Nutrient digestibility of multi-enzyme supplemented low-energy and AA diets for grower pigs[1]

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ABSTRACT: A study was conducted to determine effects of supplementing multi-enzyme on apparent ileal digestibility (**AID**) of energy and AA; and apparent total tract digestibility (**ATTD**) of energy for pigs fed low-energy and AA diets. Eight ileal-cannulated barrows (initial BW: 38.7 ± 2.75 kg) were fed four diets in a replicated 4 × 4 Latin square design to give 8 replicates per diet. Diets were positive control (**PC**) diet, negative control (**NC**) diet without or with multi-enzyme at 0.5 or 1.0 g/kg. The PC diet was formulated to meet or exceed NRC (2012) nutrient recommendations for grower pigs (25 to 50 kg), except for Ca and digestible P, which were lower than NRC (2012) recommendations by 0.13 and 0.17 percentage points, respectively, due to phytase supplementation at 1,000 FTU/kg. The NC diet was formulated to be lower in NE by 75 kcal/kg and standardized ileal digestible AA content by a mean of 3%. These reductions were achieved by partial replacement of corn and soybean meal (**SBM**) and complete replacement of soybean oil and monocalcium phosphate in PC diet with 25% corn distillers dried grains with solubles (**DDGS**) and 3.6% soybean hulls. Multi-enzyme at 1.0 g/kg supplied 1,900 U of xylanase, 300 U of β-glucanase, 1,300 U of cellulase, 11,500 U of amylase, 120 U of mannanase, 850 U of pectinase, 6,000 U of protease, and 700 U of invertase per kilogram of diet. The AID of GE, N, most AA, most component sugars of nonstarch polysaccharides (**NSP**) and P; ATTD of GE for PC diet was greater ($P < 0.05$) than those for NC diets. An increase in dietary level of multi-enzyme from 0 to 1.0 g/kg resulted in a linear increase ($P < 0.05$) in AID of Ile by 4.3%, and tended to linearly increase ($P < 0.10$) AID of Leu, Met, Phe, and Val by a mean of 3.4%. Increasing dietary multi-enzyme from 0 to 1.0 g/kg linearly increased ($P < 0.05$) AID of total NSP and P by 53.7% and 19.2%, respectively; ATTD of GE by 8.4% and DE and NE values by 8.8% and 8.2%, respectively; tended to linearly increase ($P < 0.10$) AID of GE by 8.1%. The NE values for NC diet with multi-enzyme at 1.0 g/kg tended to be greater ($P < 0.10$) than that for PC diet (2,337 vs. 2,222 kcal/kg of DM). In conclusion, multi-enzyme supplementation improved energy and nutrient digestibilities of a corn–SBM–corn DDGS-based diet, implying that the multi-enzyme fed in the current study can be used to enhance energy and nutrient utilization of low-energy AA diets for grower pigs.

Key words: corn DDGS, multi-enzyme, nutrient digestibility, pig

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INTRODUCTION

Although corn distillers dried grains with solubles (**DDGS**) is widely used to formulate swine diets (Stein and Shurson, 2009), it has a high content of nonstarch polysaccharides (**NSP**; Jaworski et al., 2015), which reduces nutrient and energy

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utilization by pigs (Woyengo et al., 2014). The NSP-degrading enzymes can potentially hydrolyze dietary NSP (Adeola and Cowieson, 2011), thereby increasing energy and nutrient digestibilities of fibrous diets for pigs (Zijlstra et al., 2010). However, the effects of adding NSP-degrading enzymes to corn DDGS-based diets for pigs have been inconsistent; most studies did not report increased energy and nutrient digestibilities in pigs fed corn DDGS-based diets due to supplemental NSP-degrading enzymes. For instance, addition of xylanase (Asmus et al., 2012; Ndou et al., 2015; Jang et al., 2016; Moran et al., 2016) or a product that contained xylanase, β-glucanase, and cellulase activities (Rho et al., 2017, 2018) to corn–soybean meal (**SBM**)–corn DDGS-based diets for pigs did not affect apparent total tract digestibility (**ATTD**) of GE.

Notably, both corn and corn DDGS contain arabinoxylans and cellulose; corn DDGS additionally contain mannans and β-glucans that are endogenous and also originate from residual yeast biomass (Pedersen et al., 2014), while SBM contains several NSP including cellulose, pectins, and xyloglucans (Knudsen, 2014). Thus, the composition and structure of NSP in corn–SBM–corn DDGS-based diets are expected to be more complex than that in corn–SBM-based diets. Enzyme supplements that were used in previous studies (Moran et al., 2016; Rho et al., 2018) in which there were limited effects of enzyme supplementation on nutrient digestibility of corn DDGS did not contain enzyme activities that targeted more than 3 major NSP including arabinoxylans, cellulose, and pectins present in corn–SBM–corn DDGS-based diets for pigs. Additionally, during the production of corn DDGS from cereal grains, protein interacts with NSP to form protein–NSP complexes (Jha et al., 2015), implying that the protein in the NSP–protein matrix can reduce the accessibility of NSP-degrading enzymes to NSP. Indeed, Pedersen et al. (2015) observed increased in vitro degradation of arabinoxylans due to the addition of protease to xylanase-supplemented corn DDGS. Thus, it was hypothesized that nutrient digestibility of corn–SBM–corn DDGS-based diets for pigs would be improved by supplemental enzyme products that contain protease and fiber-degrading enzymes that target most of the NSP in corn, corn DDGS, and SBM. The objective of this study was to determine the effects of dietary supplementation of a multi-enzyme product that contains xylanase, β-glucanase, cellulase, mannanase, pectinase, amylase, and protease to a low-energy-AA diet that is based on corn, SBM, and corn DDGS on energy and nutrient digestibilities in grower pigs.

MATERIALS AND METHODS

The experimental animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (#15-029A).

Experimental Animals

Eight crossbred ileal-cannulated barrows (initial BW of 38.7 ± 2.75 kg; Lance-Large White female × Duroc male; Pig Improvement Company) were used in the current study. Pigs had been surgically fitted with a simple T-cannula at the distal ileum according to Sauer and Ozimek (1986). Pigs were individually housed in metabolic crates (1.52 × 0.61 × 0.85 m) with smooth polyvinyl chloride walls and plastic-covered expanded metal flooring in a temperature-controlled room (22 ± 2 °C). Each crate was equipped with a single-space dry feeder and a nipple drinker.

Experimental Diets

Four experimental diets fed in this study were a positive control (**PC**) diet and negative control (**NC**) diet without or with multi-enzyme (Superzyme-CS, Canadian Bio-System Inc., Calgary, AB, Canada) at 0.5 or 1.0 g/kg (Table 1). The PC diet was formulated to meet or exceed the NRC (2012) recommended requirements of all nutrients for growing pigs except Ca and digestible P, which were lower than the NRC recommendations by 0.13 and 0.17 percentage points, respectively, due to phytase supplementation. The NC diet was same as the PC diet except for NE value that was reduced by 75 kcal/kg, and standardized ileal digestible AA content that was reduced by a mean of 3%. The reduction in NE value and standardized ileal digestible AA content in the NC diet was achieved by a partial replacement of corn and SBM and a complete replacement of soybean oil and monocalcium phosphate in the PC diet with 25% corn DDGS and 3.6% soybean hulls. All diets contained phytase (Bio-Phytase, Canadian Bio-System Inc., Calgary, AB, Canada) at 1,000 FTU/kg of diet and titanium dioxide (0.4%) as an indigestible marker. Multi-enzyme supplementation at 1.0 g/kg supplied 1,900 U of xylanase, 300 U of β-glucanase, 1,300 U of cellulase, 11,500 U of amylase, 120 U of mannanase, 850 U of pectinase, 6,000 U of protease, and 700 U of invertase per kilogram of diet.

Table 1. Ingredient and calculated nutrient composition of positive and negative control (**PC** and **NC**) diets (as-fed basis)[1]

Item	PC diet[2]	NC diet[3]
Ingredient, %		
Corn	76.29	60.25
Soybean meal	20.75	8.11
Corn DDGS, 7% oil	0.00	25.00
Soybean hulls	0.00	3.57
Soybean oil	0.05	0.00
Limestone	1.09	1.18
Monocalcium phosphate	0.20	0.00
Salt	0.50	0.50
Lys HCl	0.37	0.60
DL-Met	0.04	0.01
L-Thr	0.10	0.13
Vitamin premix[4]	0.05	0.05
Mineral premix[5]	0.15	0.15
Phytase premix	0.01	0.01
Titanium dioxide	0.40	0.40
Calculated nutrient content		
NE, kcal/kg	2,475	2,400
CP, %	16.61	16.75
Ca, %	0.53	0.53
Total P, %	0.39	0.37
Digestible P, %	0.19	0.19
Standardized ileal digestible AA, %		
Lys	0.98	0.95
Met	0.28	0.27
Thr	0.59	0.57
Trp	0.17	0.16

[1]Experimental diets were PC diet, NC diet without or with multi-enzyme (Superzyme-CS at 0.5 or 1.0 g/kg of diet, Canadian Bio-System Inc., Calgary, AB, Canada). The multi-enzyme supplementation at 1.0 g/kg supplied 1,900 U of xylanase, 300 U of β-glucanase, 1,300 U of cellulase, 11,500 U of amylase, 120 U of mannanase, 850 U of pectinase, 6,000 U of protease and 700 U of invertase per kilogram of diet.

[2]PC diet was a corn–SBM-based diet, which was formulated to be adequate in all nutrients except for Ca and digestible P, which were lower than NRC (2012) recommendations for grower pigs by 0.13 and 0.17 percentage points.

[3]NC diet was the same as the PC diet except that it was lower in NE by 75 kcal/kg and standardized ileal digestible AA content by a mean of 3%. This was achieved by a partial replacement of corn and SBM and a complete replacement of soybean oil and monocalcium phosphate with 25% corn DDGS and 3% soybean hulls.

[4]Provided the following per kilogram of diet: 2,226 IU vitamin A, 340 IU vitamin D_3, 11.3 IU vitamin E, 0.01 mg vitamin B_{12}, 0.91 mg menadione, 2.04 mg riboflavin, 12.5 mg pantothenic acid, 11.3 mg niacin, 0.23 mg folic acid, 0.68 mg pyridoxine, 0.68 mg thiamine, and 0.04 mg biotin.

[5]Provided the following per kilogram of diet: 75 mg Zn as $ZnSO_4$, 75 mg Fe as $FeSO_4$; 7 mg Cu as $CuSO_4$, and 20 mg Mn as $MnSO_4$.

Experimental Design and Procedure

The 8 pigs were fed the 4 diets in a replicated 4 × 4 Latin square design to give 8 replicates per diet. Each period consisted of 9 d; the first 5 d were for adaptation, followed by 2 d of fecal collection and 2 d of ileal digesta collection. Pigs were fed diets at 3 times maintenance energy requirement (3 × 197 kcal of metabolizable energy/kg of $BW^{0.60}$; NRC, 2012) based on BW at the beginning of each period. Daily feed allowance was offered in 2 equal portions at 0800 and 1600 hours. Representative fecal samples were collected from each pen between 0800 and 1700 hours daily. Ileal digesta was collected continuously for 12 h from 0800 to 2000 hours daily (Nyachoti et al., 2002). Collected feces and digesta were pooled for each pig and period, and stored frozen at −20 °C for further analyses.

Sample Preparation and Analyses

At the termination of each period, pooled ileal digesta samples were freeze-dried, whereas pooled fecal samples were ovendried for 96 h at 60 °C. The dried ileal digesta, fecal samples, and experimental diets were ground to pass through a 0.75-mm screen using a centrifugal mill (model ZM200; Retsch GmbH, Haan, Germany). The ground diets, ileal digesta, and feces were analyzed for DM, GE, CP, and titanium dioxide. Diets and ileal digesta samples were additionally analyzed for AA, Ca, P, NSP, and starch. Diets were analyzed for ADF, NDF, and EE. The samples were analyzed for DM by oven drying at 135 °C for 2 h (method 930.15), CP by a combustion procedure (method 990.03), EE (method 2003.06) on a Soxtec 2050 (FOSS North America, Eden Prairie, MN) as per AOAC International (2007); and for ADF and NDF (Van Soest et al., 1991) on a Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Samples were analyzed for AA (method 982.30 E [a, b, and c]; AOAC, 2006) at the University of Missouri Experiment Station laboratories (Columbia, MO). The GE was analyzed using an adiabatic bomb calorimeter (model AC600, Leco, St. Joseph, MI). Titanium dioxide in samples was determined by spectrophotometry (model Spectra MAX 190, Molecular Devices, Sunnyvale, CA) at 408 nm after ashing at 525 °C for 10 h (Myers et al., 2004). The NSP were determined by gas–liquid chromatography (component neutral sugars) using SP-2340 column and Varian CP-3380 Gas Chromatograph (Agilent Technologies, Mississauga, ON, Canada) and by colorimetry (uronic acids) using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK) as described by Englyst and Cummings (1988) with modifications (Slominski and Campbell, 1990). Starch was determined on RFA using glucose trinder (glucose reagent set, Amresco, Solon, OH).

Samples were analyzed for Ca and P using method 985.01 of AOAC (1999). Xylanase activity in diets was assayed using Xylazyme AX tablets (Megazyme International LTD., Bray, Ireland).

Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) and ATTD values of the experimental diets were calculated using the indicator method (equation 2; Stein et al., 2007). The DE value for the diets was calculated by multiplying GE by its ATTD values. The NE values of the diets were calculated using the determined DE value and analyzed macronutrient content using equation 5 that was developed by Noblet et al. (1994) and has been adopted by NRC (2012):

$$NE = 0.700 \times DE + 1.61 \times EE + 0.48 \times starch - 0.91 \times CP - 0.87 \times ADF$$

The apparent hindgut digestibility (**AHD**) of GE as proportion of GE in diet was determined by subtracting the AID of GE from the ATTD of GE. The AHD of GE as proportion of GE in ileal digesta was determined by dividing the AHD of GE (as proportion of GE in diet) by the GE in ileal digesta in pigs multiplied by 100 (Woyengo et al., 2016b).

Data were subjected to ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC) with the diet as a fixed factor, and pig and period as random factors. Pig was the experiment unit. Means were separated by probability of difference. Linear and quadratic contrasts for equally spaced levels were performed to determine the effect of increasing the level of multi-enzyme in the NC diet. To test the hypotheses, $P < 0.05$ was considered significant. If pertinent, trends ($0.05 \leq P < 0.10$) are also reported.

RESULTS

All animals used in the current study remained healthy and consumed all the feed fed throughout the study. The analyzed xylanase activity values in the NC diet with multi-enzyme at 0.5 and 1.0 g/kg were close to those that were anticipated (Table 2). Xylanase activity was only determined in the experimental diets due to potential interference from dietary carbohydrates in many enzyme assays. The analyzed CP, Ca, and P values of the PC and NC diet shown in Table 2 were slightly higher than the calculated values shown in Table 1. The NC diet had a greater content of total NSP, and hence arabinose, glucose, mannose, uronic acids, and xylose sugars that constituted NSP than the PC diet.

Table 2. Analyzed nutrient content of positive and negative control (**PC** and **NC**) diets (on DM basis)[1]

Item, %	PC diet[2]	NC diet[3]
Moisture	12.50	11.32
CP	18.33	17.28
EE	1.83	2.81
Starch	51.28	49.63
NDF	10.66	18.52
ADF	4.13	6.81
Total nonstarch polysaccharides	10.50	14.89
Component sugar content in NSP[4]		
Arabinose	1.65	2.34
Xylose	1.82	3.34
Mannose	0.29	0.61
Galactose	1.39	0.94
Glucose	3.69	5.78
Uronic acids	1.67	1.88
Indispensable AA		
Arg	1.01	0.86
His	0.46	0.46
Ile	0.71	0.68
Leu	1.54	1.76
Lys	1.23	1.20
Met	0.29	0.27
Phe	0.83	0.82
Thr	0.72	0.70
Trp	0.18	0.16
Val	0.81	0.81
Dispensable AA		
Ala	0.93	1.06
Asp	1.58	1.36
Cys	0.29	0.34
Glu	2.91	2.75
Gly	0.71	0.72
Pro	1.09	1.26
Ser	0.72	0.73
Tyr	0.53	0.56
Ca	0.60	0.45
P	0.63	0.45

[1]The analyzed xylanase activities for the PC and NC diet were 187 and 160 U of xylanase per kilogram of diet, respectively. The xylanase activities for the NC diets with multi-enzyme at 0.5 or 1.0 g/kg of diet were 1,037 and 2,113 U of xylanase per kilogram of diet, respectively.

[2]PC diet was a corn–SBM-based diet, which was formulated to be adequate in all nutrients except for Ca and digestible P, which were lower than NRC (2012) recommendations for grower pigs by 0.13 and 0.17 percentage points.

[3]Negative control diet was the same as the PC diet except that it was lower in NE by 75 kcal/kg, and standardized ileal digestible AA content by a mean of 3%; this was achieved by a partial replacement of corn and SBM and a complete replacement of soybean oil and monocalcium phosphate with 25% corn DDGS and 3% soybean hulls.

[4]NSP = nonstarch polysaccharides.

The AID of GE, starch, NSP, N, AA, Ca, and P for the experimental diets is presented (Table 3). The AID of GE for the PC diet was greater ($P < 0.05$) than that for the NC diet. The AID of starch for

Table 3. Apparent ileal digestibility (**AID**) of GE, starch, **NSP**, CP, AA, Ca, and P for the experimental diets

						P-value		
Item, %	PC[1]	NC[2]	NC + 0.5E[3]	NC + 1.0E[4]	SEM	Diet	Linear	Quadratic
GE	76.4[a]	67.7[c]	70.4[bc]	73.2[ab]	2.219	<0.001	0.069	0.920
Starch	97.4	96.7	96.7	97.1	0.414	0.279	0.436	0.659
Total NSP	27.5[ab]	21.4[b]	26.3[ab]	32.9[a]	3.382	0.022	0.007	0.948
Component sugars of NSP								
Arabinose	19.1[b]	20.9[ab]	23.7[ab]	27.6[a]	2.881	0.055	0.041	0.902
Xylose	13.4[b]	15.9[ab]	18.3[ab]	22.5[a]	3.078	0.154	0.099	0.801
Mannose	42.5[a]	29.0[b]	33.5[b]	43.9[a]	3.608	<0.001	0.004	0.297
Galactose	39.1[a]	29.2[b]	31.5[b]	33.7[b]	2.613	0.002	0.239	0.978
Glucose	35.5[ab]	26.3[c]	30.9[bc]	40.2[a]	3.803	0.010	0.008	0.510
Uronic acids	33.9[a]	25.6[bc]	21.7[c]	30.4[ab]	2.740	0.003	0.200	0.026
CP	83.4[a]	75.5[c]	78.7[b]	78.0[bc]	1.771	<0.001	0.300	0.305
Indispensable AA								
Arg	89.1[a]	83.8[b]	84.8[b]	84.8[b]	1.231	<0.001	0.553	0.679
His	86.0[a]	78.2[c]	80.8[b]	80.5[bc]	1.215	<0.001	0.178	0.302
Ile	84.4[a]	77.0[c]	81.1[b]	80.3[bc]	1.068	<0.001	0.028	0.046
Leu	86.3[a]	82.4[c]	85.0[ab]	84.7[b]	0.824	<0.001	0.051	0.138
Lys	87.4[a]	81.4[c]	83.9[b]	83.3[bc]	1.461	<0.001	0.277	0.419
Met	88.3[a]	81.1[c]	85.4[b]	83.8[b]	0.989	<0.001	0.060	0.020
Phe	85.6[a]	80.1[c]	83.1[b]	82.4[b]	0.887	<0.001	0.065	0.081
Thr	81.4[a]	74.9[c]	77.2[bc]	77.4[b]	1.674	<0.001	0.283	0.566
Trp	86.4[a]	80.0[b]	85.2[a]	81.7[b]	1.400	<0.001	0.350	0.007
Val	82.5[a]	74.5[c]	78.5[b]	78.0[b]	1.521	<0.001	0.090	0.183
Dispensable AA								
Ala	82.1[a]	77.1[b]	80.8[a]	80.2[a]	1.314	0.002	0.061	0.085
Asp	84.6[a]	74.9[c]	78.3[b]	78.0[b]	1.715	<0.001	0.236	0.363
Cys	79.0[a]	72.8[b]	75.6[b]	75.4[b]	1.655	0.003	0.356	0.477
Glu	88.9[a]	83.0[c]	85.3[b]	85.1[b]	0.936	<0.001	0.116	0.239
Gly	71.1[a]	65.4[b]	65.4[b]	65.4[b]	2.405	0.015	0.656	0.806
Pro	68.3	68.3	70.8	68.2	5.179	0.945	0.981	0.638
Ser	84.1[a]	77.3[c]	78.7[bc]	80.2[b]	1.289	<0.001	0.099	0.878
Tyr	85.0[a]	80.4[b]	79.4[b]	80.9[b]	1.131	<0.001	0.742	0.478
Ca	66.3	62.1	66.1	68.4	4.289	0.533	0.224	0.810
P	73.9[a]	60.0[b]	73.0[a]	71.5[a]	3.589	0.001	0.020	0.136

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]PC = positive control diet, which was formulated to be adequate in all nutrients except for Ca and digestible P, which were lower than NRC (2012) recommendations for grower pigs by 0.13 and 0.17 percentage points.

[2]NC = negative control diet, which was the same as the PC diet except that it was lower in NE by 75 kcal/kg, and standardized ileal digestible AA content by a mean of 3%; this was achieved by a partial replacement of corn and SBM and a complete replacement of soybean oil and monocalcium phosphate in PC diet with 25% corn DDGS and 3% soybean hulls.

[3]NC diet was supplemented with Superzyme-CS at 0.5 g/kg of diet.

[4]NC diet was supplemented with Superzyme-CS at 1.0 g/kg of diet.

pigs fed the PC diet did not differ from that for pigs fed the NC diet. The AID of component sugars of NSP (galactose, glucose, mannose, and uronic acids) for pigs fed the PC diet were greater ($P < 0.05$) than those for the NC diet. The AID of total NSP for pigs fed the PC diet also tended to be greater ($P < 0.10$) than that for the NC diet. However, the AID of arabinose and xylose sugars of NSP for pigs fed the PC diet were similar to those for pigs fed the NC diet. The AID of N and all AA (except Pro) for the PC diet were greater ($P < 0.05$) than those for the NC diet; the AID of Pro for the PC diet did not differ from that for the NC diet. The AID of Ca for pigs fed the PC diet did not differ from that for pigs fed the NC diet, whereas the AID of P for pigs fed the PC diet was greater ($P < 0.05$) than that for pigs fed the NC diet. An increase in the level of supplemental multi-enzyme in the NC diet from 0 to 1.0 g/kg tended to linearly increase ($P < 0.10$) the AID of GE. Increasing the level of supplemental multi-enzyme in the NC diet from 0 to 1.0 g/kg did not affect the AID of starch,

but resulted in a linear increase ($P < 0.05$) in the AID of total NSP; arabinose, mannose, and glucose sugars of NSP; and tended to linearly increase ($P < 0.10$) the AID of xylose sugar in NSP. Also, an increase in the level of supplemental multi-enzyme in the NC diet from 0 to 1.0 g/kg resulted in a quadratic increase ($P < 0.05$) in the AID of uronic acid sugar in NSP such that an increase in the level of multi-enzyme from 0 to 0.5 g/kg did not affect the AID of uronic acid in NSP, but an increase in the level of multi-enzyme from 0.5 to 1.0 g/kg resulted in an increase ($P < 0.05$) in the AID of uronic acid in NSP. Dietary multi-enzyme linearly increased ($P < 0.05$) the AID of Ile; and tended to linearly increase ($P < 0.10$) the AID of Ala, Leu, Met, Phe, Ser, and Val. Increasing the level of supplemental multi-enzyme in the NC diet from 0 to 1.0 g/kg quadratically increased ($P < 0.05$) the AID of Met and Trp such that an increase in the level of multi-enzyme from 0 to 0.5 g/kg increased ($P < 0.05$) the AID of Met and Trp, but a further increase in the level of multi-enzyme to 1.0 g/kg did not result in a further increase in the AID of these AA. Multi-enzyme supplementation tended to quadratically increase ($P < 0.10$) the AID of Phe and Ala such that an increase in the level of multi-enzyme from 0 to 0.5 g/kg resulted in an increase ($P < 0.05$) in the AID of Phe and Ala, but a further increase in the level of multi-enzyme to 1.0 g/kg did not result in a further increase in the AID of these AA. An increase in the level of multi-enzyme in the NC diet from 0 to 1.0 g/kg did not affect the AID of Ca but linearly increased ($P < 0.05$) the AID of P.

The ATTD of CP and GE, AHD of GE, and DE and NE values for the diets are presented (Table 4). The ATTD of GE and CP for the PC diet was greater ($P < 0.05$) than that for the NC diet. The AHD of GE (as proportion of GE in diet or in ileal digesta) for pigs fed the PC diet did not differ from those for pigs fed the NC diet. The DE and NE values for the PC diet did not differ from those for the NC diet. An increase in the level of multi-enzyme in the NC diet from 0 to 1.0 g/kg resulted in a linear increase ($P < 0.05$) in the ATTD of GE and N; and DE and NE values. Increasing the level of supplemental multi-enzyme in the NC diet from 0 to 1.0 g/kg did not affect the AHD of GE as proportion of GE in diet or in ileal digesta. The DE and NE values for the NC diet with multi-enzyme at 1.0 g/kg tended to be greater ($P < 0.10$) than those for the PC diet.

DISCUSSION

The PC and NC diets were formulated to be deficient in Ca and digestible P to maximize the response to phytase supplementation. As expected, the NC diet had a higher content of NSP than the PC diet because the NC diet was generated from the PC diet by a partial replacement of corn and SBM in the PC diet with corn DDGS and soybean hulls. Corn DDGS has a higher content of fiber than corn or SBM (NRC, 2012; Pedersen et al., 2014; Jaworski et al., 2015). Also, soybean hulls have a higher content of fiber than SBM (Karr-Lilienthal et al., 2005; NRC, 2012). However, the

Table 4. Apparent total tract digestibility (**ATTD**) of CP and GE; apparent hindgut digestibility (**AHD**) of GE; and DE and NE values for the experimental diets

						P-value		
Item	PC[1]	NC[2]	NC + 0.5E[3]	NC + 1.0E[4]	SEM	Diet	Linear	Quadratic
ATTD of CP, %	80.5[a]	73.1[b]	76.9[ab]	80.5[a]	2.439	0.097	0.031	0.970
ATTD of GE, %	78.8[a]	73.8[b]	76.7[ab]	80.2[a]	1.622	0.045	0.006	0.823
AHD of GE[5], %	2.4	6.0	6.3	7.1	2.268	0.241	0.640	0.807
AHD of GE[6], %	7.9	16.9	19.5	27.8	7.047	0.250	0.217	0.667
DE, kcal/kg of DM	3,068[ab]	2,985[b]	3,101[ab]	3,247[a]	63.5	0.040	0.004	0.815
NE, kcal/kg of DM	2,222[ab]	2,159[b]	2,225[ab]	2,337[a]	44.4	0.047	0.004	0.623

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]PC = positive control diet, which was formulated to be adequate in all nutrients except for Ca and digestible P, which were lower than NRC 2012 recommendations for grower pigs by 0.13 and 0.17 percentage points.

[2]NC = negative control diet, which was the same as the PC diet except that it was lower in NE by 75 kcal/kg, and standardized ileal digestible AA content by a mean of 3%. This was achieved by a partial replacement of corn and SBM and a complete replacement of soybean oil and monocalcium phosphate with 25% corn DDGS and 3% soybean hulls.

[3]NC diet was supplemented with Superzyme-CS at 0.5 g/kg of diet.

[4]NC diet was supplemented with Superzyme-CS at 1.0 g/kg of diet.

[5]Apparent hindgut digestibility of GE expressed as proportion of GE in diet.

[6]Apparent hindgut digestibility of GE expressed as proportion of GE in ileal digesta.

NSP of the PC diet had a greater content of galactose than the NSP of the NC diet, which could have been due to the greater content of SBM in the PC diet than in the NC diet. Pectin, which is rich in galactose (Drochner et al., 2004), constitutes a high proportion of NSP in SBM (van Laar et al., 1999), whereas arabinoxylans constitute a high proportion of NSP in corn, and hence corn DDGS (Jaworski et al., 2015).

The AID of P for the PC diet was greater than that for the NC diet, which was surprising as it had been assumed that the AID of P for the PC diet would not differ from that of the NC diet because these diets were formulated to be similar in digestible P and were all supplemented with the same levels of phytase. The lower AID of P for the NC diet than for the PC diet could, however, has been due to the higher fiber content in the NC diet when compared with the PC diet. Fiber present in corn DDGS (Jaworski et al., 2015) and soybean hulls (Karr-Lilienthal et al., 2005) is mostly insoluble. Insoluble fiber can reduce nutrient digestibility by increasing the passage rate of digesta in the small intestine (Wilfart et al., 2007). Woyengo et al. (2016a) reported a reduction in ATTD of P by grower pigs due to replacement of corn and SBM with 10% wheat DDGS, implying that NSP of corn or wheat DDGS reduce nutrient utilization by pigs. The AID of GE, N, and most AA and ATTD of GE for the NC diet were lower than those for the PC diet, which was expected because the NC diet was formulated to be lower in energy value and digestible AA contents than the PC diet. Also, the lower AID of GE, N, and most AA and ATTD of GE for the NC diet than for the PC diet could partly have been due to the higher content of insoluble fiber (and hence greater digesta passage rate) in the former diet than in the latter diet. The AID of total NSP and of most component sugars of NSP for the NC diet was lower than those of the PC diet, which could be attributed to the presence of corn DDGS in the NC diet. During the production of DDGS from cereal grains, NSP combine with other components of DDGS to form complexes that can be indigestible by fiber-degrading enzymes (Jha et al., 2015). Also, NSP in SBM is more digestible than those in corn DDGS. For instance, Jaworski and Stein (2017) reported that the AID of NSP for diets containing 29% corn DDGS was lower than that for corn–SBM-based diet in pigs (21.4% vs. 42.0%, respectively), implying that the NSP of corn DDGS is poorly digested by pigs than those of corn and SBM.

Multi-enzyme supplementation improved the AID of most component sugars of NSP, hence the total NSP for the NC diet, which was attributable to the increased degradation of NSP in the NC diet by the multi-enzyme. As previously mentioned, the major NSP present in corn and corn DDGS is arabinoxylans and cellulose (Jaworski et al., 2015), whereas the major NSP present in SBM is cellulose, pectins, and xyloglucans (Knudsen, 2014). In addition, corn DDGS contains mannans and β-glucans that are endogenous and also originate from residual yeast that was added to corn during the production of ethanol and DDGS from corn. The multi-enzyme used in the current study contained xylanase, β-glucanase, cellulase, mannanase, pectinase, and protease activities, and hence it targeted most of NSP present in the NC diet, leading to the increased digestibility of NSP. The improvement in the AID of NSP for the NC diet by the multi-enzyme supplementation is in agreement with the results from the study of Jakobsen et al. (2015), who reported an increase in the AID of total NSP in grower pigs fed diets containing 60% fermented DDGS that had been derived from 80% wheat and 20% barley and supplemented with enzyme product that contained xylanase and β-glucanase or cellulase and xylanase prior to feeding. Furthermore, Ndou et al. (2015) also reported that xylanase supplementation to corn–SBM-based diets containing 40% corn DDGS resulted in an increase in the AID of total NSP in grower pigs. In the current study, the AID of NSP was linearly increased by an increase in the level of multi-enzyme supplementation from 0 to 1.0 g/kg, indicating that the optimal dietary level of the multi-enzyme with regard to ileal digestibility of NSP is either ≥1.0 g/kg.

Multi-enzyme supplementation did not affect the AID of Ca in the NC diet but increased the AID of P. In the cells of plants, phytate is located in protein bodies within the cells, whereas most of the NSP is located within the cell walls, implying that the NSP can reduce the accessibility of phytase to phytate (Woyengo and Nyachoti, 2011). Thus, the improvement in P digestibility by the multi-enzyme supplementation could have been due to hydrolysis of NSP in the cell walls by the multi-enzyme, leading to increased accessibility of phytate by phytase. The increased AID of P due to supplemental multi-enzyme is in disagreement with the result reported by Ndou et al. (2015), who observed nonsignificant effect of multi-enzyme supplementation to corn–SBM–corn DDGS-based diets for pigs on the AID of P. However, it should be noted that the NC diet fed in the current study

contained more corn (60% vs. 41%) and less corn DDGS (25% vs. 40%) than the basal diet that was fed in the study of Ndou et al. (2015). Corn contains more phytate than corn DDGS (NRC, 2012). Most of the cells in DDGS are ruptured to release starch during ethanol production, whereas most cells in corn are intact, implying corn has a higher content of NSP-encapsulated phytate than corn DDGS. Thus, the NC diet fed in the current study had a higher content of NSP-encapsulated phytate than the basal diet fed in the study of Ndou et al. (2015). Zeng et al. (2018) reported an improvement in AID of total P by grower pigs due to addition of NSP-degrading enzymes to phytase-supplemented diets that contained 20% wheat bran. Woyengo et al. (2010, 2019) also reported an improvement in AID of P due to addition of NSP-degrading enzymes to phytase-supplemented corn–SBM-based diets for broilers. The magnitude of the improvement in P digestibility due to addition of NSP-degrading enzymes to phytase-supplemented diets for pigs is expected to increase with an increase in the dietary level of NSP-encapsulated phytate. Thus, a significant increase in the AID of P in the current study, but not in the study of Ndou et al. (2015), by supplementation of diets with NSP-degrading enzymes could partly be explained by the differences in the content of corn and corn DDGS among the studies.

Supplemental multi-enzyme increased AID of most AA for the NC diet, which could be attributed to the increased degradation of NSP by the multi-enzyme, thereby increasing the availability of NSP-encapsulated AA for enzymatic digestion and reducing the NSP-induced ileal endogenous AA losses in pigs. Dietary fiber reduces AA digestibility by encapsulating the AA (Jha et al., 2015), and by increasing endogenous gut AA losses of pigs (Schulze et al., 1994). An increase in the AID of most AA for the NC diet due to multi-enzyme supplementation in the current study is in agreement with the results from the study of Agyekum et al. (2016), who observed an increase in the AID of most indispensable AA in pigs due to the addition of an enzyme product containing xylanase, β-glucanase, protease, amylase, and pectinase to corn-based diet that contained 30% DDGS that had been produced from co-fermentation of corn and wheat in equal portions.

An increase in the dietary level of multi-enzyme from 0 to 1.0 g/kg of diet resulted in the improved AID of GE for the NC diet, which could be attributed to the increased AID of NSP and most AA by the multi-enzyme supplementation. Similarly, Agyekum et al. (2016) reported increased AID of GE in pigs due to addition of an enzyme product containing xylanase, β-glucanase, protease, amylase, and pectinase to corn-based diet that contained 30% DDGS that had been produced from co-fermentation of corn and wheat in equal portions. However, Ndou et al. (2015) supplemented corn–SBM-based diets for pigs that contained 40% corn DDGS with 5 different xylanase products and observed increased AID of GE by 2 xylanase products. Moran et al. (2016) did not report an increase in the AID of GE due to xylanase supplementation to corn–SBM-based diets for pigs that contained 30% dry or liquid corn DDGS, implying that some xylanase products alone did not hydrolyze most of the dietary NSP to liberate most of the energy-yielding nutrients for digestion in the small intestine. In the current study, the AID of GE for the NC diet was linearly improved by multi-enzyme supplementation from 0 to 1.0 g/kg in the NC diet, implying that the optimal dietary level of the multi-enzyme with regard to ileal digestibility of energy is either ≥1.0 g/kg.

Multi-enzyme supplementation at 0.5 or 1.0 g/kg did not affect the AHD of GE, implying that the multi-enzyme did not affect organic matter fermentation in the hindgut. Multi-enzyme supplementation increased the ATTD of GE, which was due to the increased AID of GE by the multi-enzyme supplementation because the multi-enzyme supplementation did not affect the AHD of GE. Similarly, addition of an enzyme product containing xylanase, β-glucanase, protease, amylase, and pectinase to corn-based diet that contained 30% DDGS that had been produced from co-fermentation of corn and wheat in equal portions resulted in increased ATTD of GE (Agyekum et al., 2016). However, addition of xylanase to corn–SBM-based diets for pigs that contained 40% corn DDGS (Ndou et al., 2015) or 30% corn DDGS (Moran et al., 2016) did not affect the ATTD of GE. The differences between the results from the current study and those from the studies of Ndou et al. (2015) and Moran et al. (2016) with regard to the effects of enzyme supplementation on ATTD of GE in pigs could be explained by the differences in the effects of the enzymes on the AID of GE among the studies. An increase in the dietary level of multi-enzyme from 0 to 1.0 g/kg of diet resulted in the improved DE and NE values for the NC diet, which was attributed to the increased ATTD of GE by the multi-enzyme supplementation because the NC diet and multi-enzyme-supplemented diets did not differ in the macronutrient composition.

In conclusion, an increase in the level of multi-enzyme supplementation from 0 to 1.0 g/kg of diet resulted in a linear increase in the digestibilities of NSP, most AA, energy, and P in grower pigs fed corn–SBM-based diet that contained 25% corn DDGS. Thus, the multi-enzyme fed in the current study can be used to enhance energy and nutrient digestibilities and utilization of low-energy-AA diets for grower pigs. An increase in the energy digestibility, and hence DE and NE values of low-energy AA diet due to the multi-enzyme supplementation implies that the dietary energy value can be reduced when the multi-enzyme is supplemented to corn–SBM-corn DDGS-based diets for grower pigs. However, the extent of reduction in the dietary NE value of corn–SBM–corn DDGS-based diets for grower pigs needs to be established because energy digestibility, and hence the NE value of the corn–SBM–corn DDGS-based diet were linearly improved by an increase in the dietary level of the multi-enzyme from 0 to 1.0 g/kg, implying that the optimal dietary level of the multi-enzyme could be ≥1.0 g/kg.

Conflict of interest statement. None declared.

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