Published January 9, 2015

The efficacy of a new 6-phytase obtained from *Buttiauxella* spp. expressed in *Trichoderma reesei* on digestibility of amino acids, energy, and nutrients in pigs fed a diet based on corn, soybean meal, wheat middlings, and corn distillers' dried grains with solubles

S. A. Adedokun,* A. Owusu-Asiedu,† D. Ragland,‡ P. Plumstead,† and O. Adeola*[1]

*Department of Animal Sciences, Purdue University, West Lafayette, IN 47907;
†DuPont Industrial Biosciences– Danisco Animal Nutrition, Marlborough, Wiltshire, UK SN8 1XN;
and ‡Department of Veterinary Clinical Science, Purdue University, West Lafayette, IN 47907

ABSTRACT Sixteen cannulated pigs were used to evaluate the effect of a new 6-phytase derived from *Buttiauxella* spp. and expressed in *Trichoderma reesei* on apparent ileal digestibility (AID) of AA and apparent total tract digestibility (ATTD) of DM, N, Ca, P, Na, Mg, K, Cl, and energy. Pigs were fed 4 diets for 2 periods in a crossover design. Within each period, there were 4 blocks of 4 pigs per block with each diet represented within each block. The average initial BW in periods 1 and 2 were 22 and 30 kg, respectively. Each period lasted 9 d with fecal collection on d 5 and 6 and a 12-h ileal digesta collection on d 7, 8, and 9. Pigs received a daily feed allowance of approximately 4.5% of their BW. The experimental diets were based on corn, soybean meal, wheat middlings, and corn distillers dried grain with solubles. Phytase was added at 0; 500; 1,000; or 2,000 phytase units/kg of diet to a basal diet that contained 205, 15, 5.4, and 10 g of CP, Lys, total P (1.6 g of nonphytate P), and Ca/kg diet, respectively. The addition of phytase improved ($P < 0.05$) AID of DM, N, Ca, and P. Increasing phytase supplementation linearly and quadratically increased ($P < 0.05$) AID of P and Ca, respectively, with AID of Ca showing a tendency for a linear increase ($P = 0.053$). Phytase supplementation of the basal diet improved ($P < 0.05$) AID of P from 46 to 62%. Phytase supplementation increased ($P < 0.05$) ATTD of DM, N, Ca, P, Mg, K, and energy. Contrasts showed that phytase supplementation of the basal diet increased ($P < 0.05$) AID for 8 indispensable AA (Arg, His, Ile, Leu, Lys, Phe, Thr, and Val), 6 dispensable AA (Ala, Asp, Cys, Glu, Ser, and Tyr), as well as for total AA. Furthermore, phytase supplementation to the basal diet showed a tendency ($P < 0.10$) to increase ileal digestibility of Gly. Ileal digestibility of Met, Trp, and Pro were not affected by phytase supplementation. Increasing the level of phytase supplementation resulted in linear increases ($P < 0.05$) in AID of 6 indispensable AA (Arg, Ile, Leu, Lys, Phe, and Val) and 1 dispensable AA (Asp) with 4 AA (His, Cys, Glu, and Tyr) showing a tendency for linear increase ($P < 0.10$) in AID of AA. The results from this study showed that in addition to increasing P and Ca utilization, the new *Buttiauxella* 6-phytase expressed in *Trichoderma reesei* enhanced ileal digestibility of N and several AA in growing pigs in a dose-dependent manner.

Key words: amino acid digestibility, cannulated pig, mineral, phosphorus, phytase

 J. Anim. Sci. 2015.93:168–175
doi:10.2527/jas2014-7912

INTRODUCTION

The rising cost of corn and soybean meal may necessitate an increase in the use of alternative sources of feed ingredient such as corn distillers' dried grain with solubles **(DDGS)**, bakery byproducts, and wheat middlings. Earlier studies in broilers and pigs fed diets containing corn and soybean meal plus DDGS or wheat bran have shown the effectiveness of phytase in releasing phytate-P (Olukosi et al., 2010; Almeida and Stein, 2012; Rutherfurd et al., 2014), which subsequently led to a reduction in the quantity of P that is excreted into the environment. The effect of phytase on ileal AA digestibility in pigs has not been

[1]Corresponding author: ladeola@purdue.edu
Received April 6, 2014.
Accepted November 9, 2014.

consistent (Liao et al., 2005; Adeola and Cowieson, 2011) with effects of phytase supplementation on ileal AA digestibility ranging from no improvements (Bruce and Sundstol, 1995; Traylor et al., 2001; Liao et al., 2005) to between 2 and 4% (Mroz et al., 1994; Kemme et al., 1999; Liao et al., 2005) improvement. Based on the structure of phytate and the level of phytate in swine diets, tremendous opportunities exist for an improvement in apparent ileal AA digestibility with exogenous phytase supplementation. However, to achieve consistency in the efficacy of phytase in improving ileal AA digestibility, conscious efforts must be made to develop a phytase that is able to improve not only phytate-bound P but also increase N and AA digestibility. Development of such a phytase may lead to a reduction in feed cost, an improvement in growth uniformity, and a reduction in nutrient (especially P and N) excretion into the environment. One of the avenues to achieving this goal is to develop a phytase that is able to commence and sufficiently hydrolyze most of the phytate-bound nutrients in the upper region of the digestive tract, especially in the stomach, where the environment is acidic (low pH) with protein and phytic acid possessing a net positive and negative charge, respectively, resulting in protein–phytic acid interaction (Okubu et al., 1976; Cheryan and Rackis, 1980; Selle et al., 2000).

Phytin is the main storage form of P in cereals and oil seeds (Lott et al., 2000). A significant amount of the phytate-P is excreted into the environment because phytate-bound P is poorly utilized by nonruminant animals (Nelson, 1967). The roles of phytic acid in nonruminant nutrition and post excretion consequences have been extensively documented (Ravindran et al., 1995; Jongbloed and Lenis, 1998; Selle et al., 2000). There are several types of microbial phytase. The 3-phytase is able to hydrolyze the ester bond at carbon 3 whereas the 6-phytase starts the hydrolysis of the ester bond at carbon 6 resulting in the release of phytate-bound P.

The ability of phytase to release other nutrients alongside P during the hydrolysis process is thought to result in higher digestibility of other minerals as well as AA, N, and energy (Kornegay et al., 1999; Namkung and Leeson, 1999). Meeting the AA requirements of nonruminant animals is the second most expensive portion of the diets and the ability to increase AA digestibility and utilization from feed ingredients would reduce feed cost and minimize environmental pollution. The hypothesis for this study was that a new 6-phytase derived from *Buttiauxella* spp. expressed in *Trichoderma reesei* with pH profile of 2.0 to 6.5 will improve apparent ileal digestibility **(AID)** and total tract digestibility **(ATTD)** of AA, N, Ca, and P. The objective of this study was to evaluate the efficacy of graded levels of a new 6-phytase derived from *Buttiauxella* spp. expressed in *Trichoderma reesei* on AID and ATTD of N, energy, Ca, and P and AID of AA in cannulated weaned piglets.

MATERIALS AND METHODS

Pigs and Experimental Diets

Sixteen weanling pigs with an average weight of 15 kg were fitted with simple T cannula approximately 6-cm anterior to the ileo-cecal-colonic junction as described by Dilger et al. (2004). Twelve hours post-surgery, all the cannulated pigs were placed on a diet that was adequate in energy and all nutrients (NRC, 1998) until the start of the study. Each pig was weighed individually at the start and end of each period. The average initial BW (mean ± SD) at the start of periods 1 and 2 were 22.1 ± 1.5 and 30.3 ± 2.3 kg, respectively. Each period consisted of 4 blocks of pigs with 4 pigs/block. Each period lasted 9 d with fecal collection in the morning and evening of d 5 and 6 and a 12-h ileal digesta collection on d 7, 8, and 9. The experimental diets were based on corn, soybean meal, wheat middlings, and corn DDGS and were formulated to be marginally deficient in nonphytate P but adequate in other nutrients (Table 1). Phytase was added at 0; 500; 1,000; or 2,000 phytase units/kg diet. Titanium dioxide was mixed into the diet at 5 g/kg of diet. The phytase used in the current trial was a 6-phytase from *Buttiauxella* spp. expressed in *Trichoderma reesei*. The pH profile of the phytase ranged from 2.0 to 6.5 with high affinity for IP_6. All protocols used in this study , including the care of all animals, were approved by the Purdue University (West Lafayette, IN 47906) Animal Care and Use Committee.

Each diet was represented within each block and each pig received a different diet in each of the 2 periods. Pigs received a daily feed allowance of approximately 4.5% BW of the lightest pig within each block. Daily feed intake was divided into 2 equal allotments and fed at 0700 and 1900 h each day. At the end of period 1, all pigs were fed a rest (wash-out) diet (Table 1) for 5 d after which the second period was commenced. The rest diet contained a calculated ME of 3,123 kcal/kg and 183, 5.9, 4.5, and 1.33 g, respectively, of CP, Ca, P, and nPP/kg.

Sample Collection, Processing, and Analyses

Fresh fecal samples were collected in the morning and evening of d 5 and 6 of the study. Fecal samples were stored at −20°C immediately after each collection. The 2-d fecal collections were pooled for each pig and dried in a forced air oven at 55°C. Ileal digesta were collected between 0700 and 1900 on d 7, 8, and 9 by

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

Ingredient, g/kg	Phytase level, phytase units/kg diet 0	500	1,000	2,000	Rest diet[1]
Corn	506.3	496.3	496.3	496.3	533.8
Wheat middling	71.9	71.9	71.9	71.9	73
Corn distillers dried grain with solubles	72.4	72.4	72.4	72.4	72.4
Soybean meal (48% CP)	271	271	271	271	258
L-Lysine.HCl	2.0	2.0	2.0	2.0	2.0
DL-Methionine	0.6	0.6	0.6	0.6	0.1
L-Threonine	1.5	1.5	1.5	1.5	0.9
Soy oil	28	28	28	28	28
Monocalcium phosphate	0	0	0	0	11.3
Sodium chloride	4.0	4.0	4.0	4.0	4.0
Limestone	13	13	13	13	12.2
Vitamin premix[2]	2.5	2.5	2.5	2.5	2.5
Mineral premix[3]	1.3	1.3	1.3	1.3	1.3
Selenium premix[4]	0.5	0.5	0.5	0.5	0.5
Titanium dioxide[5]	25	25	25	25	0
Phytase premix[6]	0	10	10	10	0
Total	1,000	1,000	1,000	1,000	1,000

[1]The rest diet was fed between the end of period I and start of period II for 5 consecutive days.

[2]Vitamin premix supplied per kilogram of diet: vitamin A, 3630 IU; vitamin D3, 363 IU; vitamin E, 36.4 IU; menadione, 1.3 mg; vitamin B12, 23.1 µg; riboflavin, 5.3 mg; D-pantothenic acid, 13.1 mg; and niacin, 19.8 mg.

[3]Mineral premix supplied per kilogram of diet: Cu (as copper chloride), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as iron carbonate), 121 mg; Mn (as manganese oxide), 15 mg; and Zn (as zinc oxide), 121 mg.

[4]Supplied 300 µg of Se per kilogram of diet.

[5]Prepared by mixing 1 g of titanium dioxide with 4 g of ground corn.

[6]Phytase premix was added to the basal diet (0 phytase unit) at 10 g/kg of diet to produce diets containing 500; 1,000; or 2,000 phytase unit phytase/kg diet. The concentration of the phytase enzyme in each premix was different. Premix of 10 g/kg of diet supplied the respective phytase concentration for each diet.

attaching a plastic bag to the O-ring of the cannula. Each bag was changed frequently as needed but not longer than 2 h and contained 10 mL of 10% formic acid to minimize microbial and enzymatic activity postcollection. Each bag was immediately placed in the freezer until processed. All the ileal digesta collected for the 3-d within each period were thawed, pooled for each pig, homogenized, subsampled, and freeze-dried. Diets, ileal digesta, and fecal samples were ground to pass through a 0.5-mm screen using a mill grinder (Retsch ZM 100, Retsch GmbH and Co., K.G., Haan, Germany).

Duplicate proximate analyses were performed on diets, ileal digesta, and fecal samples. Dry matter analysis of samples was determined by drying the samples in a drying oven at 105°C for 24 h (AOAC, 2006; Method 934.01). Diets, feces, and ileal digesta were analyzed for titanium, minerals, and N concentration and ileal digest was also analyzed for AA concentration at the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO). Samples were digested as described by Myers et al. (2004), after which titanium concentration was determined by flame atomic absorption spectroscopy. Nitrogen was determined by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC, 2000; Method 990.03), with EDTA serving as the internal standard. Samples for AA analysis were prepared using a 24-h hydrolysis in 6 *N* hydrochloric acid at 110°C under an atmosphere of N. For methionine and cysteine, performic acid oxidation was done before acid hydrolysis. Samples for tryptophan analysis were hydrolyzed using barium hydroxide. Amino acids in hydrolyzates were determined by HPLC after postcolumn derivatization (AOAC, 2000; Method 982.30 E [a, b, c]). Calcium and P concentrations were determined following nitric and perchloric acid wet-ash digestion (AOAC, 2000; Method 946.06). Samples were digested according to AOAC (2006; method 975.03[b]) and sodium, magnesium, and potassium were determined (AOAC, 2006; method 985.01) by inductively coupled plasma–optical emission spectroscopy. Chloride was determined by manual titration (AOAC, 2006; method 9.15.01, 943.01). Phytase activities in diets were determined as described by Engelen et al. (2001) and Yu et al. (2014), and the concentration of phytic acid in the diets was determined as described by Ellis et al. (1977).

Calculations and Statistical Analysis

Apparent nutrient and energy digestibility (ileal or total tract) were determined by the index method according to the following equation:

$$\text{AND } (\%) = 100 - [(Ti_I/Ti_O) \times (N_O/N_I) \times 100],$$

where AND is the apparent (ileal or total tract) nutrient or energy digestibility expressed in percentage, Ti_I is the titanium concentration in the diet, Ti_O is the titanium concentration in the output (ileal or feces), N_O is the nutrient or gross energy in the diet, and N_I is the nutrient or gross energy in the output (ileal or feces).

Data were analyzed as a randomized complete block design using SAS (SAS Institute Inc., Cary, NC). Phytase effect was tested using a contrast of 0 vs. average of 500, 1,000, and 2,000 phytase unit/kg, as well as linear and quadratic contrasts. Least squares means are presented, $P \leq 0.05$ was considered significant, and $0.05 < P < 0.1$ was considered a trend.

RESULTS

Analyzed N, minerals, energy and AA contents of the experimental diets are presented in Table 2. The

Table 2. Determined nutrient and energy composition of the experimental diets (as-fed)

Item	Phytase level, phytase unit /kg diet 0	500	1,000	2,000
Dry matter, g/kg	878	877	882	881
Nitrogen, g/kg	32.9	33.7	32.3	32.9
Calcium, g/kg	10.0	10.0	9.5	8.9
Phosphorus, g/kg	5.4	5.4	5.5	5.5
Sodium, g/kg	1.9	1.9	1.7	1.6
Chloride, g/kg	3.3	3.3	2.9	2.8
Iron, g/kg	0.4	0.3	0.3	0.3
Magnesium, g/kg	2.1	2.2	2.2	2.1
Potassium, g/kg	11.6	11.2	11.4	11.1
Zinc, g/kg	0.1	0.2	0.1	0.1
Phytic acid, g/kg	11.1	11.2	11.0	11.0
Gross energy, kcal/kg	4,114	4,105	4,168	4,135
Phytase activity, phytase unit/kg	187	596	939	2081
Indispensable amino acid, g/kg				
Arg	13.9	13.7	13.2	13.8
His	5.5	5.5	5.4	5.6
Ile	8.9	8.9	8.8	9.2
Leu	18.9	18.7	18.2	18.9
Lys	13.4	13.2	12.9	13.1
Met	4.1	3.9	3.7	4.0
Phe	10.6	10.5	10.1	10.5
Thr	9.3	9.2	8.7	8.9
Trp	2.3	2.5	2.3	2.2
Val	10.2	10.2	10.1	10.5
Dispensable amino acid, g/kg				
Ala	11.0	10.9	10.6	10.9
Asp	20.4	20.2	19.3	20.3
Cys	4.0	4.1	4.1	4.3
Glu	34.7	34.4	32.9	34.9
Gly	8.8	8.8	8.6	8.8
Pro	12.9	13.0	12.5	13.0
Ser	9.6	9.4	8.8	9.1
Tyr	7.5	7.4	7.1	7.5
Total amino acid, g/kg	207.7	206.1	199.1	207.1

analyzed value of phytic acid in each diet was 11 g/kg diet. Analyzed phytase activities of the experimental diets were 187; 596; 939; and 2,081 phytase unit/kg for 0; 500; 1,000 and 2,000 phytase unit/kg diet, respectively, and after correcting for intrinsic phytase activity, the recoveries were 82, 75, and 95% for 500; 1,000; and 2,000 phytase unit/kg diet, respectively, and are within the expected range of the formulation. All pigs were in good health throughout the duration of the study. The respective weight gain (kg) and gain/feed (kg/kg) were 4.3 ± 1.20 and 0.49 ± 0.141 for period 1 and 7.3 ± 0.85 and 0.59 ± 0.074 for period 2.

The addition of phytase improved ($P < 0.05$) AID of DM, N, Ca, and P (Table 3). Increasing phytase supplementation linearly increased ($P < 0.05$) AID of P and quadratically increased ($P < 0.05$) AID of Ca, with AID of Ca showing a tendency for a linear increase ($P = 0.053$; Table 3). Apparent ileal digestibility of DM and N showed a tendency ($P < 0.05$) for quadratic effect of phytase supplementation. Phytase supplementation of the basal diet improved ($P < 0.05$) AID of P from 46 to 62%. Phytase supplementation increased ($P < 0.05$) ATTD of DM, N, Ca, P, Mg, K, and energy (Table 4). Both linear and quadratic effects ($P < 0.05$) were observed for ATTD of DM, minerals, and energy. Contrasts showed that phytase supplementation of the basal diet increased ($P < 0.05$) AID of Arg, His, Ile, Leu, Lys, Phe, Thr, and Val; the dispensable AA Ala, Asp, Cys, Glu, Ser, and Tyr; as well as for total AA (Table 5). Phytase supplementation of the basal diet showed a tendency ($P < 0.075$) to increase AID of Gly. The AID of Met, Trp, and Pro were not affected by phytase supplementation (Table 5). Increasing level of phytase supplementation resulted in linear increases ($P < 0.05$) in AID of Arg, Ile, Leu, Lys, Phe, Val, and Asp. Four AA (His, Cys, Glu, and Tyr) showed a tendency for a linear increase ($P < 0.10$) in apparent ileal AA digestibility (Table 5).

DISCUSSION

The economic and environmental consequences of P in nonruminant nutrition have been ameliorated with the development and use of exogenous phytase in nonruminant animal diets. Economically, the reduction in the quantity of inorganic P added to the diet as a result

Table 3. Apparent ileal dry matter, nutrient, and energy digestibility of pigs fed graded levels of phytase

Item	Phytase level, phytase units/kg diet 0	500	1,000	2,000	SD	Probability of contrast 0 vs. phytase	Linear	Quadratic
Number of replicates[1]	7	8	7	8				
Dry matter, %	62.9	67.8	66.7	66.2	2.29	0.012	0.169	0.070
Nitrogen, %	73.8	78.4	76.6	76.6	1.90	0.011	0.218	0.075
Calcium, %	62.5	73.9	70.5	70.4	3.64	0.002	0.053	0.025
Phosphorus, %	46.4	57.8	64.2	62.9	4.44	<0.001	<0.001	0.102
Energy, %	66.4	69.5	68.6	70.4	2.77	0.068	0.179	0.302
Ileal digestible energy, kcal/kg	2,820	3,008	2,977	2,930	120.21	0.053	0.415	0.126

[1]Data from a pig each for diet containing 0 and 1,000 phytase units/kg diet were outliers and were removed from the data before statistical analysis.

Table 4. Apparent total tract dry matter, nutrient, and energy digestibility of pigs fed diets containing graded levels of phytase

	Phytase level, phytase units/kg diet					Probability of contrast		
	0	500	1,000	2,000	SD	0 vs. Phytase	Linear	Quadratic
Number of replicates	8	8	8	8				
Dry matter, %	79.4	83.2	82.3	82.3	1.23	<0.001	0.022	0.009
Nitrogen, %	80.2	85.2	84.2	83.7	1.54	<0.001	0.033	0.004
Calcium, %	45.4	60.7	60.3	64.6	3.60	<0.001	0.001	0.022
Phosphorus, %	30.0	52.6	59.2	66.1	4.194	<0.001	<0.001	0.005
Sodium, %	84.0	92.3	89.0	87.3	5.86	0.100	0.774	0.144
Magnesium, %	5.5	21.6	16.8	17.4	5.09	<0.001	0.029	0.011
Potassium, %	72.3	79.3	81.2	80.6	2.47	<0.001	0.001	0.003
Chloride, %	94.3	95.1	93.8	92.4	1.47	0.530	0.030	0.349
Energy, %	80.2	83.3	82.8	82.9	1.08	<0.001	0.012	0.012
Digestible energy, kcal/kg	3,607	3,864	3,789	3,748	110	0.006	0.284	0.023

of exogenous phytase supplementation has resulted in a decrease in feed cost as the cost of meeting the requirements for nonphytate P is third only to those of energy and CP/AA. The reduction in inorganic P supplementation and the ability of the animal to better utilize phytate-bound P has resulted in a reduction in P output (Ketaren et al., 1993; Mroz et al., 1994), hence a reduction in environmental consequences. The effects of phytase and other enzymes that are commercially available on N/AA digestibility have been inconsistent (Adeola and Cowieson, 2011). The increase in demand for lean meat and an increase in the cost of corn and soybean meal may necessitate an increase in the use of alternative feed ingredients in pig diets. However, the use of alternative feed ingredients brings its own challenges such as poor nutrient availability and high variability of nutrients. Hence, it is important to explore ways in which minerals and N/AA digestibility of nonruminant diets could be enhanced. One of the available routes is the development of exogenous enzymes that could increase N/AA digestibility in nonruminant diets. With recent advances in enzyme technology, including the screening and selection for candidate microbes, it is possible to select, isolate, and express enzymes with

Table 5. Apparent ileal amino acid digestibility of pigs fed graded levels of phytase

	Phytase level, phytase units/kg diet					Probability of contrast		
	0	500	1,000	2,000				
Number of replicates[1]	7	8	7	8	SD	0 vs. phytase	Linear	Quadratic
Indispensable amino acid, %								
Arg	87.6	89.5	89.1	90.3	0.55	<0.001	<0.001	0.187
His	81.9	84.9	83.6	84.7	1.56	0.002	0.067	0.331
Ile	80.7	83.6	83.4	84.4	1.34	0.003	0.005	0.177
Leu	81.5	84.6	84.2	84.7	1.36	0.003	0.015	0.106
Lys	84.3	86.9	86.1	87.0	1.06	0.004	0.015	0.192
Met	86.6	87.1	86.8	88.2	1.74	0.489	0.221	0.715
Phe	81.5	85.0	84.2	84.8	1.24	0.001	0.010	0.060
Thr	75.9	79.4	77.2	77.6	1.69	0.047	0.516	0.226
Trp	77.3	81.6	79.0	77.7	2.30	0.142	0.559	0.119
Val	77.3	80.9	80.0	81.2	2.03	0.016	0.038	0.308
Dispensable amino acid, %								
Ala	77.4	80.4	79.6	80.2	1.91	0.033	0.111	0.289
Asp	77.8	80.9	79.5	81.2	1.39	0.006	0.014	0.395
Cys	71.9	76.5	75.4	76.7	2.87	0.025	0.068	0.299
Glu	83.1	86.2	84.6	85.9	1.53	0.017	0.066	0.355
Gly	68.2	72.2	71.3	71.3	3.01	0.075	0.283	0.268
Pro	79.9	82.1	81.3	81.7	1.81	0.116	0.309	0.422
Ser	79.6	83.9	81.5	81.3	1.35	0.008	0.626	0.037
Tyr	82.6	85.4	84.3	85.0	1.25	0.011	0.066	0.199
Total amino acid, %	80.3	83.2	82.1	83.1	1.57	<0.001	0.059	0.305

[1]Data from a pig each for diet containing 0 and 1,000 phytase units/kg diet were outliers and were removed from the data before statistical analysis.

high activity levels in acidic environments as it is the case in the stomach of pigs or to develop enzymes with more than 1 active sites having the potential to release not just 1 nutrient but several. For instance, phytate binds not just P but also other nutrients (Selle et al., 2000). Likewise, the process of releasing phytate-bound P with exogenous phytase presents opportunities for releasing other nutrients as long as the hydrolytic process occurs well before the formation of binary protein–phytate complex (Selle et al., 2000), which is refractory to pepsin digestion. Additionally, more minerals, AA, and energy from the phytate molecules could be made available to the animal for absorption, thereby increasing the feeding value of the diet. Achieving this using only 1 candidate enzyme, such as phytase, may improve efficiency as well as reduce cost of feed via a reduction in the nutrient density of the diet but also through a reduction in the cost of having to add 2 or more different enzymes to a diet or having to adopt superdosing as a means of maximizing the release of nutrients that are bound to the phytate molecule. Hence, the objective of this study was to evaluate the effect of a new 6-phytase from *Buttiauxella* spp. expressed in *Trichoderma reesei* with a pH profile range of 2.0 to 6.5 on AA, energy, and mineral digestibility in cannulated pigs.

Results from the current study show that this new 6-phytase derived from *Buttiauxella* spp. and expressed in *Trichoderma reesei* improved ileal nutrient and energy digestibility in cannulated weaned pigs. Phytase supplementation of the basal diet improved AID of DM, N, Ca, P, and energy by 4.0, 3.4, 9.1, 15.2, and 3.1% units and the digestible energy was improved by 152 kcal/kg diet. Unlike earlier reports for phytase, this new phytase was able to significantly improve ileal digestibility for all of the variables examined in this study with the highest percentage point increase obtained for P (15.2) and Ca (9.1).

Earlier studies in swine showed that Cys, Thr, Pro, and Gly digestibility usually improve with exogenous phytase supplementation but Met, Arg, Glu, and Lys respond much less as reviewed in Adeola and Cowieson (2011). In the current study, all the AA, with the exception of Met, Trp, and Pro, responded positively to phytase supplementation. This observation is similar to what has been reported in broilers (Kornegay et al., 1999; Namkung and Leeson, 1999). This positive effect of phytase on ileal AA digestibility in the current study occurred despite the fact that all the diets were adequate in AA and CP. Improvement in ileal AA digestibility in broilers has also been reported for this phytase (Amerah et al., 2014).This shows that pigs still have the potential to digest and absorb more AA from diets as long as they are available in forms that can be easily utilized. The overall improvement in AID of AA with phytase supplementation was 2.6% units with 2.4 and 2.9% units for indispensable and dispensable AA, respectively. The greatest and lowest effects of phytase supplementation were seen in Cys (4.3% units difference) and Met (0.8% units difference), respectively. Methionine and Trp were unaffected by phytase supplementation. Increasing levels of phytase supplementation resulted in a linear increase in AID of 7 AA (6 indispensable and 1 dispensable) with an additional 4 AA (His, Cys, Glu, and Tyr) showing a tendency for a linear increase in AID. Based on this result, pigs may benefit from increasing levels of phytase supplementation whereas Met, Thr, Trp, Gly, Pro, and Ser digestibility may not improve much from higher supplementation levels of the phytase beyond 500 phytase units/kg diet. What was observed in this study is different from what Yáñez et al. (2011) reported for 37-kg pigs where phytase (6-phytase derived from *Escherichia coli*, 0 and 250 phytase units/kg diet) supplementation did not result in improvement in apparent ileal AA digestibility. The pigs in this study were fed daily feed allowance of 2.8 times the maintenance requirement for DE of diets containing 16% CP. Phosphorus digestibility was significantly improved (Yáñez et al. (2011) by 6.8% units but Ca digestibility was not significantly improved (49.4 vs. 55.9%). However, the improvements in P and Ca digestibilities in the current study with phytase supplementation were higher (15.2 and 9.1% units, respectively) compared with what was reported for 37-kg pigs by Yáñez et al. (2011). Results from this study confirmed what Olukosi et al. (2010) reported for 21-d-old broilers fed corn–soybean meal based diets. They reported an 8 and 4% increase in DM and N digestibility with phytase supplementation (6.4 and 4.6% increase for DM and N digestibility in the current study). They also reported an improvement of 320 kcal/kg diet (1.34 MJ/kg diet) in ileal digestible energy. The reason for the huge effect of phytase on ileal digestible energy as reported by Olukosi et al. (2010) may be due to the fact that the diets used by Olukosi et al. (2010) were marginally deficient in energy.

The stepwise hydrolysis of myo-inositol hexakisdihydrogen phosphate (phytic acid) by phytase to release phytate bound-P has been well documented with several recently published reviews summarizing thousands of published data on the effects of exogenous phytase supplementation on nutrient and energy digestibility and performance of swine and poultry (Adeola and Sands, 2003; Selle and Ravindran, 2007; Cowieson et al., 2009). Based on the current results, it could be inferred that phytate hydrolysis by this enzyme resulted in a significant extraphosphoric effect with significant improvement in apparent N, AA, energy (total tract only), and minerals digestibility. However, this is not always the case. One of the reasons for this may be the formation of phytate–protein complex well before the effect of the exogenous phytase kicks in. To circumvent this phenomenon, it is important for the exogenous phytase to be resistant to the low pH in the stomach of pigs and to be able to initiate phytate hydrolysis early enough in the stomach so as to minimize the formation of binary protein–phytate (at acidic pH) and ternary protein–mineral–phytate (close to neutral pH) complexes (Selle et al., 2000). The development of phytase with high activity level in the foregut, especially in the stomach, and its ability to survive the harsh acidic environment in the stomach as well as protease degradation will go a long way in enhancing N/AA digestibility in nonruminant animals. The pH profile of this phytase (2.0 to 6.5) and its high affinity for IP_6 may explain the reason why the phytase used in the current study was able to significantly improve AID DM, P, Ca, N, AA, and digestible energy in pigs. With the exception of sodium and chloride, phytase supplementation resulted in significant improvements in ATTD for all the variables examined in this study. Total tract N and DM digestibility were improved by 4.2 and 3.2% units, respectively. The greatest improvements in ATTD of 32.4, 14.7, and 13.1% units were obtained for P, Ca, and Mg, respectively. Our data confirmed an improvement in ATTD of P and Ca as a result of phytase (Ronozyme HiPhos) supplementation as it has been reported for 22-kg intact male pigs by Rutherfurd et al. (2014). This may be an indication of increasing phytate hydrolysis in the hindgut as a result of phytase supplementation. The effects of increasing phytase supplementation from 500 to 2,000 phytase units/kg diet on apparent ATTD for DM, N, Ca, P, Mg, K, and energy were both linear and quadratic. This may indicate that the ability of this new phytase to hydrolyze phytate in the stomach may have minimized the formation of binary protein–phytate complex with subsequent release of phytate-bound nutrients which otherwise would have been unavailable to the pig.

In conclusion, exogenous phytase has the potential to enhance digestibility of energy, N, and AA and many other nutrients other than P. The development of phytase that can tolerate the acidic gastric environment and initiate the hydrolysis of phytic acid under this condition is essential in improving the effects of phytase in nutrients and energy digestibility in swine.

LITERATURE CITED

Adeola, O., and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. Board Invited Review. J. Anim. Sci. 89:3189–3218.

Adeola, O., and J. S. Sands. 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J. Anim. Sci. 81:E78–E85.

Almeida, F. N., and H. H. Stein. 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs. J. Anim. Sci. 90:1262–1269.

Amerah, A. M., P. W. Plumstead, L. P. Barnard, and A. Kumar. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93:906–915.

AOAC. 2000. Official methods of analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Bruce, J. A. M., and F. Sundstol. 1995. The effect of microbial phytase in diets for pigs on apparent ileal and faecal digestibility, pH and flow of digesta measurements in growing pigs fed a high fibre diet. Can. J. Anim. Sci. 75:121–127.

Cheryan, M., and J. J. Rackis. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 13:297–335.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: Implications for endogenous nitrogen losses and nutrient availability. Worlds Poult. Sci. J. 65:401–418.

Dilger, R. N., J. S. Sands, D. Ragland, and O. Adeola. 2004. Digestibility of nitrogen and amino acids in soybean meal with added soyhulls. J. Anim. Sci. 82:715–724.

Ellis, R., E. R. Morris, and C. Philpot. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal. Biochem. 77:536–539.

Engelen, A. J., F. C. van der Heeft, P. H. Randsdorp, and W. A. Somers. 2001. Determination of phytase activity in feed by a colorimetric enzymatic method: collaborative interlaboratory study. J. AOAC Int. 84:629–633.

Jongbloed, A.W., and N.P. Lenis. 1998. Environmental concerns about animal manure. J. Anim. Sci. 76:2641–2648.

Kemme, P. A., A. W. Jongbloed, Z. Mroz, and A. C. Beynen. 1999. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate, and lactic acid levels. 1. Apparent ileal digestibility of amino acids. Livest. Prod. Sci. 58:107–117.

Ketaren, P. P., E. S. Batterham, E. B. Dettmann, and D. J. Farrell. 1993. Phosphorus studies in pigs. 3. Effect of phytase supplementation on the digestibility and availability of phosphorus in soyabean meal for grower pigs. Br. J. Nutr. 70:289–311.

Kornegay, E. T., Z. Zhang, and D. M. Denbow. 1999. Influence of microbial phytase supplementation of a low protein/amino acid diet on performance, ileal digestibility of protein and amino acids, and carcass measurements of finishing broilers In: Phytase in animal nutrition and waste management. 2nd ed. BASF Corporation, Mount Olive, NJ. p. 557–572.

Liao, S. F., W. C. Sauer, A. K. Kies, Y. C. Zhang, M. Cervantes, and J. M. He. 2005. Effect of phytase supplementation to diets for weanling pigs on the digestibilities of crude protein, amino acids, and energy. J. Anim. Sci. 83:625–633.

Lott, J. N. A., I. Ockenden, V. Raboy, and G. D. Batten. 2000. Phytic acid and phosphorus in crops seeds and fruits: A global estimate. Seed Sci. Res. 10:11–33.

Mroz, Z., A. W. Jongbloed, and P. A. Kemme. 1994. Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. J. Anim. Sci. 72:126–132.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179–183.

Nelson, T. S. 1967. The utilization of phytate phosphorus by poultry—A review. Poult. Sci. 46:862-871.

Namkung, H., and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids. Poult. Sci. 78:1317–1319.

NRC. 1998. Nutrient requirements of swine. 10th ed. Natl. Acad. Press, Washington, DC.

Okubu, K., D. V. Meyers, and G. A. Iacobucci. 1976. Binding of phytic acid to glycinin. Cereal Chem. 53:513–524.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2010. Broiler responses to supplementation of phytase and admixture of carbohydrases and protease in maize-soyabean meal diets with or without maize distillers' dried grain with solubles. Br. Poult. Sci. 51:434–443.

Ravindran, V., W. L. Bryden, and E. T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. Rev. 6:125–143.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2014. Effect of microbial phytase on phytate P degradation and apparent digestibility of total P and Ca throughout the gastrointestinal tract of the growing pig. J. Anim. Sci. 92:189–197.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilization. Nutr. Res. Rev. 13:255–278.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Traylor, S. L., G. L. Cromwell, M. D. Lindemann, and D. A. Knabe. 2001. Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium and phosphorus in dehulled soybean meal for growing pigs. J. Anim. Sci. 79:2634–2642.

Yáñez, J. L., E. Beltranena, M. Cervantes, and R. T. Zijlstra. 2011. Effect of phytase and xylanase supplementation or particle size on nutrient digestibility of diets containing distillers dried grains with solubles co-fermented from wheat and corn in ileal-cannulated grower pigs. J. Anim. Sci. 89:113–123.

Yu, S., M. F. Kvidtgaard, M. F. Isaksen, and S. Dalsgaard. 2014. Characterization of a mutant Buttiauxella phytase using phytic acid and phytic acid-protein complex as substrates. Anim. Sci. 1:18–32.