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Effects of a mixture of xylanase and glucanase on digestibility of energy and dietary fiber in corn- or sorghum based diets fed to growing pigs

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ABSTRACT

An experiment was conducted to test the hypothesis that an enzyme premix containing xylanase and glucanase improves the apparent total tract digestibility (ATTD) of energy and total dietary fiber (TDF) and the concentration of digestible energy (DE) and metabolizable energy (ME) in diets fed to growing pigs. A corn-soybean meal diet and a sorghum-soybean meal diet were formulated, and 4 additional diets were formulated by adding 400 g/kg distillers dried grains with solubles (DDGS) or 400 g/kg wheat middlings to the corn-based diet and the sorghum-based diet. Six additional diets were prepared by adding an enzyme premix including xylanase and β-glucanase to each of these diets. One hundred and forty-four growing pigs (61.7 \pm 5.3 kg) were allotted to a randomized complete block design with 12 diets and 12 replicate pigs per diet. Pigs were adapted to the diets for 12 days before being moved to metabolism crates. Individual pig weights and feed consumption were recorded, and average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) were calculated for each dietary treatment. After 4 days of adaptation to the metabolism crates, urine and fecal materials were collected during the following 4 days according to the marker to marker approach. Results indicated that the ATTD of GE, and DE and ME increased (P < 0.01) if enzymes were added to the diets regardless of grain source or co-product inclusion, but no effect of enzymes on ATTD of TDF was observed. However, ATTD of TDF was greater in the corn based diet containing DDGS compared with the diet containing wheat middlings, but in the sorghum-based diet, no difference in ATTD of TDF was observed (grain source \times co-product interaction, P < 0.05). However, growth performance were not affected by inclusion of enzymes, but inclusion of co-products to the diets decreased (P < 0.05) the ADG and G:F in pigs. In conclusion, mixture of enzyme including xylanase and β-glucanase used in this experiment has the potential to increase the ATTD of GE, and DE and ME, in both corn-based and sorghum-based diets without or with fiber containing co-products.

Abbreviations: AEE, acid-hydrolyzed ether extract; ATTD, apparent total tract digestibility; DDGS, distillers dried grains with solubles; DE, digestible energy; DM, dry matter; GE, gross energy; ME, metabolizable energy; NSP, non-starch polysaccharide; SBM, soybean meal; TDF, total dietary fiber.

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1. Introduction

Swine diets often contain substantial levels of non-starch polysaccharides (NSP), which are present in cereal grains and cereal grain co-products (Petry and Patience, 2020). The two main sources of NSP in cereal grains and cereal grain co-products are arabinoxylans and cellulose (Jaworski et al., 2015; Navarro et al., 2019), but the fermentability is different between these types of fiber. One possible strategy to improve the energetic contribution of fiber is to include xylanase and/or β -glucanase in the diets. Xylanase hydrolyzes the β -(1–4) glycosidic bonds of arabinoxylan by releasing a mixture of xylose and xylooligosaccharides that can be either absorbed or fermented by the pig (Casas and Stein, 2016; Abelilla and Stein, 2019; Petry et al., 2020). In contrast, endo-glucanase and exo-glucanase hydrolyze beta-1,4 linkages in the amorphous cellulose chain of glucose units, which results in release of oligomers of glucose. These oligomers may be digested by beta-glucosidase and cellodextrinase with a subsequent release of glucose, which can also be absorbed in the small intestine or fermented in the hindgut (Stein, 2019).

Energy digestibility may be increased if xylanase is added to wheat-based diets, whereas the efficacy of xylanase in corn-based diets is inconsistent, indicating that the structure and physicochemical characteristics of fiber, especially the contents of soluble NSP and insoluble NSP, may vary among cereal grains (Jaworski et al., 2015; Abelilla and Stein, 2019; Petry et al., 2020). An increase in the apparent total tract digestibility (ATTD) of energy and fiber in corn-based diets has been observed if a mixture of carbohydrases, including cellulase, xylanase, and beta-glucanase, was added to the diets (Zhang et al., 2020). However, data demonstrating positive effects of a mixture of enzymes on the digestibility of fiber in sorghum-based diets are limited. It is, therefore, possible that a mixture of enzymes rather than single enzymes are needed to increase fermentation of fiber in corn- or sorghum based diets (Stein, 2019). Therefore, an experiment was conducted to test the hypothesis that a mixture of enzymes including xylanase and β -glucanase aimed at hydrolyzing arabinoxylans and cellulose may increase the digestibility of energy by fermenting total dietary fiber in diets based on corn or sorghum and fed to growing pigs.

2. Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois, Urbana, USA, reviewed and approved the protocol for this experiment. Pigs used in the experiment were the offspring of Line 359 boars mated to Camborough sows (Pig Improvement Company, Hendersonville, TN, USA).

2.1. Ingredients, animals, diets, housing, and experimental design

The enzyme premix used in this experiment was provided by Archer Daniels Midland Company (Decatur, IL, USA). The premix was derived from the fermentation of *Trichoderma reesei*, with an enzyme activity of a minimum of 3200 xylanase units per gram and 1600 β -glucanase units per gram. The xylanase hydrolyze the xylose-xylose glycosidic bonds in the backbone of the arabinoxylans and β -glucanase hydrolyze the β 1–4 glycosidic bonds between glucose units in the amorphous part of the cellulose.

One hundred forty four growing pigs (initial body weight: 61.7 ± 5.3 kg) were allotted to a randomized complete block design with 4 blocks of 36 pigs, 12 diets, and 3 pigs per diet in each block. Therefore, there were 12 replicate pigs per diet. Corn, sorghum, soybean meal (SBM), distillers dried grains with solubles (DDGS), and wheat middlings were sourced locally (Table 1). A corn-SBM diet and a sorghum-SBM diet were formulated, and 2 diets based on corn, SBM, and 400 g/kg DDGS or corn, SBM, and 400 g/kg wheat middlings were also formulated. Two additional diets based on sorghum, SBM, and 400 g/kg DDGS, or sorghum, SBM, and 400 g/kg wheat middlings were also formulated (Tables 2 and 3). Six additional diets were formulated by adding 0.50 g/kg of the enzyme mix to each of the above diets to provide at least 1600 xylanase units and 800 β -glucanase units /kg in the diet. Vitamins and minerals were

 Table 1

 Analyzed nutrient composition of ingredients, as-fed basis.

Item	Corn	Sorghum	Soybean meal	Corn-DDGS ^a	Wheat middlings
Gross energy, MJ/kg	15.1	16.6	17.3	19.2	17.0
Dry matter, g/kg	832.4	887.2	879.4	890.8	893.5
Ash, g/kg	10.7	13.6	60.7	70.0	53.4
Crude protein, g/kg	71.7	107.2	469.8	275.8	158.3
Acid hydrolyzed ether extract, g/kg	17.0	21.6	13.4	68.4	34.8
Insoluble dietary fiber, g/kg	85.0	98.0	196.0	383.0	405.0
Soluble dietary fiber, g/kg	8.0	2.0	9.0	31.0	26.0
Total dietary fiber, g/kg	93.0	100.0	205.0	414.0	431.0
Carbohydrates, g/kg	0.0	0.0	0.0	0.0	0.0
Glucose	3.0	4.6	0.8	2.1	3.9
Fructose	1.2	2.1	1.2	1.1	2.3
Maltose	-	1.2	_	3.0	8.7
Sucrose	-	5.0	84.7	_	21.3
Raffinose	-	1.5	16.6	1.0	11.8
Stachyose	-	_	55.6	_	_
Tannic acid, g/kg	-	1.9	-	-	-

^a DDGS = distillers dried grains with solubles.

included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012).

The amount of feed supplied daily to the pigs was calculated as 3.2 times the estimated requirement for maintenance energy (i.e., 0.824 MJ per kg body weight of NRC, 2012). Water was available at all times. Individual pig body weights were recorded at the beginning and at the end of the experiment, and the amount of feed supplied each day was also recorded.

Pigs were fed experimental diets for 24 days. During the initial 12 days, pigs were housed individually in fully slatted pens $(0.9 \times 1.8 \text{ m})$ and diets were provided on an ad libitum basis during this period. Individual pig weights were recorded on days 1 and 12. Feed addition was recorded daily and the weight of feed left in the feeder was recorded on day 12. Data collected during the first 12 days were summarized to calculate average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) within dietary treatments. On day 13, pigs were moved to individual metabolism crates and they were adopted to the crates for 4 days. Fecal markers were fed in the morning meal on day 17 and in the morning meal on day 22, and fecal collections were initiated when ferric oxide appeared in the feces and ceased when chromic oxide appeared (Adeola, 2001). Feces were collected twice daily and stored at $-20\,^{\circ}$ C immediately after collection. Urine buckets were placed under the metabolism crates to allow total collection. Urines buckets were emptied every morning during the collection period and a preservative of 50 mL of 6 N HCL was added to each bucket when they were emptied. The collected urine was weighed and a subsample was stored at $-20\,^{\circ}$ C.

2.2. Chemical analyses

All samples were analyzed in duplicate. After completing sample collections, urine samples were thawed and mixed within animal and diet, and a subsample was collected for analysis. Fecal samples were dried at 65 °C in a forced-air oven and ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ, USA) before analyses. Urine samples were prepared and lyophilized before energy analysis as previously described (Kim et al., 2009). Ingredients, diets, and fecal samples were analyzed for dry matter (**DM**; method 930.1; AOAC Int, 2019) and fecal, urine, diet, and ingredient samples were analyzed for gross energy (**GE**) using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). All diets and ingredients were analyzed for ash (method 942.05; AOAC Int, 2019), and acid hydrolyzed ether extract (**AEE**) was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl, Ankom Technology, Macedon, NY, USA). Diets and ingredients were also analyzed for N and crude protein was calculated as N × 6.25. Nitrogen was measured according to the combustion procedure (method 990.03; AOAC Int, 2019) using a LECO FP628 N analyzer (LECO Corp., Saint Joseph, MI, USA). Insoluble dietary fiber and soluble dietary fiber were analyzed in diets, ingredients, and fecal samples according to method 991.43 (AOAC Int, 2019) using the Ankom Technology, Macedon, NY), and total dietary fiber (TDF) was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. Ingredients were also analyzed for sugars including

Table 2 Ingredient composition of experimental diets without enzyme premix, as-fed basis^{a, b}.

	-					
Ingredients, g/kg	Corn- SBM	Corn-SBM- DDGS	Corn-SBM-Wheat middlings	Sorghum- SBM	Sorghum-SBM- DDGS	Sorghum-SBM-Wheat middlings
Corn	638.2	461.7	460.3	_	_	_
Sorghum	_	_	_	637.2	461.1	459.9
DDGS	_	400.0	_	_	400.0	_
Wheat middlings	_	_	400.0	_	_	400.0
Soybean meal	330.0	100.0	100.0	330.0	100.0	100.0
Soybean oil	10.0	10.0	10.0	10.0	10.0	10.0
Limestone	13.0	15.0	16.0	12.0	15.0	16.0
Dicalcium phosphate	3.0	1.5	0.0	5.0	2.0	_
Salt	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix ^c	1.5	1.5	1.5	1.5	1.5	1.5
L-lysine HCl	_	5.2	5.3	0.0	5.4	5.6
DL-methionine	_	0.0	0.6	0.0	0.0	0.7
L-threonine	_	0.5	1.7	0.0	0.4	1.7
L-tryptophan	_	0.3	0.3	0.0	0.3	0.3
Phytase concentrate ^d	0.3	0.3	0.3	0.3	0.3	0.3

^a The six diets were produced without enzymes and also with inclusion of 0.50 g/kg of an enzyme premix containing xylanase and glucanase (Archer Daniels Midland Company, Decatur, IL, USA). The enzyme premix was included at the expense of corn or sorghum.

^b SBM = soybean meal; DDGS = distillers dried grains with solubles.

 $^{^{\}rm c}$ The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D₃ as cholecalciferol, 2210 IU; vitamin E as $_{\rm DL}$ -alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B₁₂, 0.03 mg; $_{\rm DL}$ -pantothenic acid as $_{\rm DL}$ -calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganoussulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

^d The phytase concentrate (Optiphos 2000, Huvepharma, Sofia, Bulgaria) contained 2000 phytase units per gram. At 0.3 g/kg inclusion, the concentrate provided 600 units of phytase per kg in the complete diet.

Table 3 Analyzed composition of experimental diets, as-fed basis^a.

Item	Corn- SBM	Corn-SBM- DDGS	Corn-SBM-wheat middlings	Sorghum- SBM	Sorghum-SBM- DDGS	Sorghum-SBM-wheat middlings
Diets without enzymes						
Dry matter, g/kg	878.4	883.7	894.2	896.0	897.4	900.5
Ash, g/kg	35.4	56.7	45.2	50.5	64.2	53.7
Gross energy, MJ/kg	15.9	16.7	16.4	16.5	17.0	16.5
AEE, g/kg	41.0	50.3	43.4	28.3	52.6	43.9
Crude protein, g/kg	207.4	194.6	153.3	213.9	202.7	170.9
Soluble dietary fiber, g/	1.0	15.0	16.0	-3.0	2.0	-1.0
Insoluble dietary fiber, g/	136.0	220.0	240.0	133.0	196.0	234.0
Total dietary fiber, g/kg	137.0	235.0	256.0	130.0	198.0	233.0
Xylanase, units/kg	< 100	< 100	< 100	162	146	< 100
Diets with enzymes						
Dry matter, g/kg	874.2	888.3	887.3	896.7	895.5	894.9
Ash, g/kg	39.6	66.6	55.3	49.7	61.7	56.5
Gross energy, MJ/kg	16.1	16.5	16.2	16.6	17.1	16.5
AEE, g/kg	39.1	49.7	32.6	45.0	56.2	54.4
Crude protein, g/kg	211.3	191.8	162.7	230.0	211.4	161.4
Soluble dietary fiber, g/	-	8.0	3.0	14.0	21.0	21.0
Insoluble dietary fiber, g/	124.0	189.0	235.0	141.0	207.0	243.0
Total dietary fiber, g/kg	124.0	197.0	238.0	155.0	228.0	264.0
Xylanase, units/kg	2804	2521	2407	2410	2710	1670

^a AEE = acid-hydrolyzed ether extract; SBM = soybean meal; DDGS = distillers dried grains with solubles.

glucose, fructose, maltose, sucrose, stachyose, and raffinose using high-performance liquid chromatography (Dionex App Notes 21 and 92). Tannic acid was analyzed in sorghum as described by Taylor et al. (2007).

Xylanase activity in the premix and feed was analyzed following the megazyme assay kit (Megazyme International Ireland, Product code T-XAX-200 T). An internal sample, assayed by the DNS method, was utilized as a standard for enzyme activity (Bailey et al., 1992). One unit of xylanase was expressed as the amount of enzyme releasing one micromole of reducing sugar from wheat arabinoxylan per milliliter of sample per second and determined from a xylose standard curve. Endo-1,4- β -glucanase activity in the premix was analyzed using the DNS method described by Ghose (1987). One unit of endo-1,4- β -glucanase was expressed as the amount of enzyme releasing one micromole of reducing sugar from carboxymethyl cellulose or Hydroxymethyl cellulose per milliliter of sample per second and determined from a glucose standard curve. Endo-1,4- β -glucanase activity was not analyzed in diets due to the high background concentration of reducing sugars in the diets, which prevents the usage of the analysis in mixed diets.

2.3. Calculations and statistical analyses

Following analysis, ATTD of DM, GE, insoluble dietary fiber, and TDF was calculated for each diet and the digestible energy (**DE**) and metabolizable energy (**ME**) in all diets was calculated (Adeola, 2001).

Data were analyzed using the MIXED procedure of SAS (SAS-Institute Inc, 2016). Normality of residuals and outliers was tested using the UNIVARIATE procedure of SAS. Means that deviated from the treatment mean by more than 3 times the interquartile range were considered outliers. Data were analyzed following a $2 \times 2 \times 3$ design with two types of diets (corn based or sorghum based), 2 microbial enzyme treatments (none or enzyme inclusion), and 3 co-product inclusions (none, DDGS, or wheat middlings). The pig was the experimental unit for all analyses. The model included grain, enzyme premix, co-product, grain \times enzyme premix, grain \times co-product, co-product \times enzyme premix, and grain \times co-product \times enzyme premix as fixed effects, and block and pig within block as random effects. Least square means were calculated for each independent variable, and means were separated using the PDIFF option in SAS. Statistical significance among dietary treatments was considered at P < 0.05 and tendencies were considered at P < 0.10.

3. Results

Daily feed intake was greater (P < 0.01) for pigs fed corn-based diets compared with pigs fed sorghum-based diets (Table 4), but GE intake was not different among treatments. Inclusion of a co-product in the diets increased (P < 0.01) excretion of GE in feces, but the increase was greater (P < 0.01) for pigs fed corn-based diets than for pigs fed sorghum-based diets (grain source \times co-product interaction, P < 0.01). Pigs fed diets with inclusion of wheat middlings had lower (P < 0.01) urine excretion of GE than pigs fed diets without co-products or pigs fed diets containing DDGS, regardless of grain source. Likewise, the ATTD of DM and insoluble dietary fiber was less (P < 0.01) in diets with inclusion of wheat middlings than in diets without co-products or diets containing DDGS. The ATTD of TDF was also reduced (P < 0.01) if co-products were added to the diets. However, the ATTD of TDF in corn-based diets

Table 4Apparent total tract digestibility (ATTD) of energy and nutrients and concentrations of digestible and metabolizable energy in experimental diets^{3,2}

Diet	Daily feed intake (kg)	GE ^b intake, MJ/day	Fecal GE output, MJ/day	Urinary GE output, MJ/ day	ATTD of DM ^b	ATTD of insoluble dietary fiber	ATTD of TDF ^b	ATTD of GE	DE ^b in diet, MJ/ kg	ME ^b in diet, MJ/ kg
Corn-based diets										
Corn-SBM	1.74 ^{ab}	27.92	2.79 ^f	0.98^{a}	0.91^{a}	0.76 ^b	$0.76^{\rm b}$	0.90^{ab}	14.41 ^b	13.82^{bc}
Corn-SBM-DDGS	1.72^{abc}	28.47	4.67 ^e	0.95 ^a	0.84 ^b	0.65 ^{cd}	0.66 ^c	0.83^{c}	13.86 ^c	13.19^{de}
Corn-SBM-wheat middlings	1.76 ^a	28.69	6.23 ^a	0.64 ^b	0.80 ^e	0.60 ^f	0.60 ^d	0.78 ^g	12.65 ^f	12.18 ^h
Corn-SBM- Enzyme	1.74 ^{ab}	27.90	2.47 ^f	0.99 ^a	0.92 ^a	0.79 ^{ab}	0.80 ^{ab}	0.91 ^a	14.61 ^{ab}	13.93 ^b
Corn-SBM- DDGS- Enzyme	1.73 ^{abc}	28.64	4.86 ^{de}	1.00 ^a	0.84 ^{bc}	0.66 ^c	0.67 ^c	0.83 ^{cd}	13.77 ^c	13.21 ^{de}
Corn-SBM-wheat middlings- Enzyme Sorghum-based	1.72 ^{abc}	28.10	5.86 ^{ab}	0.69 ^b	0.80 ^e	0.60 ^{ef}	0.61 ^d	0.79 ^f	12.95 ^e	12.45 ^g
diets										
Sorghum-SBM	1.68^{bc}	27.82	2.94 ^f	0.96 ^a	0.91 ^a	0.81 ^a	0.81 ^a	0.90 ^{ab}	14.84 ^a	14.15 ^{ab}
Sorghum-SBM- DDGS	1.66 ^{bc}	28.40	5.20 ^{cde}	1.04 ^a	0.82 ^{cd}	0.63 ^{cdef}	0.64 ^{cd}	0.82 ^{de}	13.95°	13.25 ^d
Sorghum-SBM- wheat middlings	1.68 ^{bc}	27.70	5.55 ^{bc}	0.67 ^b	0.80 ^e	0.60 ^f	0.60 ^d	0.79 ^{fg}	12.97 ^e	12.69 ^{fg}
Sorghum-SBM- Enzyme	1.65 ^c	27.37	2.94 ^f	0.92 ^a	0.91 ^a	0.81 ^a	0.80 ^{ab}	0.89 ^b	14.82 ^a	14.37 ^a
Sorghum-SBM- DDGS- Enzyme	1.68 ^{bc}	28.64	4.61 ^e	1.00 ^a	0.84 ^b	0.64 ^{cde}	0.63 ^{cd}	0.84 ^c	14.33 ^b	13.66 ^c
Sorghum-SBM- wheat middlings- Enzyme	1.74 ^{ab}	28.69	5.30 ^{bcd}	0.74 ^b	0.81 ^{de}	0.61 ^{def}	0.61 ^d	0.81 ^e	13.40 ^d	12.88 ^{ef}
SEM P-values	0.036	0.619	0.255	0.088	0.010	0.022	0.023	0.008	0.126	0.159
Grain source	0.002	0.516	0.645	0.680	0.133	0.484	0.928	0.939	< 0.001	< 0.001
Co-product inclusion	0.342	0.065	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Enzyme	0.839	0.833	0.074	0.579	0.142	0.167	0.317	0.001	0.001	0.007
Grain source × co-product inclusion	0.503	0.917	0.005	0.494	0.397	0.051	0.045	0.010	0.891	0.487
Grain source × enzyme	0.463	0.465	0.646	0.616	0.144	0.485	0.203	0.295	0.270	0.352
Co-product inclusion × enzyme	0.769	0.765	0.872	0.698	0.876	0.968	0.688	0.145	0.151	0.931
Grain source × co-product × enzyme	0.305	0.308	0.158	0.746	0.163	0.460	0.604	0.070	0.068	0.437

^a Data are least squares means of 12 observations per treatment.

were less affected by the inclusion of co-products than if co-products were added to the sorghum-based diets (grain source \times co-product interaction, P < 0.05). The ATTD of GE was lower (P < 0.01) if co-products were included in the diets, than if no co-products were used, but the reduction was greater if wheat middlings was added to the corn-based diet than if added to the sorghum based diet (grain source \times co-product interaction, P < 0.01). In addition, the ATTD of GE tended to increase (P = 0.07) if the enzyme mixture was added to the sorghum based diets containing DDGS or wheat middlings, but for corn based diets the increases was observed only in corn diets containing wheat middlings (grain source \times co-product \times enzyme interaction, P = 0.07). The DE and ME were greater (P < 0.01) in sorghum-based diets than in corn-based diets, but inclusion of co-products reduced (P < 0.01) DE and ME of diets. However, inclusion of the enzyme mixture in diets had a tendency to increase (P = 0.06) DE if added to the sorghum diets containing DDGS or wheat middlings, but for corn based diets the increases was observed only in diets containing wheat middlings (grain source \times co-product \times enzyme interaction, P = 0.06).

Supplementation of enzymes in diets did not affect growth performance parameters. However, pigs fed corn-based diets had greater (P < 0.05) ADFI and ADG compared with pigs fed sorghum-based diets (Table 5). In addition, pigs fed diets containing wheat middlings

^b DM = dry matter; DDGS = distillers dried grains with solubles; DE = digestible energy; GE = gross energy; ME = metabolizable energy; SBM = soybean meal; TDF = total dietary fiber.

Table 5Growth performance of pigs fed corn or sorghum based diets supplemented with enzyme premix^a.

Diet	Initial body weight	Final body weight	Average daily gain	Average daily feed intake	Gain to feed,
	(kg)	(kg)	(kg)	(kg)	ratio
Corn-based diets					
Corn-SBM	50.32	60.72 ^{ab}	0.92 ^{ab}	2.47 ^{ab}	0.38^{ab}
Corn-SBM-DDGS	50.14	59.09 ^{abc}	0.80 ^{abc}	2.60 ^a	0.32^{bcd}
Corn-SBM-wheat middlings	49.45	56.94 ^{bc}	0.67 ^c	2.38 ^{ab}	0.29 ^{cd}
Corn-SBM-Enzyme	49.95	61.07 ^a	0.98 ^a	2.48 ^{ab}	0.40^{a}
Corn-SBM-DDGS-Enzyme	48.79	58.03 ^{abc}	0.82 ^{abc}	2.41 ^{ab}	0.36 ^{abc}
Corn-SBM-wheat middlings-	49.23	56.68 ^{bc}	0.67 ^c	2.38 ^{ab}	0.30 ^{cd}
Enzyme					
Sorghum-based diets		aha	aba	b	ab
Sorghum-SBM	50.47	59.49 ^{abc}	0.80 ^{abc}	2.20 ^b	0.38 ^{ab}
Sorghum-SBM-DDGS	50.07	58.03 ^{abc}	0.71 ^{bc}	2.36 ^{ab}	0.31 ^{bcd}
Sorghum-SBM-wheat middlings	49.32	56.13 ^c	0.62^{c}	2.37 ^{ab}	0.27^{d}
Sorghum-SBM-Enzyme	50.94	59.07 ^{abc}	0.72^{bc}	2.18 ^b	0.35 ^{abcd}
Sorghum-SBM-DDGS-Enzyme	49.60	58.21 ^{abc}	0.77 ^{abc}	2.25 ^{ab}	0.35 ^{abc}
Sorghum-SBM-wheat middlings-	49.27	56.68 ^{bc}	0.67 ^c	2.18 ^b	0.32^{bcd}
Enzyme					
SEM	2.148	1.814	0.095	0.197	0.033
P-values					
Grain source	0.732	0.338	0.040	0.020	0.523
Co-product inclusion	0.568	0.005	0.003	0.688	0.001
Enzyme	0.702	0.897	0.689	0.323	0.198
Grain source × co-product inclusion	0.956	0.804	0.330	0.674	0.745
Grain source × enzyme	0.719	0.802	0.852	0.821	0.901
Co-product inclusion × enzyme	0.889	0.959	0.909	0.757	0.515
$\begin{array}{c} \text{Grain source} \times \text{co-product} \times \\ \text{enzyme} \end{array}$	0.982	0.875	0.649	0.805	0.406

^a Data are least squares means of 12 observations per treatment.

had reduced (P < 0.05) ADG compared with pigs fed diets containing DDGS or pigs fed diets without co-products. Likewise, the G:F and final body weight of pigs fed diets containing co-products were lower (P < 0.05) than pigs fed diets without co-products.

4. Discussion

The DE and ME in the corn-SBM diet without the enzyme mixture were within the range of reported values (Oliveira and Stein, 2016; Abelilla and Stein, 2019), and ME in the sorghum-SBM diet was within the range of ME values that have been published (Pan et al., 2017). Low-tannin sorghum may be an alternative to corn in diets for pigs (Thomas et al., 2020), and DE and ME in sorghum are not different from values in corn (Cervantes-Pahm et al., 2014; Pan et al., 2017). However, the observation that sorghum-based diets used in this experiment had greater DE and ME than corn-based diets is likely a result of a greater concentration of dry matter, crude protein, GE, and fat in sorghum than in corn, which contributed to an increase in energy digestibility.

The reduction in the ATTD of GE and TDF as well as in DE and ME that was observed if DDGS or wheat middlings was added to the diets is likely a result of the greater concentration of dietary fiber in DDGS and wheat middlings than in corn, sorghum, and SBM. Dietary fiber may serve as a structural barrier for digestion because it may hinder access of digestive enzymes to starch, crude protein, and possibly other nutrients (Abelilla and Stein, 2019). Wheat middlings had a larger concentration of insoluble dietary fiber compared with DDGS, whereas DDGS had a larger concentration of soluble dietary fiber than wheat middlings. Fiber in wheat middlings has lower fermentability in the hindgut than fiber in DDGS (Jaworski and Stein, 2017), which is the reason diets with wheat middlings had lower ATTD of DM and insoluble dietary fiber compared with diets with DDGS. It was expected that diets with DDGS provided more DE and ME per kilogram of diet than diets with wheat middlings, because DE and ME in DDGS are greater than in wheat middlings (NRC, 2012), but this was not observed. The reason for this may be that the DDGS used in this experiment was a low-oil DDGS, which contains less ME than conventional DDGS (NRC, 2012; Curry et al., 2016; Espinosa and Stein, 2018).

Arabinoxylans and cellulose are the fiber components present in the largest quantities in dietary fiber in cereal grains and cereal grain co-products (Jaworski et al., 2015; Stein, 2019). Non-starch polysaccharides in corn contain 480 g/kg arabinoxylans and 210 g/kg cellulose, whereas the NSP in sorghum contain of 440 g/kg arabinoxylans and 220 g/kg cellulose (Jaworski et al., 2015). Because arabinoxylans are present at the greatest quantities in cereal grains, xylanase is the most common NSP degrading enzyme that is added to pig diets. The mode of action of xylanase in pigs is believed to be that xylanase hydrolyzes the xylose backbone in arabinoxylans into lower molecular weight fragments that can either be absorbed or fermented in the intestinal tract of pigs (Petry and Patience, 2020). Xylanase supplemented to a corn-based diet may also increase soluble NSP in the ileum, which may be a result of soluble arabinoxylan fragments being released from the insoluble arabinoxylans in corn (Pedersen et al., 2015; Tiwari et al., 2018). The efficiency of xylanase to increase nutrient digestibility has been demonstrated (Ndou et al., 2015; Casas and Stein, 2016; Kiarie et al., 2016; Petry et al., 2019, 2020), but responses to inclusion of xylanase in corn-based diets are variable (Yañez et al., 2011; Zhang et al., 2018;

Abelilla and Stein, 2019). The lack of responses to xylanase in corn-based diets may be a result of arabinoxylans in corn having a structural complexity that prevents enzymes from gaining access to the hydrolytic site on the xylose backbone (Petry and Patience, 2020).

However, the observed improvement for DE in sorghum diets containing corn-DDGS as a mixture of enzymes was added, demonstrate that xylanase along with glucanase was effective in hydrolyzing fiber in corn DDGS. This observation is in agreement with recent data (Zhang et al., 2020), and may be a result of the β -glucanase included in the enzyme mixture, which may have fermented cellulose, and therefore, liberated glucose, which may be metabolized by hindgut microbes with subsequent synthesis of short chain fatty acids that can be absorbed and metabolized for energy (Stein, 2019).

Data demonstrating responses to carbohydrases in sorghum-based diets are limited. Sorghum contains less soluble NSP compared with wheat and corn, which results in lower fermentability by the end of the large intestine (Knudsen, 2014; Jaworski et al., 2015). However, recent data demonstrated that xylanase increased the concentration of arabinoxylo-oligosaccharides in the gastrointestinal tract in weaned pigs fed sorghum-based diets (González-Ortiz et al., 2020). These oligosaccharides may act as substrates for fermentation by intestinal microbes, and therefore, stimulate fermentation, which may contribute to an increase in nutrient or energy digestibility (Petry and Patience, 2020; Petry et al., 2021). As a result, supplementation of xylanase to sorghum-based diets fed to weaned pigs improved feed efficiency (González-Ortiz et al., 2020). The current data demonstrating an increase in ATTD of GE in sorghum based diets indicate that feed efficiency may also be improved in growing-finishing pigs if xylanase and glucanases are used.

The overall increase in ATTD of GE and in the DE and ME of diets as a result of adding a mixture of enzymes to the diets indicates that the mixture of xylanase and glucanases that were used in this experiment was effective in hydrolyzing some of the glycosidic bonds in the arabonoxylans and (or) in cellulose in the ingredients. However, because a mixture of enzymes was used, it is not possible to determine if the positive response was a result of the action of xylanase or one of the glucanases, or possibly some of the side activities in the enzyme preparation.

The lack of a response to enzyme addition on ATTD of TDF despite the increased DE and ME that were observed may be a result of carbohydrases changing hydrolysis of some fiber components from the hindgut to the small intestine, with a subsequent absorption of energy in the small intestine rather than in the large intestine. Addition of an enzyme mixture to diets increased the apparent ileal digestibility, but not ATTD of neutral detergent fiber and NSP (Moran et al., 2016; Li et al., 2018; Zeng et al., 2018). Absorption of glucose from cellulose hydrolysis in the small intestine rather than absorption of volatile fatty acids from the hindgut will result in increased energy absorption because only around 600 g/kg of the energy in glucose is captured in volatile fatty acids absorbed from the hindgut (NRC, 2012; Stein, 2019). As a consequence, absorption of glucose is energetically more advantageous than absorption of volatile fatty acids. However, because the apparent ileal digestibility of nutrients was not measured in this experiment, we cannot confirm if energy absorption was changed from the hindgut to the small intestine in pigs fed diets containing enzymes.

The decrease in growth performance of pigs as co-product was included to the diets was expected because of greater concentration of dietary fiber. However, the lack of responses to enzyme premix in growth performance of pigs may be due to the short adaptation time of pigs to the enzymes. Previous data indicated that xylanase improved ADG and G:F in pigs after 14 days of supplementation, and these parameters were even greater after day 27, which indicate that adaptation time play a role in xylanase efficacy (Petry and Patience, 2020; Petry et al., 2020). Although growth performance did not improve with enzyme supplementation, addition of enzymes improved the energetic contribution of fiber, which potentially reduced the negative effects of inclusion of co-products to the diets (Stein, 2019; Petry et al., 2020).

5. Conclusion

Pigs fed corn- or sorghum based diets containing high fiber ingredients and supplemented with a mixture of xylanase and glucanases had improved apparent total tract digestibility of energy compared with pigs fed diets without the enzymes. An improvement in the digestibility of dietary fiber was, however, not observed in this experiment.

CRediT authorship contribution statement

MSFO, FNA, and HHS conceptualized the experiment. MSFO and CDE summarized and analyzed the data. CDE, MM, FNA, and HHS contributed with data interpretation. MSFO wrote the first draft of the manuscript. CDE, MM, FNA, and HHS edited the final version of the manuscript. HHS supervised the project.

Conflict of interest

MM is an employer at Archer Daniels Midland Company, a global supplier of feed additives including enzymes. The other authors have no conflicts of interest.

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