Effects of a novel corn-expressed *E. coli* phytase on digestibility of calcium and phosphorous, growth performance, and bone ash in young growing pigs[1]

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ABSTRACT: Two experiments were conducted to test the hypothesis that a corn-expressed phytase increases growth performance, bone measurements, and nutrient digestibility by young growing pigs, if added to diets that are deficient in Ca and P. In Exp. 1, 60 pigs (initial BW: 10.78 ± 0.67 kg) were randomly allotted to 6 dietary treatments that included a positive control diet (**PC**; 0.70% total Ca and 0.60% total P) and a negative control diet (**NC**; 0.50% total Ca and 0.42% total P). Four additional diets were formulated by supplementing the NC diet with 250, 500, 1,000, or 1,500 phytase units (**FTU**)/kg. Diets were fed for 28 d and the individual BW of pigs on days 1 and 28 were recorded. Fecal samples were collected from days 25 to 27 to calculate apparent total tract digestibility (**ATTD**) of Ca and P. On the last day of the experiment, all pigs were euthanized, and the left femur was removed and analyzed for ash, Ca, and P. Results indicated that growth performance, ATTD of Ca and P, and bone ash measurements were reduced ($P < 0.05$) in NC fed pigs compared with PC fed pigs. However, growth performance, ATTD of Ca and P, and bone ash measurements were improved (linear and quadratic, $P < 0.05$) by including increasing concentrations of phytase to the NC diet. In Exp. 2, experimental procedures were similar to those used in Exp. 1. Forty-eight pigs (initial BW: 11.15 ± 0.85 kg) were randomly allotted to 6 dietary treatments in a 28-d experiment. Treatments included a PC diet, an NC diet, and 4 diets in which 500 or 1,000 FTU/kg of either the corn-expressed phytase or a commercial microbial phytase were added to the NC diet. Pigs fed the NC diet had reduced ($P < 0.01$) final BW, ADG, G:F, and bone ash concentrations compared with pigs fed the PC diet. When 500 FTU/kg phytase was fed, no differences were observed in growth performance or bone ash measurements between phytase sources, and there were no differences in growth performance among pigs fed 1,000 FTU/kg of either phytase source or the PC diet. However, regardless of concentration or source of phytase, pigs fed the PC diet had greater ($P < 0.001$) amount of bone ash, bone Ca, and bone P compared with pigs fed phytase diets. In conclusion, the corn-expressed phytase is effective in improving growth performance, Ca and P digestibility, and bone measurements in pigs fed diets that are deficient in Ca and P.

Key words: bone ash, digestibility, phosphorus, phytase, pig

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INTRODUCTION

Phytase is an enzyme that hydrolyzes the phosphomonoester bonds of phytic acid [myo-inositol, 1,2,3,4,5,6-hexakisphosphate (Dersjant-Li et al., 2015)] releasing bound P, and therefore, produces lower forms of myo-inositol phosphates (IP5, IP4, IP3, IP2, and inositol; Wyss et al., 1999;

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Lassen et al., 2001). Phytases are present in plants, microorganisms, and animal tissues (Greiner and Konietzny, 2010).

Microbial phytase is the most commonly used exogenous phytase in diets for pigs (Dersjant-li et al., 2015) and is usually produced by fermentation (Gontia et al., 2012), but an alternative option is to grow a transgenic plant that expresses phytase. Plant-expressed phytases are generated by inserting phytase-encoding genes into plants, which results in the plants producing seed with increased concentrations of phytase (Greiner and Konietzny, 2006). Advantages of using a genetically modified phytase compared with microbial phytase include the fact that foreign genes can be easily transferred and expressed in plants and plants have large biomass accumulation and use solar energy (Zhang et al., 2000). Thus, producing transgenic plants to express phytase may be an option to improve the bioavailability of P in feeds (Gontia et al., 2012).

A novel corn-expressed phytase (GraINzyme, Agrivida Inc., Woburn, MA) is effective in poultry and contains an engineered *Escherichia coli* phytase called Phy02. Phy02 improved weight gain, feed efficiency, bone mineralization and strength, and P digestibility when young pigs were fed reduced Ca and/or P diets (Lee et al., 2017; Knapp et al., 2018a, 2018b; Broomhead et al., 2019). It is, however, not known how the corn-expressed phytase may influence Ca digestibility and concentration of Ca in bone, and if the corn-expressed phytase has the same efficacy as commercial microbial phytases. Therefore, 2 experiments were conducted to test the hypothesis that the corn-expressed phytase increases growth performance, bone ash measurements, and digestibility of Ca and P when added to Ca- and P-deficient diets fed to growing pigs, and that the efficiency of the corn-expressed phytase is not different from that of a commercial phytase.

MATERIALS AND METHODS

The institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for 2 experiments. Pigs used in the experiments were the offspring of L-359 boars mated to Camborough females (PIC, Hendersonville, TN).

Experiment 1

Animals, experimental design, and diets. Sixty growing pigs (30 barrows and 30 gilts, initial average BW: 10.78 ± 0.67 kg) were randomly allotted to 6 dietary treatments and 60 pens with 10 replicate pens per treatment in a completely randomized design. Therefore, there was 1 pig per pen and 5 barrows and 5 gilts per treatment. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. Pens had fully slatted floors and were equipped with a feeder and a nipple drinker in an environmentally controlled room.

The six experimental diets were based on corn and soybean meal and the positive control (**PC**) diet supplied all nutrients according to current recommendations (NRC, 2012; Tables 1 and 2) and offered as mash. The negative control (**NC**) diet was similar to the PC diet with the exception that inclusion of total Ca was reduced by 0.20 percentage units, and inclusion of total P was reduced by 0.18 percentage units. Four additional diets were formulated by adding 250, 500, 1,000, or 1,500 phytase units (**FTU**)/kg of the corn-expressed phytase to the NC diet. All diets contained titanium dioxide as an indigestible marker.

Table 1. Composition of experimental diets, as-fed basis, Exp. 1[1,2]

Ingredient, %	Positive control	Negative control
Ground corn	64.35	66.05
Soybean meal, 48% CP	29.75	29.75
Choice white grease	2.00	1.35
Limestone	1.16	1.01
Monocalcium phosphate	1.00	0.10
L-Lys HCL, 78% Lys	0.42	0.42
DL-Met	0.10	0.10
L-Thr	0.12	0.12
Titanium dioxide	0.40	0.40
Sodium chloride	0.40	0.40
Vitamin–mineral premix[3]	0.30	0.30
Total	100.00	100.00

[1]Four additional diets were formulated by adding 0.01%, 0.02%, 0.04%, or 0.06% of a phytase premix to the NC diet. The phytase premix was added at the expense of corn and contained 2,500 units of phytase (FTU) per gram (GraINzyme, Agrivida, Inc. Woburn, MA). Thus, the 4 diets contained 250, 500, 1,000, or 1,500 FTU per kilogram of complete diet.

[2]Both diets were formulated to contain 2,483 kcal NE per kg complete diet. Diets also were formulated to contain the following quantities of standardized ileal digestible Lys, Met, Thr, and Trp: Lys, 1.23%; Met, 0.37%; Thr, 0.73%, and Trp, 0.21%.

[3]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 2. Analyzed composition of experimental diets, as-fed basis, Exp. 1

Item	Positive control	Negative control	250 FTU[1]	500 FTU	1,000 FTU	1,500 FTU
DM, %	86.54	86.61	86.55	86.49	86.56	86.41
Ash, %	5.60	5.18	4.65	4.62	4.64	4.63
GE, kcal/kg	3,817	3,870	3,923	3,886	3,874	3,851
CP, %	19.00	18.60	18.11	18.18	18.55	19.56
NDF, %	7.22	8.36	7.20	7.96	7.33	7.67
ADF, %	1.53	1.65	1.54	1.43	1.58	1.45
Ca, %	0.84	0.52	0.58	0.62	0.58	0.57
P, %	0.58	0.44	0.41	0.38	0.41	0.39
Phytase, FTU/kg	0	0	170	440	1,200	1,500

[1]FTU = phytase units.

Growth performance, bone measurements, and fecal collection. Pigs were offered their respective diets on an ad libitum basis, and water was freely available throughout the experiment. Daily feed allotments were recorded and on the last day of the 28 d experiment, feeders were emptied and the amount of feed left in each feeder was recorded and subtracted from total feed allotments to calculate feed disappearance for each pen. Pig weights were recorded at the beginning of the experiment and on the last day of the experiment. During the last 3 d of the experiment, fecal samples were collected from all pigs by anal stimulation. Samples from the 3 d were pooled within each pig and were dried in a forced air oven at 65 °C and then ground using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ) with a 1-mm screen.

On the last day of the experiment, all pigs were euthanized via captive bolt penetration. The right rear leg was removed and autoclaved at 125 °C for 55 min, and the femur was extracted. The marrow of the broken femur was removed, and bones were dried and soaked in petroleum ether under a chemical hood for 72 h to remove the remaining marrow and fat. Bones were dried overnight at 130 °C and ashed at 600 °C for 24 h to calculate the concentration of bone ash.

Chemical analysis. All diets and fecal samples were analyzed for DM (method 930.15; AOAC International, 2007) and for ash (Method 942.05: AOAC International, 2007). Diets were also analyzed for GE on an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) using benzoic acid as the internal standard. The concentration of N in all diets was measured using the combustion procedure (method 999.03: AOAC International, 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. Diets were also analyzed for ADF and NDF using Ankom Technology method 12 and 13 (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY), and for phytase activity (Phytex Method, Version 1; Eurofins, Des Moines, IA). Calcium and P were analyzed in bone ash, diets, and fecal samples using inductively coupled plasma-optical emission spectrometry (**ICP-OES**; Method 985.01 A, B, and C; AOAC International, 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC International, 2007). Diets and fecal samples were analyzed for titanium as well (Myers et al., 2004).

Calculations and statistical analyses. The ADG, ADFI, and G:F were calculated for each pig and treatment group. Bone ash percentage was calculated by dividing the quantity of bone ash by the quantity of fat free, dried bone, and multiplied by 100. The quantity of bone P or Ca in grams was calculated by multiplying the bone P or Ca percentage by the quantity of bone ash and dividing by 100. The apparent total tract digestibility (**ATTD**) of Ca and P were calculated in each diet (Adeola, 2001).

Normality of data was verified, and outliers were identified using UNIVARIATE procedure (version 9.3, SAS Institute, Cary, USA). Outliers were identified as values that deviated from the treatment mean by more than 3 times the interquartile range. No outliers were identified for growth performance or bone mineralization data, but 2 outliers were removed from the data for ATTD of P. Data were analyzed using the MIXED procedure of SAS. Contrast statements were used to determine linear and quadratic effects of inclusion of graded levels of phytase to the NC diet. Additionally, the PC diet was compared with NC and with the NC diet supplemented with 1,500 FTU/kg by using a Student's *t*-test procedure of the statistical package SAS. The main effect of sex was included in the initial analysis, but because no effects of sex were observed, the final model did not include the effect of sex. Means were calculated using the LS means statement in SAS. The pig was the experimental unit, and statistical significance and tendency were considered at $P < 0.05$ and $0.05 \le P < 0.10$, respectively.

Experiment 2

Animals, experimental design, and diets. Forty-eight weanling pigs (24 barrows and 24 gilts, average initial BW: 11.15 ± 0.85 kg) were randomly allotted to 6 diets and placed in 48 pens with 8 replicate pens per treatment in a completely randomized design. Pigs were housed individually in the same facility as used for Exp. 1.

The PC diet was formulated to contain nutrients according to current recommendations (NRC, 2012), and the NC diet was similar to the PC diet with the exception that inclusion of Ca was reduced by 0.20 percentage units, and inclusion of digestible P was reduced by 0.18 percentage units (Tables 3 and 4). Four additional diets were formulated by adding 500 or 1,000 FTU/kg of the corn-expressed phytase or 500 or 1,000 FTU/kg of a commercial *Buttiauxella* spp. phytase expressed in *Trichoderma reesei* (AxtraPhy, Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI) to the NC diet. All diets were provided as mash.

Diets were fed for 28 d and daily feed allotments as well as the weight of feed left in the feeder on day 28 were recorded. The BW of each pig was recorded at the beginning of the experiment and on day 28, and on the last day of the experiment, pigs were euthanized and the right hind leg was removed and processed as explained for Exp. 1. All procedures to calculate growth performance and bone measurements were as describe for Exp. 1. Diets were analyzed for DM, ash, GE, CP, Ca, P, and phytase activity, and bones were analyzed for DM, ash, Ca, and P. All analyses were conducted as in Exp. 1.

Statistical analyses. Normality of data was verified and outliers were identified using UNIVARIATE procedure (version 9.3, SAS Institute) as explained for Exp. 1. Data were analyzed using the Proc Mixed procedure of SAS. An ANOVA was conducted with diet, sex, and the interaction between sex and diet as main effects and replicate as random effect. However, there were no interactions between treatment and sex and there were no main effects of sex on any variables. The final model, therefore, only analyzed effects of diet. Means were calculated using the LS means statement in SAS. The pig was the experimental unit and statistical significance and tendency were considered at $P < 0.05$ and $0.05 \le P < 0.10$, respectively.

Table 3. Composition of experimental diets, as-fed basis, Exp. 2

			Corn-expressed phytase[1]		Commercial phytase[2]	
Ingredient, %	Positive control	Negative control	500 FTU[3]	1,000 FTU	500 FTU	1,000 FTU
Ground corn	64.75	66.45	66.43	66.41	66.445	66.44
Soybean meal	29.75	29.75	29.75	29.75	29.75	29.75
Choice white grease	2.00	1.35	1.35	1.35	1.35	1.35
Limestone	1.16	1.01	1.01	1.01	1.01	1.01
Monocalcium phosphate	1.00	0.10	0.10	0.10	0.10	0.10
L-Lys HCl, 78% Lys	0.42	0.42	0.42	0.42	0.42	0.42
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10
L-Thr	0.12	0.12	0.12	0.12	0.12	0.12
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix[4]	0.30	0.30	0.30	0.30	0.30	0.30
Phytase premix[5]	—	—	0.02	0.04	0.005	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]Corn-expressed phytase (GraINzyme; Agrivida, Inc., Woburn, MA).

[2]Commercial phytase (AxtraPhy; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI).

[3]FTU = units of phytase.

[4]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[5]The GraINzyme phytase premix contained 2,500 FTU per gram, whereas the AxtraPhy phytase premix contained 10,000 FTU per gram.

Table 4. Analyzed nutrient composition of experimental diets, as-fed basis, Exp. 2

			Corn-expressed phytase[1]		Commercial phytase[2]	
Item	Positive control	Negative control	500 FTU[3]	1,000 FTU	500 FTU	1,000 FTU
DM, %	88.35	87.97	87.76	87.77	87.79	87.81
Ash, %	2.53	2.50	2.77	2.46	2.65	2.48
GE, kcal/kg	3,942	3,933	4,012	4,092	3,955	3,968
CP, %	18.55	17.45	16.23	19.11	19.28	18.71
Ca, %	0.76	0.56	0.48	0.55	0.60	0.58
P, %	0.59	0.40	0.39	0.41	0.40	0.41
Phytase, FTU/kg	82	<70	350	700	390	810

[1]Corn-expressed phytase (GraINzyme; Agrivida, Inc., Woburn, MA).

[2]Commercial phytase (AxtraPhy; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI).

[3]FTU = phytase units.

Table 5. Growth performance and apparent total tract digestibility (**ATTD**) of Ca and P for pigs fed the positive control diet (**PC**), the negative control diet (**NC**), or the NC diet supplemented with 250, 5060, 1,000, or 1,500 FTU[1]/kg diets, Exp. 1[2]

	Treatments							*P*-value			
Item	PC	NC	250 FTU	500 FTU	1,000 FTU	1,500 FTU	Pooled SEM	PC vs. NC	Linear	Quadratic	PC vs. 1,500 FTU
Initial BW, kg	10.77	10.81	10.82	10.81	10.74	10.74	0.22	0.903	0.766	0.955	0.927
Final BW, kg	28.40	21.54	24.41	26.55	26.87	29.40	0.84	0.001	0.001	0.027	0.492
ADG, g/d	600	383	480	562	576	637	30.40	0.001	0.001	0.009	0.415
ADFI, g/d	1,029	848	956	1,025	1,070	1,118	47.73	0.016	0.001	0.054	0.199
G:F	0.584	0.482	0.529	0.549	0.553	0.568	0.015	0.001	0.001	0.020	0.740
ATTD Ca, %	65.96	51.61	65.49	72.36	74.96	73.53	2.88	0.009	0.001	0.001	0.033
ATTD P, %	53.64	40.82	52.53	50.29	63.82	63.05	1.64	0.001	0.001	0.001	0.001

[1]FTU = phytase units.

[2]Data are means of 10 observations per treatment, except for the NC diet, which had 9 observations.

RESULTS

Experiment 1

There were no differences in initial BW among pigs assigned to the different treatments (Table 5). However, pigs fed the NC diet had lower ($P < 0.05$) final BW, ADG, ADFI, and G:F compared with pigs fed the PC diet. Final BW, ADG, and G:F increased linearly ($P < 0.01$) and quadratically ($P < 0.05$) as the concentration of phytase in the diet increased. Addition of phytase to the NC diet also increased ADFI linearly ($P < 0.01$). Therefore, there was no difference in final BW, ADG, ADFI, and G:F between pigs fed the PC diet and pigs fed the diet containing 1,500 FTU/kg of the corn-expressed phytase.

Pigs fed the NC diet had lower ($P < 0.01$) ATTD of Ca and P compared with pigs fed the PC diet. Addition of phytase to the NC diet increased (linear and quadratic, $P < 0.01$) the ATTD of Ca and P. However, pigs fed the diet containing 1,500 FTU/kg of the corn-expressed phytase had greater ($P < 0.05$) ATTD of Ca and P than pigs fed the PC diet.

Pigs fed the NC diet had less ($P < 0.01$) bone ash (% and g) compared with pigs fed the PC diet (Table 6). Addition of phytase increased bone ash percentage and total amount of bone ash (linear and quadratic, $P < 0.05$), and there was no difference between pigs fed the PC diet and pigs fed the diet containing 1,500 FTU/kg phytase.

The percentages of Ca and P in bone ash were not affected by dietary treatments. However, the total amount (g) of Ca and P was less ($P < 0.01$) in bone ash from pigs fed the NC diet compared with pigs fed the PC diet, but addition of phytase to the NC diet increased (linear, $P < 0.01$; quadratic, $P < 0.05$) the amount of Ca and P in bone ash. Pigs fed the diet containing 1,500 FTU/kg of phytase had concentrations of bone Ca and bone P that were not different from pigs fed the PC diet.

Experiment 2

There were no differences in initial BW among pigs assigned to dietary treatments (Table 7), but pigs fed the NC diet had lower ($P < 0.01$) final BW

Table 6. Bone ash, bone Ca, and bone P for pigs fed the positive control diet (**PC**), the negative control diet (**NC**), or the NC diet supplemented with 250, 500, 1,000, or 1,500 FTU[1]/kg diets, Exp. 1[2]

	Treatments							*P*-value			
Item	PC	NC	250 FTU	500 FTU	1,000 FTU	1,500 FTU	Pooled SEM	PC vs. NC	Linear	Quadratic	PC vs. 1,500 FTU
Bone ash[3], %	42.71	33.48	35.79	37.73	40.30	42.58	0.79	0.001	0.001	0.008	0.898
Bone ash, g/femur	14.80	7.49	8.89	10.58	12.26	14.31	0.57	0.001	0.001	0.018	0.608
Bone Ca, %	34.50	34.13	34.15	34.31	34.80	34.16	0.51	0.619	0.852	0.327	0.605
Bone Ca, g/femur	5.10	2.57	3.05	3.64	4.27	4.91	0.22	0.001	0.001	0.025	0.604
Bone P, %	16.96	16.48	16.76	16.64	16.94	16.81	0.25	0.161	0.370	0.411	0.673
Bone P, g/femur	2.51	1.24	1.50	1.76	2.08	2.42	0.11	0.001	0.001	0.029	0.617

[1]FTU = phytase units.

[2]Data are means of 10 observations per treatment, except for the NC, which had 9 observations.

[3]Bone ash as percent of the weight of dried, defatted bone.

Table 7. Growth performance for pigs fed the positive control diet (**PC**), the negative control diet (**NC**), or the NC diet supplemented with 500 or 1,000 FTU[1]/kg diets of the corn-expressed phytase[2] or the commercial phytase[3], Exp. 2[4]

			Corn-expressed phytase		Commercial phytase			
Item	PC	NC	500 FTU	1,000 FTU	500 FTU	1,000 FTU	Pooled SEM	*P*-value
Initial BW, kg	11.26	11.28	11.21	11.18	11.04	11.23	0.33	0.996
Final BW, kg	32.26[a]	27.17[b]	27.87[b]	29.81[ab]	30.01[ab]	29.53[ab]	1.42	0.002
ADG, g/d	749[a]	564[c]	596[bc]	650[abc]	685[ab]	654[ab]	48	0.001
ADFI, g/d	1,254	1,201	1,085	1,257	1,309	1,185	61	0.151
G:F	0.60[a]	0.47[b]	0.56[a]	0.52[ab]	0.53[ab]	0.55[ab]	0.04	0.001

[a–c]Means within a row without a common superscript are different ($P < 0.05$).

[1]FTU = phytase units.

[2]Corn-expressed phytase (GraINzyme; Agrivida, Inc., Woburn, MA).

[3]Commercial phytase (AxtraPhy; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI).

[4]Data are means of 8 observations per treatment.

and ADG compared with pigs fed the PC diet. There was no difference in ADG between pigs fed 500 or 1,000 FTU/kg phytase from the commercial phytase and pigs fed 1,000 FTU/kg phytase from the corn-expressed phytase or pigs fed the PC diet. No differences among treatments in ADFI were observed. Pigs fed the NC diet had lower ($P < 0.01$) G:F compared with pigs fed the PC diet, but addition of 500 FTU/kg phytase from either the corn-expressed phytase or the commercial phytase to the NC increased G:F to the same level as in the PC diet.

Pigs fed the NC diet had less ($P < 0.01$) bone ash (% and g per femur) compared with pigs fed the PC diet (Table 8). However, addition of 1,000 FTU/kg phytase from either the corn-expressed phytase or the commercial phytase to the NC diet was not enough to achieve the same amount of bone ash (g; $P < 0.01$) as observed for pigs fed the PC diet. There were no differences between pigs fed the PC diet and pigs fed 1,000 FTU/kg phytase from the commercial phytase in bone ash percentage, but pigs fed 1,000 FTU/kg phytase from the corn-expressed phytase had lower ($P < 0.01$) bone ash percentage than pigs fed the PC diet.

There were no differences among treatments in the percentage of Ca and P in bone ash. Pigs fed the NC diet had less ($P < 0.01$) Ca and P (g) in bone ash compared with pigs fed the PC diet. Pigs fed the NC diet with 1,000 FTU/kg phytase from either the corn-expressed phytase or the commercial phytase also had less ($P < 0.01$) Ca and P (g) in bone ash compared with pigs fed the PC diet, but greater ($P < 0.01$) quantities than pigs fed the NC diet.

DISCUSSION

Microbial phytase may be expressed in plants such as tobacco (Pen et al., 1993), canola (Ponstein et al., 2002), soybean (Li et al., 1997), wheat (Brinch-Pedersen et al., 2000), alfalfa (Ullah et al., 2002), and potato (Hong et al., 2008). Phytases produced in soybean (Denbow et al., 1998) and canola seeds (Zhang et al., 2000) had the same performance as

Table 8. Bone mineralization for pigs fed the positive control diet (**PC**), the negative control diet (**NC**), or the NC diet supplemented with 500 or 1,000 FTU[1]/kg diets of the corn-expressed phytase[2] or the commercial phytase[3], Exp. 2[4]

			Corn-expressed phytase		Commercial phytase			
Item	PC	NC	500 FTU	1,000 FTU	500 FTU	1,000 FTU	Pooled SEM	*P*-value
Bone ash[5], %	50.25[a]	41.44[e]	42.84[de]	46.11[bc]	45.09[cd]	48.57[ab]	0.81	0.001
Bone ash, g/femur	16.71[a]	7.24[d]	9.89[c]	11.16[c]	11.15[c]	13.60[b]	0.64	0.001
Bone Ca, %	36.53	36.38	36.28	36.28	36.08	36.75	0.34	0.774
Bone Ca, g/femur	6.10[a]	2.63[d]	3.47[c]	4.05[c]	4.03[c]	4.99[b]	0.23	0.001
Bone P, %	16.96	16.62	16.72	16.74	16.85	17.11	0.17	0.379
Bone P, g/femur	2.83[a]	1.22[d]	1.60[cd]	1.87[c]	1.88[c]	2.32[b]	0.09	0.001

[a–e]Means within a row without a common superscript are different ($P < 0.05$).

[1]FTU = phytase units.

[2]Corn-expressed phytase (GraINzyme; Agrivida, Inc., Woburn, MA).

[3]Commercial phytase (AxtraPhy; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI).

[4]Data are means of 8 observations per treatment.

[5]Bone ash as percent of the weight of dried, defatted bone.

microbial phytases, but the thermo-tolerance of these phytases might not be sufficient to survive postharvest heating that is used during toasting of the defatted meals (Haefner et al., 2005). In contrast, phytase in corn is expected to maintain activity after harvest (Nyannor et al., 2007) because the temperature used in drying of the corn is less than the temperature used in toasting of oilseed meals. Therefore, the efficacy of an *E. coli* phytase expressed in corn was evaluated in the present experiments.

Beneficial effects of supplementing microbial phytase to P and Ca deficient diets have been reported since 1991 (Dersjant-Li et al., 2015) and in numerous peer-reviewed publications (Selle and Ravindran, 2008; Cowieson et al., 2009). The ADG, ADFI, and G:F obtained in the present experiments are consistent with those reported previously (Knapp et al., 2018a, 2018b; Broomhead et al., 2019) using the same *E. coli* phytase expressed in corn. Likewise, the improvement in ATTD of P and quantity of bone ash observed in both experiments are in agreement with Broomhead et al. (2019), who also reported an improved bone breaking strength in pigs fed diets supplemented with the corn-expressed phytase. These observations are most likely the result of liberation of phytate-bound P and Ca by phytase (Lei et al., 1993), which likely reduced the P deficiency in the NC diet. The lack of a difference in Exp. 1 in bone mineralization between pigs fed the NC diet with 1,500 FTU corn-expressed phytase and pigs fed the control diet demonstrates that the increased ATTD of P that was the result of inclusion of phytase in the diet compensated for the reduced concentration of P in the diet. The improvement in growth performance and digestibility of Ca and P that were observed is consistent with responses to other corn-expressed phytases (Nyannor et al., 2007, 2009; Wang et al., 2011).

The dietary reduction of 0.20 and 0.18 percentage units of Ca and P in the NC diets from the recommended requirements (NRC, 2012) reduced growth performance and bone characteristics as was expected. This is likely because a diet deficient in P and Ca restricts growth of young pigs who have high requirements for P to synthesize bone and soft tissue (Mahan, 1982). The decrease in weight, but not percentage, of Ca and P in bone ash when Ca and P was removed from the diets indicate that the composition of bone ash is relatively stable regardless of the dietary provision of Ca and P (González-Vega et al., 2016), whereas the size of the bone is directly affected by dietary Ca and P.

The commercial *Buttiauxella* spp. phytase (AxtraPhy) that was used in Exp. 2 has high activity toward phytic acid at low pH (Yu et al., 2014). *Buttiauxella* phytase improves growth performance and nutrient utilization in pigs (Adedokun et al., 2015) and poultry (Amerah et al., 2014; Kiarie et al., 2015). The observation that the weight of bone ash, bone Ca, and bone P were less in pigs fed the corn-expressed phytase compared with pigs fed the commercial phytase indicates that inclusion of the corn-expressed phytase may need to be greater to obtain similar bone mineralization. The fact that there were no differences in growth performance between pigs fed diets containing 1,000 FTU/kg of either of the two phytases is likely a result of the fact that the requirement for P to maximize body weight gain is less than the requirement to maximize bone ash (NRC, 2012). The observation that bone ash measurements were greater for pigs fed the PC diet than for pigs fed either of the 2 phytases indicate that the reduction in Ca and P (i.e., 0.20% and 0.18%, respectively) in the NC diet was greater than the total amounts of Ca and P that were released from phytate. This was true not only if 500 FTU of phytase was used but also if 1,000 FTU was used. It appears that to achieve the same levels of bone mineralization as pigs fed the PC diet, the corn-expressed phytase needs to be included at 1,500 FTU/kg, as observed in Exp.1.

In conclusion, the corn-expressed phytase is effective in improving growth performance, Ca and P digestibility, and bone measurements in pigs fed diets that are deficient in Ca and P. No differences in growth performance data were observed between pigs fed the corn-expressed phytase and pigs fed a commercial phytase. However, pigs fed a diet containing 1,000 FTU of the commercial phytase had greater bone ash concentration than pigs fed 1,000 FTU of the corn-expressed phytase, indicating that a greater inclusion rate of the corn-expressed phytase may be needed to obtain a response that is similar to that obtained from the commercial phytase.

Conflict of interest statement. None declared.

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