 1994). Diets, fecal samples, and CM for Ca and P analysis were ash at 600 °C for 12 h, digested according to the AOAC (2005) method 985.01, and determined using a Varian Inductively Coupled Plasma Mass Spectrometer (Varian, Inc., Palo Alto, CA). The concentration of phytate-bound P in the CM was calculated as 28.2% of analyzed phytate (Tran and Sauvant, 2004). Non-phytate P was calculated by subtracting phytate-bound P from total P. Titanium dioxide concentration in feed and fecal samples was measured according to the revised protocol (Lomer et al., 2000) and submitted to analyses using a Varian Inductively Coupled Plasma Mass Spectrometer (Varian, Inc., Palo Alto, CA). Diets were analyzed for xylanase activity by ELISA method using Quantiplate Kits for Econase XT (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK) and phytase activity by an ELISA method, using Quantiplate Kits for Quantum Blue supplied by Envirologix (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK).

Short-chain fatty acids, digesta pH, and organ weight

On day 35 of the trial, one pig per pen was randomly selected, weighed, then sedated by an intramuscular injection of azaperone and xylazine (2.2 and 2 mg/kg, respectively; Elanco, Division Eli Lilly Canada Inc., Guelph, ON, Canada and Bimeda MTC Animal Health Inc., Cambridge, ON, Canada, respectively) and euthanized by penetration of captive bolt. Subsequently, pigs were eviscerated from sternum to pubis, the gastrointestinal tract and organs (heart and lungs without trachea) were removed, emptied from blood and digesta, and weighed. Approximately 20 mL of digesta samples from the ileum (30 cm immediately before the ileocecal junction) and the colon (medial colon, 30 cm) were collected for pH analysis using a digital pH meter (Accumet, Fisher Scientific, Hampton, NH, USA). Additionally, 1 g of colonic digesta per pig was obtained for SCFA. The SCFA were analyzed as free acids by gas chromatography, using pivalic acid as an internal standard (Holben et al., 2002). Briefly, 1 mL of H_2O was mixed with 1 g of cecal content, and then 1 mL of 20 mM pivalic acid solution was added as an internal standard. After mixing, 1 mL of perchloric acid was added, and SCFA were extracted by shaking the mixture for 5 min. After centrifugation, perchloric acid in the supernatant was precipitated by adding 50 µL of 4 M KOH in 500 µL of supernatant. After 5 min, saturated oxalic acid was added, and the mixture incubated at 4 °C for 60 min and then centrifuged. Samples were analyzed by gas chromatography using a glass column packed with 80/120 Carbopack B-DA/4% Carbowax 20 M stationary phase (Supelco Inc., Bellefonte, PA, USA), using helium as the carrier gas and a flame ionization detector. The acids measured were acetic, propionic, butyric, valeric, isobutyric, 2-methyl-butyric, isovaleric, and lactic acid.

Calculations and statistical analysis

The ATTD (%) of nutrients (CP, NDF, DM, P, and Ca) was calculated using the following equation:

$$\text{ATTD (\%)} = [1 - (\text{TiO}_2\ \%\ \text{diet} / \text{TiO}_2\ \%\ \text{feces}) \times (\text{Nutrient feces} / \text{Nutrient diet})] \times 100$$

Where TiO_2 % diet is the concentration of titanium dioxide in the diet and TiO_2 % feces is the concentration of titanium dioxide in the excreta. Nutrient diet is the nutrient concentration in the diet. Nutrient feces is the nutrient concentration in the feces.

Data were analyzed using the Mixed Procedure of SAS (SAS software 9.4, SAS Institute, 2013). Treatments were randomly assigned to two rooms (blocks) with 24 pens per room, for a total of 48 pens. Single-sex pens of three pigs served as the experimental unit (three replicates per sex). Pig weight, feed intake, and disappearance were recorded weekly to calculate average daily feed intake (**ADFI**), feed efficiency (G:F), and average daily gain (**ADG**). Random effects of sex, room, sex–treatment interaction, and block–treatment–sex interaction were considered. The model included factorial effects as follows: protein source main effect, enzyme main effect, sex main effect, and two- and three-way interactions from protein source–xylanase and sex. To determine the significance of factorial effects, contrasts were applied, and a *t*-test for the main effect was used. All statements of significance are based on $P < 0.05$, and trends were observed at $0.05 < P < 0.10$.

Results

After 2 wk of the study, one pen (three pigs) was removed from the trial due to health concerns; all other pigs remained healthy during the duration of the study.

Chemical composition of canola meal and diets

The analyzed chemical composition (as-fed basis) of the wheat, SBM, RCM, and its corresponding fractions (DCM and CCM) used in the formulation of the experimental diets are presented in Table 1. Soybean meal was one of the protein sources used in the experiment and had 47.6% CP content. The other protein

sources were RCM, DCM, and CCM. Lower total dietary fiber content was observed in DCM (24.57%) compared with CCM (37.25%) and RCM (33.01%), respectively, whereas NSPs' content was 20.54%, 16.97%, and 20.99% for RCM, DCM, and CCM, respectively. Additionally, starch was higher for diets containing SBM compared with diets containing RCM, DCM, and CCM. The composition, calculated, and analyzed nutrient contents of the experimental diets fed to weaned pigs in phase I are presented in Tables 2 and 3, respectively. The composition, calculated, and analyzed nutrient contents of phase II diets are shown in Tables 4 and 5, respectively. Analyzed enzyme activities in feed samples of phase I and II diets were all close to expected.

Effect of protein source and the use of xylanase on growth performance, organ weight, nutrient digestibility, and digesta pH

The effect of protein source, xylanase supplementation, sex, and their two- and three-way interactions on the ADG, ADFI, and G:F ratio is shown in Table 6. At the beginning of the experiment, barrows were heavier than gilts ($P < 0.05$; 6.32 vs. 6.19 kg), which resulted in the sex effect ($P < 0.05$) and a two-way interaction ($P < 0.05$) for protein source and sex when the treatments were assigned. However, such differences were not maintained for the rest of the experiment. Protein source, xylanase supplementation, or sex did not affect ($P > 0.10$) overall ADG. Likewise, protein source, xylanase supplementation, or sex had no effect ($P > 0.10$) on the overall G:F ratio. However, a tendency ($P = 0.054$) for a higher G:F ratio (0.66 vs. 0.63) with xylanase supplementation on week 4 was observed. Likewise, a tendency ($P = 0.054$) for higher G:F ratio when pigs were fed diets containing DCM as the protein source in week 5 was observed. Protein source, xylanase supplementation, or sex did not affect ($P > 0.10$) the overall ADFI. However, in the first week of the study, a significant two-way interaction ($P < 0.05$) for protein source and xylanase was observed.

The effect of protein source and xylanase supplementation on the ileal and colonic pH and the ATTD of nutrients is shown in Table 7. Ileum pH was higher ($P < 0.05$) for diets containing xylanase (6.66 vs. 6.27). Additionally, a tendency ($P = 0.092$) for higher ileal pH in diets fed to barrow was observed. A three-way interaction for protein source–xylanase–sex for colonic digesta pH was observed. The pH means in the digesta of pigs fed either protein source (CCM, DCM, RCM, or SBM) behave differently in barrows compared with gilts; colonic pH decreased in the case of feeding diets supplemented with xylanase and DCM as protein source to barrows, whereas, feeding diets supplemented with xylanase and RCM as the protein source resulted in decrease in colonic pH, in the case of gilts. Supplementing xylanase in all other protein sources increased colonic pH. Furthermore, colonic pH was higher ($P < 0.05$) in diets containing xylanase (5.93 vs. 5.74). Protein source affected colonic pH, and diets containing DCM and SBM had higher pH ($P < 0.05$; 6.02 and 5.92, respectively) compared with diets containing RCM and CCM (5.68 and 5.73, respectively).

The addition of xylanase resulted in higher ($P < 0.05$) ATTD of protein, DM, P, and Ca, compared with diets without xylanase (75% vs. 70%, 80% vs. 78%, 80% vs. 77%, 49% vs. 45%, and 62% vs. 52%, respectively). The effect of protein source was significant ($P < 0.05$) for DM and Ca digestibility. A tendency ($P = 0.059$) for higher protein digestibility when pigs were fed SBM-containing diets was observed. A two-way interaction protein–xylanase ($P < 0.05$) for ATTD of NDF was observed, and xylanase supplementation to diets containing RCM and DCM resulted in increased ATTD of NDF from 28% to 31% and from 30% to 38%, respectively. Additionally, a two-way interaction xylanase–sex ($P < 0.05$) for ATTD of NDF was also observed, and ATTD of NDF increased when xylanase was fed to gilts from 30% to 35%, whereas it decreased from 36% to 30% when fed to barrows. However, in general, diets containing SBM as protein sources had higher digestibility coefficients than the others.

The effect of protein source and the use of xylanase on organ weights are shown in Table 7. Protein source had no effect ($P > 0.10$) on the relative weight of all organs measured, except ($P < 0.05$) liver and spleen. The liver of pigs fed RCM and DCM had higher ($P < 0.05$) relative weight (36.63 and 37.12 g/kg/BW) than pigs fed diets containing SBM and CCM (34 and 34.96 g/kg/BW). Whereas the relative weight of spleens in pigs fed diets containing SBM and RCM was 2.57 and 2.28 g/kg/BW, compared with 1.96 and 1.92 g/kg/BW in pigs fed DCM and CCM, respectively. The relative weights of the liver and spleen were increased ($P < 0.05$) by xylanase supplementation. Xylanase supplementation tended to increase the relative weights of the kidney ($P = 0.092$; 6.39 vs. 6.02).

Effect of protein source and xylanase supplementation on the levels of SCFA

The effect of protein source, sex, the use of xylanase, and their two- and three-way interactions on the levels of SCFA is also shown in Table 7. A three-way interaction protein–xylanase–sex for propionic acid was observed; the concentration of propionic acid differed not just according to protein source and xylanase supplementation but also according to sex. Supplementing xylanase to diets containing DCM fed to barrows significantly increased the concentration of propionic acid, whereas supplementing xylanase to diets containing CCM, RCM, and SBM as the protein source decreased propionic acid concentration. However, in the case of gilts, supplementing xylanase to diets containing CCM and RCM significantly increased the concentration of propionic acid, whereas supplementing xylanase to diets containing DCM and SBM resulted in decreased concentration of propionic acid. For acetic acid, a significant effect ($P < 0.05$) for the three-way interaction protein–xylanase–sex was also observed. Feeding xylanase-supplemented diets containing CCM, RCM, and SBM to barrows significantly increased the concentration of acetic acid. Whereas feeding gilts diets supplemented with xylanase and using CCM and DCM as the protein source significantly increased the concentration of acetic acid.

Valeric acid concentrations were influenced by protein sources ($P < 0.01$). Pigs fed with RCM and DCM had higher levels; CCM had intermediate concentrations, while SBM had the lowest (3.7, 3.6, 2.5, and 1.7 mM, respectively). Overall, xylanase supplementation reduced butyric acid concentration in diets (18.8 vs. 15.5 mM; $P = 0.085$).

Discussion

Chemical composition of canola meal and diets

The fiber present in fibrous feed ingredients can reduce nutrient digestibility through encapsulation, resulting in lower feed efficiency and growth (Kerr and Shurson, 2013). However, with the incorporation of NSP-degrading enzymes (NSPases) to the diet, those effects can be reduced. A systematic review and meta-analysis of the impact of feed enzymes on growth and nutrient digestibility in growing and finisher pigs found that G:F was improved in 32% of the studies, including growth data

Table 6. Effect of protein source and xylanase supplementation on the ADG, ADFI, and gain to feed ratio (G:F) of weaned pigs[1]

	Least square means for main effects															
							Protein × xylanase interaction									
	Protein source effect				Xylanase effect		Without xylanase				With xylanase					
Traits	SBM	RCM	DCM	CCM	with	w/o	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM	SEM	P-value
Initial BW[2,3]	6.27	6.19	6.23	6.32	6.26	6.24	6.28	6.17	6.15	6.26	6.22	6.28	6.29	6.10	0.07	0.391
Final BW	22.22	21.39	21.42	21.26	21.55	21.61	22.62	21.31	21.66	21.88	22.72	21.12	22.84	21.17	0.86	0.865
ADG, g/d/pig																
Week 1	103	98	86	83	92	93	105	100	92	76	102	96	81	90	12.12	0.775
Week 2	344	314	308	314	322	318	349	302	301	319	343	325	314	311	31.16	0.925
Week 3	505	467	431	453	453	453	526	456	399	475	515	454	472	450	19.61	0.885
Week 4	561	581	564	578	570	572	558	597	554	579	571	567	581	578	41.77	0.938
Week 5	744	733	762	693	713	752	729	776	782	720	787	691	792	665	58.14	0.648
ADFI, g/day/pig																
Week 1	151	158	137	141	142	151	160	161	159	130	143	155	118	153	10.11	0.012
Week 2	417	393	384	408	394	407	425	388	396	419	414	398	372	399	41.79	0.953
Week 3	746	697	670	696	702	703	778	686	629	719	750	682	741	702	37.78	0.862
Week 4	853	893	877	926	916	858	839	946	933	950	880	840	830	896	61.72	0.491
Week 5	1,160	1,162	1,135	1,190	1,153	1,171	1,164	1,195	1,112	1,226	1,215	1,124	1,175	1,156	90.01	0.812
G:F																
Week 1	0.67	0.62	0.64	0.60	0.61	0.65	0.65	0.63	0.60	0.58	0.72	0.61	0.68	0.61	0.09	0.922
Week 2[4]	0.82	0.81	0.79	0.77	0.81	0.78	0.82	0.79	0.76	0.76	0.83	0.82	0.85	0.78	0.04	0.532
Week 3	0.68	0.67	0.65	0.65	0.66	0.66	0.69	0.66	0.62	0.67	0.68	0.67	0.69	0.64	0.03	0.752
Week 4[5]	0.66	0.65	0.65	0.63	0.66	0.63	0.66	0.63	0.61	0.61	0.65	0.68	0.69	0.64	0.02	0.387
Week 5	0.64	0.63	0.69	0.59	0.63	0.65	0.64	0.63	0.71	0.60	0.66	0.62	0.66	0.58	0.02	0.791

[1]Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, sex, two- and three-way interactions. Random effects included block and interactions of block with the three fixed effects. Single-sex pens were used, 3 pens per sex.
[2]Sex effect for initial BW (6.32 and 6.19 for barrows and gilts, respectively $P < 0.05$; SEM 0.03).
[3]Protein × sex interaction for initial BW ($P < 0.005$).
[4]Tendency ($P < 0.10$) in the G:F interaction xylanase × sex in week 2.
[5]Tendency ($P < 0.10$) in the G:F xylanase effect in week 4.

Table 7. Effect of protein source and xylanase supplementation on ileum and colon pH, ATTD of nutrients, relative organ weight, and SCFA in the mid colon of nursery pigs[1]

	Least square means for main effects															
							Protein × xylanase interaction									
	Protein source effect				Xylanase effect		Without xylanase				With xylanase					
Traits	SBM	RCM	DCM	CCM	with	without	SBM	RCM	DCM	CCM	SBM	RCM	DCM	CCM	SEM	*P*-value
pH																
Ileum[2]	6.35	6.36	6.50	6.66	6.66^{x}	6.27^{y}	6.08	6.18	6.25	6.54	6.61	6.55	6.73	6.76	0.16	0.733
Colon[3,5]	5.92^{x}	5.68^{y}	6.02^{x}	5.73^{y}	5.93^{x}	5.74^{y}	5.73	5.78	5.76	5.65	6.05	5.78	6.54	5.80	0.12	0.300
Digestibility, %																
CP	75	71	72	72	75^{x}	70^{y}	73	68	70	70	78	73	75	73	1.54	0.471
NDF[4]	37	30	33	30	33	33	37	28	30	36	37	31	38	24	3.19	0.042
DM	81^{x}	76^{y}	77^{y}	78^{xy}	80^{x}	77^{y}	79	74	75	77	83	77	79	80	1.51	0.937
P	51	44	47	46	49^{x}	45^{y}	52	42	41	44	51	47	51	48	2.82	0.075
Ca	66^{x}	52^{y}	57^{xy}	54^{y}	62^{x}	52^{y}	64	49	48	46	67	56	62	63	3.49	0.265
Organ weight, g/kg/BW																
Heart[6]	5.64	5.63	5.53	5.27	5.58	5.45	5.76	5.50	5.33	5.18	5.69	5.77	6.08	5.28	0.33	0.845
Lungs	12.88	12.82	12.64	12.85	13.34	12.26	12.33	13.05	12.15	11.45	13.25	12.60	15.18	14.09	0.61	0.494
Thyroid	0.09	0.09	0.07	0.05	0.07	0.07	0.07	0.12	0.04	0.04	0.10	0.05	0.08	0.06	0.03	0.252
Liver	34.0^{y}	36.6^{x}	37.1^{x}	35.0^{y}	36.3^{x}	35.1^{y}	33.9	36.7	36.0	33.7	33.5	37.0	37.3	36.1	0.68	0.175
Spleen[7]	2.57^{x}	2.28^{x}	1.96^{y}	1.92^{y}	2.36^{x}	2.01^{y}	2.28	2.25	1.72	1.76	2.87	2.32	2.20	2.90	0.27	0.484
Kidney[15]	6.09	6.02	6.74	5.96	6.39	6.02	6.13	6.12	6.60	5.34	5.92	6.03	6.70	6.45	0.26	0.132
Stomach	8.04	7.90	8.49	7.62	7.94	8.09	8.47	8.20	8.03	7.40	7.55	7.64	8.41	7.98	0.40	0.093
Small intestine[8]	44.48	44.33	46.31	44.94	44.26	45.27	46.20	44.22	44.88	40.82	42.79	41.52	45.31	40.25	1.78	0.330
Large intestine	20.06	19.87	20.37	19.63	18.85	21.12	20.80	19.42	22.36	21.24	18.99	19.13	19.80	18.40	1.47	0.954
SCFA[14], mM																
Total SCFA	135.2	138.9	126.0	125.3	129.3	133.4	141.4	139.1	130.2	122.6	129.1	138.4	120.4	128.3	9.62	0.647
Acetic acid[12,13]	54.4	56.3	50.2	53.8	55.2	52.2	52.9	53.8	51.5	47.4	56.2	52.9	49.3	55.3	1.19	0.089
Propionic acid[11]	36.2^{y}	41.0^{x}	36.2^{y}	32.9^{z}	34.7^{y}	38.4^{x}	38.2	40.3	39.1	34.3	32.6	39.7	32.3	33.0	3.11	0.018
Butyric acid[16]	17.6	17.5	16.3	17.2	15.5	18.8	20.4	19.4	16.0	20.0	14.3	16.4	16.0	14.9	2.17	0.449
Valeric acid	1.7^{y}	3.7^{x}	3.6^{x}	2.5^{xy}	2.7	3.1	1.7	4.1	3.3	2.9	1.5	3.2	3.4	2.0	0.63	0.277
Lactic acid[9]	5.2	3.0	5.0	4.7	5.1	3.9	5.8	2.0	3.4	4.6	5.3	3.9	6.8	5.1	1.05	0.698
BCFA[10]	2.3	1.5	1.7	1.6	1.6	1.9	2.6	1.8	1.8	1.6	2.0	1.1	1.6	1.7	0.40	0.650
VFA	130.0	135.7	120.6	120.8	124.1	129.5	136.2	136.3	126.0	118.8	123.7	134.9	113.6	123.2	9.77	0.648

[1]Based on the MIXED procedure analysis. Fixed effects included protein source, xylanase, sex, two- and three-way interactions. Random effects included block and interactions of block with the three fixed effects. Single-sex pens were used, 3 pens per sex.
[2]Tendency for higher ($P < 0.10$; SEM 0.12) ileal pH in barrows (6.56 vs. 6.37) compared with gilts.
[3]Tendency ($P < 0.10$) in the interaction protein × sex for colon pH.
[4]Xylanase × sex interaction ($P < 0.05$) for NDF digestibility.
[5]Three-way interaction protein–xylanase–sex ($P < 0.05$) for colon pH.
[6]Protein × sex interaction ($P < 0.05$) for relative weight of heart.
[7]Tendency in the protein × sex interaction ($P < 0.10$) for the relative weight of spleen.
[8]Tendency in the xylanase × sex interaction ($P < 0.10$) for the relative weight of the small intestine.
[9]Nonvolatile.
[10]Branched-chain fatty acids include isovaleric, isobutyric, and 2-methyl-butyric acids.
[11]Protein × sex interaction ($P < 0.0001$) for propionic acid.
[12]Tendency in the interaction protein × sex ($P < 0.10$) for the levels of acetic and lactic acid.
[13]Tendency in the interaction of xylanase × sex, protein source, and xylanase effect ($P < 0.10$) for the levels of acetic acid.
[14]Three-way interaction protein–xylanase–sex ($P < 0.05$) for acetic and propionic acid.
[15]Tendency for protein source and xylanase effect ($P < 0.10$) for relative weight of kidney.
[16]Tendency for xylanase effect ($P < 0.10$) for butyric acid concentration.
[x–z]Main effect means sharing a common letter within a factor are not significantly different using a *t*-test ($P < 0.05$).

(Torres-Pitarch et al., 2019). Additionally, DM, gross energy, apparent ileal digestibility, and ATTD were improved by xylanase, xylanase + ß-glucanase, mannanase, and protease dietary supplementation (Torres-Pitarch et al., 2019). Dietary supplementation of xylanase + ß-glucanase did not affect the ADG, ADFI, and G:F (Torres-Pitarch et al., 2019). Given the extensive use of phytase in swine nutrition, all diets in the study contained phytase at 500 FTU/kg; therefore, any improvement in growth performance or digestibility of nutrients would be attributed solely to the use of xylanase. In the formulation of the diets for the current study, protein sources with distinctive nutrient values were utilized, such as the case of DCM, which was higher in CP, total P, non-phytate, and phytate P, and NSP contents, compared with the RCM and CCM. Higher efficiency derived from the use of xylanase has been shown for diets containing a high amount of fiber and NSPs (Zhang et al., 2018).

Effect of protein source and xylanase supplementation on growth performance, organ weight, nutrient digestibility, and digesta pH

The protein source and the use of xylanase or sex did not affect pig growth performance for the 5 wk of the trial. Nevertheless, it is important to mention that the piglets used in the current study were weaned at 18 ± 2 d of age, a very immature microbiota may imbalance the ability of the animal to handle the fermentation activity, which may have influenced the ability of the gastrointestinal tract (**GIT**) microbiota of the piglets to face the fermentable oligosaccharides produced through the action of xylanase. It can be hypothesized that if the piglets had been weaned at 21 d of age or older, they could have performed better with xylanase supplementation. Furthermore, the piglets were fed for only 35 d, and having the pigs on trial for a more extended period would help to know better about the potential of the enzyme on growth performance. Additionally, the diets met the nutrient requirements of swine, according to NRC (2012).

In the present study, the use of xylanase resulted in a significant increase in the ATTD of protein (from 70% to 75%). It has been determined that higher CP digestibility is associated with higher AA digestibility, as can be observed from a study by Stein et al. (2001) that reported SID of CP and AA of 75.2% ± 2.6 and 83.6% ± 2.1, respectively, for wheat fed to growing pigs. However, higher SID of CP and corresponding increase in AA digestibility (82.8% ± 2.5 and 89.5% ± 2.7, respectively), when wheat was fed to gestating sows, was observed; implicating that to maximize the use of phytase and xylanase in the formulation of swine diets, it is essential to determine the SID of CP and AA of the energy source (cereal) as well as the protein source in the diets, when supplemented with exogenous enzymes. A two-way interaction of protein–xylanase for the ATTD of NDF was observed, and xylanase supplementation increased NDF digestibility from 28% to 31% and from 30% to 38% for RCM and DCM, respectively. A two-way interaction of enzyme–sex for the ATTD of NDF was also observed, and overall, supplementing xylanase to diets fed to gilts resulted in higher NDF digestibility.

Results in the present study are consistent with findings by Nortey et al. (2007), indicating increased energy, AA, and P digestibility with the use of xylanase and phytase in wheat byproducts. Weiland (2017) determined that the use of xylanase increased the ATTD of ADF in high-fiber diets, increasing the hindgut disappearance of NDF, ADF, and hemicellulose, increased the AID of DM, starch, and nitrogen, and tended to increase the AID of GE in low-fiber diets. The results are also consistent with findings by Dong et al. (2018) indicating increased nutrient digestibility with xylanase supplementation; however, the author also found that supplemental xylanase (2,000 U/kg) increased growth performance and decreased the richness of gut bacteria while diminishing the growth of pathogenic bacteria.

The pH of colonic digesta was affected by protein source and xylanase supplementation; furthermore, a three-way interaction for protein–xylanase–sex and a tendency for a two-way interaction for protein–sex were observed. Whereas, for ileal pH, a xylanase supplementation effect and a tendency for gender were observed. The effects of xylanase increasing colonic pH are consistent with results by Taylor et al. (2018) that showed higher pH values when diets were supplemented with 8,000 and 16,000 BXU/kg xylanase. However, the increase in pH in colon and ileal digesta with the use of xylanase is challenging to explain, since less Ca and P in solution would result in an increase in acidity rather than a decrease, because higher Ca and P in solution would have a buffer effect (Metzler-Zebeli et al., 2010). The breakdown of fiber in the colon indicated by higher ATTD of Ca and P when diets were supplemented with xylanase (from 52% to 62% and from 45% to 49%, respectively) could result in increased Ca absorption in the colon of pigs. Calcium absorption could happen along the digestive tract. Zhao et al. (2019) studied the molecular distribution of porcine Ca-sensing receptors (**pCaSR**); finding that pCaSR are distributed along the longitudinal axis of the digestive tract, but mostly located in the epithelia of the stomach, duodenum, jejunum, ileum, and colon. The pCaSR are responsive to changes in the extracellular Ca concentration and it is involved in Ca homeostasis (Saidak et al., 2009). The small intestine accounts for approximately 90% of the Ca absorption (Wasserman, 2004; Schröder and Breves, 2006), while the stomach or the large intestine can take up 10% of the total Ca absorbed (Barger-Lux et al., 1989). However, the large intestine may become the leading site for Ca absorption if NSP fractions are included in the diet that interferes with cation absorption in the small intestine (Metzler-Zebeli et al., 2010). Additionally, higher ATTD of nutrients and DM observed when xylanase was supplemented, without improved growth performance confirms the importance of determining not just SID or AA but also its effect on NE, Ca, and P digestibility, when diets are supplemented with phytase plus xylanase. Higher digestibility of Ca in swine diets supplemented with phytase and xylanase, as observed in the present study, can represent an excess of available Ca, which has been indicated as detrimental to the growth performance of pigs unless P is also included above the requirements (Merriman et al., 2017).

Furthermore, a recent extensive survey analyzing commercial pig and broiler diets indicated an excess of 0.22 percentage units of Ca (Walk, 2016). An excess of Ca in diets can have a detrimental effect on feed intake and ADG in finishing pigs when diets are balanced according to P requirements. The addition of phytase plus xylanase in the present experiment further increased the digestibility of Ca and P in diets containing CM or its fractions as a protein source. Therefore, it is crucial to determine the impact of supplementing diets containing CM with microbial phytase and xylanase not just on growth performance but also on the apparent and STTD of Ca and P, to properly formulate diets and to maximize the growth performance of growing pigs, and, thereby, preventing adverse effects derived from excess Ca in diets (Lee et al., 2019).

Protein source and the addition of xylanase affected the liver and spleen size without affecting the growth performance. A significant increase in the relative size of the liver and spleen when xylanase was added to the diets (from 35.06 to

36.29 and from 2.01 to2.36 g/kg BW, respectively) compared with unsupplemented diets regardless of protein source was observed. Therefore, such increases can be attributed to the effect of xylanase in the release of anti-nutritive factors such as glucosinolates in CM, which can be broken down into toxic products, such as thiocyanate, isothiocyanate, oxazolidinethione (goitrin), and nitriles, which may not only reduce feed intake and growth performance but also affect thyroid function by constraining thyroid hormone production and impair liver and kidney function (Bell, 1993). Higher glucosinolates (**GSL**) content in DCM compared with its parent meal (9.2 vs. 9.6; Mejicanos et al., 2017) has been observed.

Pigs fed diets containing SBM and RCM had heavier spleens than pigs fed diets containing DCM and CCM; furthermore, the addition of xylanase to diets fed to pigs also resulted on increased relative size of spleens which can be related to an over-activity of the spleen's function of destroying old red blood cells; furthermore, it suggests a relation between xylanase supplementation and increase in the development of the spleen which is an immune-related organ that produces antibodies (Jia and Pamer, 2009) positively impacting the immune capacity of the pig. However, the spleen also can store healthy erythrocytes and serve as a reservoir place for platelets (that can be important in case of hemorrhagic shock) and monocytes (Clendening, 1930; Sherwood, 1997; Mebius and Kraal, 2005; Swirski et al., 2009). Additionally, the amount of fiber in the diet has been related to increases in the relative weights of organs. In a study feeding pigs high-fiber diets, Anugwa et al. (1989) observed increased stomach, liver, and kidneys size. Feeding high-fiber diets would increase the secretion of digestive juices and enzymes, with implications on the increase in the workload of secretory organs, leading to hypertrophy (Agyekum and Nyachoti, 2017).

Effect of protein source and xylanase supplementation on the levels of SCFA

The total SCFA production was not affected by the protein source, sex, or the addition of xylanase. The total concentrations of SCFA were in the range of 120.4 to 141.4 mM, which is consistent with observations by Topping and Clifton (2001), indicating that depending on the diet, the total concentration of SCFA in the proximal colon decreases from 70 to 140 mM to 20 to 70 mM in the distal colon due to absorption. However, in the present study, total SCFA concentration was higher than those reported by Cardona et al. (2005) for piglets 35-d of age raised indoors (30.3 to 75.5 mM). In the present study, the concentration of volatile fatty acid (**VFA**) in the colon of the pigs were in the range of 113.6 to 136.3 mM, whereas, in a study by Agyekum et al. (2016), the total VFA in the ileum of pigs were between 20.93 and 28.28 mmol/L, when feeding DDGS supplemented with multienzyme. The three-way interactions observed for acetic and propionic acid showed the influence of protein source, gender, and xylanase supplementation in microbial fermentation.

Xylanase supplementation in CCM diets increased acetic acid concentration from 47.4 to 55.3 mM; however, no increase was observed in RCM diets, having similar NDF content, indicating that the type of fiber present in each CM fraction influences SCFA production. Xylanase tended to reduce butyric acid concentrations, which can be associated with increased pH with xylanase supplementation, as pH has a direct effect on bacteria composition in the gut (Palframan et al., 2002). Furthermore, xylanase can increase nutrient digestibility in the small intestine, leading to reduced availability of substrate in the hindgut for fermentation. The reduced fermentation in the hindgut due to xylanase supplementation can result in increased hindgut pH. Additionally, xylanase supplementation can increase starch digestibility in the small intestine, leading to its reduced availability in the large intestine for the production of the butyric acid. However, in the current study, it cannot be discarded an effect on butyric acid concentrations or other VFA compounds. The evaluation of any feed additive through its fermentation capacity by measuring VFA needs to be cautious by several reasons, as pointed byGonzalez-Ortiz et al. (2019): 1) rate of production and absorption; 2) the turnover rate of VFA from the intestine into the blood is extremely fast; 3) the repeatability and reproducibility of such volatile parameters measured at one single point in time question their relevance; and 4) measurements of the presence of enzymes and genes involved in butyrate production would have helped to clarify the impacts of xylanase and different diets according to their fiber content on the intestinal microbiota and fermentability pattern.

In the present study, protein source affected valeric acid concentration in colonic digesta of pigs, and feeding diets containing RCM and DCM resulted in a higher level of valeric acid than feeding diets containing SBM, whereas CCM was intermediate, which suggests increased fermentation of branched-chain AA in the colon of the pigs. In the formulation of the diets, branched-chain AA did not exceed the recommended concentrations (NRC, 2012). The lower levels in valeric acid were observed in SBM as a protein source, which can be related to the high digestibility of protein in SBM (75%); therefore, lower availability of protein for fermentation in the hindgut due to less protein arriving into the hindgut. Additionally, protein digestibility in RCM and DCM was 71% and 72%, respectively. However, the addition of xylanase to RCM and DCM resulted in increases in the ATTD of NDF from 28% to 31% and from 30% to 38%, respectively. Therefore, it is speculated that the breakdown of the fiber due to xylanase supplementation resulted in increased AA availability in the colon of pigs, thus higher concentrations of valeric acid.

Furthermore, the ATTD of protein in xylanase-supplemented diets increased from 70% to 75%, which could have resulted in lower protein fermentability in the hindgut; therefore, lower concentration of branched-chain fatty acids with xylanase supplementation. Lower pH in ileum and colon when xylanase was supplemented to diets may be related to numerically smaller total SCFA (129.3 vs. 133.4 for diets with xylanase and without xylanase, respectively). Higher absorption of butyric acid in xylanase-supplemented diets fed to pigs can be speculated.

In conclusion, the use of xylanase improved nutrient digestibilities in all diets. However, a two-way interaction of protein–xylanase in the NDF digestibility indicates a greater influence of the use of xylanase in wheat–RCM and wheat–DCM-based diets. Xylanase supplementation increased ileal and colonic digesta pH without influencing the growth performance of weaned pigs fed wheat and CM-based diets for 35 d. All diets were balanced according to SID of AA, STTD of P, and NE requirements; therefore, equal growth performance was observed. Xylanase supplementation reduced the concentration of butyric acid in the colon of pigs. A three-way interaction of protein–xylanase–sex in the concentration of acetic and propionic acid indicates that the combined effect of protein source and xylanase could be different according to sex. However, supplementing xylanase increased the relative weight of the liver and spleen and tended to increase the size of the kidney.

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Conflict of interest statement

The authors have no conflicts of interest to disclose.

Literature Cited

Agyekum, A. K., and C. M. Nyachoti. 2017. Nutritional and metabolic consequences of feeding high-fiber diets to swine: a review. *Eng.* **3**:716–725. doi:10.1016/J.Eng.2017.03.010

Agyekum, A. K., A. Regassa, E. Kiarie, and C. M. Nyachoti. 2016. Nutrient digestibility, digesta volatile fatty acids, and intestinal bacterial profile in growing pigs fed a distillers dried grains with solubles containing diet supplemented with multi-enzyme cocktail. *Anim. Feed Sci. Technol.* **212**:70–80. doi:10.1016/j.anifeedsci.2015.12.006

Anugwa, F. O., V. H. Varel, J. S. Dickson, W. G. Pond, and L. P. Krook. 1989. Effects of dietary fiber and protein concentration on growth, feed efficiency, visceral organ weights and large intestine microbial populations of swine. *J. Nutr.* **119**:879–886. doi:10.1093/jn/119.6.879

AOAC. 2000. *Official Methods of Analysis of AOAC International Method 2000.12. Phytase activity in feed: colorimetric enzymatic method*. 17th ed. Arlington (VA): AOAC International.

AOAC. 2005. *Official Methods of Analysis of AOAC International*. 18th ed. Gaithersburg (MD): AOAC International.

Baily, M. J., and K. Poulanen. 1989. Production of xylanases by strains of *Aspergillus*. *App. Microbiol. Biotechnol.* **30**:5–10. doi:10.1007/BF00255989

Barger-Lux, M. J., R. P. Heaney, and R. R. Recker. 1989. Time course of calcium absorption in humans: evidence for a colonic component. *Calcif. Tissue Int.* **44**:308–311. doi:10.1007/bf02556309

Bell, J. M., 1993. Factors affecting the nutritional value of canola meal: a review. *Can J Anim Sci.* **73**:689–697. doi:10.4141/cjas93-075.

Bedford, M. R. and A. J. Morgan. 1995. The use of enzymes in canola-based diets. In: van Hartingsveldt, W., M. Hessing, J. P. van der Lugt, and W. A. C. Somers, editors. Proceedings of the 2nd European Symposium on Feed Enzymes; Noordwijkerhout (The Netherlands); October 25–27, 1995; Zeist: TNO Nutrition and Food Research Institute; p. 125–131.

Canadian Council on Animal Care. 2009. *Guide to the Care and Use of Experimental Animals*. Vol. **1**. 2nd ed. Ottawa (ON), Canada: Canadian Council on Animal Care.

Canola Council of Canada, 2015. *Canola Meal, Feed Industry Guide*. 5th ed. Available from http://www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf [accessed April 1, 2018].

Cardona, M. E., E. Collinder, S. Stern, B. Tjellström, E. Norin, and T. Midtvedt. 2005. Correlation between faecal iso-butyric and iso-valeric acids in different species. *Microb. Ecol. Health Dis.* **17**:177–182. doi.10.1080/08910600500356265

Carré, P., M. Citeau, G. Robin, and M. Estorges. 2016. Hull content and chemical composition of whole seeds, hulls and germs in cultivars of rapeseed (*Brassica napus*). *OCL* **23**:8 doi:10.1051/ocl/2016013

Clendening, L. 1930. *The human body*. Garden City (NY): Garden City Publishing Company, Inc.

Disetlhe, A. R. P., U. Marume, and V. Mlambo. 2018. Humic acid and enzymes inclusion in canola-based diets generate different responses in growth performance, protein utilization dynamics, and hemato-biochemical parameters in broiler chickens. *Poult. Sci.* **97**:2745–2753. doi:10.3382/ps/pey047

Dong, B., S. Liu, C. Wang, and Y. Cao. 2018. Effects of xylanase supplementation to wheat-based diets on growth performance, nutrient digestibility and gut microbes in weanling pigs. *Asian-Australas. J. Anim. Sci.* **31**:1491–1499. doi:10.5713/ajas.17.0867

Englyst, H. N., and J. H. Cummings. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* **109**:937–942. doi:10.1039/AN9840900937

Englyst, H. N., and J. H. Cummings. 1988. Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. *J. Assoc. Off. Anal. Chem.* **71**:808–814. doi:10.1093/jaoac/71.4.808

Fang, Z. F., J. Peng, T. J. Tang, Z. L. Liu, J. J. Dai, and L. Z. Jin. 2007. Xylanase supplementation improved digestibility and performance of growing pigs fed Chinese double-low rapeseed meal inclusion diets: in vitro and in vivo studies. *Asian-Australas. J. Anim. Sci.* **20**:1721–1728. doi:10.5713/ajas.2007.1721

Frese, S. A., K. Parker, C. C. Calvert, and D. A. Mills. 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome* **3**:28. doi:10.1186/s40168-015-0091-8

Gonzáles-Ortiz, G., T. T. dos Santos, K. Vienola, S. Vartiainen, J. Apajalahti, and M. R. Bedford. 2019. Response of broiler chickens to xylanase and butyrate supplementation. *Poult. Sci.* **0**:1–11. doi:10.3382/ps/pez113

Hansen, J. Ø., A. Skrede, L. T. Mydland, and M. Øverland. 2017. Fractionation of rapeseed meal by milling, sieving and air classification – effect on crude protein, amino acids and fiber content and digestibility. *Anim. Feed Sci. Technol.* **230**:143–153 doi:10.1016/j.anifeedsci.2017.05.007

Haug, W., and H. J. Landtzch. 1983. Sensitive method for the rapid determination of phytate in cereal products. *J. Sci. Food Agric.* **34**:1423–1426. doi:10.1002/jsfa.2740341217

Holben, W. E., P. Williams, M. Gilbert, M. Saarinen, L. K. Särkilahti, and J. H. Apajalahti. 2002. Phylogenetic analysis of intestinal microflora indicates a novel *Mycoplasma* phylotype in farmed and wild salmon. *Microb. Ecol.* **44**:175–185. doi:10.1007/s00248-002-1011-6

Jia, T. and E. G. Pamer, 2009. Dispensable but not irrelevant. *Science* **325**:612–616. doi:10.1126/science.1178329

Kerr, B. J., and G. C. Shurson. 2013. Strategies to improve fiber utilization in swine. *J. Anim. Sci. Biotechnol.* **4**:11. doi:10.1186/2049-1891-4-11

Lee, S. A., L. V. Lagos, C. L. Walk, and H. H. Stein. 2019. Standardized total tract digestibility of calcium varies among sources of calcium carbonate, but not among sources of dicalcium phosphate, but microbial phytase increases calcium digestibility in calcium carbonate1. *J. Anim. Sci.* **97**:3440–3450. doi:10.1093/jas/skz176

Lomer, M. C. E., R. P. H. Thompson, J. Commisso, C. L. Keen, and J. J. Powel. 2000. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. *Analyst* **125**:2339–2343. doi:10.1039/B006285P

Mebius, R. E., and G. Kraal. 2005. Structure and function of the spleen. *Nat. Rev. Immunol.* **5**:606–616. doi:10.1038/nri1669

Mejicanos, G. A., J. W. Kim, and C. M. Nyachoti. 2018. Tail-end dehulling of canola meal improves apparent and standardized total tract digestibility of phosphorus when fed to growing pigs. *J. Anim. Sci.* **96**:1430–1440. doi:10.1093/jas/sky040

Mejicanos, G., and C. M. Nyachoti. 2018. Effect of tail-end dehulling of canola meal on apparent and standardized ileal digestibility of amino acids when fed to growing. *Anim. Feed Sci. Technol.* **243**:102–111. doi:10.1016/j.anifeedsci.2018.07.011

Mejicanos, G. A., A. Rogiewicz, C. M. Nyachoti, and B. A. Slominski. 2017. Fractionation of canola meal using sieving technology. *Can. J. Anim. Sci.* **97**:613–621. doi:10.1139/cjas-2016-0229

Merriman, L. A., C. L. Walk, M. R. Murphy, C. M. Parsons, and H. H. Stein. 2017. Inclusion of excess dietary calcium in diets for 100- to 130-kg growing pigs reduces feed intake and daily gain if dietary phosphorus is at or below the requirement. *J. Anim. Sci.* **95**:5439–5446. doi:10.2527/jas2017.1995

Metzler-Zebeli, B. U., S. Hooda, R. Mosenthin, M. G. Gänzle, and R. T. Zijlstra. 2010. Bacterial fermentation affects net mineral flux in the large intestine of pigs fed diets with viscous and fermentable nonstarch polysaccharides. *J. Anim. Sci.* **88**:3351–3362. doi:10.2527/jas.2010-2906

Ndou, S., E. Kiarie, A. Agyekum. J. Heo, L. Romero, S. Arent, R. Lorentsen, and C. Nyachoti. 2015. Comparative efficacy of xylanases on growth performance and digestibility in growing pigs fed wheat and wheat bran- or corn and corn DDGS-based diets supplemented with phytase. *Anim. Feed Sci. Technol.* **209**:230–239. doi:10.1016/j.anifeedsci.2015.08.011

Nortey, T. N., J. F. Patience, P. H. Simmins, N. L. Trottier, and R. T. Zijlstra. 2007. Effects of individual or combined xylanase and phytase supplementation on energy, amino acid, and phosphorus digestibility and growth performance of grower pigs fed wheat-based diets containing wheat millrun. *J. Anim. Sci.* **85**:1432–1443. doi:10.2527/jas.2006-613

NRC. 2012. *Nutrient requirements of swine*. 11th rev. ed.s Washington (DC): The National Academies Press.

Palframan, R. J., G. R. Gibson, and R. A. Rastall. 2002. Effect of pH and dose on the growth of gut bacteria on prebiotic carbohydrates in vitro. *Anaerobe* **8**:287–292. doi:10.1006/anae.2002.0434

Saidak, Z., R. Mentaverri, and E. M. Brown. 2009. The role of the calcium-sensing receptor in the development and progression of cancer. *Endocr. Rev.* **30**:178–195. doi:10.1210/er.2008-0041

SAS Institute. 2013. SAS® Software Release 9.4. Cary, NC: SAS Institute, Inc.

Schröder, B., and G. Breves. 2006. Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. *Anim. Health Res. Rev.* **7**:31–41. doi:10.1017/S1466252307001144

Sherwood, L., 1997. *Human physiology: from cells to systems*. 3rd ed. Belmont, CA: Wadsworth Publishing Company.

Slominski, B. A., and L. D. Campbell, 1987. Gas chromatographic determination of indole glucosinolates – a re-examination. *J. Sci. Food Agric.* **40**:131–143. doi:10.1002/jsfa.2740400205

Slominski, B. A., and L. D. Campbell. 1990. Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. *J. Sci. Food Agric.* **53**:175–184. doi:10.1002/jsfa.2740530205

Slominski, B. A., L. D. Campbell, and W. Guenter. 1994. Carbohydrates and dietary fiber components of yellow and brown seeded canola. *J. Agric. Food Chem.* **42**:704–707. doi:10.1021/jf00039a020

Statistics Canada. 2018. The daily: production of principal field crops, November 2018. Available from https://www150.statcan.gc.ca/n1/daily-quotidien/181206/dq181206b-eng.htm [accessed May 31, 2019].

Stein, H. H., S. W. Kim, T. T. Nielsen, and R. A. Easter. 2001. Standardized ileal protein and amino acid digestibility by growing pigs and sows. *J. Anim. Sci.* **79**:2113–2122. doi:10.2527/2001.7982113x

Swirski, F. K., M. Nahrendorf, M. Etzrodt, M. Wildgruber, V. Cortez-Retamozo, P. Panizzi, J. L. Figueiredo, R. H. Kohler, A. Chudnovskiy, P. Waterman, *et al.* 2009. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* **325**:612–616. doi:10.1126/science.1175202

Taylor, A. E., M. R. Bedford, and H. M. Miller. 2018. The effects of xylanase on grower pig performance, concentrations of volatile fatty acids and peptide YY in portal and peripheral blood. *Animal* **12**:2499–2504. doi:10.1017/S1751731118000277

Topping, D. L., and P. M. Clifton. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* **81**:1031–1064. doi:10.1152/physrev.2001.81.3.1031

Torres-Pitarch, A., E. G. Manzanilla, G. E. Gardiner, J. V. O'Doherty, and P. G. Lawlor. 2019. Systematic review and meta-analysis of the effect of feed enzymes on growth and nutrient digestibility in grow-finisher pigs: effect of enzyme type and cereal source. *Anim. Feed Sci. Technol.* **251**:153–165. doi:10.1016/j.anifeedsci.2018.12.007

Tran, G., and D. Sauvant. 2004. Chemical data and nutritional value. In: Sauvant, D., J. M. Pérez, and G. Tran, editors. *Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses, fish*. Paris-Grignon (France): Institut National de la Recherche Agronomique (INRA), Association Française de Zootechnie (AFZ), Institute National Agronomique; p. 17–24.

Walk, C. L. 2016. The influence of calcium on phytase efficacy in non-ruminant animals. *Anim. Prod. Sci.* **56**:1345–1349. doi:10.1071/AN15341

Wasserman, R. H. 2004. Vitamin D and the dual processes of intestinal calcium absorption. *J. Nutr.* **134**:3137–3139. doi:10.1093/jn/134.11.3137

Weiland, S. A. 2017. *The impact of xylanase and body weight, and their interaction, on the utilization of dietary components in swine* [master's thesis]. Ames (IA): Iowa State University. Available from https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=6464&context=etd

Zhang, Z., H. M. Tun, R. Li, B. J. M. Gonzalez, H. C. Keenes, C. M. Nyachoti, E. Kiarie, and E. Khafipour. 2018. Impact of xylanases on gut microbiota of growing pigs fed corn- or wheat-based diets. *Anim. Nutr.* **4**:339–350. doi:10.1016/j.aninu.2018.06.007

Zhao, X., B. Schindell, W. Li, L. Ni, S. Liu, C. U. B. Wijerathne, J. Gong, C. M. Nyachoti, K. O, and C. Yang. 2019. Distribution and localization of porcine calcium sensing receptor in different tissues of weaned piglets. *J. Anim. Sci.* **97**:2402–2413. doi:10.1093/jas/skz096