Effects of increasing concentrations of an *Escherichia coli* phytase on the apparent ileal digestibility of amino acids and the apparent total tract digestibility of energy and nutrients in corn-soybean meal diets fed to growing pigs[1]

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ABSTRACT: An experiment was conducted to test the hypothesis that inclusion of increasing concentrations of an *Escherichia coli* phytase to a corn–soybean meal (**SBM**) diet results in improved digestibility of DM, GE, CP, NDF, ADF, macrominerals, microminerals, and AA. Twenty-four growing barrows (initial BW: 37.0 ± 1.4 kg) were equipped with a T-cannula in the distal ileum and placed individually in metabolism crates, and allotted to a 2-period switch-back design with 6 diets and 4 replicate pigs per diet in each period. The positive control diet was a corn–SBM diet that contained limestone and dicalcium phosphate to meet the requirement for standardized total tract digestible (**STTD**) P and Ca (0.31% STTD P and 0.70% Ca). A negative control diet that was similar to the positive control diet, with the exception that no dicalcium phosphate was used, was also formulated, and this diet contained 0.16% STTD P and 0.43% Ca. Four additional diets were formulated by adding 500, 1,000, 2,000, or 4,000 units of microbial phytase (**FTU**) to the negative control diet. Each period lasted 14 d. Fecal and urine samples were collected from the feed provided from days 6 to 11 of each period following 5 d of adaptation to the diets. Ileal digesta were collected for 8 h on days 13 and 14. Results indicated that addition of the *E. coli* phytase to the negative control diet tended to quadratically improve the apparent ileal digestibility of Phe ($P = 0.086$) and Asp ($P = 0.054$), and linearly increased ($P < 0.05$) the apparent total tract digestibility (**ATTD**) of ADF, K, and Fe. Microbial phytase also quadratically increased ($P < 0.05$) the ATTD of NDF and Mg, and linearly and quadratically increased ($P < 0.05$) the ATTD and retention of Ca and P. However, no effects of the phytase on ATTD of GE or the concentration of DE were observed. In conclusion, the increased absorption of several minerals including Ca, P, K, Mg, and Fe that was observed as increasing concentrations of an *E. coli* phytase was added to a corn–SBM meal diet indicates that the dietary provision of these minerals may be reduced if phytase is fed.

Key words: digestibility, energy digestibility, *Escherichia coli* phytase, minerals, pigs

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INTRODUCTION

Escherichia coli phytase has been used commercially for approximately the last 15 yr to improve profitability and reduce the environmental impact from pig and poultry production (Cowieson et al., 2015). Effects of microbial phytase on improving the apparent total tract digestibility (**ATTD**) of P and Ca in plant

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feed ingredients fed to pigs have been demonstrated (Rodríguez et al., 2013; Almaguer et al., 2014; She et al., 2015). The concentration of microbial phytase needed to maximize the ATTD of Ca and P is 800 to 1,000 units if measured using the official AOAC procedure (Method 2000.12; AOAC International, 2007; Almeida et al., 2013). However, benefits beyond the release of Ca and P may be obtained by feeding greater doses of microbial phytase (Cowieson et al., 2011; Walk et al., 2014).

Phytate may act as an antinutritional factor in diets (Kies et al., 2005; Adeola and Cowieson, 2011), and inclusion of greater levels of phytase than what is needed to maximize the ATTD of P and Ca may improve the utilization of energy, AA, and ATTD of other minerals in poultry (Brenes et al., 2003; Pirgozliev and Bedford, 2013). However, the mechanism that results in these benefits has not been elucidated, and it has not been demonstrated if similar advantages are consistently obtained in pigs (Adeola and Sands, 2003; Zeng et al., 2014; Holloway et al., 2015). The dose of microbial phytase needed to obtain possible extra-phosphoric effects also needs to be established. Therefore, the objective of this experiment was to test the hypothesis that addition of increasing concentrations of an *E. coli* phytase to a corn–soybean meal (**SBM**)–based diet fed to growing pigs will increase the apparent ileal digestibility (**AID**) of CP and AA, and the ATTD of GE, DM, Ca, P, Na, K, Mg, S, Cu, Fe, and Mn.

MATERIALS AND METHODS

The protocol for the experiment was reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois (Urbana-Champaign, IL). Pigs used in the experiments were the offspring of L 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Twenty-four growing barrows (initial BW: 37.0 ± 1.4 kg) were equipped with a T-cannula in the distal ileum and allotted to a 2-period switch back design with 6 diets and 4 replicate pigs per diet in each period resulting in 8 replicate pigs per diet. Pigs were housed individually in stainless-steel metabolism crates in an environmentally controlled room. Crates had smooth sidings, a fully slatted floor, a screen floor, and a urine tray, which allowed for total, but separate, collection of feces and urine. A feeder and a cup drinker were installed in each crate.

The same batches of corn and SBM were used to prepare all diets (Table 1). A positive control diet based on corn and SBM was formulated (Table 2). This diet contained 1.00% dicalcium phosphate and 0.83% limestone and was calculated to contain 0.70% Ca and 0.31% standardized total tract digestible (**STTD**) P. Cornstarch was added to the diet at 0.40%, but no microbial phytase was included in the positive control diet. A negative control diet that was similar to the positive control diet with the exception that no dicalcium phosphate, but 0.90% limestone, was included, was also used. This diet was formulated to contain 0.43% Ca and 0.16% STTD P; thus, it provided 0.27% Ca and 0.15% STTD P less than the positive control diet. Four additional diets were formulated by adding 500, 1,000, 2,000, or 4,000 units of phytase

Table 1. Analyzed composition of ingredients, as-fed basis

Item	Corn	Soybean meal
GE, kcal/kg	3,764	4,183
DM, %	84.92	88.59
CP (N × 6.25), %	6.45	47.79
Ash, %	1.24	6.60
NDF, %	4.43	10.05
ADF, %	3.74	8.19
Ca, %	0.03	0.30
P, %	0.22	0.61
Phytase, FTU[1]/kg	88	180
K, %	0.30	2.15
S, %	0.09	0.40
Na, %	0.004	0.001
Mg, %	0.08	0.27
Fe, mg/kg	18.9	81.9
Zn, mg/kg	20.4	47.2
Mn, mg/kg	4.48	33.9
Cu, mg/kg	0.82	11.9
Indispensable AA, %		
Arg	0.28	3.31
His	0.20	1.35
Ile	0.23	2.30
Leu	0.71	3.70
Lys	0.23	3.04
Met	0.12	0.69
Phe	0.30	2.44
Thr	0.21	1.78
Trp	0.06	0.76
Val	0.30	2.35
Dispensable AA, %		
Ala	0.43	2.05
Asp	0.41	5.30
Cys	0.13	0.64
Glu	1.06	8.52
Gly	0.25	2.01
Pro	0.47	2.12
Ser	0.25	1.89
Tyr	0.16	1.62

[1]FTU = phytase units.

Table 2. Ingredient composition of experimental diets, as-fed basis

			Phytase, FTU[1] per kilogram complete feed			
Ingredient, %	PC[2]	NC	500	1,000	2,000	4,000
Corn	64.65	65.58	65.58	65.58	65.58	65.58
Soybean meal	29.00	29.00	29.00	29.00	29.00	29.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Cornstarch	0.40	0.40	0.375	0.35	0.30	0.20
Limestone	0.83	0.90	0.90	0.90	0.90	0.90
Dicalcium phosphate	1.00	–	–	–	–	–
L-Lys HCL	0.12	0.12	0.12	0.12	0.12	0.12
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.20	0.20	0.20	0.20	0.20	0.20
Phytase premix[4]	–	–	0.025	0.05	0.10	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]FTU = phytase units.

[2]PC = positive control; NC = negative control.

[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-α 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; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite (0.125 mg) and selenium yeast (0.125 mg); and Zn, 124.9 mg as zinc sulfate.

[4]The phytase premix (Optiphos, Huvepharma, Sofia, Bulgaria) contained 2,000 units of phytase per gram.

(**FTU**) per kg of diet to the negative control diet. The *E. coli* phytase (Optiphos 2000; Huvepharma, Sofia, Bulgaria) was provided as a phytase premix containing 2,000 FTU per gram, and this premix was included in the diets at the expense of cornstarch. Vitamins and all minerals except Ca and P were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.40% titanium dioxide as an indigestible marker.

Pigs were provided feed at 3.3 times the maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012), and water was available at all times throughout the experiment. Pig weights were recorded at the beginning and at the conclusion of each period, and the amount of feed supplied each day was recorded. The initial 5 d of each period was considered an adaptation period to the diet. Feces were collected quantitatively from the feed provided from days 6 to 11 according to the marker to marker approach (Adeola, 2001). Thus, on day 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection, and on day 11, ferric oxide was added to the morning meal to mark the conclusion of fecal collection. Urine was collected from days 6 to 11 in urine buckets over a preservative of 50 mL of 6 *N* HCl. Buckets were covered by gauze to prevent solids from contaminating the urine. Fecal samples and 20% of the collected urine were stored at −20 °C immediately after collection. Ileal digesta were collected for 8 h on days 13 and 14 by attaching a plastic bag to the cannula barrel. Bags were removed whenever they were filled with digesta, or at least once every 30 min, and immediately frozen at −20 °C to prevent bacterial degradation of the AA in the digesta. On the completion of the first experimental period, animals were deprived of feed overnight, and the following morning, a new experimental diet was offered. At the conclusion of the experiment, ileal samples were thawed, mixed within animal and diet, and a subsample was collected. Urine samples were thawed and mixed within animal, and a 200-mL subsample was collected for analysis. Ileal digesta samples were lyophilized using a Gamma 1–16 LSC Freeze Drier (Ima Life North America, Tonawanda, NY). Lyophilized samples of digesta were finely ground using a coffee grinder with a stainless steel blade. All fecal samples were dried at 65 °C in a forced air oven to approximately 95% DM and finely ground using a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ).

Samples of corn, SBM, and diets were analyzed for DM (Method 927.05; AOAC International, 2007), ash (Method 942.05; AOAC International, 2007), GE using an isoperibolic bomb calorimeter (Model 6300; Parr Instruments, Moline, IL), and for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY).

These samples were also analyzed for CP (N × 6.25; Method 990.03; AOAC International, 2007) and for AA on a Hitachi Amino Acid Analyzer Model L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, using ninhydrin for postcolumn derivatization and norleucine as the internal standard [Method 982.30 E (a, b, c); AOAC International, 2007]. Corn, SBM, and diets were also analyzed for Ca, P, Na, K, Mg, Cu, Fe, Mn, and Zn by inductively coupled plasma optical emissions spectrometry (**ICP-OES**) using an internally validated method (Method 985.01 A, B, and C; AOAC, 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC, 2007) and S (Method 956.01; AOAC 2006). Standard reference material Tomato Leaves (Standard Reference Material [SRM] number 1573a; National Institute of Standards and Technology, Gaithersburg, MD) and Industrial Sludge (SRM number 2782; National Institute of Standards and Technology) were used as reference standards to validate micromineral analyses. The inductively coupled plasma calibration standard (ICM-103; Ultra Scientific Analytical Solutions, Kingstown, RI) was diluted with 5% nitric acid and used as calibration curves, and Plasma CAL (QC 19; Qmx Laboratories, Thaxted, UK) was included for every 20 samples to serve as quality control standards. Corn and SBM were also analyzed for phytase (Engelen et al., 2001), and diets were analyzed for Ti (Myers et al., 2004). Ileal digesta samples were analyzed for DM, CP, Ti, and AA as explained for diet samples and all fecal samples were analyzed as diets with the exception that Ti, CP, and AA were not analyzed in fecal samples.

The AID (%) of CP and AA were calculated for each diet according to the following equation (Stein et al., 2007):

$$\text{AID} = \left[1 - \left(\text{AA}_{\text{digesta}} / \text{AA}_{\text{diet}}\right) \times \left(\text{M}_{\text{diet}} / \text{M}_{\text{digesta}}\right)\right] \times 100,$$

where $\text{AA}_{\text{digesta}}$ and AA_{diet} represent the AA concentrations (g/kg) in digesta and diet DM, respectively, and M_{diet} and $\text{M}_{\text{digesta}}$ represent the marker concentrations (g/kg) in diet and digesta DM, respectively.

The daily amount of energy that was excreted in the feces was determined, and the concentration of DE in each diet was calculated (Adeola, 2001).

The ATTD (%) of GE, DM, Ca, P, Na, K, Mg, S, Cu, Fe, and Mn were calculated for each diet according to the following equation (Liu et al., 2014):

$$\text{ATTD} = [(\text{intake} - \text{output}) / \text{intake}] \times 100.$$

The retention (%) of Ca and P were calculated using the following equation (Liu et al., 2014):

$$\text{Retention} = [\text{intake} - (\text{fecal excretion} + \text{urine excretion}) / \text{intake}] \times 100.$$

Outliers were identified as values that deviated from the treatment mean by more than 3 times the interquartile range. Data were analyzed by ANOVA using the PROC MIXED of SAS in a randomized complete block design. The pig was the experimental unit for all analyses. The statistical model included the fixed effects of phytase level and the random effects of pig and period. Least square means were calculated for each independent variable. Contrast statements were used to compare the positive control diet and the negative control diet, and linear and quadratic effects of supplementing the graded level of phytase to the negative control diet were also analyzed using contrast statements. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

The analyzed nutrient concentrations in all diets were in agreement with calculated values. The analyzed Ca concentration in all diets ranged from 98% to 112% of calculated values, and analyzed P ranged from 84% to 91% of calculated values (Table 3). The positive and the negative control diets had concentrations of phytase that were below the detection limits, but the 4 diets that contained phytase analyzed 595, 1,120, 2,030, and 3,885 FTU of phytase, respectively.

Addition of phytase from 0 to 4,000 FTU tended to quadratically increase the AID of Phe ($P = 0.086$) and Asp ($P = 0.054$; Table 4), but no other effects of phytase on the AID of AA were observed, and there were no differences between the positive and the negative control diets. No differences were observed in ADFI or GE intake among treatments (Table 5). However, there were greater ($P < 0.05$) dry feces output from pigs fed the positive control diet than from pigs fed the negative control diet. There was also a tendency ($P = 0.084$) for fecal GE output from pigs fed the positive control diet to be greater than from pigs fed the negative control diet, and there was a tendency for the ATTD of GE to be greater ($P = 0.05$) for the negative control diet than for the positive control diet. There was greater

Table 3. Chemical composition of experimental diets, as-fed basis

			Phytase, FTU[1] per kilogram complete feed			
Item	PC[2]	NC	500	1,000	2,000	4,000
GE, kcal/kg	3,946	4,025	4,032	4,015	3,992	4,005
DM, %	86.91	86.84	86.75	87.06	87.02	86.80
CP (N × 6.25), %	17.88	18.08	16.81	18.51	18.40	19.29
Ash, %	5.07	4.65	4.39	4.76	4.69	4.80
NDF, %	8.91	7.96	7.87	8.36	9.64	8.46
ADF, %	2.91	2.70	2.61	2.68	3.42	3.96
Ca, %	0.70	0.43	0.42	0.46	0.48	0.48
P, %	0.51	0.34	0.32	0.32	0.33	0.33
STTD[3] P, %	0.31	0.16	0.16	0.16	0.16	0.16
Phytase, FTU/kg	<70	<70	595	1,120	2,030	3,885
K, %	0.81	0.80	0.77	0.82	0.78	0.82
S, %	0.21	0.20	0.20	0.21	0.21	0.20
Na, %	0.16	0.13	0.15	0.15	0.16	0.14
Mg, %	0.13	0.14	0.13	0.13	0.13	0.13
Ti, %	0.24	0.22	0.23	0.23	0.24	0.24
Fe, mg/kg	273	179	168	156	173	162
Zn, mg/kg	125	138	118	147	155	114
Mn, mg/kg	79	73	69	74	81	72
Cu, mg/kg	15	12	13	12	12	13
Indispensable AA, %						
Arg	1.16	1.28	1.20	1.19	1.25	1.17
His	0.52	0.55	0.54	0.53	0.54	0.52
Ile	0.80	0.88	0.84	0.83	0.86	0.81
Leu	1.54	1.70	1.61	1.60	1.66	1.57
Lys	1.10	1.19	1.17	1.17	1.20	1.13
Met	0.28	0.30	0.30	0.28	0.29	0.27
Phe	0.89	1.00	0.92	0.94	0.97	0.92
Thr	0.66	0.75	0.71	0.70	0.73	0.70
Trp	0.23	0.23	0.22	0.25	0.27	0.26
Val	0.88	0.98	0.93	0.91	0.96	0.88
Dispensable AA, %						
Ala	0.89	0.98	0.92	0.91	0.96	0.90
Asp	1.78	2.00	1.89	1.87	1.95	1.84
Cys	0.27	0.31	0.29	0.28	0.29	0.28
Glu	3.14	3.51	3.31	3.29	3.43	3.22
Gly	0.76	0.84	0.79	0.78	0.82	0.77
Pro	0.91	1.11	0.96	0.94	1.11	0.94
Ser	0.73	0.84	0.79	0.78	0.83	0.78
Tyr	0.58	0.63	0.59	0.61	0.63	0.60

[1]FTU = phytase units.

[2]PC = positive control; NC = negative control.

[3]STTD = standardized total tract digestible; values for STTD P are calculated (NRC, 2012); values for STTD P do not include effects of microbial phytase.

($P < 0.05$) ATTD of DM from pigs fed the negative control diet than from pigs fed the positive control diet, but no effects of phytase on ATTD of DM were observed. Phytase supplementation quadratically increased ($P < 0.01$) the ATTD of NDF, and linearly increased ($P < 0.001$) the ATTD of ADF.

The DE in the negative control diet was greater ($P < 0.001$) than in the positive control diet, but no effects of microbial phytase on DE were observed. Pigs fed the positive control diet had greater ($P < 0.001$) Ca intake, fecal Ca output, and absorption of Ca than pigs fed the negative control diet (Table 6). There were both linear ($P < 0.001$) and quadratic ($P < 0.01$) increases in Ca intake as phytase was added to the negative control diet, and both linear and quadratic reductions ($P < 0.001$) in fecal Ca output were observed as phytase was included in the diets. There were also linear and quadratic increases ($P < 0.001$) in absorbed Ca and ATTD of Ca in response to increased phytase in the diets and the ATTD of Ca tended ($P = 0.065$) to

Table 4. Effects of increasing concentrations of an *E. coli* phytase on the apparent ileal digestibility of DM, CP, and AA in corn-soybean meal–based diets fed to growing pigs, %

			Phytase, FTU[1] per kilogram complete feed					*P*-value[2]		
Item	PC[3]	NC	500	1,000	2,000	4,000	SEM	PC vs. NC	Linear	Quadratic
DM	70.80	71.95	70.92	70.70	73.52	70.95	1.43	0.566	0.989	0.465
CP	77.41	77.31	76.41	76.16	79.04	76.53	1.13	0.952	0.914	0.357
Indispensable AA										
Arg	89.85	90.35	89.99	89.84	90.88	89.89	0.44	0.443	0.861	0.439
His	83.58	85.28	83.28	82.83	84.89	82.49	0.77	0.121	0.115	0.881
Ile	81.93	82.35	82.31	82.12	84.21	82.62	0.79	0.700	0.484	0.196
Leu	83.51	84.08	84.12	84.02	85.79	84.29	0.72	0.571	0.540	0.162
Lys	81.26	80.74	80.17	78.95	82.25	79.59	1.20	0.758	0.865	0.471
Met	86.76	85.44	86.41	81.42	88.24	80.66	3.25	0.699	0.249	0.348
Phe	83.14	83.52	83.95	83.84	85.77	84.41	0.69	0.683	0.205	0.086
Thr	74.41	74.86	74.11	73.82	76.58	73.70	1.18	0.778	0.830	0.263
Trp	80.91	80.62	80.77	81.00	83.34	81.18	1.16	0.861	0.519	0.163
Val	78.74	79.58	79.26	78.59	81.21	79.02	1.00	0.543	0.943	0.324
Mean	82.73	82.87	82.84	82.31	84.62	82.56	0.83	0.895	0.877	0.185
Dispensable AA										
Ala	78.86	79.40	78.18	78.36	80.85	78.53	1.19	0.752	0.937	0.399
Asp	79.86	78.88	79.12	78.72	82.04	79.06	0.92	0.457	0.543	0.054
Cys	74.23	75.69	71.80	69.65	74.05	73.98	2.48	0.673	0.826	0.391
Glu	84.76	83.91	83.17	82.16	87.12	83.78	1.22	0.624	0.482	0.225
Gly	64.67	63.31	60.05	61.52	66.81	63.64	2.88	0.740	0.480	0.581
Pro	82.10	80.67	81.94	78.24	81.45	80.60	1.92	0.599	0.977	0.873
Ser	83.29	83.85	83.70	83.75	85.46	83.81	0.87	0.601	0.703	0.173
Tyr	83.54	83.83	83.60	83.68	85.47	83.96	0.74	0.782	0.529	0.184
Mean	80.87	80.30	80.71	78.95	82.20	80.18	1.00	0.691	0.784	0.430
All AA	81.71	81.47	82.05	80.47	83.29	81.26	0.83	0.837	0.912	0.286

Data are means of 8 observations per treatment, except for the negative control diet that had only 6 observations, and diets containing 500 and 2,000 FTU of phytase only had 7 observations.

[1]FTU = phytase units.

[2]Linear and quadratic contrasts of adding 500, 1,000, 2,000, or 4,000 FTU/kg phytase to the NC diet.

[3]PC = positive control; NC = negative control.

be greater for the positive control diet than for the negative control diet. Urinary Ca output for pigs fed the negative control diet was greater ($P < 0.01$) than for pigs fed the positive control diet, and there were linear and quadratic decreases ($P < 0.05$) in urinary Ca output as increasing concentrations of phytase were fed. Calcium retention (calculated as g/d and as percent of intake) was greater ($P < 0.01$) for pigs fed the positive control diet compared with pigs fed the negative control diet, and there were both linear ($P < 0.01$) and quadratic ($P < 0.01$) increases in the retention of Ca as phytase addition to the diets increased.

Pigs fed the positive control diet had greater ($P < 0.01$) P intake, fecal P output, absorbed P, and ATTD of P than pigs fed the negative control diet, but addition of phytase to the diets increased (quadratic, $P < 0.01$) P intake, absorbed P, and ATTD of P. In contrast, addition of phytase decreased fecal P output (linear and quadratic, $P < 0.001$), but phytase quadratically increased ($P < 0.05$) urinary P output. If calculated as g/d or percent of intake, P retention in pigs fed the positive control diet was greater ($P < 0.001$) than in pigs fed the negative control diet, but retention of P increased (linear and quadratic, $P < 0.001$) as phytase supplementation increased.

Pigs fed the positive control diet had greater ($P < 0.001$) Na intake than pigs fed the negative control diet, but linear and quadratic increases ($P < 0.01$) in Na intake were observed as phytase inclusion increased. Addition of phytase increased absorbed Na (quadratic, $P < 0.05$), but no effect of phytase on ATTD of Na was observed.

The inclusion of phytase linearly and quadratically increased ($P < 0.05$) K intake, but decreased (quadratic, $P < 0.05$) fecal K output. Absorbed K increased (linear, $P < 0.01$) as phytase was added to the diets and a linear increase ($P < 0.01$) in the ATTD of K was observed with increasing phytase

Table 5. Effects of increasing concentrations of an *E. coli* phytase on the apparent total tract digestibility (ATTD) of DM, GE, NDF, and ADF, and concentration of DE in corn-soybean meal–based diets fed to growing pigs

			Phytase, FTU[1] per kilogram complete feed					*P*-value[2]		
Item	PC[3]	NC	500	1,000	2,000	4,000	SEM	PC vs. NC	Linear	Quadratic
ADFI, kg/d	1.75	1.73	1.76	1.73	1.72	1.75	0.15	0.452	0.963	0.513
GE intake, kcal/d	6,922	6,974	7,090	6,949	6,876	6,993	597.48	0.645	0.690	0.314
Dry feces output, kg/d	0.20	0.17	0.16	0.16	0.16	0.16	0.02	0.033	0.450	0.348
Fecal GE output, kcal/d	913	813	795	810	782	806	85.82	0.084	0.919	0.657
ATTD, GE %	86.88	88.34	88.80	88.38	88.68	88.44	0.51	0.050	0.966	0.721
ATTD, DM %	89.64	90.91	91.64	91.35	91.70	91.85	0.38	0.020	0.142	0.476
ATTD, NDF %	64.37	60.14	61.05	61.44	68.12	61.29	1.73	0.091	0.363	0.008
ATTD, ADF %	65.05	66.09	64.91	62.02	71.93	73.75	1.65	0.658	<0.001	0.719
DE in diet, kcal/kg										
As-fed basis	3,429	3,559	3,580	3,549	3,540	3,542	20.42	<0.001	0.327	0.686
DM basis	3,945	4,095	4,127	4,076	4,068	4,081	23.49	<0.001	0.340	0.407

Data are means of 8 observations per treatment.

[1]FTU = phytase units.

[2]Linear and quadratic contrasts of adding 500, 1,000, 2,000, or 4,000 FTU/kg phytase to the negative control diet.

[3]PC = positive control; NC = negative control.

addition. There was a quadratic decrease ($P < 0.05$) in fecal Mg output with increasing phytase concentration in the diets and phytase tended to improve absorbed Mg (quadratic, $P = 0.05$). A quadratic increase ($P < 0.05$) in the ATTD of Mg was also observed as phytase supplementation increased.

Intake of S by pigs fed the positive control diet was greater ($P < 0.001$) than by pigs fed the negative control diet, and there was a quadratic ($P < 0.01$) increase in S intake with increasing phytase addition. However, fecal S output tended ($P = 0.06$) to be less from pigs fed the negative control diet than from pigs fed the positive control diet. Pigs fed the positive control diet absorbed more ($P < 0.001$) S than pigs fed the negative control diet, and there was a quadratic increase ($P < 0.001$) in absorbed S as phytase inclusion in the diets increased from 0 to 4,000 FTU. However, the ATTD of S was not affected by dietary treatment.

A quadratic decrease ($P < 0.05$) was observed in fecal Cu output as phytase supplementation increased and a tendency (quadratic, $P = 0.080$) for increased ATTD of Cu was observed with increasing addition of phytase (Table 7). Pigs fed the negative control diet had less ($P < 0.001$) Fe intake than pigs fed the positive control diet, and there were linear ($P < 0.001$) and quadratic ($P < 0.01$) reductions in Fe intake as phytase supplementation increased. Reduced ($P < 0.001$) fecal Fe output was also observed from pigs fed the negative control diet compared with pigs fed the positive control diet. There was a linear reduction ($P < 0.05$) in absorbed Fe as phytase supplementation increased. Pigs fed the negative control diet had greater ($P < 0.05$) ATTD of Fe than pigs fed the positive control diet, but a linear decrease ($P < 0.05$) in the ATTD of Fe was observed as the inclusion of phytase increased. There were no differences among treatments for any of the variables calculated for Mn.

DISCUSSION

The efficacy of phytase in dephosphorylating phytic acid in plant feed ingredients and therefore enhancing digestibility by pigs of P has been demonstrated (Selle et al., 2012). Phytases are classified as 3- or 6- phytases on the basis of the site on the phytic acid molecule where the initial dephosphorylation takes place (Adeola and Sands, 2003). Fungal phytases start hydrolysis of the P-bond at the 3 carbon atom of the inositol ring, whereas *E. coli* phytases start hydrolysis of the phytate-P bond at the 6 carbon atom of the inositol ring (Cowieson et al., 2011).

The analyzed concentrations of *E. coli* phytase in the positive and negative control diets were <70 FTU/kg indicating that no phytase was added to these diets. The analyzed concentrations of *E. coli* phytase in the other diets were as expected.

Apparent Ileal Digestibility of AA

The hypothesis that phytase may improve the utilization of dietary N and AA is a result of speculations that phytate-protein complexes may form in the intestinal tract of pigs, which may result in reduced protein digestibility (Pomar et al., 2008). Protein or AA that are complexed with phytate

Table 6. Effects of increasing concentrations of an *E. coli* phytase on the apparent total tract digestibility (ATTD) of macrominerals, and retention of Ca and P in corn-soybean meal based diets fed to growing pigs

			Phytase, FTU[1] per kilogram complete feed					*P*-value[2]		
Item	PC[3]	NC	500	1,000	2,000	4,000	SEM	PC vs. NC	Linear	Quadratic
Ca										
Intake, g/d	12.35	7.36	7.33	7.93	8.22	8.40	0.73	<0.001	<0.001	0.002
Fecal output, g/d	3.75	2.63	1.40	1.19	1.15	1.21	0.20	<0.001	<0.001	<0.001
Absorbed, g/d	8.60	4.73	5.94	6.73	7.06	7.18	0.66	<0.001	<0.001	<0.001
ATTD, Ca %	69.61	64.14	80.94	85.07	85.90	85.37	2.04	0.065	<0.001	<0.001
Urine output, g/d	3.91	17.21	4.83	6.90	4.04	4.99	3.71	0.003	0.046	0.034
Retention, g/d	7.17	1.30	4.97	5.35	6.26	6.18	1.17	<0.001	<0.001	<0.001
Retention, %	59.94	16.65	67.38	71.46	75.71	73.28	10.09	0.001	0.002	0.001
P										
Intake, g/d	8.89	5.82	5.57	5.52	5.61	5.73	0.52	<0.001	0.789	0.007
Fecal output, g/d	4.08	3.35	1.71	1.56	1.30	1.39	0.27	0.001	<0.001	<0.001
Absorbed, g/d	4.81	2.36	3.86	3.96	4.32	4.34	0.29	<0.001	<0.001	<0.001
ATTD, P %	54.34	42.44	69.36	71.82	76.93	75.65	1.93	<0.001	<0.001	<0.001
Urine output, mg/d	115.40	92.97	62.13	106.72	183.14	63.08	36.23	0.656	0.960	0.038
Retention, g/d	4.69	2.26	3.80	3.85	4.13	4.27	0.31	<0.001	<0.001	<0.001
Retention, %	53.05	40.77	68.23	69.72	73.59	74.51	1.90	<0.001	<0.001	<0.001
Na										
Intake, g/d	2.74	2.24	2.62	2.65	2.79	2.48	0.22	<0.001	0.008	<0.001
Fecal output, g/d	1.20	1.06	1.07	0.94	0.64	0.68	0.55	0.704	0.148	0.492
Absorbed, g/d	1.54	1.17	1.55	1.71	2.15	1.80	0.37	0.318	0.076	0.029
ATTD, Na %	57.75	54.04	61.20	65.20	77.85	73.65	18.94	0.779	0.101	0.229
K										
Intake, g/d	14.24	13.91	13.54	14.26	13.45	14.39	1.19	0.147	0.032	0.033
Fecal output, g/d	2.69	2.28	1.85	1.81	1.46	1.82	0.29	0.063	0.050	0.002
Absorbed, g/d	11.55	11.63	11.69	12.46	11.83	12.57	0.92	0.789	0.005	0.890
ATTD, K %	81.27	83.66	86.36	87.36	88.05	88.45	1.18	0.113	0.006	0.050
Mg										
Intake, g/d	2.35	2.34	2.29	2.27	2.29	2.34	0.20	0.762	0.446	0.054
Fecal output, g/d	1.69	1.63	1.38	1.41	1.34	1.46	0.21	0.608	0.426	0.025
Absorbed, g/d	0.66	0.71	0.91	0.86	0.96	0.88	0.07	0.627	0.206	0.050
ATTD, Mg %	28.67	30.50	36.53	38.43	42.24	37.43	3.60	0.668	0.142	0.014
S										
Intake, g/d	3.72	3.41	3.43	3.62	3.57	3.53	0.30	<0.001	0.078	0.007
Fecal output, g/d	0.82	0.73	0.70	0.74	0.70	0.77	0.07	0.060	0.334	0.505
Absorbed, g/d	2.90	2.69	2.73	2.88	2.86	2.76	0.24	<0.001	0.242	<0.001
ATTD, S %	78.07	78.75	79.59	79.50	80.31	78.23	0.85	0.576	0.525	0.101

Data are means of 8 observations per treatment, except for the positive and negative control diets that had only 6 observations, and diets containing 2,000 and 4,000 FTU of phytase only had 7 observations.

[1]FTU = phytase units.

[2]Linear and quadratic contrasts of adding 500, 1,000, 2,000, or 4,000 FTU/kg phytase to the negative control diet.

[3]PC = positive control; NC = negative control.

may be less accessible to proteolytic enzymes in the small intestine because of formation of ternary complexes of phytin, cations, and protein (Kies et al., 2006). Thus, when phytase is supplemented to the diets, it is possible that these complexes, if they exist, may be hydrolyzed, with a subsequent release of not only P, but also CP, AA, and other nutrients that were bound in the complex. However, results of the present experiment did not support this hypothesis and the lack of improvements in the AID of AA, except for the tendency for improvement in the AID of Phe and Asp, as phytase was added to the diet, indicates that there probably was no protein bound to the phytate complex. This observation is in agreement with some previous data (Bruce and Sundstøl, 1995; Traylor et al., 2001; Zeng et al., 2016), whereas Mroz et al. (1994), Kemme et al. (1999), and Adedokun et al. (2015) reported improved AID of some AA as a consequence of inclusion of phytase in the diets. It is

Table 7. Effects of increasing concentrations of an *E. coli* phytase on the apparent total tract digestibility (ATTD) of microminerals in corn-soybean meal–based diets fed to growing pigs

			Phytase, FTU[1] per kilogram complete feed					*P*-value[2]		
Item	PC[3]	NC	500	1,000	2,000	4,000	SEM	PC vs. NC	Linear	Quadratic
Cu										
Intake, mg/d	35.08	34.65	35.17	34.62	34.45	34.92	2.98	0.452	0.963	0.513
Fecal output, mg/d	23.72	23.03	20.97	21.26	20.59	23.50	2.25	0.651	0.404	0.043
Absorbed, mg/d	11.36	11.62	14.20	11.99	13.85	11.42	1.18	0.861	0.525	0.148
ATTD, Cu %	32.66	33.68	40.56	37.77	40.68	34.46	2.85	0.803	0.721	0.080
Fe										
Intake, mg/d	478.82	310.13	295.43	270.02	297.95	282.84	27.33	<0.001	<0.001	0.007
Fecal output, mg/d	416.57	232.14	227.62	232.81	226.02	252.58	33.11	<0.001	0.185	0.337
Absorbed, mg/d	62.26	78.00	67.81	37.22	71.93	30.25	12.29	0.370	0.010	0.844
ATTD, Fe %	13.44	25.41	22.72	14.23	25.04	10.62	4.13	0.029	0.021	0.579
Mn										
Intake, mg/d	127.86	126.31	128.19	126.18	125.55	127.28	10.88	0.452	0.963	0.513
Fecal output, mg/d	113.45	102.97	98.69	100.97	93.52	106.18	10.30	0.127	0.589	0.109
Absorbed, mg/d	14.41	23.33	35.15	25.21	32.03	21.10	4.53	0.171	0.349	0.154
ATTD, Mn %	11.59	18.61	27.76	20.32	25.92	16.40	3.79	0.198	0.346	0.141

Data are means of 8 observations per treatment, except for the diets containing 500 and 1,000 FTU of phytase only had 7 observations.

[1]FTU = phytase units.

[2]Linear and quadratic contrasts of adding 500, 1,000, 2,000, or 4,000 FTU/kg phytase to the negative control diet.

[3]PC = positive control; NC = negative control.

not clear why different responses were obtained from different experiments using pigs. In contrast, in poultry, it appears that microbial phytase more consistently increases the AID of AA (Yi et al., 1996; Cowieson et al., 2015). It has been hypothesized that the impact of microbial phytase on the AID of AA is greater in Lys-deficient diets that are fed on an ad libitum *basis* rather than in corn–SBM diets that are fed at a restricted level twice daily as was done in this experiment (Selle and Ravindran, 2008). Nevertheless, under the conditions of this experiment, the hypothesis that microbial phytase increases the AID of AA has to be rejected. As a consequence, it appears that there is no basis for applying an AA value to the phytase premix.

Apparent Total Tract Digestibility of DM, GE, NDF, ADF, and on DE

Pirgozliev and Bedford (2013) reported that there was no significant response of microbial phytase on apparent ME in broiler chickens and the same observation was reported from studies with pigs (Adeola et al., 2004; 2006; Zeng et al., 2016). Thus, the results of this experiment indicating that microbial phytase did not increase the ATTD of DM and GE or the DE in the diet are in agreement with previous data. However, data from other experiments indicated that phytase may improve the ATTD of GE (Adedokun et al., 2015; Velayudhan et al., 2015). It is possible that the different responses may be due to the use of different phytases or being a result of different Ca:P ratios in the diets, but research to address these hypotheses has not been reported.

Limited data have been reported on the impact of microbial phytase on the utilization of fiber by pigs. However, the observation that the ATTD of NDF and ADF was increased by phytase is in contrast with recent data (Zeng et al., 2016). The observation that the ATTD of DM and GE was greater in the negative control diet than in the positive control diet was not expected, but the increased DE in the negative control diet confirmed that pigs digested the energy in the negative control diet better than in the positive control diet. The main difference between the 2 diets is that 1% dicalcium phosphate in the positive control diet was replaced by ground corn in the negative control diet. The GE in the negative control diet, therefore, was greater than in the positive control diet and this increase in GE in combination with the greater ATTD of GE resulted in an increase in DE.

Apparent Total Tract Digestibility of Ca and P

The small, but significant, changes that were observed for the intake of Ca and P as phytase was added to the diets are a result of small, but

nonsignificant, differences among diets in daily feed intake in combination with small differences among diets in analyzed concentrations of Ca and P. These differences in intake of the minerals, which were also observed for Na, K, and S, were not intended, but reflect analytical inaccuracies and animal variation. The increase in digestibility and absorption of P that was observed when phytase was added to the diets is in agreement with results of numerous experiments (Kerr et al., 2010; Almeida and Stein, 2012; Almeida et al., 2013). This observation demonstrates that the *E. coli* phytase that was used in this experiment was effective in hydrolyzing phytate P in corn and SBM. In addition, both linear and quadratic increases for the ATTD and retention of P were observed as the dietary concentration of *E. coli* phytase increased. The linear and quadratic increases in ATTD of P that were observed as increasing concentrations of phytase were added to the diets are probably a result of increased degradation of phytate in the stomach because the main site of phytase activity is the stomach (Yi and Kornegay, 1996). Phytate may be degraded faster or to a greater extent if dietary phytase is included at a greater concentration compared with a lower concentration (Kies et al., 2006) and thus results in an overall increase in P digestibility.

Calcium is an important dietary determinant of phytase efficacy (Selle and Ravindran, 2008) and up to 35% of dietary Ca may be bound to phytate, which reduces the ATTD of Ca (González-Vega and Stein, 2014). Dietary Ca influences the ATTD of P, and if Ca is provided in excess of the requirement, P digestibility will be reduced (Stein et al., 2011). Thus, when comparing effects of phytases among experiments, the concentration of dietary Ca needs to be taken into account. The observation that the ATTD of Ca in diets with phytase was greater than in the diet without phytase indicates that phytic acid likely bound some of the dietary Ca in the control diet. It is, therefore, likely that Ca-phytate complexes may have been formed in the diets, but when phytase was added, the complex was hydrolyzed, which is the reason the ATTD of Ca increased as phytase was added to the diets. Values for the ATTD of Ca that were observed when pigs were fed the negative or the positive control diets were in agreement with previous data reported for a corn–SBM based diet (Almeida et al., 2013; Zeng et al., 2016). Results of several experiments have indicated that microbial phytase may improve the ATTD of Ca in pigs (González-Vega et al., 2013; 2015a; 2015b), and the present results confirmed this observation.

Apparent Total Tract Digestibility of Na, K, Mg, and S

Phytate may increase Na excretion in birds, but this effect may be counteracted by phytase (Cowieson et al., 2004). Dietary Na and phytase may increase absorption, ATTD, and retention of P (Merriman, 2016) and phytase may improve ileal Na digestibility (Selle et al., 2009; Truong et al., 2015). In the present experiment, absorption of Na was also increased as phytase was used. It is possible that phytate induces the movement of Na into the small intestinal lumen in pigs, and therefore, phytase may improve Na retention and effectively balance dietary cation–anion difference (DCAD = [(Na × 10,000)/23] + [(K × 10,000)/39] − [(Cl × 10,000)/35.5]; González-Vega et al., 2016). Phytic acid associates with K^+ and Mg^{2+} to form phytin in plants (González-Vega et al., 2014), which is likely the reason for the increased ATTD of K and Mg as phytase was added to the diets. We are not aware of other experiments in which the effect of phytase on the ATTD of S was determined, but the observation that the ATTD of S was not changed by phytase indicates that S is not bound to the phytate complex, which may be because S is an anion like P.

Apparent Total Tract Digestibility of Cu, Fe, and Mn

In addition to the negative impact on ATTD of P, phytate has been associated with reduced ATTD of Cu and Fe (Adeola, 1995). Phytic acid readily forms complexes with multivalent cations, with Cu^{2+}, Mn^{2+}, and Fe^{2+} forming complexes in a decreasing order of stability (Maenz et al., 1999). Inclusion of 1,500 FTU/kg microbial phytase in a corn–SBM–based diet may improve the ATTD of Cu (Kies et al., 2005) and the tendency for phytase to increase the ATTD of Cu that was observed in this experiment supports previous data.

Formation of Fe-phytate complexes is expected to make Fe less available for digestion and absorption. The linear increase in the ATTD of Fe as phytase was used that was demonstrated in this experiment is in agreement with results of studies with humans and rats (Pallauf et al., 1999; Hurrell et al., 2003). In contrast, the lack of a response to microbial phytase on the ATTD of Mn was surprising because Mn content of bone and kidney has been reported to be increased by inclusion of 20,000 FTU of phytase by Zeng et al., 2015. However, it is possible that a greater concentration of phytase than was used in this experiment is needed to increase the ATTD of Mn in pigs.

In conclusion, results of this experiment confirmed that increasing concentrations of an *E. coli* phytase in a corn–SBM diet improve the ATTD of Ca and P and several other minerals that may have been bound in the phytate complex in the intestinal tract of pigs. It also appears that the ATTD of ADF and NDF is increased by phytase indicating that fiber may also be bound in the phytate complex in diets that contain no phytase. However, under the condition of this experiment, it was not possible to confirm the hypothesis that phytase also increases the ATTD of AA and GE.

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