

8-2019

Insoluble dietary fiber does not affect the ability of phytase to release phosphorus from phytate in the diet of nursery pigs

Jesus A. Acosta
Iowa State University

John F. Patience
Iowa State University, jfp@iastate.edu

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/812. 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.

Insoluble dietary fiber does not affect the ability of phytase to release phosphorus from phytate in the diet of nursery pigs

Abstract

Phytase is added to swine diets to improve the utilization of phytate-bound P. This provides financial and environmental benefits to the pig industry. It is unclear if phytase works equally well under all dietary circumstances. The objective was to determine if insoluble fiber impacts the efficacy of the phytase enzyme in nursery pigs when fed diets limiting in P content. A total of 480 pigs (initial BW 5.48 ± 0.14 kg) were blocked by BW and randomly assigned to treatment within block. A common nutrient-adequate diet was fed from d -14 to -5, and 2 basal P deficient diets (either a corn-soy diet containing 0.16% standardized total tract digestible [STTD] P [LF], or a corn-soybean meal plus 20% corn bran containing 0.14% STTD P [HF]) were fed from d -5 to 0 to acclimate pigs to a P deficient diet. From d 0-21, pigs received 8 dietary treatments (6 pens per treatment: n=6). Experimental diets consisted of LF supplemented with one of 4 levels of added phytase (0, 109, 218, and 327 phytase units [FTU]/kg; Quantum Blue 5 G, AB Vista, Wiltshire, UK) expected to provide 0.16, 0.21, 0.26, and 0.31% STTD P, respectively, or HF supplemented with one of the same 4 levels of added phytase. Titanium dioxide was added to the diet at 0.4% as an indigestible marker. On d 21, one pig representing the average BW for each pen was euthanized, and fibulae were collected and analyzed for bone ash. Fecal samples were collected from each pen on d 19-20. Data were analyzed using PROC MIXED of SAS. There were no interactions between insoluble fiber and phytase for any of the variables evaluated. For d 0 - 21, adding phytase increased ADG ($P < 0.001$) with the response being linear ($P < 0.001$), whereas insoluble fiber decreased ADG ($P = 0.033$). There were no effects of phytase or insoluble fiber on ADFI ($P = 0.381$ and $P = 0.632$, respectively). Phytase improved G:F ratio ($P < 0.001$) with the response being linear ($P < 0.001$). Insoluble fiber tended to decrease G:F ratio ($P = 0.097$). Phytase increased bone ash ($P = 0.005$) with the response being linear ($P = 0.001$), but there was no effect of insoluble fiber ($P = 0.949$). Phytase did not affect the apparent total tract digestibility of DM, NDF, or ADF ($P > 0.050$), whereas insoluble fiber decreased the ATTD of DM ($P < 0.001$), NDF ($P < 0.001$), and ADF ($P < 0.001$). In conclusion, the addition of insoluble fiber did not affect the ability of phytase to improve growth performance and bone mineralization in nursery pigs fed a P deficient diet.

Keywords

corn bran, insoluble fiber, swine, bone ash, digestibility

Disciplines

Agriculture | Animal Experimentation and Research | Animal Sciences

Comments

This is a manuscript of an article published as Acosta, Jesus A., and John F. Patience. "Insoluble dietary fiber does not affect the ability of phytase to release phosphorus from phytate in the diet of nursery pigs." *Journal of animal science* 97, no. 8 (2019): 3451-3459. doi:[10.1093/jas/skz194](https://doi.org/10.1093/jas/skz194). Posted with permission.

Running head: Insoluble fiber and phytase efficacy in pigs

**Insoluble dietary fiber does not affect the ability of phytase to release phosphorus from
phytate in the diet of nursery pigs¹**

Jesus A. Acosta* and John F. Patience^{*2}

*Iowa State University, Department of Animal Science, Ames, IA, 50011

¹The authors thank the Iowa Pork Producers Association for financial support of this experiment. Appreciation is also expressed to AB Vista, Iowa Corn Processors, DSM and Ajinomoto Heartland Inc., for in-kind contributions.

²Corresponding author: jfp@iastate.edu

ABSTRACT: Phytase is added to swine diets to improve the utilization of phytate-bound P. This provides financial and environmental benefits to the pig industry. It is unclear if phytase works equally well under all dietary circumstances. The objective was to determine if insoluble fiber impacts the efficacy of the phytase enzyme in nursery pigs when fed diets limiting in P content. A total of 480 pigs (initial BW 5.48 ± 0.14 kg) were blocked by BW and randomly assigned to treatment within block. A common nutrient-adequate diet was fed from d -14 to -5, and 2 basal P deficient diets (either a corn-soy diet containing 0.16% standardized total tract digestible [STTD] P [LF], or a corn-soybean meal plus 20% corn bran containing 0.14% STTD P [HF]) were fed from d -5 to 0 to acclimate pigs to a P deficient diet. From d 0-21, pigs received 8 dietary treatments (6 pens per treatment: $n=6$). Experimental diets consisted of LF supplemented with one of 4 levels of added phytase (0, 109, 218, and 327 phytase units [FTU]/kg; Quantum Blue 5 G, AB Vista, Wiltshire, UK) expected to provide 0.16, 0.21, 0.26, and 0.31% STTD P, respectively, or HF supplemented with one of the same 4 levels of added phytase. Titanium dioxide was added to the diet at 0.4% as an indigestible marker. On d 21, one pig representing the average BW for each pen was euthanized, and fibulae were collected and analyzed for bone ash. Fecal samples were collected from each pen on d 19-20. Data were analyzed using PROC MIXED of SAS. There were no interactions between insoluble fiber and phytase for any of the variables evaluated. For d 0 - 21, adding phytase increased ADG ($P < 0.001$) with the response being linear ($P < 0.001$), whereas insoluble fiber decreased ADG ($P = 0.033$). There were no effects of phytase or insoluble fiber on ADFI ($P = 0.381$ and $P = 0.632$, respectively). Phytase improved G:F ratio ($P < 0.001$) with the response being linear ($P < 0.001$). Insoluble fiber tended to decrease G:F ratio ($P = 0.097$). Phytase increased bone ash ($P = 0.005$) with the response being linear ($P = 0.001$), but there was no effect of insoluble fiber ($P = 0.949$).

Phytase did not affect the apparent total tract digestibility of DM, NDF, or ADF ($P > 0.050$), whereas insoluble fiber decreased the ATTD of DM ($P < 0.001$), NDF ($P < 0.001$), and ADF ($P < 0.001$). In conclusion, the addition of insoluble fiber did not affect the ability of phytase to improve growth performance and bone mineralization in nursery pigs fed a P deficient diet.

Keywords: corn bran, insoluble fiber, swine, bone ash, digestibility.

INTRODUCTION

The P level of swine diets is carefully controlled because of its cost and essentiality for achieving growth performance outcomes, and because excesses increase the quantity of land required to sustainably apply manure to farm land. Although P is present in acceptable concentrations in most plant feedstuffs, only a fraction can be used by the pig (Beaulieu et al., 2007). The reason for poor P utilization from plant feedstuffs is the significant proportion that is present in the form of inositol hexakisphosphate also known as phytate (Vohra and Satyanarayana, 2003) and the fact that the gastrointestinal tract of the pig does not secrete phytase, the enzyme necessary to hydrolyze phytate.

Therefore, more expensive inorganic sources of P have to be included to meet the desired diet specifications for available P. Phytase is used in swine rations as an exogenous enzyme to release the P of phytate origin. Consequently, the use of phytase improves the digestibility of P and reduces fecal P excretion (Johnston et al., 2004; Veum et al., 2006; Jang et al., 2017), which has positive production and environmental implications.

However, it is unclear if phytase works equally well under all dietary circumstances. Inclusion of ingredients with high insoluble fiber content is a common strategy to lower the cost of swine diets and has at least two potential mechanisms for interacting with phytase. First, insoluble fiber can reduce the time for enzyme-substrate interaction by increasing gastrointestinal rate of passage and stomach emptying (Wenk, 2001). Therefore, there are fewer chances for the released inorganic P to be absorbed by the small intestine. Second, insoluble fiber can delay the enzyme-substrate interaction by physically isolating or trapping the substrate (Meng et al., 2005; Grundy et al., 2015), decreasing the efficacy of phytate degradation. Although evaluating these mechanisms is important, as a first step, there is a need to determine if

the phytase-fiber interaction affects production parameters as well as bone mineralization. Therefore, the objective of this study was to determine if insoluble fiber affects the efficacy of the phytase enzyme's ability to improve growth performance and increase bone ash of nursery pigs when fed diets limiting in P content.

MATERIALS AND METHODS

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Laboratory Animals (FASS, 2010) and were approved by the Institutional Animal Care and Use committee at Iowa State University (12-17-8657-S).

Animals, Diets, and Experimental Design

A total of 480 crossbreed weaned pigs (5.48 ± 0.14 kg BW; progeny of Camborough terminal sows \times 337 terminal sires; PIC Inc., Hendersonville, TN) were blocked by initial BW (6 blocks; 10 pigs per pen) and then randomly assigned to treatment within block. Pens were also randomly assigned to treatment (n=6). Within each pen, pigs were not separated by sex, but pens within a block had equal numbers of barrows and gilts.

A common nutrient-adequate diet was fed from d -14 to -5, and 2 basal P deficient diets (either a low insoluble fiber [LF] corn-soybean meal diet containing 0.16% standardized total tract digestible [STTD] P, or a high insoluble fiber [HF] corn-soybean meal plus 20% corn bran diet containing 0.14% STTD P; **Table 1**) were fed from d -5 to 0 to acclimate pigs to the P deficient diets (Jones et al., 2010). Across both basal diets, only corn, bran and to a minor extent soybean meal differed, to simplify the interpretation of experimental outcomes, free from confounding due to variation in ingredient composition. From d 0 to 21, pigs received 1 of 8

dietary treatments. One set of treatments was based on the LF basal diet with 4 levels of added phytase (0, 109, 218, and 327 phytase units [FTU]/kg). The second set was based on the HF basal with the same 4 levels of added phytase. All diets were formulated to meet the nutrient requirements of nursery pigs except for total Ca and the STTD P (NRC, 2012). The 4 levels of estimated STTD P in the LF diets (0.16, 0.21, 0.26, and 0.31%) were formulated to meet 46, 60, 75, and 88%, respectively, of requirement. The 4 levels of STTD P in the HF diets (0.14, 0.19, 0.24, and 0.29%) were formulated to meet 41, 55, 69, and 83%, respectively, of requirement.

Total Ca levels were formulated to be below requirement (0.50% or about 65% of the estimated requirement; NRC, 2012) to ensure that excesses did not impair phytase efficacy and thus confound experimental outcomes (Angel et al., 2002; Beaulieu et al., 2007; Selle et al., 2009), especially at the relatively low phytase levels tested. Furthermore, a recent study in the same facility using the same genetics revealed that levels of Ca as low as 0.41% did not impair growth performance or levels of bone mineral density compared to higher levels (Soto et al., 2019). Notably, calcium levels did not increase as phytase levels increased to avoid a confounding effect of changing Ca content of the diet (Wu et al., 2018). Diets contained 0.4% titanium dioxide to allow determination of apparent total tract digestibility (**ATTD**) of DM, acid detergent fiber (**ADF**) and neutral detergent fiber (**NDF**). All diets were provided *ad libitum* as a mash.

Diets were manufactured at the Iowa State University Swine Nutrition Farm feed mill. Other than corn and soybean meal, all ingredients were weighed on an analytical scale which was calibrated with a standard weight. These ingredients were blended in a dough mixer before being added into the main mixer, to ensure precision in mixing and homogeneity of the final mixed feed.

Sample Collection Handling and Chemical Analyses

Before mixing diets, ingredient subsamples used in the formulation were assayed for total Ca and P (modified method 985.01; AOAC, 1996) at Mid-west labs (Omaha, NE). Enzyme activity of the phytase (Quantum Blue 5 G, AB Vista, Wiltshire, UK) was determined to be 7,600 μmol of phosphate per min at pH 5.5 at 37°C (7,600 FTU/g) at the AB Vista laboratory (Ystrad Mynach, UK). Phytase was used within the labeled “best before date” to assure the stability of enzyme activity even after feed manufacturing (De Jong et al., 2016).

Ten diet subsamples were collected at the time of mixing; these samples were carefully homogenized and pooled into one subsample. From d 18 to 20, fresh fecal subsamples were collected via grab sampling from each pen. All subsamples were stored at -20°C to avoid bacterial degradation. Before being assayed, fecal subsamples were thawed and oven-dried in a convection oven at 65°C until subsamples reached a constant weight (Jacobs et al., 2011). Diets and dried fecal subsamples were ground in a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ) through a 1 mm screen and stored in desiccators to maintain a constant percentage of DM. At d 21, one pig with a BW close to the pen average was euthanized via captive bolt stunning to collect the fibula of the right leg.

Feed and fecal samples were assayed at the Monogastric Nutrition Laboratory (Iowa State University, Ames, IA). Assays included DM using a drying oven (method 930.15; AOAC, 2007). Acid detergent fiber and NDF were determined using an Ankom automated fiber analyzer (model 2000, Macedon, NY; a modified method from Van Soest and Robertson, 1979). Titanium dioxide was determined colorimetrically using a spectrophotometer (model Synergy 4, BioTek, Winooski, VT; Leone, 1973). Additionally, concentrations of Ca and P of the experimental diets were determined as previously described at Mid-west labs (Omaha, NE).

Fibulae were assayed for bone ash content (600°C for 16 h; Veum et al., 2006). Before ashing, the bones were autoclaved and all soft tissue was removed. They were then oven dried at 103°C for 36 h before and after being soaked in fresh hexane until clear to remove ether extract.

Calculations

Pig weights and feed disappearance were measured by pen on d 0, and 21 to calculate ADG, ADFI, and G:F. The ATTD of DM, NDF and ADF was determined using the following equation (Adeola, 2001):

$$\text{ATTD, \%} = [100 - [100 \times (\% \text{ titanium dioxide in feed} / \% \text{ titanium dioxide in feces} \times (\text{concentration of component in feces} / \text{concentration of component in feed}))]]$$

Fibula bone ash percentage was expressed in relation to of the initial dry fat-free bone weight. The quantity of STTD P released was calculated according to Gourley et al. (2018). The average daily phytase release of STTD P intake for each pen was calculated as dietary STTD P, % minus 0.16% (the STTD P in the 0 FTU/kg diet) multiplied by ADFI. Subsequently, a standard curve was developed for each response criteria (ADG, G:F, percentage of bone ash, and bone ash weight) using intake of STTD P released by phytase as the predictor variable. The equation for the standard curve was then used to calculate phytase-released STTD P for each pen fed the different phytase treatments based on the observed value for each response criteria. The calculated value was then adjusted using the pen ADFI.

Statistical Analysis

The ROBUSTREG procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC) was used to analyze for outliers. Pen was the experimental unit in all analyses. Data were analyzed using PROC

MIXED of SAS according to the following model: main (fixed) effects, phytase, insoluble fiber and the interaction between phytase and insoluble fiber; block was a random effect. Additionally, to test the response to phytase, linear and quadratic polynomial orthogonal contrasts were tested. Effects were considered statistically significant with P -values ≤ 0.05 and P -values between 0.05 and 0.10 were considered trends.

RESULTS

Calcium levels of the experimental diets averaged 0.55%, or about 10% above formulation (**Table 2**). Total P content of the LF and HF experimental diets averaged 0.39% and 0.34%, respectively, or 100% and 97% of expected values based on the formulations. As a result, the average Ca:P ratio was 1.4 in the LF diets, and 1.6 in the HF diets, compared with the formulated values of 1.3 and 1.5 for the LF and HF diets, respectively.

No interactions between phytase and insoluble fiber addition were observed for final BW, growth performance, bone ash, or digestibility variables (individual means of the LF and HF experimental diets are presented in Supplementary Tables 1-3). By design, initial BW was not affected by the addition of phytase or insoluble fiber. In contrast, a main effect of phytase on final BW was observed ($P = 0.001$; **Table 3**), with a linear increase as phytase level increased ($P < 0.001$). Insoluble fiber decreased final BW ($P = 0.007$). There was a main effect of phytase on ADG ($P < 0.001$), with a linear increase as phytase level increased ($P < 0.001$). Increasing the insoluble fiber level of the diet decreased ADG ($P = 0.033$). No main effects of phytase or insoluble fiber were observed for ADFI. A main effect of phytase for G:F was observed ($P < 0.001$), with linear increase as phytase level increased ($P < 0.001$). Increasing the insoluble fiber level of the diet tended to decrease G:F ($P = 0.097$). There was a main effect of phytase on bone

ash percentage ($P = 0.005$), with a linear increase as phytase level increased ($P = 0.001$). Insoluble fiber level in the diet did not affect mean bone ash percentage. A main effect of phytase on fibula bone ash weight was observed ($P = 0.037$), with linear increase for as phytase level increased ($P = 0.004$). Fiber level had no effect on bone ash weight.

The calculated STTD P released by the addition of phytase increased in all response variables as phytase increased in the diet (**Table 4**). The average release of all response variables was 0.050, 0.094, and 0.139% STTD P with the addition of 109, 217, and 327 FTU/kg respectively.

The addition of phytase did not influence the ATTD of DM (**Table 5**). Although no main effect of phytase was observed for the ATTD of NDF and ADF, a tendency for linear decrease was observed as phytase levels increased ($P = 0.071$ and $P = 0.081$, for NDF and ADF respectively). Insoluble fiber decreased the ATTD of DM ($P < 0.001$), NDF ($P < 0.001$), and ADF ($P < 0.001$).

DISCUSSION

Phosphorus is an essential nutrient for mammals and has ubiquitous biological functions (Calvo and Lamberg-Allardt, 2015) including being a structural component of bones (Clarke, 2008) and participating in vital cellular processes such as energy metabolism and signal transduction (June et al., 1990; Huskisson et al., 2007). Therefore, failure to supply adequate P in the diet results in decreased growth performance and impaired skeletal development (Pokharel et al., 2017). Herein, analysis of the experimental diets confirmed that total P levels were deficient, which translated into impaired growth performance in pigs fed the two basal diets. However, the

rate of removals during the entire experimental period (8 pigs; 1.6% of total) was within the normal range for this facility and was not more heavily associated with any one treatment.

The main objective of adding phytase to swine diets was to meet P specifications through the release of P from phytate. The results of this experiment indicated a clear improvement in growth performance, bone mineralization and the calculated P released in response to the addition of phytase to the basal diets, suggesting an effective role of phytase in releasing P from phytate *in-vivo*. These results agree with Veum et al. (2006), Veum and Ellersieck (2008), Jones et al. (2010), and Gourley et al. (2018) who also reported increased growth performance and bone mineralization by adding phytase using P deficient diets. Overall, scientific data supports the effectiveness of adding phytase to release digestible P to pigs.

On the other hand, phytase did not influence DM digestibility. Dry matter digestibility is influenced by the disappearance in the gastrointestinal tract of all dietary components. Phytase has been shown to improve mineral (Madrid et al., 2013) and amino acid digestibility through the decrease of antinutritional effects of phytate and its derivatives (Cowieson et al., 2017; Zouaoui et al., 2018). Zeng et al., (2014) reported an increase in the apparent ileal digestibility of DM (using 1,000 to 20,000 FTU/kg), but Zeng et al. (2016) using 0 to 20,000 FTU/ kg and She et al. (2018) using 0 to 4,000 FTU/ kg, did not. However, these studies used much higher levels (super-dosed levels) of phytase than those used herein (0-327 FTU/kg), and investigated ileal as opposed to total tract digestibility, the focus of this study. Thus, although the effect of phytase on DM is possible at high levels, the results of this experiment suggest it is not likely to occur at low inclusion rates.

Despite the numerical decrease, this experiment did not find any effects of phytase (0 – 327 FTU) on fiber digestibility, suggesting that phytase does not substantially affect the ability

of microbiota to ferment insoluble fiber. No comparable studies investigating the effect of phytase on the digestibility of fiber components were found.

This experiment also investigated the effect of adding insoluble fiber (in the form of corn bran) on growth performance, bone mineralization, and the digestibility of dry matter and fiber. Final BW, ADG, and feed efficiency were reduced in the higher fiber diets. At lower inclusion levels, high insoluble fiber ingredients do not always affect growth performance (5% corn DDGS, Tran et al., 2012; 5% corn bran, Liu et al., 2018). This may be more an effect of experiments not having sufficient power to detect small differences in performance. At higher levels, ingredients rich in insoluble fiber have been shown to decrease growth performance (Berrocoso et al., 2015), and markedly reduce feed efficiency (Asmus et al., 2014) unless dietary net energy is equalized (Weber et al., 2015). The decrease in growth performance has been mainly attributed to a lower NE concentration in high fiber ingredients (Gutierrez et al., 2013; Acosta et al., 2016a), and the potential difficulty nursery pigs have in adjusting feed intake to compensate for the lower dietary energy concentration of high fiber diets. In this experiment, no attempt was made to equalize NE across treatments (2.56 vs 2.43 Mcal NE/kg for the LF and HF diets, respectively) as this would have required the addition of fat which could confound the utilization of Ca due to the formation of calcium soaps in the gastrointestinal tract.

Although there was no effect of fiber on bone mineralization, results also suggest a strong effect of insoluble fiber on the digestibility of DM, NDF, and ADF. The effect of insoluble fiber on the digestibility of dry matter and insoluble fiber constituents is supported by the literature (Le Goff and Noblet, 2001; Liu et al., 2014; Acosta et al., 2017), including fiber of corn bran origin (Gutierrez et al., 2013). The decrease in fiber digestibility can be the result of a limited capacity of the pig to ferment insoluble fiber (Stanogias and Pearce, 1985), especially at early

growth stages. Alternatively, the markedly low digestibility of fiber in the high fiber diets can be the result of the additional weight of intestinal endogenous materials in the determination of NDF and ADF in fecal samples (Montoya et al., 2015).

Interactive effects between phytase and fiber were not observed in this experiment; there are at least two explanations to consider. First, insoluble fiber has the potential to increase digesta passage rate in the proximal gastrointestinal tract, which allows less time for an enzyme-substrate interaction (Wenk, 2001), thus decreasing the efficacy of phytate catabolism into phytate products (Laird et al., 2016), a key mechanism of action for phytase (Blaabjerg et al., 2011; Holloway et al., 2016). Fortunately, it appears that phytase action takes place before or at the gastrointestinal tract locations in which P can be absorbed, regardless of the increased passage rate resulting from insoluble fiber addition. Moreover, Bournazel et al. (2017) suggested that insoluble fiber (in the form of rapeseed hulls; 13.1% NDF) does not affect the release of inorganic P from phytate in the stomach.

Second, the insoluble fiber matrix can isolate or trap feed components including phytate (Bedford and Schulze, 1998). Based on the results of this experiment, it is possible that the insoluble fiber matrix is not affecting the enzyme-substrate interaction. A possible explanation in this case is that most phytate in corn is concentrated in the germ (Hídvégi and Lásztity, 2003) and therefore not directly associated with the fiber matrix. Therefore, although theoretically possible, the interactive mechanisms between phytase and fiber are absent or not of sufficient magnitude to affect production outcomes and bone mineralization.

In conclusion, the addition of insoluble fiber did not affect the ability of phytase to improve growth performance and bone mineralization in pigs fed P deficient diets; therefore,

phytase can be used with confidence in high and low insoluble fiber diets, as it is equally effective in both.

LITERATURE CITED

- Acosta, J., J. F. Patience, and R. D. Boyd. 2016a. Comparison of growth and efficiency of dietary energy utilization by growing pigs offered feeding programs based on the metabolizable energy system. *J. Anim. Sci.* 94:1520–1530. doi:10.2527/jas2015-9321
- Acosta, J. A., R. D. Boyd, and J. F. Patience. 2017. Digestion and nitrogen balance using swine diets containing increasing proportions of coproduct ingredients and formulated using the net energy system. *J. Anim. Sci.* 95:1243–1252. doi:10.2527/jas2016.1161
- Adeola, O. 2001. Digestion and balance techniques in pigs. In: Lewis A. L. Southern L. L. editors, *Swine nutrition*. CRC Press, Boca Raton, FL. p. 903–916.
- 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. 1996. *Official Methods of Analysis*. Assoc. Off. Anal. Chem., Washington, DC.
- AOAC. 2007. *Official methods of analysis*. 18th ed. AOAC Int., Arlington, VA.
- Asmus, M. D., J. M. DeRouchey, M. D. Tokach, S. S. Dritz, T. A. Houser, J. L. Nelssen, and R. D. Goodband. 2014. Effects of lowering dietary fiber before marketing on finishing pig growth performance, carcass characteristics, carcass fat quality, and intestinal weights. *J. Anim. Sci.* 92:119–128. doi:10.2527/jas2013-6679

- 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.* 87:353-364. doi:10.4141/CJAS06032
- Bedford, M. R., and H. Schulze. 1998. Exogenous enzymes for pigs and poultry. *Nutr. Res. Rev.* 11:91-114. doi:10.1079/NRR19980007
- Berrocoso, J. D., D. Menoyo, P. Guzmán, B. Saldaña, L. Cámara, and G. G. Mateos. 2015. Effects of fiber inclusion on growth performance and nutrient digestibility of piglets reared under optimal or poor hygienic conditions. *J. Anim. Sci.* 93:3919-3931. doi:10.2527/jas2015-9137
- Blaabjerg, K., H. Jørgensen, A. -H. Tauson, and H. D. Poulsen. 2011. The presence of inositol phosphates in gastric pig digesta is affected by time after feeding a nonfermented or fermented liquid wheat- and barley-based diet. *J. Anim. Sci.* 89:3153-3162. doi:10.2527/jas.2010-3358
- Bournazel, M., M. Lessire, M. J. Duclos, M. Magnin, N. Mème, C. Peyronnet, E. Recoules, A. Quinsac, E. Labussière, and A. Narcy. 2017. Effects of rapeseed meal fiber content on phosphorus and calcium digestibility in growing pigs fed diets without or with microbial phytase. *Anim.* 12: 34-42. doi:10.1017/S1751731117001343
- Calvo, M. S., and C. J. Lamberg-Allardt. 2015. Phosphorus. *Adv. Nutr.* 6: 860-862. doi:10.3945/an.115.008516
- Clarke, B. 2008. Normal Bone Anatomy and Physiology. *Clin. J. Am. Soc. Nephrol.* 3(Suppl 3): 131-S139. doi:10.2215/CJN.04151206

Cowieson, A. J., J. -P. Ruckebusch, J. O. B. Sorbara, J. W. Wilson, P. Guggenbuhl, L. Tanadini, and F. F. Roos. 2017. A systematic view on the effect of microbial phytase on ileal amino acid digestibility in pigs. *Anim. Feed Sci. Tech.* 231: 138–149.

doi:10.1016/j.anifeedsci.2017.07.007

De Jong, J. A., J. M. DeRouche, M. D. Tokach, S. S. Dritz, R. D. Goodband, J. C. Woodworth, C. K. Jones, and C. R. Stark. 2016. Stability of commercial phytase sources under different environmental conditions. *J. Anim. Sci.* 94:4259–4266. doi:10.2527/jas2016-0742

FASS. 2010. *Guide for the Care and Use of Agricultural Animals in Research and Teaching: Third Edition*. Champaign, IL: Federation of Animal Science Societies. 169 pp.

Gourley, K. M., J. C. Woodworth, J. M. DeRouche, 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

Grundy, M. M. L., P. J. Wilde, P. J. Butterworth, R. Gray, and P. R. Ellis. 2015. Impact of cell wall encapsulation of almonds on in vitro duodenal lipolysis. *Food Chem.* 185: 405–412. doi: 10.1016/j.foodchem.2015.04.013

Gutierrez, N. A., B. J. Kerr, and J. F. Patience. 2013. Effect of insoluble-low fermentable fiber from corn-ethanol distillation origin on energy, fiber, and amino acid digestibility, hindgut degradability of fiber, and growth performance of pigs. *J. Anim. Sci.* 91:5314–5325. doi:10.2527/jas2013-6328

Hídvégi, M., and R. Lásztity. 2003. Phytic acid content of cereals and legumes and interaction with proteins. *Periodica Polytech., Chem. Eng.* 46: 59-64.

- Holloway, C. L., R. D. Boyd, C. L. Walk, and J. F. Patience. 2016. Impact of super-dosing phytase in diets fed to 40 kg, 60 kg and 80 kg pigs on phytate catabolism. *J. Anim. Sci.* 94(Suppl. 2): 112-113. doi:10.2527/msasas2016-238
- Huskisson, E., S. Maggini, and M. Ruf. 2007. The role of vitamins and minerals in energy metabolism and well-being. *J. Int. Med. Res.* 35: 277-289.
doi:10.1177/147323000703500301
- Jacobs, B. M., J. F. Patience, W. A. Dozier, K. J. Stalder, and B. J. Kerr. 2011. Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta. *J. Anim. Sci.* 89:2624–2630. doi:10.2527/jas.2010-3768
- 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/jas2017.1781
- 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
- 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. doi:10.2527/2004.823705x

- June, C. H., M. C. Fletcher, J. A. Ledbetter, G. L. Schieven, J. N. Siegel, A. F. Phillips, and L. E. Samelson. 1990. Inhibition of tyrosine phosphorylation prevents T-cell receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. USA.* 87:7722-7726.
doi:10.1073/pnas.87.19.7722
- Laird, S., I. Kühn, P. Wilcock, and H. M. Miller. 2016. The effects of phytase on grower pig growth performance and ileal inositol phosphate degradation. *J. Anim. Sci.*94:142–145.
doi:10.2527/jas2015-9762
- Le Goff, G., and J. Noblet. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. *J. Anim. Sci.* 79:2418–2427.
- Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. *J. Assoc. Off. Anal. Chem.* 56:535–537.
- Liu, P., J. Zhao, W. Wang, P. Guo, W. Lu, C. Wang, L. Liu, L. J. Johnston, Y. Zhao, X. Wu, C. Xu, J. Zhang, and X. Ma. 2018. Dietary Corn Bran Altered the Diversity of Microbial Communities and Cytokine Production in Weaned Pigs. *Front. Microbiol.* 9:2090.
doi:10.3389/fmicb.2018.02090
- Liu, Y., M. Song, F. N. Almeida, S. L. Tilton, M. J. Cecava, and H. H. Stein. 2014. Energy concentration and amino acid digestibility in corn and corn coproducts from the wet-milling industry fed to growing pigs. *J. Anim. Sci.* 92:4557–4565. doi:10.2527/jas2014-6747
- Madrid, J., S. Martínez, C. López, and F. Hernández. 2013. Effect of phytase on nutrient digestibility, mineral utilization and performance in growing pigs. *Livest. Sci.* 154:144–151. doi:10.1016/j.livsci.2013.03.003

- Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell, and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. *Poult. Sci.* 84:37–47. doi: 10.1093/ps/84.1.37
- Montoya, C. A., S. M. Rutherford, and P. J. Moughan. 2015. Nondietary gut materials interfere with the determination of dietary fiber digestibility in growing pigs when using the Prosky method. *J. Nutr.* 145:1966–72. doi:10.3945/jn.115.212639.
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.
- Pokharel, B. B., A. Regassa, C. M. Nyachoti, and W. K. Kim. 2017. Effect of low levels of dietary available phosphorus on phosphorus utilization, bone mineralization, phosphorus transporter mRNA expression and performance in growing pigs. *J. Environ. Sci. Health B.* 52:395–401. doi:10.1080/03601234.2017.1292096
- 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.
- She, Y, J. C. Sparks, and H. H. Stein. 2018. Effects of increasing concentrations of an *Escherichia coli* phytase on the apparent ileal digestibility of amino acids and the apparent total tract digestibility of energy and nutrients in corn-soybean meal diets fed to growing pigs. *J. Anim. Sci.* 96:2804–2816. doi:10.1093/jas/sky152
- Soto, J. A., D. D. Koehler, L. K. Kellesvig Geising, S. Becker, S. A. Gould, and J.F. Patience. 2019. Effects of increasing calcium to available phosphorus ratios in diets containing

- phytase on growth performance and bone mineral content of nursery pigs. *J. Anim. Sci.* (accepted).
- Stanogias, G., and G. R. Pearce. 1985. The digestion of fibre by pigs. 1. The effects of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage. *Br. J. Nutr.* 53: 205:225. doi:10.1079/BJN19850144
- Tran, H., R. Moreno, E. E. Hinkle, J. W. Bundy, J. Walter, T. E. Burkey, and P. S. Miller. 2012. Effect of corn distillers dried grains with solubles on growth performance and health status indicators in weanling pigs. *J. Anim. Sci.* 90:790–801. doi:10.2527/jas.2011-4196
- Van Soest, P. J., and J. B. Robertson. 1979. Systems of analysis for evaluating fibrous feeds. In: Workshop standardization of analytical methodology for feeds. Int. Dev. Res Center, Ottawa, Canada. p. 49–60.
- Veum, T. L., D. W. Bollinger, C. E. Buff and M. R. Bedford. 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.* 84:1147-1558. doi:10.2527/2006.8451147x
- Veum, T. L., and M. R. Ellersieck. 2008. Effect of low doses of *Aspergillus niger* phytase on growth performance, bone strength, and nutrient absorption and excretion by growing and finishing swine fed corn-soybean meal diets deficient in available phosphorus and calcium. *J. Anim. Sci.* 86:858–870. doi:10.2527/jas.2007-0312
- Vohra, A., and T. Satyanarayana. 2003. Phytases: Microbial sources, production, purification, and potential biotechnological applications, *Critical Rev. Biotech.* 23:29-60. doi: 10.1080/713609297

- Weber, E. K., J. F. Patience, and K. J. Stalder. 2015. Wean-to-finish feeder space availability effects on nursery and finishing pig performance and total tract digestibility in a commercial setting when feeding dried distillers grains with solubles. *J. Anim. Sci.* 93:1905-1915. doi:10.2527/jas.2014-8136
- Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. *Anim. Feed Sci. Technol.* 90: 21-23. doi:10.1016/S0377-8401(01)00194-8
- Wu, F., M. D. Tokach, J. M. DeRouchey, S. S. Dritz, J. C. Woodworth, and R. D. Goodband. 2018. Effects of Dietary P Concentrations in Response to Increasing Dietary Ca Concentrations on Growth Performance of Nursery Pigs. *J. Anim. Sci.* 96: (Suppl. 2): 129. doi:10.1093/jas/sky073.239
- Zeng, Z. K., D. Wang, X. S. Piao, P. F. Li, H. Y. Zhang, C. X. Shi, and S. K. Yu. 2014. Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals and amino acids digestibilities. *Asian-Aust. J. Anim. Sci.* 27:237-246. doi:10.5713/ajas.2013.13370
- 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. Tech.* 238: 18-28. doi:10.1016/j.anifeedsci.2018.01.019

Table 1. Ingredient and chemical composition of the low and high fiber basal diets, as fed basis¹.

Item	Low fiber	High fiber
Ingredient composition, %		
Corn	61.51	42.10
Soybean meal	32.68	32.08
Corn bran	-	20.00
Soybean oil	3.00	3.00
Limestone	0.72	0.73
L-Lysine HCl	0.45	0.45
DL-Methionine	0.16	0.16
L-Threonine	0.13	0.13
Salt	0.35	0.35
Vitamin premix ²	0.25	0.25
Trace mineral premix ³	0.15	0.15
Titanium dioxide	0.40	0.40
Zinc oxide	0.20	0.20
Chemical composition, analyzed		
DM, %	88.1	88.5
Acid detergent fiber, %	2.8	3.8
Neutral detergent fiber, %	7.5	15.2
Chemical composition, calculated		
NE, Mcal/kg	2.56	2.43
ME, Mcal/kg	3.44	3.27
Acid hydrolyzed ether extract, %	5.7	6.7
Crude protein, %	20.7	20.7
SID ⁵ Lys, %	1.33	1.33
SID AA:Lys		
Lys	1.00	1.00
Met	0.34	0.34
TSAA	0.55	0.55
Thr	0.59	0.59
Trp	0.17	0.17
Total P, % ⁴	0.39	0.35

STTD ⁶ P, %	0.16	0.14
Ca, % ⁴	0.50	0.50

¹Quantum Blue 5G phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK) was added to the low and high fiber basal diets at 0.00148% to create the 109 FTU/kg dietary treatments, added at 0.00297% to create the 218 FTU/kg dietary treatments, and added at 0.004445% to create the 327 FTU/kg dietary treatments.

²Vitamin premix provided the following (per kg diet): 7,656 IU of vitamin A; 875 IU of vitamin D3; 40 IU of vitamin E; 4 mg of menadione (to provide vitamin K); 14 mg of riboflavin; 34 mg of d-pantothenic acid; 0.06mg of vitamin B12, and 70 mg of niacin.

³Mineral premix provided the following (/kg diet): 165 mg of Fe (ferrous sulfate); 165 mg of Zn (zinc sulfate); 39 mg of Mn (manganese sulfate); 16.5 mg of Cu (cooper sulfate); 0.3 mg/kg of I (calcium iodate); and 0.3 mg/kg of Se (sodium selenite).

⁴Analyzed Ca and P content of experimental diets is presented in Table 2.

⁵SID = Standardized ileal digestible.

⁶STTD = Standardized total tract digestible.

Table 2. Analyzed Ca and P content of the experimental diets, as fed basis

Item	Low fiber ¹				High fiber ²			
	Phytase ³ FTU/kg							
	0	109	217	327	0	109	217	327
Ca, %	0.59	0.52	0.57	0.51	0.55	0.55	0.52	0.62
P, %	0.39	0.39	0.38	0.38	0.33	0.34	0.35	0.32
Ca: P	1.51	1.33	1.50	1.34	1.67	1.62	1.49	1.94

¹Corn-soybean-meal-based diet. Total Ca content was formulated to 50%; Total P content was formulated to be 0.39%.

²Corn-soybean meal plus 20% corn bran-based diet. Ca content was formulated to 50%; Total P content was formulated to be 0.35%.

³Quantum Blue 5G phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK).

Table 3. Main effects of the addition of phytase¹ and of fiber level² on growth performance and bone mineralization³ in nursery pigs

Item	Phytase, FTU/kg				Fiber level		SEM	<i>P</i> -value ⁴			
	0	109	217	327	Low	High		Phytase	Fiber	Phytase	
										L	Q
d 0 BW, kg	6.92	6.67	6.77	6.80	6.83	6.75	0.48	0.140	0.295	-	-
d 21 BW, kg	12.33	12.38	12.58	12.94	12.71	12.41	0.84	0.001	0.007	<0.001	0.139
ADG, kg	0.268	0.282	0.291	0.306	0.291	0.282	0.018	<0.001	0.033	<0.001	0.778
ADFI, kg	0.514	0.512	0.519	0.527	0.519	0.516	0.020	0.381	0.632	0.127	0.431
Gain:Feed	0.520	0.548	0.559	0.580	0.559	0.544	0.018	<0.001	0.097	<0.001	0.652
Bone ash, %	44.6	45.8	47.0	47.4	46.2	46.2	0.6	0.005	0.949	0.001	0.469
Bone ash weight, g	0.387	0.406	0.426	0.447	0.407	0.427	0.032	0.037	0.179	0.004	0.948

¹Quantum Blue 5G phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK).

²Either low (corn-soybean-meal-based diets) or high (corn-soybean meal plus 20% corn bran-based diets).

³One pig per pen was euthanized and fibulas were used to determine bone ash weight and percentage bone ash.

⁴*P*-Values are: Phytase = main effect of phytase, Fiber=main effect of fiber; Phytase, L= Orthogonal linear contrast of Phytase, Q=orthogonal quadratic contrast of Phytase.

Table 4. Quantity of standardized total tract digestible P released (%) based on different response criteria

Item	Phytase, FTU/kg			SEM
	109	217	327	
Response criteria variables				
ADG	0.04	0.09	0.15	0.02
G:F	0.06	0.09	0.14	0.04
Percent bone ash	0.05	0.11	0.14	0.04
Bone ash weight	0.04	0.09	0.13	0.04
Average variables	0.050	0.09	0.14	-

¹Quantum Blue 5G phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK).

Table 5. Effects of phytase¹ and fiber level² on the apparent total tract digestibility (ATTD) of DM and fiber components.

Item	Phytase, FTU/kg				Fiber level			<i>P</i> -value ³			
	0	109	217	327	Low	High	SEM	Phytase	Fiber	Phytase	
										L	Q
ATTD, %											
DM	80.2	80.0	79.9	79.7	85.1	74.8	0.3	0.670	<0.001	0.257	0.772
NDF	35.8	32.4	31.9	32.0	54.7	11.4	1.5	0.187	<0.001	0.071	0.228
ADF	31.7	29.5	28.9	27.7	54.5	4.5	1.9	0.343	<0.001	0.081	0.743

¹Quantum Blue 5G phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK).

²Either low (corn-soybean-meal-based diets) or high (corn-soybean meal plus 20% corn bran-based diets).

³*P*-Values are: Phytase = main effect of phytase, Fiber=main effect of fiber; Phytase, L= Orthogonal linear contrast of Phytase, Q=orthogonal quadratic contrast of Phytase.