Animal Feed Science and Technology 232 (2017) 102–109

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of graded levels of an *Escherichia coli* phytase on growth performance, apparent total tract digestibility of phosphorus, and on bone parameters of weanling pigs fed phosphorus-deficient corn-soybean meal based diets

Yue She[a,b], Yanhong Liu[c], J. Caroline González-Vega[a], Hans H. Stein[a,*]

[a] Department of Animal Sciences, University of Illinois, Urbana 61801, United States
[b] Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China
[c] Department of Animal Science, University of California, Davis 95616, United States

ARTICLE INFO

Keywords:
Apparent total tract digestibility
Bone parameters
Escherichia coli phytase
Growth performance
Phosphorus
Pigs

ABSTRACT

An experiment was conducted to evaluate the efficiency of a novel *Escherichia (E.) coli* phytase on improving growth performance, calcium (Ca) and phosphorus (P) digestibility, and bone ash concentration of weanling pigs fed P-deficient corn-soybean meal based diets. Sixty weanling pigs with an initial body weight of 11.2 ± 1.2 kg were randomly allotted to 1 of 6 dietary treatments with 10 replicate pigs per treatment. The dietary treatments were: 1) positive control (PC), 2) negative control (NC), 3) NC + 250 phytase units (FTU)/kg diet, 4) NC + 500 FTU/kg diet, 5) NC + 1000 FTU/kg diet, and 6) NC + 2500 FTU/kg diet. Pigs were fed phase I diets during the initial 6 days and phase II diets from day 7–27. At the end of the experiment, all pigs were euthanized to collect the 3rd and 4th metacarpals from each front foot. Results indicated that in both phase I and II, pigs fed the NC diet had reduced ($P < 0.05$) apparent total tract digestibility (ATTD) of P compared with pigs fed the PC diet. However, inclusion of graded levels of *E. coli* phytase to the NC diet increased (linear and quadratic, $P < 0.01$) the ATTD of P. No differences were observed in the ATTD of Ca or the ATTD of dry matter as *E. coli* phytase inclusion increased in the diets. Pigs fed the PC diet had greater ($P < 0.05$) fat-free dried bone weight (g), bone ash weight (g), bone ash concentration (g/kg), bone Ca (g), and bone P (g and g/kg) and greater ($P < 0.05$) average daily gain (ADG), average daily feed intake (ADFI), and gain: feed ratio (G:F) during phase II and during the overall experimental period than pigs fed the NC diet. Graded levels of *E. coli* phytase linearly and quadratically increased ($P < 0.05$) fat-free dried bone weight (g), bone ash weight (g), bone ash (g/kg), bone Ca (g), and bone P (g). Inclusion of *E. coli* phytase also increased (linear, $P < 0.05$) final body weight, ADG, ADFI and G:F during phase II and during the entire experimental period. No differences were observed between diets supplemented with 1000 or 2500 FTU/kg of *E. coli* phytase for fat-free dried bone (g), bone ash weight (g), bone ash (g/kg), bone Ca (g and g/kg), bone P (g) or final BW, ADG, ADFI, or G:F during phase II or during the overall experimental period. In conclusion, adding up to 1000 FTU/

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; Ca, calcium; FTU, phytase units; *E.*, *coliEscherichia coli*; GF, gain to feed ratio; NC, negative control; P, phosphorus; PC, positive control; SBM, soybean meal

* Corresponding author.
E-mail address: hstein@illinois.edu (H.H. Stein).

http://dx.doi.org/10.1016/j.anifeedsci.2017.08.005
Received 29 April 2017; Received in revised form 2 August 2017; Accepted 3 August 2017
0377-8401/

kg of the novel *E. coli* phytase to P-deficient diets increased growth performance and P utilization in weanling pigs.

1. Introduction

The major ingredients in most commercial swine diets are cereal grains, cereal grain co-products, and oilseed meals (Stein et al., 2016). Approximately 70% of the total phosphorus (P) in these plant ingredients is bound to phytate, and, therefore, has low digestibility by pigs (Omogbenigun et al., 2003; Jeong et al., 2015). Therefore, inorganic P sources, such as dicalcium phosphate and monocalcium phosphate are often added to diets for pigs to provide sufficient quantities of digestible P (Fan et al., 2001). However, inclusion of *Escherichia (E.) coli* phytase in diets for pigs improves phytate P utilization and reduces P excretion (Rodríguez et al., 2013; Rojas et al., 2013; Gao et al., 2013; Almaguer et al., 2014; She et al., 2015).

It is important that P supplementation is optimized during the weanling period to provide bone mineral reserves for the growing-finishing phases (Varley et al., 2011). In contrast, a diet deficient in digestible P may restrict the growth of weanling pigs, and potentially lead to skeletal disease (Bühler et al., 2010). When *E. coli* phytase is added to low-P diets, P utilization and bone health is improved (Simons et al., 1990).

In a previous study, effects of adding graded levels of an *E. coli* phytase to diets based on corn or corn co-products fed to growing pigs were determined (Almeida and Stein, 2012). However, the inclusion rate of *E. coli* phytase that is needed to maximize P digestibility, average daily gain (ADG), gain to feed ratio (G:F), and bone ash for weanling pigs fed a corn-soybean meal (SBM) diet has not been reported. The objective of this experiment, therefore, was to test the hypothesis that inclusion of graded levels of a novel

Table 1
Composition of experimental diets (g/kg, unless otherwise indicated; as fed basis)[a].

	Phase I		Phase II	
Item	Negative control (NC)	Positive control (PC)	Negative control (NC)	Positive control (PC)
Ingredients				
Corn	626.5	621.3	645.5	642.5
Soybean meal, 48%	300.0	300.0	297.5	297.5
Animal fat	30.0	30.0	20.0	20.0
Cornstarch	2.5	–	2.5	–
Limestone	18.9	12.5	16.1	11.6
Monocalcium phosphate	–	14.0	–	10.0
Lysine HCl	5.7	5.7	4.2	4.2
DL-Methionine	1.7	1.7	1.0	1.0
Threonine	1.8	1.8	1.2	1.2
L-Tryptophan	0.1	0.1	–	–
L-Valine	0.8	0.9	–	–
Salt	4.0	4.0	4.0	4.0
Vitamin mineral premix[b]	3.0	3.0	3.0	3.0
Titanium oxide	5.0	5.0	5.0	5.0
Calculated energy and nutrients				
Digestibility energy, MJ/kg	14.64	14.57	14.53	14.49
Metabolizable energy, MJ/kg	14.11	13.99	14.00	13.92
Crude protein, g/kg	194.8	194.4	195.2	194.9
Lysine[c], g/kg	13.5	13.5	12.3	12.3
Methionine[c], g/kg	4.4	4.4	3.7	3.7
Threonine[c], g/kg	7.9	7.9	7.3	7.3
Acid detergent fibre, g/kg	33.9	33.7	34.3	34.2
Neutral detergent fibre, g/kg	81.7	81.2	83.2	83.0
Calcium[d], g/kg	8.0	8.0	7.0	7.0
Digestible phosphorus[d], g/kg	1.6	4.0	1.5	3.3

[a] Within each phase, four additional diets were formulated by adding 250, 500, 1000 or 2500 units of *E. coli* phytase to the negative control diet. Phase I diets were fed from day 0–6 of experiment.

[b] The vitamin-micromineral premix will provide 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, 2208 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.

[c] Values for amino acids are indicated as standardized ileal digestible amino acids.

[d] Analyzed values in the PC and NC diets in phase 1 were 0.98 and 0.93% Ca and 0.62 and 0.42% P, respectively. Analyzed Ca and P in phase 1 diets containing 250, 500, 1000 or 2500 units of phytase were 1.11, 0.92. 0.93, and 0.96% Ca, and 0.38, 0.40, 0.40, and 0.39% P. For phase 2 diets, Ca analyzed 1.03, 0.88, 0.82, 0.86, 0.84, and 0.85 for PC, NC, and diets containing 250, 500, 1000, or 2500 units of phytase, whereas the corresponding values for analyzed P were 0.59, 0.37, 0.37, 0.36, 0.35, and 0.35.

E. coli phytase to P-deficient corn-SBM based diets will increase growth performance, digestibility of P and bone ash concentration in weanling pigs.

2. Materials and methods

The protocol for this experiment was reviewed and approved by The Institutional Animal Care stand Use Committee at the University of Illinois (Urbana-Champaign, IL). No health problems were observed during the experiment with the exception that one pig fed the negative control diet was removed from the experiment on day 7 because of an apparent problem with one hind leg.

2.1. Diets

The experiment was conducted over a 27 day period with phase I diets being fed from day 1–6 and phase II diets being fed from day 7–27. There were 6 diets in each phase, therefore, a total of 12 diets were formulated (Table 1). The 6 dietary treatments in each phase included: 1) positive control diet (PC); 2) negative control diet (NC); 3) NC + 250 phytase units (FTU)/kg feed; 4) NC + 500 FTU/kg feed; 5) NC + 1000 FTU/kg feed; and 6) NC + 2500 FTU/kg feed. The PC diets were formulated to support maximum growth performance, and contained 4.0 and 3.3 g/kg standardized total tract digestible P in phase I and II, respectively (NRC, 2012). The NC phase I diet contained 1.6 g/kg standardized total tract digestible P and the NC phase II diet contained 1.5 g/kg standardized total tract digestible P. The calcium (Ca) concentration in both PC and NC phase I diets was 8.0 g/kg, whereas in phase II diets, the Ca concentration was 7.0 g/kg. Four additional diets were formulated within each phase by adding 250, 500, 1000 or 2500 FTU/kg of *E. coli* phytase to the NC diets. The enzyme was premixed with a small amount of corn and then mixed with the other ingredients. All diets were based on corn and SBM, and vitamins and minerals other than P were included in all diets to meet or exceed current requirements for weanling pigs (NRC, 2012). All diets also contained 5.0 g/kg titanium dioxide as an indigestible marker. Diets were fed in a meal form. The *E. coli* phytase used in this experiment was a new phytase provided by Origination Inc. (Minneapolis, MN).

2.2. Animals, maintenance conditions and experimental design

A total of 60 weanling gilts with an average initial body weight of 11.2 ± 1.2 kg were allotted to 1 of 6 treatment groups. Ten pigs were assigned to each treatment group in a randomized complete block design with 2 blocks of 30 pigs and 5 replicate pigs per diet within each block. All pigs were the offspring of L 359 boars that were mated to F-46 females (Pig Improvement Company, Hendersonville, TN). The room temperature was maintained between 23.0 and 24.0 °C throughout the experiment. Animals were fed a conventional post-weaning diet from weaning and until initiation of the experiment two weeks post-weaning. Animals were individually housed in pens that were equipped with a single-space feeder and a nipple drinker. Water was provided through individual nipple drinkers and pigs had free access to water throughout the experiment.

2.3. Observations, sample collections and chemical analysis

The body weight of each pig was recorded at the beginning of the experiment, at the end of phase I and at the conclusion of the experiment. Daily feed allotments were recorded as well and feed left in the feeder was recorded at the end of phase I and at the conclusion of the experiment. At the conclusion of the experiment, data for feed intake and pig body weights were summarized to calculate ADG, average daily feed intake (ADFI) and G:F. Fecal samples were collected twice daily from all pigs at the last two days of phase I (days 5 and 6) and the last two days of phase II (days 26 and 27) and all fecal samples were mixed within pig and phase, dried

Table 2
Expected and analyzed activities of enzymes.

	Activity		
Treatment	Expected	Analyzed	% Recovery
Phase I			
1. Positive control (PC)	0	110	–
2. Negative control (NC)	0	< 70	–
3. NC + 250 FTU phytase/kg feed	250	340	136
4. NC + 500 FTU phytase/kg feed	500	460	92
5. NC + 1000 FTU phytase/kg feed	1000	880	88
6. NC + 2500 FTU phytase/kg feed	2500	2100	84
Phase II			
1. PC	0	< 70	–
2. NC	0	< 70	–
3. NC + 250 FTU phytase/kg feed	250	230	92
4. NC + 500 FTU phytase/kg feed	500	380	76
5. NC + 1000 FTU phytase/kg feed	1000	820	82
6. NC + 2500 FTU phytase/kg feed	2500	2100	84

and finely ground at the end of the experiment. At the conclusion of the experiment, all pigs were euthanized and the third and fourth metacarpals from both front legs were collected.

Diet and fecal samples were analyzed in duplicate for dry matter (Method 927.05; AOAC International, 2007), Ca and P (Method 985.01; AOAC International, 2007), and titanium (Method 990.08; AOAC International, 2007). Diet samples were also analyzed for phytase (Method 2000.12; AOAC International, 2007; Table 2). Metacarpals were cleaned to remove soft tissue, defatted in ether extract for 3 days, and ashed in a muffle furnace at 600 °C for 16 h to determine total bone ash. Bone ash was subsequently analyzed for Ca and P.

2.4. Calculations and statistical analyses

Values for ATTD of dry matter, Ca and P were calculated according to the following equation (NRC, 2012):

$$ATTD = 1 - (N_{feces}/N_{diet}) \times (M_{diet}/M_{feces})$$

where N_{feces} and N_{diet} represent dry matter, Ca or P concentration (g/kg) in feces and diet dry matter, respectively, and M_{diet} and M_{feces} represent the titanium concentration (g/kg) in diet and feces dry matter, respectively.

Outliers were determined as values that deviated from the treatment mean by more than 3 times the interquartile range, but no outliers were identified. Data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS Inst. Inc., Cary, NC) in a randomized complete block design with the pig as the experimental unit. The statistical model included dietary treatment as the fixed effect and block and replicate as random effects. Polynomial contrasts were used to examine responses to addition of graded levels of phytase to the NC diets. Coefficients used for linear and quadratic contrasts were determined using PROC Interactive Matrix Language. Least square means were calculated for each independent variable. If diet was a significant source of variation, means were separated using the PDIFF option of SAS. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

3. Results

3.1. Growth performance

No differences among dietary treatments were observed for initial body weight of pigs (Table 3). Pigs fed the PC diet had greater ($P < 0.05$) final BW, ADG, ADFI, and G:F in phase II and for the overall experimental period than pigs fed the NC diet. However, as *E. coli* phytase was added to the NC diet in increasing quantities, ADG and final body weight increased (linear and quadratic, $P < 0.01$) in phase II, and ADFI and G:F also increased (linear, $P < 0.01$) during phase II. For the overall experimental period, ADG, ADFI, and G:F increased (linear, $P < 0.05$) as *E. coli* phytase was added to the NC diet and there was a tendency ($P = 0.06$) for a

Table 3
Effects of a novel *E. coli* phytase on growth performance of weaned pigs fed corn-soybean meal based diets[e].

	Dietary treatments							P-value[f]		
			Phytase (units/kg diet)							
Item	PC[g]	NC[g]	250	500	1000	2500	SEM	Diet	Linear	Quadratic
Initial body weight, kg	11.21	11.23	11.28	11.20	11.30	11.17	0.39	0.80	0.45	0.41
Phase I (day 0–6)										
Final body weight, kg	13.30[a,b]	12.19[b]	14.36[a]	13.06[b]	13.25[a,b]	12.93[b]	1.08	0.03	0.79	0.21
Average daily gain, g/d	380	224	546	342	356	358	66	0.05	0.96	0.44
Average daily feed intake, g/d	805	709	799	752	744	778	73	0.69	0.59	0.91
Gain: Feed, g/kg	462[b]	291[b]	662[a]	455[b]	475[a,b]	401[b]	73	0.02	0.61	0.14
Phase II (day 7–27)										
Final body weight, kg	27.74[a]	19.93[d]	24.18[b,c]	22.66[c]	24.18[b,c]	25.16[b]	1.83	< 0.01	< 0.01	0.04
Average daily gain, g/d	687[a]	372[d]	467[c]	456[c]	520[b,c]	581[b]	43	< 0.01	< 0.01	0.03
Average daily feed intake, g/d	1205[a]	896[c]	1009[b,c]	979[b,c]	1098[a,b]	1168[a]	89	< 0.01	< 0.01	0.15
Gain: Feed, g/kg	570[a]	419[c]	461[b,c]	467[b]	474[b]	497[b]	16	< 0.01	< 0.01	0.16
Overall (day 0–27)										
Average daily gain, g/d	618[a]	335[d]	484[b,c]	431[c]	483[b,c]	531[b]	41	< 0.01	< 0.01	0.06
Average daily feed intake, g/d	1117[a]	848[c]	963[b,c]	929[b,c]	1020[a,b]	1083[a]	80	< 0.01	< 0.01	0.18
Gain: Feed, g/kg	553[a]	396[c]	496[b]	466[b]	474[b]	489[b]	19	< 0.01	< 0.05	0.08

[a] Within a row, means without a common superscript letter are different ($P < 0.05$).
[b] Within a row, means without a common superscript letter are different ($P < 0.05$).
[c] Within a row, means without a common superscript letter are different ($P < 0.05$).
[d] Within a row, means without a common superscript letter are different ($P < 0.05$).
[e] Each least squares mean represents 10 observations, with the exception that the least square mean for the negative control diet from day 7–27 had only 9 observations.
[f] Linear = linear effect of adding microbial phytase to the NC diet; Quadratic = quadratic effect of adding microbial phytase to the NC diet.
[g] PC = positive control diet; NC = negative control diet.

quadratic increase in ADG for the overall period. However, regardless of the level of *E. coli* phytase in the diet, final body weight on day 27 of pigs fed phytase supplemented diets was less ($P < 0.05$) than that of pigs fed the PC diet. Likewise, ADG in phase II and for the overall period was less ($P < 0.05$) for pigs fed either level of *E. coli* phytase compared with pigs fed the PC diet. For both experimental phases and for the overall period, no differences in final body weight, ADG, ADFI, or G:F between pigs fed diets with 1000 units of *E. coli* phytase and pigs fed diets with 2500 units.

3.2. Apparent total tract digestibility of dry matter, calcium and phosphorus

Compared with PC, pigs fed the NC diet had less ($P < 0.05$) ATTD of P in phase I and II (Table 4). However, pigs fed the NC diet had greater ($P < 0.05$) ATTD of Ca and ATTD of dry matter than pigs fed the PC diet in phase II, but not in phase I. Inclusion of *E. coli* phytase to the NC diet increased (linear and quadratic, $P < 0.01$) the ATTD of P in phase I and phase II, but no increase in the ATTD of Ca or dry matter was observed as *E. coli* phytase was added to the NC diet.

3.3. Bone ash and mineralization

Pigs fed the PC diet had greater ($P < 0.05$) fat-free dried bone weight, metacarpal bone ash weight, bone ash concentration, bone Ca in g, bone P in g and bone P concentration compared with pigs fed the NC diet (Table 5). Inclusion of graded levels of *E. coli* phytase to the NC diet increased (linear and quadratic, $P < 0.01$) fat-free dried bone weight, metacarpal bone ash weight, bone ash concentration, bone Ca in g and bone P in g. Increasing levels of *E. coli* phytase also linearly increased ($P < 0.01$) bone P concentration.

4. Discussion

The novel phytase that was used in this experiment (Smizyme, Origination, Minneapolis, MN, USA) is a novel heat stable phytase that has improved thermo stability compared with older types of *E.coli* phytases due to a strong enzyme confirmation that prevents unfolding of the protein. The analyzed *E. coli* phytase activities of all diets were in good agreement with expected values. The analyzed values for Ca in most diets were greater than calculated, which may be a result of the soybean meal and possibly the limestone containing more Ca than calculated. Calcium carbonate is sometimes used as a flow agent in soybean meal and there is, therefore, some variability in the concentration of Ca in soybean meal (Sotak-Peper et al., 2016). Likewise, the concentration of Ca in limestone may be greater than 38% depending on the source although the value reported by NRC (2012), which was used in diet formulation, is only 35.85% (Merriman and Stein, 2016).

Improvements in growth performance for pigs fed phytase-supplemented corn-SBM diets have been demonstrated (Almeida and Stein, 2010), and the current results are consistent with these observations. This is likely a result of phytase liberating P that is bound in the phytate complex (Braña et al., 2006). The reason that no differences in growth performance were observed among treatments in phase I is most likely that pigs had sufficient Ca and P stored in bones to meet the need during the initial 6 days of the experiment. However, the fact that pigs fed the PC diet had greater final body weight, ADG, ADFI, and G:F during phase II and for the overall experimental period than pigs fed the NC diet indicates that P-deficiency will negatively affect growth performance of weanling pigs, which is also in agreement with previous data (Harper et al., 1997; Li et al., 1998; Jendza et al., 2005; Veum et al., 2006). However,

Table 4
Effects of a novel *E. coli* phytase on apparent total tract digestibility (ATTD) of dry matter, calcium (Ca) and phosphorus (P) by weaned pigs fed corn-soybean meal based diets[d].

	Dietary treatments							P-value[f]		
			Phytase (units/kg diet)							
Item	PC[e]	NC[e]	250	500	1000	2500	SEM	Diet	Linear	Quadratic
Phase I (day 0–6)										
ATTD, dry matter	0.822	0.806	0.835	0.851	0.819	0.828	0.01	0.247	0.856	0.373
ATTD, Ca	0.820	0.803	0.833	0.847	0.817	0.825	0.01	0.303	0.860	0.373
ATTD, P	0.461[a]	0.242[b]	0.251[b]	0.370[a]	0.430[a]	0.429[a]	0.04	< 0.001	< 0.001	0.005
Phase II (day 7–27)										
ATTD, dry matter	0.807[b]	0.851[a]	0.852[a]	0.849[a]	0.852[a]	0.850[a]	0.09	< 0.001	0.969	0.992
ATTD, Ca	0.812[b]	0.854[a]	0.856[a]	0.853[a]	0.856[a]	0.856[a]	0.08	< 0.001	0.829	0.997
ATTD, P	0.361[b]	0.167[d]	0.252[c]	0.303[b,c]	0.345[b]	0.458[a]	0.02	< 0.001	< 0.001	< 0.001

[a] Within a row, means without a common superscript letter are different ($P < 0.05$).
[b] Within a row, means without a common superscript letter are different ($P < 0.05$).
[c] Within a row, means without a common superscript letter are different ($P < 0.05$).
[d] Each least squares mean represents 10 observations, with the exception that the least square mean for the negative control diet from d 7–27 had only 9 observations.
[e] PC = positive control diet; NC = negative control diet.
[f] Linear = linear effect of adding microbial phytase to the NC diet; Quadratic = quadratic effect of adding microbial phytase to the NC diet.

Table 5
Effects of a novel *E. coli* phytase on metacarpal bone ash and concentrations of calcium (Ca) and phosphorus (P) in bone ash[e].

	Dietary treatments							P-value[g]		
			Phytase (units/kg diet)							
Item	PC[f]	NC[f]	250	500	1000	2500	SEM	Diet	Linear	Quadratic
Fat-free dried bone wt, g	4.62[a]	2.63[d]	3.57[b]	3.04[c]	3.63[b]	3.78[b]	0.32	< 0.01	< 0.01	0.01
Bone ash wt, g	2.57[a]	1.28[e]	1.73[c,d]	1.57[d]	1.86[b,c]	1.97[b]	0.20	< 0.01	< 0.01	< 0.01
Bone ash, g/kg	556.0[a]	489.9[c]	485.7[c]	516.1[b]	511.1[b]	520.2[b]	9.5	< 0.01	< 0.01	< 0.01
Bone Ca, g	1.00[a]	0.50[e]	0.67[c,d]	0.62[d]	0.72[b,c]	0.77[b]	0.08	< 0.01	< 0.01	< 0.01
Bone Ca, g/kg ash	389[b,c]	392[a,b]	388[c]	393[a]	390[a,b,c]	389[b,c]	1.5	0.038	0.209	0.972
Bone P, g	0.47[a]	0.23[e]	0.31[c,d]	0.28[d]	0.33[b,c]	0.36[b]	0.04	< 0.01	< 0.01	< 0.01
Bone P, g/kg ash	184[a]	177[d]	177[d]	179[c]	180[c]	181[b]	0.6	< 0.01	< 0.01	0.07

[a] Within a row, means without a common superscript letter are different ($P < 0.05$).
[b] Within a row, means without a common superscript letter are different ($P < 0.05$).
[c] Within a row, means without a common superscript letter are different ($P < 0.05$).
[d] Within a row, means without a common superscript letter are different ($P < 0.05$).
[e] Each least squares mean represents 10 observations, with the exception that the least square means for the negative control diet had only 9 observations.
[f] PC = positive control diet; NC = negative control diet.
[g] Linear = linear effect of adding microbial phytase to the NC diet; Quadratic = quadratic effect of adding microbial phytase to the NC diet.

the observation that pigs receiving 2500 FTU/kg of *E. coli* phytase had less ADG and G:F than pigs fed the PC diet indicates that the digestible P released from phytate in a corn-SBM based diet is not sufficient to meet the requirement of weanling pigs fed corn-soybean meal based diets without added feed phosphates. This observation is in contrast with data from Almeida and Stein (2010) who reported that pig growth performance was not reduced in diets containing no feed phosphate if microbial phytase was used, However, the diets by Almeida and Stein (2010) contained 200 g per kilogram of distillers dried grains with solubles, which has a greater concentration of digestible P than corn and SBM (Stein et al., 2016), and diets containing distillers dried grains with solubles are, therefore, less deficient in P than corn-SBM diets if no feed phosphate is used. As a consequence, some inorganic P needs to be added to corn-SBM diets fed to weanling pigs even if phytase is included in the diet. The lack of a difference in growth performance between pigs fed diets supplemented with 1000 and 2500 FTU/kg of phytase in phase II and for the overall experimental period indicates that 1000 FTU/kg of the *E. coli* phytase used in this experiment results in maximum growth performance and there seems to be no advantage of using more than 1000 FTU.

The increased ATTD of P in the PC diet compared with the NC diet is a result of the fact that P from monocalcium phosphate has a greater digestibility than P in corn and SBM (NRC, 2012). However, the improved P digestibility that was observed as the *E. coli* phytase was added to the NC diet indicates that phytase liberated some of the P that was bound to phytate in corn and SBM. This observation is in agreement with previous data (Kerr et al., 2010; Rodríguez et al., 2013; Maison et al., 2015; Almaguer et al., 2014; She et al., 2015; Sotak-Peper et al., 2016) and indicates that the *E. coli* phytase that was used in this experiment was effective in hydrolyzing the ester bond between phytate and P in corn and SBM. The linear improvement in P digestibility as increasing concentrations of phytase was added to the diet without an apparent plateau was unexpected, because previous data have indicated that P digestibility may be maximized at a dose of approximately 800 units/kg of phytase (Almeida et al., 2013). However, it is likely that different phytases are different in terms of effectiveness in hydrolyzing the phytate-P bond and they may also have different pH optimum in the stomach (Yi and Kornegay, 1996). Nevertheless, because of the increased digestibility of P in the diets containing the *E. coli* phytase compared with the NC diet, less inorganic P is needed in the diet if phytase is used.

Calcium may influence the effectiveness of microbial phytase (Selle and Ravindran, 2008). The majority of the P in plant feed ingredients is bound to phytate, and at least one third of dietary Ca may also be bound to this molecule, which results in a limited availability of both minerals (González-Vega and Stein, 2014). In fact, phytate in plant feed ingredients often chelates Mg^{2+} and K^{+}, and Cu as well as Ca and P (González-Vega et al., 2014), but because Ca is the mineral present in swine diets in greatest concentrations, it is likely that Ca-phytate complexes were formed in the intestinal tract. The ATTD of Ca that was observed when pigs were fed the negative or the positive control diets was in agreement with published values (Almeida et al., 2013; Zeng et al., 2016) and microbial phytase often improves the digestibility of Ca (Almeida et al., 2013; González-Vega et al., 2013, 2015a,b). However, in the current experiment, no effect of phytase on digestibility of Ca was observed, but it is not clear why the phytase used in this experiment failed to improve the ATTD of Ca.

Bone parameters are more sensitive to increasing concentrations of P if there is sufficient Ca in the diet than growth performance, and therefore, bone ash has been suggested as an indicator of availability of P (Walker et al., 1993). The total quantity of P in bone ash is a measure of P deposition in bones because P is stored in a mineral form, which is analyzed as bone ash. As more P is absorbed, more bone may be synthesized, and if that is the case, more bone ash is retained. In other words, it is the size of bone that is increased with increased availability of P or Ca, and therefore, the total amount of bone ash increases as the quantities of available Ca and P increases. As a consequence, the total amount of Ca and P deposited in bone ash increases as well. However, P is deposited in bone ash at a relatively constant concentration (Petersen et al., 2011), which is the reason there were only small differences in the concentration of P in bone ash among treatments and no differences among treatments in the concentration of Ca in bone ash were observed. As an example, the quantity of P stored in bone ash in pigs fed the NC diet was only 23 g compared with 47 g for pigs fed the PC diet, but the concentration of P calculated as g per kg ash was only reduced from 184 to 177 g per kg ash. The observation that the total quantities of Ca, P, and bone ash were increased with increasing phytase in the diets clearly indicates that inclusion of phytase in the diet made more P available for absorption. These data also demonstrate that the regulation of P deposition is at the level of total bone ash synthesis, whereas the composition of the bone ash does not change. This observation is in agreement with data reported by Petersen et al. (2011) and indicates that both total bone ash and total bone P are more accurate measures of P deposition in bones than percent bone ash or the concentration of P in bone ash.

5. Conclusions

Results of this experiment indicate that inclusion of a novel *E. coli* phytase improves P utilization of weanling pigs fed P-deficient corn-SBM diets. However, regardless of the concentration of phytase in the diet, results for the phytase supplemented diets did not equal those of the positive control diet. As a consequence, and under the conditions of this experiment, pigs from 11 to 25 kg that are fed a corn-SBM diet supplemented with the novel *E. coli* phytase will need to also receive some inorganic P to ensure sufficient provision of P to support maximum growth rate.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

AOAC International, 2007. Official Methods of Analysis. In: Hortwitz, W., Latimer, G.W. (Eds.), 18th ed. AOAC International, Gaithersburg, MD.
Almaguer, B.L., Sulabo, R.C., Liu, Y., Stein, H.H., 2014. Standardized total tract digestibility of phosphorus in copra meal, palm kernel expellers, palm kernel meal, and soybean meal fed to growing pigs. J. Anim. Sci. 92, 2473–2480.
Almeida, F.N., Stein, H.H., 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88, 2968–2977.
Almeida, F.N., Stein, H.H., 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.
Almeida, F.N., Sulabo, R.C., Stein, H.H., 2013. Effects of a novel bacterial phytase expressed in *Aspergillus Oryzae* on digestibility of calcium and phosphorus in diets fed to weanling or growing pigs. J. Anim. Sci. Biotechnol. 4, 8.
Bühler, K., Liesegang, A., Bucher, B., Wenk, C., Broz, J., 2010. Influence of benzoic acid and phytase in low-phosphorus diets on bone parameters in growing-finishing pigs. J. Anim. Sci. 88, 3363–3371.
Braña, D.V., Ellis, M., Castañeda, E.O., Sands, J.S., Baker, D.H., 2006. Effect of a novel phytase on growth performance, bone ash, and mineral digestibility in nursery and grower-finisher pigs. J. Anim. Sci. 84, 1839–1849.
Fan, M.Z., Archbold, T., Sauer, W.C., Lackeyram, D., Rideout, T., Gao, Y., de Lange, C.F., Hacker, R.R., 2001. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. J. Nutr. 131, 2388–2396.
Gao, C.Q., Ji, C., Zhang, J.Y., Zhao, L.H., Ma, Q.G., 2013. Effect of a novel plant phytase on performance, egg quality, apparent ileal nutrient digestibility and bone mineralization of laying hens fed corn-soybean diets. Anim. Feed Sci. Technol. 186, 101–105.
González-Vega, J.C., Stein, H.H., 2014. Invited review – calcium digestibility and metabolism in pigs. Asian Australas. J. Anim. Sci. 27, 1–9.
González-Vega, J.C., Walk, C.L., Liu, Y., Stein, H.H., 2013. Determination of endogenous intestinal losses of Ca and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91, 4807–4816.
González-Vega, J.C., Walk, C.L., Liu, Y., Stein, H.H., 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Arch. Anim. Nutr. 68, 126–142.
González-Vega, J.C., Walk, C.L., Stein, H.H., 2015a. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93, 2255–2264.
González-Vega, J.C., Walk, C.L., Stein, H.H., 2015b. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93, 4808–4818.
Harper, A.F., Kornegay, E.T., Schell, T.C., 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75, 3174–3186.
Jendza, J.A., Dilger, R.N., Adedokun, S.A., Sands, J.S., Adeola, O., 2005. *Escherichia coli* phytase improves growth performance of starter, grower, and finisher pigs fed phosphorus-deficient diets. J. Anim. Sci. 83, 1882–1889.
Jeong, Y.D., Lee, S.H., Park, C.S., Cho, S.B., Park, S.K., 2015. Variation in coefficient of total tract apparent digestibility of dry matter, nitrogen, and phosphorus and coefficient of total tract standardized digestibility of phosphorus in different corns fed to growing-finishing pigs. Anim. Feed Sci. Technol. 201, 66–71.
Kerr, B.J., Weber, T.E., Miller, P.S., Southern, L.L., 2010. Effect of phytase on apparent total tract digestibility of phosphorus in corn-soybean meal diets fed to finishing pigs. J. Anim. Sci. 88, 238–247.
Li, D.F., Che, X.R., Wang, Y.Q., Cao, H., Thacker, P.A., 1998. Effect of microbial phytase, vitamin D_3, and citric acid on growth performance and phosphorus: nitrogen and calcium digestibility in growing swine. Anim. Feed Sci. Technol. 73, 173–186.
Maison, T., Liu, Y., Stein, H.H., 2015. Apparent and standardized total tract digestibility by growing pigs of phosphorus in canola meal from North America and 00-rapeseed meal and 00-rapeseed expellers from Europe without and with microbial phytase. J. Anim. Sci. 93, 3494–3502.
Merriman, L.M., Stein, H.H., 2016. Particle size of calcium carbonate does not affect apparent and standardized total tract digestibility of calcium, retention of calcium, or growth performance of growing pigs. J. Anim. Sci. 94, 3844–3850.
NRC, 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl. Acad. Press, Washington, D.C.
Omogbenigun, F.O., Nyachoti, C.M., Slominski, B.A., 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci. 81, 1806–1813.
Petersen, G.I., Pedersen, C., Lindemann, M.D., Stein, H.H., 2011. Relative bioavailability of phosphorus in inorganic phosphorus sources fed to growing pigs. J. Anim. Sci. 89, 460–466.
Rodríguez, D.A., Sulabo, R.C., González-Vega, J.C., Stein, H.H., 2013. Energy concentration and phosphorus digestibility in canola cottonseed, and sunflower products fed to growing pigs. Can. J. Anim. Sci. 93, 493–503.
Rojas, O.J., Liu, Y., Stein, H.H., 2013. Phosphorus digestibility and concentration of digestible and metabolizable energy in corn, corn coproducts, and bakery meal fed to growing pigs. J. Anim. Sci. 91, 5326–5335.
Selle, P.H., Ravindran, V., 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113, 99–122.
She, Y., Su, Y.B., Liu, L., Huang, C.F., Li, J.T., Li, P., Li, D.F., Piao, X.S., 2015. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. Anim. Feed Sci. Technol. 208, 132–144.

Simons, P.C., Versteegh, H.A., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G., Beudeker, R.F., Verschoor, G.J., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64, 525–540.
Sotak-Peper, K.M., González-Vega, J.C., Stein, H.H., 2016. Effects of production area and microbial phytase on the apparent and standardized total tract digestibility of phosphorus in soybean meal fed to growing pigs. J. Anim. Sci. 94, 2397–2402.
Stein, H.H., Lagos, L.V., Casas, G.A., 2016. Invited review: nutritional value of feed ingredients of plant origin fed to pigs. Anim. Feed Sci. Technol. 218, 33–66.
Varley, P.F., Callan, J.J., O'Doherty, J.V., 2011. Effect of dietary phosphorus and calcium level and phytase addition on performance bone parameters, apparent nutrient digestibility, mineral and nitrogen utilization of weaner pigs and the subsequent effect on finisher pig bone parameters. Anim. Feed Sci. Technol. 165, 201–209.
Veum, T.L., Bollinger, D.W., Buff, C.E., Bedford, M.R., 2006. A genetically engineered *Escherichia coli* phytase improves nutrient utilization, growth performance, and bone strength of young swine fed diets deficient in available phosphorus. J. Anim. Sci. 84, 1147–1158.
Walker, G.L., Danielson, D.M., Peo Jr., E.R., Mumm, R.F., 1993. Effect of calcium source, dietary calcium concentration, and gestation phase on various bone parameters in gestating gilts. J. Anim. Sci. 71, 3003–3010.
Yi, Z., Kornegay, E.T., 1996. Sites of phytase activity in the gastrointestinal tract of young pigs. Anim. Feed Sci. Technol. 61, 361–368.
Zeng, Z.K., Li, Q.Y., Zhao, P.F., Xu, X., Tian, Q.Y., Wang, H.L., Pan, L., Yu, S., Piao, X.S., 2016. A new *Buttiauxella* phytase continuously hydrolyzes phytate and improves amino acid digestibility and mineral balance in growing pigs fed phosphorous-deficient diet. J. Anim. Sci. 94, 629–638.