IOWA STATE UNIVERSITY
Digital Repository

Animal Science Publications | Animal Science

1-2019

Evaluating phosphorus release by phytase in diets fed to growing pigs that are not deficient in phosphorus

Kristin M. Olsen
Iowa State University

Stacie A. Gould
Iowa State University

Carrie L. Walk
AB Vista

Nick V. L. Serão
Iowa State University, serao@iastate.edu

Stephanie L. Hansen
Iowa State University, slhansen@iastate.edu

See next page for additional authors

Follow this and additional works at: https://lib.dr.iastate.edu/ans_pubs

 Part of the Agriculture Commons, Animal Experimentation and Research Commons, and the Animal Sciences Commons

The complete bibliographic information for this item can be found at https://lib.dr.iastate.edu/ans_pubs/811. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Animal Science at Iowa State University Digital Repository. It has been accepted for inclusion in Animal Science Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Evaluating phosphorus release by phytase in diets fed to growing pigs that are not deficient in phosphorus

Abstract

Microbial phytase is widely used to enhance digestibility of phytate-P. By tradition, diets with P content well below requirement are used to quantify phytate-P release by phytase, but P-adequate diets may be more physiologically relevant. The objective of this study was to investigate the effects of phytase on P digestion and metabolism and develop a P release curve for phytase in Padequate diets (above requirement according to NRC, 2012), and to compare these effects in a Pdeficient diet. Three replicates of 24 barrows each (BW = 23.0 ± 1.8 kg) were randomly assigned to 1 of 8 dietary treatments, housed in individual pens for 21 d, then moved to metabolism crates for 5 d urine and fecal collections. A basal corn-soybean meal diet (P-adequate, A) was formulated at 0.36% standardized total tract digestible (STTD) P and total Ca:STTD P of 1.83. Phytase was added to A at 200 (A200), 400 (A400), 600 (A600), and 800 (A800) phytase units (FTU)/kg. A positive control diet (PC) was formulated using monocalcium phosphate (MCP) to increase STTD P by 0.16% to 0.52%, the expected STTD P release of 800 FTU/kg. A P-deficient diet (D) was formulated by reducing MCP to achieve 0.21% STTD P, and 200 FTU phytase/kg was added to D for D200. Pig was the experimental unit, and replicate and dietary treatment were fixed effects. Orthogonal polynomial contrasts were used to test linear and quadratic effects of phytase within A, A200, A400, A600, and A800. Phytase increased percent apparent total tract digestibility (ATTD) and STTD of P (quadratic $P < 0.001$), and quantity of absorbed P (linear $P < 0.001$; quadratic $P = 0.069$). Urinary P increased linearly with phytase ($P < 0.001$) and retained P also increased (linear $P = 0.001$, quadratic $P = 0.094$). Phytate-P release was estimated to be 0.049, 0.080, 0.093, and 0.09% STTD P for 200, 400, 600, and 800 FTU/kg, respectively. It appears that the effect of phytase may be lower in P-adequate diets as compared to P-deficient diets, given that there was a 12% improvement for A200 vs A, and a 28% improvement in STTD P for D200 vs D. In conclusion, phytase improved P digestibility and retention in P-adequate diets, and P digestibility was used to estimate the quantity of P released by phytase. Further research investigating P release by phytase in P-adequate diets, rather than Pdeficient diets, may be preferable.

Keywords

Calcium, phosphorus digestibility, phosphorus requirement, phytate, standardized total tract digestible phosphorus, swine

Disciplines

Agriculture | Animal Experimentation and Research | Animal Sciences

Comments

This is a manuscript of an article published as Olsen, Kristin M., Stacie A. Gould, Carrie L. Walk, Nick VL Serão, Stephanie L. Hansen, and John F. Patience. "Evaluating phosphorus release by phytase in diets fed to growing pigs that are not deficient in phosphorus." *Journal of animal science* 97, no. 1 (2019): 327-337. doi:10.1093/jas/sky402. Posted with permission.

Authors

Kristin M. Olsen, Stacie A. Gould, Carrie L. Walk, Nick V. L. Serão, Stephanie L. Hansen, and John F. Patience

This article is available at Iowa State University Digital Repository: https://lib.dr.iastate.edu/ans_pubs/811

Running head: Phytase in phosphorus adequate diets

Evaluating phosphorus release by phytase in diets fed to growing pigs that are not deficient in phosphorus[1]

K. M. Olsen, * S. A. Gould,* C. L. Walk,† N. V. L. Serão,* S. L. Hansen,* J. F. Patience*[2]

*Dept. of Animal Science, Iowa State University, Ames, IA, 50011;

† AB Vista, Marlborough, Wiltshire, UK, SN8 4AN

[1]The authors would like to thank AB Vista Feed Ingredients for financial support of this research. Appreciation is also expressed to DSM and Ajinomoto Heartland for in-kind support.

[2]Corresponding author: jfp@iastate.edu

ABSTRACT

Microbial phytase is widely used to enhance digestibility of phytate-P. By tradition, diets with P content well below requirement are used to quantify phytate-P release by phytase, but P-adequate diets may be more physiologically relevant. The objective of this study was to investigate the effects of phytase on P digestion and metabolism and develop a P release curve for phytase in P-adequate diets (above requirement according to NRC, 2012), and to compare these effects in a P-deficient diet. Three replicates of 24 barrows each (BW = 23.0 ± 1.8 kg) were randomly assigned to 1 of 8 dietary treatments, housed in individual pens for 21 d, then moved to metabolism crates for 5 d urine and fecal collections. A basal corn-soybean meal diet (P-adequate, A) was formulated at 0.36% standardized total tract digestible (STTD) P and total Ca:STTD P of 1.83. Phytase was added to A at 200 (A200), 400 (A400), 600 (A600), and 800 (A800) phytase units (FTU)/kg. A positive control diet (PC) was formulated using monocalcium phosphate (MCP) to increase STTD P by 0.16% to 0.52%, the expected STTD P release of 800 FTU/kg. A P-deficient diet (D) was formulated by reducing MCP to achieve 0.21% STTD P, and 200 FTU phytase/kg was added to D for D200. Pig was the experimental unit, and replicate and dietary treatment were fixed effects. Orthogonal polynomial contrasts were used to test linear and quadratic effects of phytase within A, A200, A400, A600, and A800. Phytase increased percent apparent total tract digestibility (ATTD) and STTD of P (quadratic $P < 0.001$), and quantity of absorbed P (linear $P < 0.001$; quadratic $P = 0.069$). Urinary P increased linearly with phytase ($P < 0.001$) and retained P also increased (linear $P = 0.001$, quadratic $P = 0.094$). Phytate-P release was estimated to be 0.049, 0.080, 0.093, and 0.09% STTD P for 200, 400, 600, and 800 FTU/kg, respectively. It appears that the effect of phytase may be lower in P-adequate diets as compared to P-deficient diets, given that there was a 12% improvement for A200 vs A, and a 28%

improvement in STTD P for D200 vs D. In conclusion, phytase improved P digestibility and retention in P-adequate diets, and P digestibility was used to estimate the quantity of P released by phytase. Further research investigating P release by phytase in P-adequate diets, rather than P-deficient diets, may be preferable.

Keywords: Calcium, phosphorus digestibility, phosphorus requirement, phytate, standardized total tract digestible phosphorus, swine

INTRODUCTION

The use of microbial phytase to increase P availability has become increasingly common in commercial pig production (Selle and Ravindran, 2008). Previous studies have predicted the release of P by phytase, and dose-response curves have been developed using growth performance or bone characteristics as response criteria (Augspurger et al., 2003; Jones et al., 2010). Bone characteristics, such as ash weight or percent ash, have generally been the most sensitive measurements for estimating P release by phytase. (Cromwell, 2005). Almost all dose-response curves have been developed using diets well below the pig's P requirement for growth (Augspurger et al., 2003; Jones et al., 2010; Kerr et al., 2010; Gourley et al., 2018). Feeding diets deficient in P may result in impaired growth performance, enhanced efficiency of absorption of dietary P, or stimulation of P release from bones (Cromwell, 2005; Berndt and Kumar, 2009). Lower phytase efficacy in P-adequate diets has been previously reported (Fan et al., 2005; Almeida et al., 2013; Rodehutscord, 2016). Thus, phytase release curves developed when P is fed at requirement may be more representative of normal physiological conditions that are occurring in commercial conditions. Undertaking such studies using diets that are above the pig's

requirement for P also reduces concerns of lameness that may occur in experimental animals fed diets deficient in P.

The primary objectives of this experiment were 1) to evaluate P digestibility and balance in response to phytase addition to a basal diet that meets the pig's requirement for P (NRC, 2012) and 2) to generate a P release curve for phytase. Gutierrez et al. (2015) demonstrated that pigs fed diets with P levels above their requirement for growth excrete excess P in the urine in a linear fashion, meaning that urinary P may be an indicator of P release by phytase in P-adequate diets. Phytase has been previously shown to improve digestibility of other nutrients such as AA and energy (Angel et al., 2002; Johnston et al., 2004; Bohlke et al., 2005). Thus the secondary objective of this experiment was to compare the effects of phytase on P and Ca metabolism and nutrient digestibility in both a P-adequate and a P-deficient diet. The hypotheses tested were that phytase would improve P digestion and absorption in the P-adequate diets, resulting in increased excretion of P in urine, and that P release by phytase would be equal in the P-adequate and the P-deficient diet.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Iowa State University (11-16-8379-S).

Animals, housing, and management

This experiment was conducted at the Iowa State University Swine Nutrition Farm (Ames, IA). Seventy-two crossbred barrows (Genetiporc 6.0 × Genetiporc F25, PIC, Inc., Hendersonville, TN) with a starting BW of 23.0 ± 1.8 kg were employed. With 24 available

crates, the experiment was repeated 3 times to achieve adequate replication, as determined from previous similar studies, starting in November 2016 and ending in March 2017. Pigs were randomly assigned to 1 of 8 dietary treatments and fed the assigned diets for the duration of the 30-d experiment. Within each replicate, pigs were assigned to dietary treatments based on a completely randomized design. For the first 21 d of the experiment, pigs were housed in individual pens (1.8 m x 2.7 m) with slatted floors, equipped with a self-feeder and nipple waterer. Pigs were given feed and water *ad libitum* until d 19, at which point pigs were limit fed at 2.85 times the daily maintenance energy requirement for the average body weight of each replicate (197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The average BW on d 19 was estimated from d 14 BW to calculate the feed allowance for d 19 and 20; when pigs were weighed on d 21, the feed allowance was recalculated using the d 21 average BW for each replicate. The daily ration of feed was given in two equal meals at 0800 and 1600 h. If a pig did not consume its entire meal, orts were collected 1 h after feeding and weighed. Ort weights were then used to calculate actual daily feed intake for each pig. Pigs were moved to metal metabolism crates equipped with slatted floors and feeders for the last 9 d of the experiment. In the metabolism crates, water was given at a 2.7:1 ratio to feed 1 h after initial feeding. Water was controlled to limit luxury water consumption (Fraser et al., 1993), but water requirements were met as described in Shaw et al. (2006). Pigs were allowed a 6-d adaptation to the limit feeding schedule and a 4-d adaptation to metabolism crates. Thus, the total adaptation time to the dietary treatments was 25 d (21 d in pens, 4 d in crates).

Experimental design and diets

Eight corn-soybean meal-based diets were formulated based on NRC requirements for growing pigs (NRC, 2012; Table 1). Basal ingredients were assayed for Ca and P content to ensure precision in final diet composition. A basal diet (A) was formulated to meet or exceed all NRC nutrient requirements, including P and Ca. The basal diet was formulated to contain 0.36% standardized total tract digestible (STTD) P, which was above the requirement of 0.31% (NRC, 2012). This level of STTD P was used to ensure that the diets would in fact be above the requirement of P for growth. To achieve the objective of estimating P-release by phytase in P-adequate diets, a microbial phytase (Quantum Blue 5G, 5,000 phytase units (FTU)/g, AB Vista, Marlborough, UK) was added to the basal diet at the expense of corn to achieve levels of 200, 400, 600, and 800 FTU/kg (A200, A400, A600, A800, respectively). The sixth diet (positive control; PC) was formulated to provide the same amount of STTD P as the diet with 800 FTU phytase/kg. Monocalcium phosphate (MCP) was added to achieve STTD P equivalent to the expected STTD P release of 800 FTU phytase/kg, which was 0.16% STTD P. Thus, this diet was formulated to contain 0.52% STTD P. To compare the effects of phytase supplementation in a P-deficient diet with phytase supplementation in a P-adequate diet, the seventh diet (D) was formulated to be slightly deficient in STTD P, at 0.21%. This diet was designed to demonstrate potential differences in phytase efficacy due to lower dietary P but not to be deficient enough to cause lameness. To maintain a constant total Ca: STTD P ratio with the P-adequate basal diet, total Ca was also reduced. Phytase was added at 200 FTU/kg to the seventh diet to achieve the eighth diet (D200). For all diets, NRC (2012) values of STTD P for ingredients were used in formulations. The bag of phytase was pre-analyzed for phytase activity (method 2000.12; AOAC, 2007) and was determined to contain 7,600 FTU/g. This analyzed activity was used to

determine the amount of phytase to add to each diet. One FTU is defined as the amount of enzyme activity that liberates 1 μmol inorganic orthophosphate per minute from 0.0051 mol/L sodium phytate at pH 5.5 and 37°C (AOAC, 2007). Titanium dioxide (TiO_2) was added at 0.4% to all the diets as an indigestible marker. To ensure uniformity of diets, ingredients with low inclusion rates including vitamin and trace mineral premixes, MCP, limestone, salt, synthetic AA, TiO_2, and phytase were weighed on an analytical scale and premixed in a small batch mixer (approximately 57 L capacity, Hobart Corporation, Troy, OH) before being added to the bulk ingredients in the main batch mixer. In addition, scales were validated with a standard check weight and precise weights of all ingredients added were obtained and recorded during mixing. All these procedures were followed to ensure the precision of the diet formulation and mixing procedures.

Sample and data collection

To give pigs extra time to adapt to limit feeding prior to entering metabolism crates, limit feeding began on d 19. Thus, feed disappearance was determined on d 14 and 19. Pigs were weighed on d 0, 14, and 21, before being moved to metabolism crates, to determine ADG, ADFI, and G:F. Pigs were also weighed at the end of the metabolism period (d 30). Representative diet samples (2 per treatment, approximately 500 g each) were collected throughout the final mixture, homogenized, and stored at -20°C until further analysis. Samples of MCP, limestone, corn, and soybean meal for Ca and P analysis were also obtained at the time of mixing and stored; samples of the vitamin and trace mineral premixes were taken after mixing, but were the same source and batch used at the time of mixing. Total urine and fecal grab samples were collected twice a day during the last 5 d of the experiment. Only barrows were used in this experiment to maximize the

accuracy with which separate urine and feces could be collected. Urine was collected in acid washed containers pre-loaded with 20 mL of HCl. Urine was filtered through glass wool, subsampled, and stored in acid washed plastic containers at -20°C until further analysis.

Chemical analysis

After each replicate was completed, urine and fecal samples were thawed at room temperature, pooled and homogenized for each pig, and subsampled for chemical analysis. This gave a total of 72 fecal and 72 urine samples. Fecal samples were dried in a convection oven at 75°C until a constant weight was achieved. Urine samples were re-stored at -20°C. Diet and dried fecal samples were ground through a 1 mm screen and stored in desiccators. Urine subsamples were thawed, mixed to ensure sample homogeneity, and filtered through Whatman 41 filter paper (GE Healthcare Life Sciences, Chicago, IL, USA) prior to analysis. Diet and fecal samples were analyzed for DM (method 990.03, AOAC 2007), TiO_2 (Leone, 1973), ash (method 942.45, AOAC, 2007), GE, N, and acid hydrolyzed ether extract (AEE). Gross energy was determined using an isoperibolic bomb calorimeter (Parr 6200 calorimeter; Parr Instruments Co., Moline, IL); benzoic acid (6,318 cal GE/kg; Parr Instruments Co.) was used as the standard for calibration and was determined to be 6,316 ± 7 cal GE/g. Acid hydrolyzed ether-extract (method 2003.06, AOAC 2007) was determined using a SoxCap SC 247 hydrolyzer and a Soxtex 255 semiautomatic extractor (FOSS North America, Eden Prairie, MN). Total N (method 990.03, AOAC 2007) was analyzed with a TruMac apparatus (Leco Corporation, St. Joseph, MI). Ethylenediaminetetraacetic acid (EDTA; 9.56% N) was used for calibration and was determined to contain 9.58 ± 0.04% N. Crude protein was calculated as N x 6.25.

Diet, fecal, and urine samples were analyzed for P and Ca by inductively coupled plasma optical emission spectrometry (ICP; Optima 7000 DV; PerkinElmer, Waltham, MA) as described by Pogge et al. (2014). Monocalcium phosphate, limestone, corn, soybean meal, and vitamin and mineral premixes were analyzed for Ca and P in the same manner. Prior to ICP analysis, ingredients, diets, and fecal samples were prepared by acid digestion as described by Richter et al. (2012), and urine samples were diluted 1:10 in 1% nitric acid. Analyses were performed in duplicate. A maximum of 1% CV between duplicates was required for GE, N, DM, and ash. A maximum CV of 5% was required for TiO_2 and AEE, and 10% for Ca and P. If the CV between duplicates of a sample exceeded these maximums, the sample was re-run in duplicate. Diets were also analyzed for phytase activity (method 2000.12; AOAC, 2007) and phytate-bound P content using the method described in the Megazyme phytic acid/total phosphorus kit (K-PHYT; Megazyme, Wicklow, Ireland).

Calculations

To determine the daily mean values for total DM intake and urine output of P and Ca, 5 d totals were recorded and divided by d within each collection period (g/d). Apparent total tract digestibility (ATTD) of nutrients and fecal output (DM) were calculated according to Oresanya et al. (2008). Standardized total tract digestibility (STTD) of P was calculated as described in NRC (2012), assuming basal endogenous losses of P (EPL) to be 190 mg/kg DMI. Apparent mineral absorption and net retention (referred to herein as absorption and retention) were calculated on a DM basis (g/d) as:

Apparent absorption = mineral intake – mineral in feces [1]

Net retention = mineral intake – mineral in feces – mineral in urine [2]

Retention of P, as a percentage of P intake and of P absorbed, were also calculated:

Retention, % of intake = retention / P intake x 100 [3]

Retention, % of absorbed = retention / absorbed P x 100 [4]

Statistical Analysis

Data were analyzed in a model including the fixed effects of dietary treatment, replicate, and their interaction. The interaction term was removed from the model when not significant ($P > 0.05$). The interaction was significant for a few traits, but this was largely driven by changes in magnitude between the differences of means, rather than changes in the pattern of treatment means across replicates. Least-squares means were separated using Tukey's method. Prior to final analyses, outliers (defined as Studentized residuals greater than 3 standard deviations from zero) were identified and removed and normality of the residuals was verified using the Shapiro-Wilk's test. Residual plots were examined to confirm the assumptions of equal variances were met. Orthogonal polynomial contrasts were performed on the 5 P-adequate treatments (A, A200, A400, A600, and A800) to test the linear and quadratic effects of phytase level on selected response variables. For these 5 treatments, the dietary treatment x replicate interaction was not significant ($P > 0.05$), so the interaction term was excluded from the model statement used to test linear and quadratic effects of phytase and to generate the appropriate regression equations. Differences were considered significant if $P < 0.05$, and trends if $0.05 < P < 0.10$. SAS 9.4 (SAS Inst. Inc., Cary, NC) was used for all analyses with GLM and UNIVARIATE procedures used for statistical analyses and outlier identification, respectively.

RESULTS

Results of diet and ingredient analysis are presented in tables 2 and 3. Across treatments, pigs performed as expected (Table 4) and no lameness was observed. During the *ad libitum* feeding period, pigs fed diets D or D200 had lower ADFI and ADG compared with pigs fed A or PC ($P < 0.05$; Table 4). A few instances of diarrhea were observed prior to the metabolism period, and affected pigs were treated with tylosin phosphate; whenever pigs were treated, all pigs in that replicate were treated in the same manner. Diarrhea was observed equally across dietary treatments and did not appear to be associated with any particular treatment. Diet analysis for phytase confirmed phytase was present in the intended treatments. Ingredient analysis confirmed expected values for Ca and P, except for greater Ca content in the vitamin and trace mineral premixes than expected (Tables 1, 2 and 3). Analyzed Ca and P values for ingredients were used to calculate final diet Ca and P content, rather than actual analyzed values, though analysis results for Ca and P in diets were within analytical tolerance of formulated values. The greater-than-expected Ca content in the vitamin and trace mineral premixes made the final total Ca:STTD P ratio 2.03 in the basal P-adequate diet, and 2.14 in the basal P-deficient diet.

Effect of phytase on P balance

In the P-adequate diets (A, A200, A400, A600 and A800) phytase improved ATTD of P in a quadratic fashion ($P < 0.001$, Table 5). Phosphorus ATTD was also greater in D200 compared to D ($P < 0.05$). The greatest ATTD of P was observed in A400, A600, A800, and PC. As expected, STTD of P also increased with the addition of phytase in a pattern similar to that of ATTD of P (treatment $P < 0.001$). Absorbed P increased with phytase level in the P-adequate diets (linear *P*

< 0.001, quadratic $P = 0.069$; Table 5). Urinary P excretion increased linearly in response to phytase in diets A, A200, A400, A600, and A800 ($P < 0.001$). Retained P also increased in response to phytase in these diets (linear $P = 0.001$, quadratic $P = 0.094$). Retention of P as a percentage of intake also increased (quadratic $P = 0.009$) and reached a maximum of 53% in A400, A600 and A800. Fecal and total excretion of P decreased with the addition of phytase in A, A200, A400, A600, and A800 (quadratic $P = 0.001$; quadratic $P = 0.001$ for fecal and total P excretion, respectively). As in the P-adequate diets, 200 FTU phytase/kg improved the absorption and retention of P ($P < 0.05$; Table 5) when added to the P-deficient diet (D200 vs D). Urine P excretion did not differ between D and D200 ($P > 0.1$. Compared to D, pigs fed D200 tended to have reduced fecal excretion of P ($P = 0.063$) and significantly reduced total excretion of P ($P < 0.05$).

Effect of phytase on Ca balance

Phytase improved ATTD of Ca in the P-adequate diets (linear $P < 0.001$; Table 6), although the mean ATTD of Ca was similar for A, A200, A400, A600, A800, and PC ($P > 0.05$). This corresponded to a linear increase in the absorption of Ca in A, A200, A400, A600, and A800 ($P < 0.001$). Retained Ca also increased with phytase inclusion in these diets (linear $P <$ 0.001, quadratic $P = 0.072$). Urine Ca excretion did not differ for pigs fed A, A200, A400, A600, A800, or PC ($P > 0.05$) Fecal and total Ca excretion decreased with the addition of phytase for pigs fed diets A, A200, A400, A600, and A800 (fecal excretion: linear $P < 0.001$; total excretion: linear $P < 0.001$, quadratic $P = 0.0935$). Compared to D, D200 resulted in numerically, but not significantly, improved ATTD of Ca and absorbed Ca ($P > 0.10$; Table 6). The addition of phytase significantly improved the retention of Ca ($P < 0.05$), likely through the slight increase

in absorption and a reduction in urinary Ca losses for D200 compared to D ($P < 0.05$). Both D and D200 resulted in elevated urinary Ca when compared to the P-adequate diets ($P < 0.05$), which is typical for pigs fed below their requirement for P.

Results of regression analysis for effect of phytase in P-adequate diets

The improvement in digestibility (STTD of P) for a given phytase level, as indicated by the corresponding regression equation (Table 5), was multiplied by the total P content of the diet to determine P release. Phytase was estimated to release 0.049, 0.080, 0.093, and 0.09% STTD P for 200, 400, 600, and 800 FTU/kg, respectively. In the same manner, Ca release by phytase was estimated using ATTD of Ca. Phytase was estimated to release 0.022, 0.045, 0.062, and 0.091% ATTD Ca for 200, 400, 600, and 800 FTU/kg, respectively.

Effect of phytase on apparent total tract digestibility of energy and other nutrients

In the P-adequate diets, there was a significant phytase effect (quadratic $P = 0.011$) on the ATTD of DM, with the greatest ATTD of DM observed in A400, A600, and A800 (Table 7). There was also an effect of phytase on ATTD of GE (quadratic $P = 0.019$), with A400 having the greatest GE ATTD, while A200, A600, and A800 had intermediate GE ATTD between A400 and A. For ATTD of N, there was a significant effect of phytase in the P-adequate diets with A200, A400, A600, and A800 having slightly greater ATTD of N than A (quadratic $P = 0.046$). There were no differences between D and D200 for ATTD of DM, GE, or N ($P > 0.10$). The ATTD of ash was significantly improved by phytase in the both the P-adequate (quadratic $P < 0.001$) and the P-deficient diets ($P < 0.05$). There was a tendency for phytase to improve the

ATTD of AEE in the P-adequate diets ($P = 0.06$), and the P-deficient diet with 200 FTU phytase/kg (D200) resulted in significantly greater ATTD of AEE than D ($P < 0.05$).

DISCUSSION

Although phytase caused a linear increase in urine P when pigs were fed P-adequate diets, retained P was also increased. Letourneau-Montminy et al. (2012) demonstrated that retained P increased with increased P availability when a consistent Ca:P ratio was used. Results from Gutierrez et al. (2015) indicated that, although pigs began excreting more P in the urine after the requirement for growth was met (at 4.96 g/d STTD P intake), femur mineral content continued to increase as dietary P and Ca increased, suggesting that pigs have a greater requirement for bone development than for growth. In other words, while 0.31% STTD P may be the amount required to maximize growth (NRC, 2012), it may not necessarily be the amount required to maximize bone development and mineral retention. Stein et al. (2008) observed a similar response for retained P supplied as MCP in the diet and Ca:P ratios remained constant, suggesting that pigs have a greater capacity to retain P in the body than what is necessary for growth. Dietary Ca was also made more available by phytase in the current experiment; ATTD Ca improved from 48.4 in the basal P-adequate diet to 60.6% with 800 FTU phytase/kg. All additional available Ca from phytase appeared to be retained in the body with no increase in urinary Ca excretion. Thus, while urinary excretion of P was increased due to phytase, some of the excess P made available by phytase was also retained in the body with a simultaneous increase in Ca retention.

Urine P excretion increased as expected due to phytase addition in the P-adequate diets, confirming that the diets were, in fact, above P requirement (Gutierrez et al., 2015). Urine Ca

excretion was constant as P absorption increased, and urine Ca was elevated when pigs were fed the P-deficient diet, which also indicates the study design was achieved (Gutierrez et al., 2015). Because retained P, as well as urinary P, increased when phytase was added to the diet, it was determined that urinary P alone could not be used to quantify the release of P by phytase in this scenario. Therefore, the increase in calculated STTD of P was used to estimate the quantity of P released by phytase (Table 5). The present estimates for STTD P release are lower than those previously estimated using diets deficient in P, based on expected values for release provided by the manufacturer, which were 0.08, 0.12, 0.14, and 0.16% for 200, 400, 600, and 800 FTU phytase/kg.

When 200 FTU phytase/kg was added to the P-deficient diet, the calculated STTD of P improved by 28%, but only by 12% when added to the P-adequate diet. This indicates that P release may be lowered when phytase is used in P-adequate diets compared to P-deficient diets, and is in agreement with previous reports (Fan et al., 2005; Almeida et al., 2013; Rodehutscord, 2016). The P-deficient diet in this study was formulated at 68% of NRC requirement, whereas many studies evaluating phytase use basal diets with P as low as 20-40% of requirement (NRC, 1998; Augspurger et al., 2003; Jones et al., 2010; Kerr et al., 2010; Gourley et al., 2018). Therefore, this discrepancy in P release may be exaggerated in diets that are even lower in P than the diets used in the present study.

Several hypotheses for decreased phytase efficacy in adequate or high-P diets have been proposed. To meet the P requirement, MCP or dicalcium phosphate is often added. Because inorganic P is the final hydrolysis product of phytase, it is possible that inorganic P inhibits phytase activity (Greiner et al., 1993; Rodehutscord, 2016). Alternatively, pigs may respond to P-deficiency by increasing the efficiency of P-uptake in the intestine (Berndt and Kumar, 2009;

Saddoris et al., 2010), or by increasing the mobilization of P from bone (Cromwell, 2005). Additionally, a buffering effect from increased Ca, usually present in the form of limestone or MCP in P-adequate diets, has been proposed as a reason for reduced the efficacy of phytase in P-adequate diets (Almeida et al., 2013). It also seems likely that the pH in the gastrointestinal tract influences the action of phytase (Selle et al., 2000). The difference in STTD P release from 200 FTU phytase/kg added to the P-adequate compared to P-deficient diet in this study was not large, but nonetheless important if diets are formulated very close to requirement.

As the substrate for phytase, phytate-P must be present in adequate quantities for phytase to be effective in improving P availability. Phytate-P should not have been a limiting factor in this study (Dersjant-Li et al., 2015; Zeng et al., 2016), although it is recognized that phytase efficacy is likely a function of dietary phytate content (Selle and Ravindran, 2008). It is also believed that a high dietary Ca:P ratio negatively impacts phytase efficacy (Selle et al., 2000; Beaulieu et al., 2007). The Ca:P ratios used in the present study (approximately 2:1 for Ca: STTD P) were likely not wide enough to cause decreases in phytase efficacy (Beaulieu et al., 2007; González-Vega et al., 2016). Furthermore, the issue of a wide Ca:P seems to be less critical when diets are at or above P-requirements (Wu et al., 2018). However, this does not necessarily rule out the potential for greater total Ca, regardless of the ratio, to inhibit phytase as previously discussed.

The formation of Ca-phytate complexes in the pig's gastrointestinal tract reduces the availability of Ca, and roughly one third of dietary Ca may be present in these complexes (Selle et al., 2009). Improvements in the ATTD of Ca due to phytase are therefore expected and have been observed in many other experiments (González-Vega et al., 2013; González-Vega et al., 2015; Zeng et al., 2016; Blavi et al., 2017). Using ATTD of Ca as the response variable, the

results predicted 0.025% Ca release for every 200 FTU phytase/kg added to the P-adequate diet. This value corresponded to similar improvements in Ca digestibility reported by Selle et al., (2009) and González-Vega et al., (2015). One phytate molecule may bind up to 5 or 6 Ca molecules (Selle et al., 2009), and most phytases are assumed to release Ca and P in a ratio of 1:1 or greater because IP6 and IP5 have greater affinities for Ca than IP4 or IP3 (Cowieson et al., 2011).

Phytate can also bind and decrease the availability of other nutrients including amino acids, starch (either through hydrogen bonds with the phosphate group or binding starch-associated proteins (Thompson, 1993)) and fatty acids (Angel et al., 2002; Johnston et al., 2004; Bohlke et al., 2005). Thus, it is not surprising to see improvements in the ATTD of GE and N due to phytase addition (Selle et al., 2000; Adeola and Cowieson, 2011; Almeida et al., 2013; Zouaoui et al., 2018). Improvements in energy utilization as reviewed by Adeola and Cowieson (2011) are variable, and most of the mechanistic effects have been observed in poultry, rather than swine. It has been suggested that phytate decreases fat digestibility, and that phytase should mitigate this effect (Camden et al., 2001; Selle et al., 2003; Vigors et al., 2014). The present results agree with this theory, as does research reported by Jang et al. (2017). Although data from Almeida et al. (2013) suggest phytase only improves N and GE digestibility when P-deficient diets are used, the current data suggest that phytase can improve digestibility of N and GE in P-adequate diets as well.

Inclusion of microbial phytase, which can hydrolyze phytate and release inorganic P, has become common practice in commercial swine diets. To effectively formulate diets with phytase, precise estimates for P release are necessary. In conclusion, while phytase is clearly a useful tool for improving digestibility of P, Ca, and other nutrients, most estimates for P release by phytase

have been obtained from experiments in which pigs are fed P-deficient diets. The results of this study indicate P-release may be lower when pigs are fed a P-adequate diet. Thus, in commercial conditions where phytase is added to diets adequate or marginally deficient in P, it is possible that P-release estimates are not entirely accurate, and could result in inadequate quantities of P being fed. This combined with the fact that current P requirements likely do not maximize mineral retention may have further implications for developing gilts; it is possible that slight underfeeding of P and sub-optimal bone development may be occurring. Further studies should be done to confirm lower P release by phytase in P-adequate diets, or diets that are marginally deficient in P. Ideally, additional studies should be done with multiple phytase sources and levels, and with pigs at different stages of growth.

LITERATURE CITED

Adeola, O. and A. J. Cowieson. 2011. Board invited reivew: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218. doi:10.2527/jas.2010-3715.

Almeida, F. N., A. A. Pahm, G. I. Petersen, N. R. Augspurger, and H. H. Stein. 2013. Effects of phytase on amino acid and energy digestibility in corn–soybean meal diets fed to growing pigs. Prof. Anim. Sci. 29:693–700. doi:10.15232/S1080-7446(15)30302-8.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471–480. doi:10.1093/japr/11.4.471.

AOAC. 2007. Official methods of analysis of AOAC International. 18th ed. AOAC

International, Gaithersburg, MD.

Augspurger, N. R., D. M. Webel, X. G. Lei, and D. H. Baker. 2003. Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. J. Anim. Sci. 81:474–483. doi:10.2527/2003.812474x.

Beaulieu, A. D., M. R. Bedford, and J. F. Patience. 2007. Supplementing corn or corn-barley diets with an *E. coli* derived phytase decreases total and soluble P output by weanling and growing pigs. Can. J. Anim. Sci. 87.

Berndt, T., and R. Kumar. 2009. Novel mechanisms in the regulation of phosphorus homeostasis. Physiol. 24:17–25. doi:10.1152/physiol.00034.2008.

Blavi, L., D. Sola-Oriol, J. F. Perez, and H. H. Stein. 2017. Effects of zinc oxide and microbial phytase on digestibility of calcium and phosphorus in maize-based diets fed to growing pigs. J. Anim. Sci. 95:847–854. doi:10.2527/jas2016.1149.

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396–2403.

Breves, G., and B. Schröder. 1991. Comparative aspects of gastrointestinal phosphorus metabolism. Nutr. Res. Rev. 4:125–140. doi:10.1079/NRR19910011.

Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. Anim. Sci. 73:289–297. doi:10.1017/S1357729800058264.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. Worlds. Poult. Sci. J. 67:225–236.

doi:10.1017/S0043933911000250.

Cromwell, G. L. 2005. Phosphorus and swine nutrition. In: A. N. Sims, J. Thomas, Sharpley, editor. Phosphorus: agriculture and the environment. Madison, WS. p. 607–631.

Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: A critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95:878–896. doi:10.1002/jsfa.6998.

Fan, M. Z., T. J. Li, Y. L. Yin, Z. Y. Tang, Z. P. Hou, R. L. Huang, Z. Y. Deng, H. Y. Zhong, R. G. Zhang, J. Zhang, B. Wang, and H. Schulze. 2005. Effect of phytase supplementation with two levels of phosphorus diets on ileal and faecal digestibilities of nutrients and phosphorus, calcium, nitrogen, and energy balances in growing pigs. Anim. Sci. 81:67–75.

Fraser, D., J. F. Patience, P. A. Phillips, and J. M. McLeese. 1993. Water for piglets and lactating sows: quantity, quality, and quandries. In: P. C. Garnsworthy, D. J. A. Cole, and W. Haresign, editors. Recent developments in pig nutrition. 2nd ed. Nottingham University Press, Loughborough, Leicestershire. p. 201–224.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807–4816. doi:10.2527/jas.2013-6410.

González-Vega, J. C., C. L. Walk, M. R. Murphy, and H. H. Stein. 2016. Requirement for digestible calcium by 25 to 50 kg pigs at different dietary concentrations of phosphorus as indicated by growth performance, bone ash concentration, and calcium and phosphorus balances. J. Anim. Sci. 94:5272–5285. doi:10.2527/jas2016-0751.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015. 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. doi:10.2527/jas2014-8215.

Gourley, K. M., J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, and R. D. Goodband. 2018. Determining the available phosphorus release of Natuphos E 5,000 G phytase for nursery pigs. J. Anim. Sci. 96:1101–1107. doi:10.1093/jas/sky006.

Greiner, R., U. Konietzny, and K. D. Jany. 1993. Purification and characterization of two phytases from *Escherichia coli*. Arch. Biochem. Biophys. 303:107–113. doi:10.1006/abbi.1993.1261.

Gutierrez, N. A., N. V. L. Serão, A. J. Elsbernd, S. L. Hansen, C. L. Walk, M. R. Bedford, and J. F. Patience. 2015. Quantitative relationships between standardized total tract digestible phosphorus and total calcium intakes and their retention and excretion in growing pigs fed corn-soybean meal diets. J. Anim. Sci. 93:2174–2182. doi:10.2527/jas2014-8623.

Jang, Y. D., P. Wilcock, R. D. Boyd, and M. D. Lindemann. 2017. Effect of combined xylanase and phytase on growth performance, apparent total tract digestibility, and carcass characteristics in growing pigs fed corn-based diets containing high-fiber coproducts. J. Anim. Sci. 95:4005–4017. doi:10.2527/jas.2017.1781.

Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews, and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. J. Anim. Sci. 82:705–714.

Jones, C. K., M. D. Tokach, S. S. Dritz, B. W. Ratliff, N. L. Horn, R. D. Goodband, J. M. DeRouchey, R. C. Sulabo, and J. L. Nelssen. 2010. Efficacy of different commercial phytase enzymes and development of an available phosphorus release curve for

Escherichia coli-derived phytases in nursery pigs. J. Anim. Sci. 88:3631–3644. doi:10.2527/jas.2010-2936.

Kerr, B. J., T. E. Weber, P. S. Miller, and L. L. Southern. 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. doi:10.2527/jas.2009-2146.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535–537.

Letourneau-Montminy, M. P., C. Jondreville, D. Sauvant, and A. Narcy. 2012. Meta-analysis of phosphorus utilization by growing pigs : effect of dietary phosphorus , calcium and exogenous phytase. Anim. 6:1590–1600. doi:10.1017/S1751731112000560.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. National Academey Press, Washington, DC.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. National Academy Press, Washington, D.C.

Oresanya, T. F., A. D. Beaulieu, and J. F. Patience. 2008. Investigations of energy metabolism in weanling barrows: The interaction of dietary energy concentration and daily feed (energy) intake. J. Anim. Sci. 86:348–363. doi:10.2527/jas.2007-0009.

Pogge, D. J., M. E. Drewnoski, and S. L. Hansen. 2014. High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. J. Anim. Sci. 92:2182–2191. doi:10.2527/jas2013-7481.

Richter, E. L., M. E. Drewnoski, and S. L. Hansen. 2012. Effects of increased dietary sulfur on beef steer mineral status, performance, and meat fatty acid composition. J. Anim. Sci. 90:3945–3953. doi:10.2527/jas2011-4512.

Rodehutscord, M. 2016. Interactions between minerals and phytate degradation in poultry – challenges for phosphorus digestibility assays. In: C. L. Walk, I. Kuhn, H. H. Stein, M. T. Kidd, M. Rodehutscord, editors, Phytate destruction – consequences for precision animal nutrition. Wageningen Academic Publishers, The Netherlands. p.167-177.

Saddoris, K. L., J. C. Fleet, and J. S. Radcliffe. 2010. Sodium-dependent phosphate uptake in the jejunum is post-transcriptionally regulated in pigs fed a low-phosphorus diet and is independent of dietary calcium concentration. J. Nutr. 140:731–736. doi:10.3945/jn.109.110080.

Selle, P. H., A. J. Cowieson, N. P. Cowieson, and V. Ravindran. 2012. Protein-phytate interactions in pig and poultry nutrition: a reappraisal. Nutr. Res. Rev. 25:1–17. doi:10.1017/S0954422411000151.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124:126–141. doi:10.1016/j.livsci.2009.01.006.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99–122. doi:10.1016/j.livsci.2007.05.014.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo, and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilisation of broilers offered wheat-based diets. Asian-Australasian J. Anim. Sci. 16:394–402. doi:10.5713/ajas.2003.394.

Selle, P. H., V. Ravindran, A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13:255–278. doi:10.1079/095442200108729098.

Shaw, M. I., A. D. Beaulieu, and J. F. Patience. 2006. Effect of diet composition on water consumption in growing pigs. J. Anim. Sci. 84:3123–3132. doi:10.2527/jas.2005-690.

Stein, H. H., C. T. Kadzere, S. W. Kim, and P. S. Miller. 2008. Influence of dietary phosphorus concentration on the digestibility of phosphorus in monocalcium phosphate by growing pigs. J. Anim. Sci. 86:1861–1867. doi:10.2527/jas.2008-0867.

Tancharoenrat, P., and V. Ravindran. 2014. Influence of tallow and calcium concentrations on the performance and energy and nutrient utilization in broiler starters. Poult. Sci. 93:1453–1462. doi:10.3382/ps.2013-03817.

Thompson, L. U. 1993. Potential health benefits and problems associated with antinutrients in foods. Food Res. Int. 26:131–149. doi:10.1016/0963-9969(93)90069-U.

Vigors, S., T. Sweeney, C. J. O'Shea, J. A. Browne, and J. V. O'Doherty. 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. Br. J. Nutr. 112:688–697. doi:10.1017/S0007114514001494.

Wu, F., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, M. A. D. Goncalves, and J. R. Bergstrom. 2018. Effects of dietary calcium to phosphorus ratio and addition of phytase on growth performance of nursery pigs. J. Anim. Sci. doi:10.1093/infdis/jiy023/4808591.

Zeng, Z. K., Q. Y. Li, P. F. Zhao, X. Xu, Q. Y. Tian, H. L. Wang, L. Pan, S. Yu, and X. S. Piao. 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. doi:10.2527/jas2015-9143.

Zouaoui, M., M. P. Létourneau-Montminy, and F. Guay. 2018. Effect of phytase on amino acid

digestibility in pig: a meta-analysis. Anim. Feed Sci. Technol. 238:18–28. doi:10.1016/j.anifeedsci.2018.01.019.

Table 1. Ingredient and nutrient composition of experimental diets (as-fed basis)

	Dietary Treatment		
	A[1]	PC	D[2]
Ingredient, %			
Corn	72.38	71.88	73.56
Soybean meal (47.5% CP)	23.20	23.20	23.20
Soybean oil	0.80	0.80	0.80
Limestone	0.92	0.51	0.60
Monocalcium phosphate	1.12	2.02	0.26
L-Lys HCl	0.30	0.30	0.30
DL-Met	0.06	0.06	0.06
L-Thr	0.07	0.07	0.07
Vitamin Premix[3]	0.20	0.20	0.20
Mineral Premix[4]	0.15	0.15	0.15
Titanium dioxide	0.40	0.40	0.40
NaCl	0.40	0.40	0.40
Calculated nutrients, %			
STTD P	0.36	0.52	0.21
Total P	0.63	0.81	0.46
Total Ca	0.66	0.66	0.38
Ca: STTD P	1.83	1.27	1.83
NE, Mcal/kg	2.49	2.47	2.52
SID Lys	0.98	0.98	0.98
SID Met	0.30	0.30	0.30
SID TSAA	0.50	0.50	0.50
SID Thr	0.59	0.59	0.59
SID Trp	0.17	0.17	0.17

[1]There were 5 diets containing 0.36% standardized total tract digestible (STTD) P. Phytase was added at the expense of corn in the following amounts: 0, 200, 400, 600, 800 phytase units (FTU) phytase/kg (Quantum Blue, AB Vista, Marlborough, U.K.; treatments A, A200, A400, A600, A800, respectively).

[2]There were 2 diets containing 0.21% STTD P (treatments D and D200). Phytase was added at the expense of corn at 0 or 200 FTU/kg phytase (Quantum Blue, AB Vista, Marlborough, U.K.).

[3]Premix provided per kg of complete diet: 6,125 IU vitamin A, 700 IU vitamin D3, 50 IU vitamin E, 3 mg vitamin K, 11 mg riboflavin, 56 mg niacin, 27 mg pantothenic acid, 24 mg vitamin B_{12}.

[4]Premix provided per kg of complete diet: 165 mg Fe (ferrous sulfate), 165 mg Zn (zinc sulfate), 39 mg Mn (manganese sulfate), 16.5 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), 0.3 mg Se (sodium selenite).

Table 2. Analyzed nutrient composition of experimental diets (as-fed basis)

	Dietary Treatment[1]							
	A	A200	A400	A600	A800	PC	D	D200
Nutrient, %								
DM	88.68	88.40	88.51	88.57	88.60	88.40	88.42	88.29
GE, Mcal/kg	3.91	3.92	3.95	3.93	3.93	3.93	3.99	4.01
CP	15.26	15.64	16.15	15.95	14.97	15.71	16.02	16.08
AEE[2]	4.16	4.14	4.12	4.20	4.06	4.09	4.04	4.30
Ash	4.66	4.84	4.86	4.79	4.81	5.22	4.00	3.94
Total Ca[3]	0.73	0.73	0.73	0.73	0.73	0.73	0.45	0.45
Total P[3]	0.63	0.63	0.63	0.63	0.63	0.80	0.46	0.46
Phytate-P	0.23	0.24	0.24	0.23	0.23	0.24	0.24	0.23

[1]There were a total of 8 dietary treatments. Diets 1-5 were P-adequate diets: (0.36 % standardized total tract digestible (STTD) P) with 0 (A), 200 (A200), 400 (A400), 600 (A600), or 800 (A800) phytase units (FTU)/kg phytase (Quantum Blue, AB Vista, Marlborough, U.K.), respectively. A sixth diet (positive control; PC) was formulated with increased monocalcium phosphate to reach 0.52% STTD P. Diets 7 and 8 were P-deficient diets (0.21% STTD P) with 0 (D) or 200 FTU/kg phytase (D200), respectively. Phytase was added at the expense of corn.
[2]AEE=Acid hydrolyzed ether extract.
[3]Calculated values based on analysis of ingredients for Ca and P.

Table 3. Analyzed total P and Ca content of ingredients[1] (as-fed basis)

Ingredient	P, %	Ca, %
Corn	0.31	0.01
Soybean meal	0.78	0.50
Limestone	-	38.90
Monocalcium phosphate	19.80	17.80
Vitamin premix	0.06	19.70
Mineral premix	0.06	7.60

[1] Samples were analyzed for P and Ca by inductively coupled plasma optical emission spectrometry (ICP; Optima 7000 DV; PerkinElmer, Waltham, MA) as described by Pogge et al. (2014).

Table 4. Effect of dietary treatment on pig BW and growth performance during *ad libitum* feeding period

	Dietary treatments[1]										
Diet STTD P, %	Adequate, 0.36					0.52	Deficient, 0.21				
	A	A200	A400	A600	A800	PC	D	D200	SEM	TRT P[2]	PHY[3]
d0 BW, kg	23.49	22.51	22.38	23.31	23.11	23.04	22.64	23.09	0.430	0.5592	0.2784
d21 BW, kg	39.27	39.27	37.53	40.02	38.64	38.22	36.60	37.60	0.792	0.0667	0.2523
d30 BW, kg	44.71^{ab}	45.80^{b}	43.42^{ab}	46.27^{b}	44.64^{ab}	44.20^{ab}	41.89^{a}	43.31^{ab}	0.838	0.0124	0.1491
d0-12 ADG, kg[4]	0.75^{ab}	0.80^{b}	0.72^{ab}	0.80^{b}	0.74^{ab}	0.72^{ab}	0.66^{a}	0.71^{ab}	0.020	0.0005	0.0338
d0-19 ADFI, kg[4]	1.63^{ab}	1.67^{ab}	1.54^{ab}	1.76^{b}	1.64^{ab}	1.56^{ab}	1.47^{a}	1.59^{ab}	0.056	0.0274	0.1124

[1] There were a total of 8 dietary treatments. Diets 1-5 were P-adequate diets: (0.36 % STTD P) with 0 (A), 200 (A200), 400 (A400), 600 (A600), or 800 (A800) FTU/kg phytase (Quantum Blue, AB Vista, Marlborough, U.K.), respectively. A sixth diet (positive control; PC) was formulated with increased monocalcium phosphate to reach 0.52% STTD P. Diets 7 and 8 were P-deficient diets (0.21% STTD P) with 0 (D) or 200 (D200) FTU/kg phytase, respectively. Phytase was added at the expense of corn.

[2] *P*-value for overall effect of dietary treatment

[3] Orthogonal polynomial contrasts were performed to test the effect of phytase level in the 5 P-adequate diets (0.36% STTD P). PHY represents the overall *P*-value for effect of phytase in these diets.

[4] Pigs were limit fed starting on d19 but were not weighed until d21. Therefore, ADG was calculated from d21, and ADFI was calculated from d19.

[a,b] Means with the same superscript are statistically the same across all 8 treatments ($P > 0.05$) based on Tukey's Method.

Table 5. Least square means for the effect of dietary treatment on digestibility (ATTD) and balance of P in growing pigs

	Dietary treatments[1]												
Diet STTD P, %	Adequate, 0.36					0.52	Deficient, 0.21			*P*-value			
	A	A200	A400	A600	A800	PC	D	D200	SEM	TRT[2]	PHY[3]	Lin[3]	Quad[3]
Feed intake (as fed), g/d	1,491	1,511	1,463	1,484	1,508	1,488	1,426	1,493	0.021	0.110	-	-	-
P intake, g/d	9.45^{b}	9.58^{b}	9.27^{b}	9.40^{b}	9.56^{b}	12.05^{c}	6.66^{a}	6.97^{a}	0.121	<0.001	-	-	-
ATTD, %	46.42^{b}	52.53^{c}	60.12^{d}	60.47^{d}	60.40^{d}	60.51^{d}	41.21^{a}	53.77^{c}	1.12	<0.001	<0.001	<0.001	0.001
STTD[4], %	49.94^{a}	56.08^{b}	63.58^{cd}	63.97^{d}	63.96^{d}	63.25^{cd}	45.75^{a}	58.56^{bc}	1.15	<0.001	<0.001	<0.001	0.001
Absorbed, g/d	4.40^{c}	5.04^{cd}	5.43^{de}	5.70^{de}	5.79^{e}	7.29^{f}	2.75^{a}	3.57^{b}	0.156	<0.001	<0.001	<0.001	0.069
Retained, g/d	4.20^{bc}	4.69^{cd}	4.95^{cd}	5.04^{d}	5.03^{d}	5.08^{d}	2.73^{a}	3.55^{b}	0.167	<0.001	0.004	0.001	0.094
Urine, g/d	0.19^{ab}	0.35^{bc}	0.48^{cd}	0.66^{de}	0.76^{e}	2.28^{f}	0.02^{a}	0.03^{a}	0.055	<0.001	<0.0001	<0.001	0.782
Fecal, g/d	5.05^{a}	4.54^{a}	3.84^{b}	3.70^{b}	3.77^{b}	4.76^{a}	3.90^{b}	3.40^{b}	0.116	<0.001	<0.001	<0.001	0.001
Total excreted, g/d	5.25^{b}	4.89^{cb}	4.32^{de}	4.36^{cde}	4.53^{cd}	6.97^{a}	3.93^{e}	3.26^{f}	0.132	<0.001	<0.001	<0.001	0.001
Retention, % of absorbed	95.67^{de}	93.13^{cd}	90.83^{bcd}	88.44^{cb}	86.62^{b}	69.76^{a}	99.19^{e}	99.08^{e}	1.093	<0.001	<0.001	<0.001	0.744
Retention, % of intake	44.35^{bc}	48.90^{cd}	53.07^{c}	53.51^{c}	52.41^{c}	42.21^{a}	40.87^{a}	53.47^{c}	1.56	<0.001	0.007	<0.001	0.009

[1] There were a total of 8 dietary treatments. Diets 1-5 were P-adequate diets: (0.36 % standardized total tract digestible (STTD) P and 0.73% total Ca) with 0 (A), 200 (A200), 400 (A400), 600 (A600), or 800 (A800) phytase units (FTU)/kg (Quantum Blue, AB Vista, Marlborough, U.K.), respectively. A sixth diet (positive control; PC) was formulated with increased monocalcium phosphate to reach 0.52% STTD P and 0.73% total Ca. Diets 7 and 8 were P-deficient diets (0.21% STTD P and 0.45% total Ca) with 0 (D) or 200 (D200) FTU/kg, respectively. Phytase was added at the expense of corn.

[2] *P*-value for overall effect of dietary treatment.

[3] Orthogonal polynomial contrasts were performed to test the effect of phytase level in the 5 P-adequate diets (0.36% STTD P). PHY represents the overall *P*-value for effect of phytase in these diets. Lin and Quad represent the *P*-values for the linear and quadratic effects of phytase level. Resulting regression equations for selected traits are as follows: ATTD of P, % = 46.16 + 0.0458 × (x) – 0.000035 × (x^2), R^2=0.60; STTD of P, % = 49.54 + 0.0457 × (x) – 0.000035 × (x^2), R^2=0.59; Urine P, g/d = 0.20 + 0.0007 × (x), R^2=0.48; Retained P, g/d = 4.38 + 0.0009 × (x), R^2=0.15; where x = phytase units (FTU/kg).

[4] STTD P was calculated assuming 190 mg endogenous P losses/kg DMI based on NRC (2012).

[a-f] Means with the same superscript are statistically the same across all 8 treatments ($P > 0.05$) based on Tukey's Method.

Table 6. Least square means for the effect of dietary treatment on apparent total tract digestibility (ATTD) and balance of Ca in growing pigs

	Dietary treatments[1]												
Diet STTD P, %	Adequate, 0.36					0.52	Deficient, 0.21			*P* - value			
	A	A200	A400	A600	A800	PC	D	D200	SEM	TRT P[2]	PHY[3]	Lin[3]	Quad[3]
Ca intake, g/d	10.71[b]	10.86[b]	10.52[b]	10.66[b]	10.82[b]	10.70[b]	6.30[a]	6.60[a]	0.124	0.001	-	-	-
ATTD, %	48.36[a]	52.93[ab]	59.89[bc]	58.87[bc]	60.64[bc]	59.57[bc]	58.64[bc]	66.53[c]	2.195	<0.001	0.001	<0.001	0.110
Absorbed, g/d	5.18[bc]	5.76[cd]	6.39[d]	6.32[cd]	6.57[d]	6.37[d]	3.70[a]	4.41[ab]	0.238	<0.001	0.001	<0.001	0.139
Retained, g/d	4.87[c]	5.57[cd]	6.24[d]	6.12[d]	6.43[d]	6.22[d]	2.33[a]	3.62[b]	0.225	<0.001	<0.001	<0.001	0.072
Urinary, g/d	0.30[a]	0.19[a]	0.14[a]	0.20[a]	0.14[a]	0.15[a]	1.37[c]	0.78[b]	0.048	<0.001	0.535	0.197	0.440
Fecal, g/d	5.53[a]	5.10[ba]	4.46[b]	4.34[b]	4.26[b]	4.33[b]	2.60[ca]	2.19[ca]	0.231	<0.001	0.001	<0.001	0.165
Total excreted, g/d	5.84[a]	5.28[ba]	4.61[bc]	4.54[bc]	4.40[bc]	4.48[bc]	3.96[dc]	2.97[d]	0.224	<0.001	<0.001	<0.001	0.094

[1] There were a total of 8 dietary treatments. Diets 1-5 were P-adequate diets: (0.36 % standardized total tract digestible (STTD) P and 0.73% total Ca) with 0 (A), 200 (A200), 400 (A400), 600 (A600), or 800 (A800) phytase units (FTU)/kg (Quantum Blue, AB Vista, Marlborough, U.K.), respectively. A sixth diet (positive control; PC) was formulated with increased monocalcium phosphate to reach 0.52% STTD P and 0.73% total Ca. Diets 7 and 8 were P-deficient diets (0.21% STTD P and 0.45% total Ca) with 0 (D) or 200 (D200) FTU/kg, respectively. Phytase was added at the expense of corn.

[2] *P*-value for overall effect of dietary treatment.

[3] Orthogonal polynomial contrasts were performed to test the effect of phytase level in the 5 P-adequate diets (0.36% STTD P). PHY represents the overall *P*-value for effect of phytase in these diets. Lin and Quad represent the *P*-values for the linear and quadratic effects of phytase level. Resulting regression equations for selected traits are as follows: ATTD of Ca, % = 50.02 + 0.01524 × (x), R^2=0.21; Retained Ca, g/d = 5.05 + 0.00183 × (x), R^2=0.23; where x = phytase units (FTU/kg).

[a-d] Means with the same superscript are statistically the same across all 8 treatments ($P > 0.05$) based on Tukey's Method.

Table 7. Effect of dietary treatment on apparent total tract nutrient digestibility (ATTD)

	Dietary treatments[1]												
Diet STTD P, %	Adequate, 0.36					0.52	Deficient, 0.21			*P* - value			
	A	A200	A400	A600	A800	PC	D	D200	SEM	TRT P[2]	PHY[3]	Lin[3]	Quad[3]
DM, %	85.00[a]	86.07[ab]	87.13[b]	86.71[b]	86.89[b]	87.33[b]	86.22[ab]	87.19[a]	0.356	<0.001	<0.001	<0.001	0.011
GE, %	84.69[a]	85.60[ab]	86.52[b]	86.05[ab]	86.14[ab]	87.34[b]	85.72[ab]	86.45[ab]	0.389	<0.001	0.004	0.003	0.019
N, %	82.07[a]	84.85[ab]	85.55[ab]	84.89[ab]	85.24[ab]	86.54[b]	84.19[ab]	84.37[ab]	0.856	0.032	0.038	0.018	0.046
Ash, %	42.17[a]	49.40[b]	56.98[d]	53.95[cd]	55.19[d]	54.16[d]	50.54[bc]	55.19[d]	0.846	<0.001	<0.001	<0.001	<0.001
AEE[4], %	50.92[a]	51.30[a]	51.66[a]	54.19[ab]	51.05[a]	59.30[c]	53.00[a]	57.58[bc]	0.934	<0.001	0.060	0.257	0.147

[1] There were a total of 8 dietary treatments. Diets 1-5 were P-adequate diets: (0.36 % standardized total tract digestible (STTD) P and 0.73% total Ca) with 0 (A), 200 (A200), 400 (A400), 600 (A600), or 800 (A800) phytase units (FTU)/kg (Quantum Blue, AB Vista, Marlborough, U.K.), respectively. A sixth diet (positive control; PC) was formulated with increased monocalcium phosphate to reach 0.52% STTD P and 0.73% total Ca. Diets 7 and 8 were P-deficient diets (0.21% STTD P and 0.45% total Ca) with 0 (D) or 200 (D200) FTU/kg phytase, respectively. Phytase was added at the expense of corn.

[2] *P*-value for overall effect of dietary treatment.

[3] Orthogonal polynomial contrasts were performed to test the effect of phytase level in the 5 P-adequate diets (0.36% STTD P). PHY represents the overall *P*-value for effect of phytase in these diets.

[4] AEE = Acid hydrolyzed ether extract.

[a-d] Means with the same superscript are statistically the same across all 8 treatments ($P > 0.05$) based on Tukey's Method.