Adverse effects on growth performance and bone development in nursery pigs fed diets marginally deficient in phosphorus with increasing calcium to available phosphorus ratios.  

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ABSTRACT

The objective of this experiment was to evaluate the growth performance and bone mineral content of nursery pigs in response to increasing total calcium (Ca) to available phosphorus (aP) ratios in diets containing phytase (250 FTU/kg; Natuphos E, BASF, Florham Park, NJ). A total of 480 nursery pigs (Body weight (BW) = 5.7 ± 0.6 kg) with 10 pigs/pen and 7 pens per treatment (6 pens fed 2.75:1 diet) were allotted to 7 treatments consisting of increasing ratios of calcium to available phosphorus (Ca:aP): 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75. From d -7 - 0, pigs were fed a common diet. They were then fed the treatment diets during two experimental phases from d 1 – 14, and d 15 – 28, respectively. Available phosphorus (P) was formulated to 0.33 and 0.27% (approximately 90% of requirement) in dietary phases 1 and 2, respectively. Body weight, average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F) were determined. Bone mineral content (BMC) of the femur was measured on d 28 on 1 pig per pen using dual x-ray absorptiometry (DXA). Data were analyzed as a linear mixed model using PROC MIXED (SAS, 9.3). Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing the Ca:aP ratio. Over the 28-d experimental period, increasing Ca:aP ratio resulted in a linear decrease in ADG (353, 338, 328, 304, 317, 291, 280 g/d; P < 0.01), ADFI (539, 528, 528, 500, 533, 512, 489 g/d; P < 0.05), and G:F (0.68, 0.66, 0.64, 0.62, 0.61, 0.59, 0.58; P < 0.01). Increasing Ca:aP ratio also resulted in decreased BW on days 14 and 28 (P < 0.01). The BMC of the femur decreased with increasing Ca:aP ratio (6.2, 6.3, 5.7, 5.9,
5.5, 5.6, 5.3 g; \( P < 0.05 \). Regression analysis explained the impact of Ca:aP as follows on ADG (ADG (g/d) = 339 - 36x; \( r^2 = 0.81 \)), G:F (G:F = 0.61 – 0.03x; \( r^2 = 0.72 \)) and BMC (BMC (g) = 6.4 – 0.27x; \( r^2 = 0.43 \)), where x is the Ca:aP ratio. In conclusion, all outcomes indicated that any level of calcium above the minimum used in this experiment impaired growth performance and skeletal development. Further research using even lower levels of dietary Ca is warranted.

**Key words:** bone mineral content, dual x-ray absorptiometry, swine
**List of Abbreviations**

Ca, calcium

aP, available phosphorus

BW, body weight

Ca:aP, calcium to available phosphorus ratio

P, phosphorus

ADG, average daily gain

ADFI, average daily feed intake

G:F, feed efficiency

DXA, dual x-ray absorptiometry

BMC, bone mineral content

Ca:tP, calcium to total phosphorus ratio

STTD, standardized total tract digestible
INTRODUCTION

Calcium (Ca) and phosphorus (P) are the two most abundant minerals in the body of the pig and are required for many important physiological functions (Oster et al., 2016). The vast majority of Ca – about 99% - is present in skeletal tissues (Nielsen, 1972), but it also fulfils other very important roles involving blood clotting, nerve impulse transmission, muscle contractility, and fluid balance, to name a few (Pravina et al., 2013). In contrast, only about 77.5% of the P in the body can be found in skeletal tissues (Nielsen, 1972); it is also involved in a wide array of metabolic functions, including energy metabolism, protein synthesis, signal transduction, acid-base homeostasis and cell membrane polarity (Oster et al., 2016). The fact that the P status of the pig may influence adaptive immune function is further evidence of the central importance of maintaining an adequate dietary supply of this critical mineral element (Heyer et al., 2015).

The ratio of calcium to phosphorus (Ca:P) in bone is about 2.1:1; this is tightly regulated by the finite chemical structure of hydroxyapatite which, along with collagen, constitutes most of the structure of bone (Cromwell, 2005). Maintenance of desirable blood levels of Ca and P is the consequence of the intricate balance between renal excretion, enteral absorption and osseous mobilization or accumulation (Berndt and Kumar, 2009). These are all under some degree of control by the endocrine system, including calcium-binding protein, parathyroid hormone, vitamin D and calcitonin.

If either Ca or P is present in the diet in excess or deficit relative to requirement, compromised utilization of the other may occur (Létourneau-Montminy et al., 2015). Specifically, overfeeding Ca impairs P absorption, at least in part due to the formation of insoluble tricalcium phosphate, leading to negative impacts on skeletal development and growth performance (Cromwell, 2005). Notably, the Ca:P in the diet is increasingly important when P is close to or below requirement (Hays, 1976; Peo, 1976; Létourneau-Montminy et al., 2015; Wu et al., 2018). This is an important concept because most practical diets are formulated to be as close as possible to
requirement, in order to minimize the quantity of P excreted in the manure (Bridges et al., 1995), to preserve finite global P reserves (Edixhoven et al., 2013) and to minimize diet cost; the latter is important as P is the third most expensive nutrient in the diet after carbohydrates (energy) and protein (Patience, 2017). It therefore begs the question as to how severely performance and skeletal development are impaired if P inadvertently falls slightly below requirement, and particularly, when Ca is present in relative excess.

There is a lack of clarity on the dose response to a wide calcium:available phosphorus ratio (Ca:aP) when available phosphorus (aP) is marginally deficient. The importance of the ratio is well known, but the quantitative relationship between Ca:aP and growth performance and bone development is much less clear (Wu et al., 2018). Therefore, the objective of this experiment was to characterize the nature of this relationship by titrating an increasingly wide Ca:aP against pig performance and bone development when the basal diet was marginally deficient in aP. This objective tested the hypothesis that even a narrow Ca:aP will negatively impact growth performance and bone development, and as this ratio widens, the affect would be amplified at an increasing rate.

**MATERIALS AND METHODS**

All experimental procedures adhered to the principles for the ethical and humane use of animals according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Iowa State University Animal Care and Use Committee (IACUC #18-99).

**Animals, housing, and experimental design**

A total of 480 weanling pigs (5.7 ± 0.6 kg body weight (BW); L337 × Camborough, PIC, Inc., Hendersonville, TN) were purchased and transported to the Iowa State University Swine Nutrition Farm (Ames, IA). Upon arrival, pigs were individually weighed, ear-tagged, and vaccinated for K88+ *Escherichia coli* via a water-delivered vaccine (Arko Laboratories, Jewell, IA). Pigs were blocked by initial weight into 7 blocks and pens were randomly assigned to 1 of 7 dietary treatments. Pigs were
housed 10 pigs per pen and 6 or 7 pens per treatment; with 48 pens available and 7 treatments, 1 treatment was randomly chosen to have one less block (pen) than the others. Pens contained mixed sexes with the same number of barrows and gilts per pen across all treatments within each block.

**Diets and feeding**

Pens (1.2 m × 2.4 m) were equipped with a four-space dry self-feeder and 2 nipple waterers to provide *ad libitum* access to feed and water. Pigs received a common standard nursery diet from day -7 to 0 during the pre-test acclimation period. They then received the experimental diets over two phases from d 1 – 14 (Phase 1), and d 14 – 28 (Phase 2), respectively. Diets were formulated to meet or exceed NRC (2012) nutrient recommendations, with the exception of Ca and P. Diets consisted of increasing Ca:aP: 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, and 2.75. Available P was kept constant in each diet and formulated to be 0.33 and 0.27% (about 90% of requirement; NRC, 2012) in dietary phases 1 and 2, respectively.

**Data and sample collection**

Pig BW and feed intake were measured on d 0 and 28 of the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). On d 28, one gilt from each pen representing the average pen weight was euthanized via captive bolt stunning followed by exsanguination. The right femur was harvested, carefully cleaned, and stored at -20º C for later analysis. Femurs were analyzed for bone mineral content (BMC) using dual-energy X-ray absorptiometry (DXA; Hologic Discovery A, Bedford, MA, USA).

Multiple diet subsamples were collected as each feed batch was unloaded from the mixer. Samples were carefully and thoroughly homogenized, subsampled, and stored at -20º C until later analysis.
Analytical methods

All ingredients containing Ca and P were sampled and analyzed for Ca and P content prior to the formulation of the experimental diets (Eurofins Scientific, Des Moines, IA; AOAC 984.27, 927.02, 985.01, 965.17 modified). The results of these assays were then utilized in the formulation of the diets (BESTMIX Feed Formulation, Adifo Software, Maldegem, Belgium). Complete feed samples were analyzed for total Ca and P (Tables 1 and 2; Eurofins Scientific, Des Moines, IA) using the same procedures as previously described.

Statistical analysis

Data were analyzed according to the following mixed model:

\[ Y_{ijk} = \mu + \tau_i + \nu_j + e_{ijk} \]

where \( Y_{ijk} \) is the observed value for \( k^{th} \) experimental unit within the \( i^{th} \) level of Ca:aP of the \( j^{th} \) block for the \( k^{th} \) pen; \( \mu \) is the general mean; \( \tau_i \) is the fixed effect of the \( i^{th} \) level of Ca:aP (\( i = 1 \) to 7); \( \nu_j \) is the random effect of the \( j^{th} \) block (\( j = 1 \) to 7); and \( e_{ijk} \) is the associated variance as described by the model for \( Y_{ijk} \) (\( l = 1 \) through 48); assuming \( \nu_j \sim N \left( 0, I\sigma^2_{\nu_j} \right) \) and \( e_{ijkl} \sim N \left( 0, I\sigma^2_e \right) \), where \( I \) is the identity matrix.

Regression parameters for overall ADG, ADFI, G:F, BMC, and final BW were estimated according to the following model:

\[ Y_{ijk} = \beta_0 + \beta_1 x_i + e_t \]

where \( Y_{ijk} \) is the observed value for \( k^{th} \) experimental unit within the \( i^{th} \) level of Ca:aP of the \( j^{th} \) block for the \( k^{th} \) pen; \( \beta_0 \) is the intercept; \( \beta_1 \) is the regression coefficient; \( x_i \) represents the value of the weighted explanatory continuous variable; and \( e_t \) is the random error associated with \( Y_{ijk} \).
The PROC UNIVARIATE procedure in SAS 9.3 (SAS Inst., Cary, NC) was used to verify normality and homogeneity of the studentized residuals. The mixed model was analyzed using PROC MIXED, and regression parameters were obtained using PROC GLM. The $r^2$ for a given a model was obtained using leave one out cross (LOOC) validation using PROC GLMSELECT, and the 95% confidence interval bands were applied from the best fit model. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing Ca:aP ratio. Least square means were separated using Fisher’s Least Significant Difference test, and treatment differences were considered significant if $P \leq 0.05$ and trends if $0.05 > P \leq 0.10$.

RESULTS AND DISCUSSION

The objective of this experiment was to characterize the nature of the relationship between an increasingly wide Ca:aP and growth performance and bone mineral content when nursery age pigs are fed diets that are marginally deficient in P. Specifically, the principal investigators wanted to determine how wide the ratio must be before impairment is observed, and if a very wide ratio had proportionately more or less impact than a narrower ratio. In other words, is the relationship completely linear or is the expected negative impact intensified at very high ratios? While numerous studies have compared narrow versus wide Ca:aP when P is deficient (Schlegel and Gutzwiller, 2020), only one other study utilized sufficient dietary treatments to define the slope of the curve (González-Vega et al., 2016b); in this instance, the evaluation involved older pigs (25 to 50 kg) than was the focus of this experiment (6 to 15 kg). In the study reported herein, 7 ratios of Ca:aP from 1.25 to 2.75 were evaluated. Available P and total Ca were used due to their common application within the commercial pig industry.

The experiment proceeded without incident; only 1.7 percent of pigs were removed from the study and none was due to impaired skeletal development or locomotion difficulties. Pretest growth performance of the pigs was within the normal range for this facility and source of pigs and resulted in similar final body weights across treatments on d0 - the start of the experimental period...
(Table 3). There were no treatment effects during the pretest period since all of the pigs received a common diet.

The results of the Ca and P assays of the experimental diets confirmed that formulation targets were met across the treatments (Table 4). For example, across all 14 diets, Ca averaged 96.8% of target and P averaged 96.1% of target; both are slightly below formulated levels but well within accepted analytical tolerances (AAFCO, 2000). Achieving assayed levels of Ca and P in the experimental diets that are close to formulated values is critical to experimental precision but can be a particular challenge for Ca and P. For this reason, extra care was given to ingredient pre-assay, thorough mixing of the diets, extensive sampling of each batch of feed and thorough homogenizing of samples prior to assay, as previously described.

The relationship between Ca:aP was linear for all growth parameters measured: final BW, ADG, ADFI and G:F (Figures 1 – 4; \( P < 0.05 \)); in no instance was the response curvilinear or quadratic. These data supported part of our hypothesis, in that as the ratio widened, performance was increasingly impaired. However, it was not supported in that the impact was clearly linear; while growth performance declined as the Ca:aP increased, the slope of the curve did not change. Additionally, the measurement of BMC supported the same conclusion; a small increase in the ratio resulted in impaired bone development and widening this ratio simply extended the range of the response without any change in the slope (Figure 5; \( P < 0.05 \)).

The ideal Ca:aP has not been definitively established for weaned pigs, but great progress has been made in the past half-decade (Gutzwiler et al., 2014; González-Vega et al., 2016b; Merriman et al., 2017; Wu et L., 2018; Lagos et al., 2019b; ). Research has shown that excess dietary Ca can negatively impact growth performance and bone ash in swine and that this is at least in part dependent on the concentration of dietary P (Qian et al., 1996; Wu et al., 2018; Lagos et al., 2019b). Previous research has demonstrated a detrimental effect of increasing Ca in P deficient diets on growth performance in nursery (Gonzalez-Vega et al., 2016a; Lagos et al, 2019a) and grow-finish
swine (Liu et al., 1998; Gonzalez-Vega et al., 2016b; Merriman et al., 2017). The reduction in growth performance is possibly explained by the formation of insoluble Ca-P complexes in the gastrointestinal tract when Ca is in excess, resulting in insufficient absorption of P and further exacerbation of the P deficiency (Heaney and Nordin, 2002; Stein et al., 2011). Increasing Ca intake leads to a linear decline in net uptake of P from the gut, irrespective of the level of P in the diet. However, at low dietary P, this decline leads to a negative uptake, which can lead to the adverse effects observed in this study (Heaney and Nordin, 2002). Notably, in pigs and poultry, the negative effects of excess Ca are somewhat mitigated when diets are above the requirement for P (Akter et al., 2017; Wu et al., 2018).

Total Ca to total P ratios (Ca:P) above 1.3:1 in swine diets with low P reduced growth performance across all stages of production (Reinhart and Mahan, 1986); however, when P was included in the diet above requirement, Ca:P up to 2:0:1 did not have negative effects on growth performance. When evaluating the Ca:P ratio in nursery pigs, Qian et al. (1996) reported linear improvements in ADG, ADFI, and feed efficiency when the ratio decreased from 2:0:1 to 1.2:1, regardless of dietary P which agrees with the results of the current study. Wu et al. (2018) observed no negative impact of Ca:P from 0.8:1 to 1.6:1 on growth performance of nursery pigs, but reported reductions in growth performance when this ratio was higher than 1.9:1 and when P was below requirement.

Recent research by Lagos et al. (2019b) demonstrated that if diets are deficient in P (50% of NRC standardized total tract digestible (STTD) requirement), then Ca must also be deficient in order to prevent reductions in growth performance in pigs weighing 11- to 25-kg. Furthermore, Lagos et al. (2019b) determined that if P is included above requirement, then Ca must also be provided above the requirement to improve growth performance, indicating that the effective use of Ca and P is dependent on their ratio as well as their individual concentrations. The authors concluded that the optimal STTD Ca:STTD P ratio to maximize growth performance is 1.40:1 or less for pigs weighing 11-
to 25-kg when STTD P is provided at requirement. In this study, reduction of transcellular Ca absorption and increased paracellular Ca absorption was suggested by decreased abundance of genes involved in Ca absorption and tight junction proteins in the small intestine. However, actual protein concentrations were not measured. Nonetheless, the effect of high dietary Ca demonstrates that it may result in impaired intestinal integrity. The authors did not observe any changes in bone ash (g per femur) by increasing dietary Ca if STTD P was deficient, which is in contrast to the current study and surprising. However, the diets utilized by Lagos et al. (2019b) were much more P-deficient than the diets utilized in the present experiment, potentially indicating that P was severely limiting bone deposition. While the Ca:aP cannot be too high, neither can it be too low, as the requirement by the pig for both minerals to support bone growth must be satisfied (Létourneau-Montminy et al., 2012).

Of course, diets are not intentionally formulated or manufactured to be deficient in P, but diet insufficiency can occur under certain circumstances. For example, when pigs experience very low feed intake, daily intake of P can fall below the minimum requirement estimated by the NRC (2012) to be about 2.4 g STTD P per day for pigs of the age employed in this study. Errors in formulation or manufacture, although infrequent, can occur with serious consequences (Crenshaw, 2001). Recently, studies have revealed that the quantity of P released by phytase may have been overestimated which could also result in P deficiencies (Olsen et al., 2019). Finally, dietary P may be rendered less available in the gastrointestinal tract in the presence of diarrhea (Crenshaw, 2001) or when there is excess Ca in the diet (Heaney and Nordin, 2002).

Our current understanding of P metabolism indicates that pigs do not increase feed intake in response to a primary P deficiency (Misiura et al., 2020). Perhaps, the greater concern is the recent revelation that during circumstances of inadequate P intake, the pig may respond by directing proportionately more of the limited supply of P to protein accretion
rather than bone development (Misiura et al., 2020). This may be the result of the pig having some flexibility in bone mass, so the greater and more urgent need for P would be that used for protein accretion.

Fortunately, a greater understanding of the interaction between Ca and P supply in the diet can be further investigated by measuring urinary and fecal excretion and retention, as well as intestinal and renal transport, such as that demonstrated by Gutierrez et al. (2015). Phosphorus absorption is achieved by both transcellular and paracellular processes; the former dominates when dietary P is below requirement, e.g. the conditions of this study (Portale et al., 1989; Saddoris et al., 2010). Enhanced P uptake from the gut when dietary intake is below requirement is a well-known physiological adjustment. When plasma P decreases, Na/P cotransport activity in the gut increases along with activation of 1,25-hydroxylase activity and an elevation in vitamin D₃ levels (Segawa et al., 2004). In terms of kinetics, the $K_m$ for P transport remains unchanged, but $V_{max}$ is elevated. Moreover, renal tissue must also adjust in order to conserve P (Levine et al., 1984; Murer et al., 2003).

In conclusion, overall growth performance and BMC of nursery pigs is impaired by a Ca:aP at least as low as 1.25:1 when the diet is deficient in P. Indeed, the reduction in growth performance, and in bone development, is progressively worsened as the ratio widens. However, the response of all parameters measured in this study was linear, indicating that while widening the ratio has negative consequences, the impact is not curvilinear. In other words, the severity of the impact did not lessen when the ratio was very wide, but neither did it escalate. Prior to this study, it was known that the Ca:aP was important when dietary P is close to requirement, and that the problem worsens when dietary P is below requirement. The results of this study clearly reveal the linear nature of the relationship between the Ca:aP and pig performance, even when the ratio is quite wide. Bone mineral concentration was similarly
affected. These results further confirm the importance of ensuring adequate P and an optimum ratio of Ca to P ratio in swine diets in order to maximize growth performance and skeletal development.

**DISCLOSURE**

The authors disclose that there was no conflict of interest.
LITERATURE CITED

AAFCO. 2000. Official Publication 2000. Association of American Feed Control Officials Incorporated. Atlanta, GA.

Akter, M. M., H. Graham, and P. A. Iji. 2018. Influence of different levels of calcium, non-phytate phosphorus and phytase on apparent metabolizable energy, nutrient utilization, plasma mineral concentration and digestive enzyme activities of broiler chickens. J. Appl. Anim. Res. 46:278–286. doi:10.1080/09712119.2017.1295972.

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

Bridges, T. C., L. W. Turner, G. L. Cromwell and J. L. Pierce. 1995. Modeling the effects of diet formulation on nitrogen and phosphorus excretion in swine waste. Appl. Engineer. Agricul. 11:731-739. doi:10.13031/2013.25797.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 196–221.

Cromwell, G. L. 2005. Phosphorus and swine nutrition. In: J.T. Sims and A.N. Sharpley, editors, Phosphorus: Agriculture and the environment. ASA, CSSA & SSSA, Madison, WI. p. 607-634. doi.org/10.2134/agronmonogr46.c20.

Edixhoven, J. D., J. Gupta and H. H. G. Savenije. 2013. Recent revisions of phosphate rock reserves and resources: reassuring or misleading? An in-depth literature review of global estimates of phosphate rock reserves and resources. Earth Syst. Dynam. Discuss. 4:1005-1034. doi:10.5194/esdd-4-1005-2013.

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. Third ed. Federation of Animal Science Societies, Champaign, IL.
González-Vega, J. C., Y. Liu, J. C. McCann, C. L. Walk, J. J. Loor, and H. H. Stein. 2016a. Requirement for digestible calcium by eleven- to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. J. Anim. Sci. 94:3321. doi:10.2527/jas.2016-0444.

González-Vega, J. C., C. L. Walk, M. R. Murphy, and H. H. Stein. 2016b. 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/jas.2016-0751.

Gutierrez, N.A., N.V.L. Serao, A.J. Elsbernd, S. Hansen, C.L. Walk, M.R. Bedford and J.F. Patience. 2015. Quantitative relationships between standardized total tract digestible phosphorus and calcium intake and its retention and excretion in growing pigs fed corn-soybean meal diets. J. Anim. Sci 93:2174-2182. doi:10.2527/jas.2014-8623.

Gutzwiller, A., P. Schlegel, D. Guggisberg and P. Stoll. 2014. Effects of benzoic acid and dietary calcium:phosphorus ratio on performance and mineral metabolism of weanling pigs. Asian-Australa J. Anim. Sci. 27:530-536. doi:10.5713/ajas.2013.13527.

Hays, V. W. 1976. NFIA Literature Review on Phosphorus in Swine Nutrition. National Feed Ingredient Association. West Des Moines, IA.

Heaney, R. P., and B. E. Nordin. 2002. Calcium effects on phosphorus absorption: implications for the prevention and co-therapy of osteoporosis. J. Am. Coll. Nutr. 21:239–244. doi:10.1080/07315724.2002.10719216.

Heyer, C. M. E., E. Weiss, S. Schmucker, M. Rodehutscord, L. E. Hoelzle, R. Mosenthin and V. Stefanski. 2015. The impact of phosphorus on the immune system and the intestinal
microbiota with special focus on the pig. Nutr. Res. Rev. 28:67-82.
doi.org/10.1017/S0954422415000049.

Lagos, L. V., C. L. Walk, M. R. Murphy, and H. H. Stein. 2019a. Effects of dietary digestible calcium on growth performance and bone ash concentration in 50- to 85-kg growing pigs fed diets with different concentrations of digestible phosphorus. Anim. Feed Sci. Technol. 247:262–272. doi:10.1016/j.anifeedsci.2018.11.019.

Lagos, L. V., S. A. Lee, G. Fondevila, C. L. Walk, M. R. Murphy, J. J. Loor, and H. H. Stein. 2019b. Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. J. Anim. Sci. Biotech. 10:47. doi:10.1186/s40104-019-0349-2.

Létourneau-Montminy, M. P., A. Narcy, M. Magin, D. Sauvant, J. F. Bernier, C. Pomar and C. Jondreville. 2010. Effect of reduced dietary calcium concentration and phytase supplementation on calcium and phosphorus utilization in weanling pigs with modified mineral status. J. Anim. Sci. 88:1706-1717. doi:10.2527/jas.2008-1615.

Létourneau-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 Animal 6:1590–1600. doi:10.1017/ S1751731112000560.

Létourneau-Montminy, M. P., A. Narcy, J. Y. Dourmad, T. D. Crenshaw, and C. Pomar. 2015. Modeling the metabolic fate of dietary phosphorus and calcium and the dynamics of body ash content in growing pigs, J. Anim. Sci. 93:1200–1217. doi:10.2527/jas.2014-8519.

Levine B. S., K. Ho, K. Kurokawa and J. W. Coburn. 1984. Early renal adaptation to dietary phosphorus restriction. Miner Electrolyte Metab. 10: 222–227.
Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 1998. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci. 76:808–813. doi:10.2527/1998.763808x.

Merriman, L. A., C. L. Walk, M. R. Murphy, C. M. Parsons, and H. H. Stein. 2017. Inclusion of excess dietary calcium in diets for 100- to 130-kg growing pigs reduces feed intake and daily gain if dietary phosphorus is at or below the requirement. J. Anim. Sci. 95:5439–5446. doi:10.2527/jas2017.1995.

Misiura, M. M., J. A. N. Filipe, C. L. Walk and I. Kyriazakis. 2020. How do pigs deal with dietary phosphorus deficiency? Brit. J. Nutr. 124:256-272. doi.org/10.1017/S0007114520000975.

Murer H, N. Hernando, I. Forster and J. Biber J. 2003. Regulation of Na/Pi transporter in the proximal tubule. Ann. Rev. Physiol. 65:531–542.

Nielson, A. J. 1972. Deposition of calcium and phosphorus in growing pigs determined by balance experiments and slaughter investigations. Acta. Agric. Scand. 22:223-237. doi:10.1080/00015127209433486.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Olsen, K. M, S. A. Gould, C. L. Walk, N. V. L. Serao, S. L. Hansen and J. F. Patience. 2019. Evaluating phosphorus release by phytase in diets fed to growing pigs that are not deficient in phosphorus. J. Anim. Sci. 97:327-337. doi.org/10.1093/jas/sky402.

Oster, M., F. Just, K. Büsing, P. Wolf, C. Polley, B. Vollmar, E. Muráni, S. Ponsuksili and K. Wimmers. 2016. Toward improved phosphorus efficiency in monogastrics—interplay of serum, minerals, bone, and immune system after divergent dietary phosphorus supply in swine. Am.
J. Physiol. Regul. Integr. Comp. Physiol. 310:R917-R925. doi:10.1152/ajpregu.00215.2015.
doi.org/10.1152/ajpregu.00215.2015.

Patience, J.F. 2017. The theory and practice of feed formulation. In: P. Moughan, K de Lange and W Hendriks, editors, Feed Evaluation Science. Wageningen Academic Press, Wageningen, The Netherlands. p. 457 – 490.

Peo, E. R., Jr. 1976. NFIA Literature Review on Calcium in Swine Nutrition. National Feed Ingredient Association, West Des Moines, IA.

Portale, A. A., B. P. Halloran and R. Curtis. Physiological regulation of the serum concentration of 1,25-dihydroxyvitamin D by phosphorous in normal men. J. Clin. Invest. 83:1494–1499.

Pravina, P., D. Sayaji and M. Avinash. 2013. Calcium and its role in the human body. Int. J. Res. Pharmaceut. Biomed. Sci. 4:659-668.

Qian, H., E. T. Kornegay, and D. E. Conner. 1996. Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74:1288–1297. doi:10.2527/1996.7461288x.

Reinhart, G. A., and D. C. Mahan. 1986. Effect of various calcium:phosphorus ratios at low and high dietary phosphorus for starter, grower and finishing swine. J. Anim. Sci. 63:457–466. doi:10.2527/jas1986.632457x.

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.org/10.3945/jn.109.110080.
Schlegel, P. and A. Gutzwiler. 2020. Dietary calcium to digestible phosphorus ratio for optimal growth performance and bone mineralization in growing and finishing pigs. Animals 10:178. doi:10.3390/ani10020178.

Segawa H, I. Kaneko, S. Tamanaka, M. Ito, M. Kuwahata, Y. Inoue, S. Kato and K. Miyamoto. 2004. Intestinal Na-Pi cotransporter adaptation to dietary Pi content in vitamin D receptor null mice. Am J Physiol Renal Physiol. 287:F39–47. doi.org/10.1152/ajprenal.00375.2003.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller; North Central Coordinating Committee on Swine Nutrition (NCCC-42). 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139–2144. doi:10.2527/jas.2010-3522.

Wise, A. 1983. Dietary factors determining the biological activities of phytate. Nutr. Abstr. Rev. 53:791–806.

Wu, F., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, M. A. D. Gonçalves, 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. 96:1825–1837. doi:10.1093/jas/sky101.
Figure 1. Relationship between d 0 – 28 average daily gain (ADG) and calcium to available phosphorus ratio. Data points represent least square means of dietary treatments.

Figure 2. Relationship between d 0 – 28 average daily gain (ADG) and calcium to available phosphorus ratio. Data points represent least square means of dietary treatments.

Figure 3. Relationship between d 0 – 28 average daily feed intake (ADFI) and calcium to available phosphorus ratio. Data points represent least square means of dietary treatments.

Figure 4. Relationship between d 0 – 28 gain to feed (G:F) and calcium to available phosphorus ratio. Data points represent least square means of dietary treatments.

Figure 5. Relationship between bone mineral content and Ca:aP ratio. Data points represent least square means of dietary treatments.
Table 1. Ingredient and nutrient composition of the experimental diets: Phase 1 (as-fed basis, %)

| Item                                    | 1.25      | 1.50      | 1.75      | 2.00      | 2.25      | 2.50      | 2.75      |
|-----------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Corn                                    | 46.85     | 46.64     | 46.44     | 46.23     | 46.02     | 45.82     | 45.61     |
| Soybean meal, 46.5 % CP                 | 22.50     | 22.50     | 22.50     | 22.50     | 22.50     | 22.50     | 22.50     |
| Oats, steam rolled                      | 10.00     | 10.00     | 10.00     | 10.00     | 10.00     | 10.00     | 10.00     |
| Whey permeate                           | 10.00     | 10.00     | 10.00     | 10.00     | 10.00     | 10.00     | 10.00     |
| Enzyme treated soybean meal             | 6.00      | 6.00      | 6.00      | 6.00      | 6.00      | 6.00      | 6.00      |
| Soybean oil                             | 2.00      | 2.00      | 2.00      | 2.00      | 2.00      | 2.00      | 2.00      |
| L-Lysine HCl                            | 0.59      | 0.59      | 0.59      | 0.59      | 0.59      | 0.59      | 0.59      |
| DL-methionine                           | 0.28      | 0.28      | 0.28      | 0.28      | 0.28      | 0.28      | 0.28      |
| L-threonine                             | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      |
| L-tryptophan                            | 0.03      | 0.03      | 0.03      | 0.03      | 0.03      | 0.03      | 0.03      |
| L-valine                                | 0.09      | 0.09      | 0.09      | 0.09      | 0.09      | 0.09      | 0.09      |
| Sodium chloride                         | 0.61      | 0.61      | 0.61      | 0.61      | 0.61      | 0.61      | 0.61      |
| Limestone                               | 0.24      | 0.45      | 0.65      | 0.86      | 1.07      | 1.27      | 1.48      |
| Monocalcium phosphate                    | 0.30      | 0.30      | 0.30      | 0.30      | 0.31      | 0.31      | 0.31      |
| Phytase2                                 | 0.01      | 0.01      | 0.01      | 0.01      | 0.01      | 0.01      | 0.01      |
| VTM premix3                             | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      | 0.25      |
| Calculated nutrient levels              |           |           |           |           |           |           |           |
| ME, kcal/kg                             | 3461      | 3452      | 3446      | 3439      | 3433      | 3426      | 3417      |
| Available P, %                          | 0.33      | 0.33      | 0.33      | 0.33      | 0.33      | 0.33      | 0.33      |
| STTD P, %                               | 0.36      | 0.36      | 0.36      | 0.36      | 0.36      | 0.36      | 0.36      |
|             | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 | 1.35 |
|-------------|------|------|------|------|------|------|------|
| SID Lys, %  |      |      |      |      |      |      |      |
| SID Met + Cys, % | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| SID Thr, %  | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| SID Trp, %  | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |

1 Phase 1 diet was fed from d 0 to 14 of the experiment.
2 Natuphos ® E, BASF, Florham Park, NJ.
3 Provided a minimum per kg of diet: 2,000 IU of vitamin A; 300 IU of vitamin D3; 25 IU of vitamin E; 0.90 mg of menadione (to provide vitamin K); 3 mg of riboflavin; 10 mg of d-pantothenic acid; 0.01 mg of vitamin B12, and 15 mg of niacin, 110 mg of Fe (ferrous sulfate); 2,400 mg of Zn (200 mg/kg as zinc sulfate and 2,200 mg/g as zinc oxide); 50 mg of Mn (manganese sulfate); 20 mg of Cu (copper sulfate); 0.9 mg of I (calcium iodate); 0.3 mg of Se (sodium selenite)
Table 2. Ingredient and nutrient composition of the experimental diets: Phase 2 (as-fed basis, %)

| Item                                | 1.25  | 1.50  | 1.75  | 2.00  | 2.25  | 2.50  | 2.75  |
|-------------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Ingredient, %                       |       |       |       |       |       |       |       |
| Corn                                | 47.62 | 47.45 | 47.28 | 47.12 | 46.95 | 46.78 | 46.61 |
| Soybean meal, 46.5 % CP             | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 |
| Oats, steam rolled                  | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Whey Permeate                       | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Enzyme-treated soybean meal         | 6.00  | 6.00  | 6.00  | 6.00  | 6.00  | 6.00  | 6.00  |
| Soybean oil                         | 2.00  | 2.00  | 2.00  | 2.00  | 2.00  | 2.00  | 2.00  |
| L-Lysine HCl                        | 0.44  | 0.44  | 0.44  | 0.44  | 0.44  | 0.44  | 0.44  |
| DL-methionine                       | 0.21  | 0.21  | 0.21  | 0.21  | 0.21  | 0.21  | 0.21  |
| L-threonine                         | 0.17  | 0.17  | 0.17  | 0.17  | 0.17  | 0.17  | 0.17  |
| L-tryptophan                        | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| L-valine                            | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| Sodium chloride                     | 0.61  | 0.61  | 0.61  | 0.61  | 0.61  | 0.61  | 0.61  |
| Limestone                           | 0.19  | 0.36  | 0.52  | 0.69  | 0.86  | 1.03  | 1.20  |
| Monocalcium phosphate               | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| Phytase $^2$                         | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  | 0.01  |
| VTM Premix $^3$                     | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  |
| Calculated nutrient levels          |       |       |       |       |       |       |       |
| ME, kcal/kg                         | 3468  | 3461  | 3457  | 3450  | 3444  | 3439  | 3433  |
| Available P, %                      | 0.27  | 0.27  | 0.27  | 0.27  | 0.27  | 0.27  | 0.27  |
| STTD P, %                           | 0.31  | 0.31  | 0.31  | 0.31  | 0.31  | 0.31  | 0.31  |
|          | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 |
|----------|------|------|------|------|------|------|------|
| SID Lys, % |      |      |      |      |      |      |      |
| SID Met + Cys. % | 0.71 | 0.71 | 0.71 | 0.71 | 0.71 | 0.71 | 0.71 |
| SID Thr, % | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| SID Trp, % | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |

1 Phase 2 diet was fed from d 14 to 28 of the experiment
2 Natuphos ® E, BASF, Florham Park, NJ
3 Provided a minimum per kg of diet: 2,000 IU of vitamin A; 300 IU of vitamin D3; 14 IU of vitamin E; 0.90 mg of menadione (to provide vitamin K); 2.5 mg of riboflavin; 8 mg of d-pantothenic acid; 0.01 mg of vitamin B₁₂, and 15 mg of niacin, 110 mg of Fe (ferrous sulfate); 200 mg of Zn (zinc sulfate); 50 mg of Mn (manganese sulfate); 200 mg of Cu (copper sulfate and tri-basic copper chloride); 0.3 mg of I (calcium iodate); 0.3 mg of Se (sodium selenite)
Table 3. Performance of pigs prior to the start of the experiment

| Item            | Ca:aP | 1.25 | 1.50 | 1.75 | 2.00 | 2.25 | 2.50 | 2.75 | SEM | Treatment |
|-----------------|-------|------|------|------|------|------|------|------|-----|-----------|
| Initial BW, kg  |       | 5.71 | 5.72 | 5.72 | 5.72 | 5.73 | 5.83 | 0.621|     | 0.999     |
| Final BW, kg    |       | 6.24 | 6.26 | 6.20 | 6.21 | 6.18 | 6.19 | 6.30 | 0.604| 0.999     |
| ADG, kg         |       | 0.08 | 0.08 | 0.07 | 0.07 | 0.07 | 0.06 | 0.007|     | 0.666     |
| ADFI, kg        |       | 0.11 | 0.11 | 0.10 | 0.11 | 0.10 | 0.10 | 0.10 | 0.006| 0.439     |
| G:F             |       | 0.67 | 0.67 | 0.66 | 0.65 | 0.64 | 0.64 | 0.62 | 0.043| 0.977     |

1 The pre-test period was initiated upon arrival of the pigs to the farm and lasted 7 days, during which a typical phase 1 nursery diet was fed ad libitum. There were 10 pigs per pen, and 6 or 7 pens per treatment, due to the availability of only 48 pens for the experiment. The treatment with 6 pens per treatment was selected randomly.
Table 4. Formulated and analyzed calcium and phosphorus content of dietary treatments

|                  | 1.25 | 1.50 | 1.75 | 2.00 | 2.25 | 2.50 | 2.75 |
|------------------|------|------|------|------|------|------|------|
| **Ca**           |      |      |      |      |      |      |      |
| Phase 1<sup>1</sup> Formulated, %  |      |      |      |      |      |      |      |
| Ca               | 0.41 | 0.49 | 0.58 | 0.66 | 0.74 | 0.83 | 0.91 |
| P                | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 |
| Analyzed, %      |      |      |      |      |      |      |      |
| Ca               | 0.41 | 0.49 | 0.54 | 0.66 | 0.71 | 0.78 | 0.85 |
| P                | 0.45 | 0.45 | 0.43 | 0.46 | 0.43 | 0.47 | 0.43 |
| Phase 2<sup>1</sup> Formulated, %  |      |      |      |      |      |      |      |
| Ca               | 0.34 | 0.41 | 0.47 | 0.54 | 0.61 | 0.68 | 0.74 |
| P                | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Analyzed, %      |      |      |      |      |      |      |      |
| Ca               | 0.33 | 0.40 | 0.45 | 0.52 | 0.60 | 0.64 | 0.74 |
| P                | 0.38 | 0.39 | 0.37 | 0.39 | 0.38 | 0.36 | 0.39 |

<sup>1</sup>Phase 1 was fed from d0 to 14 and phase 2 was fed from d14 to 28 of the experiment.
Figure 1

$r^2 = 0.92$

$y = -0.75x + 17.6$

Trt $P$-value $< 0.001$

Linear $P$-value $< 0.001$
Figure 2

$r^2 = 0.81$

$y = -36x + 339$

Trt $P$-value < 0.001

Linear $P$-value < 0.001
$r^2 = 0.74$
$y = -29x + 576$
Trt $P$-value = 0.032
Linear $P$-value = 0.006
$r^2 = 0.72$

$y = -0.03x + 0.61$

Trt $P$-value $<0.001$

Linear $P$-value $<0.001$
Figure 5

\[ r^2 = 0.43 \]
\[ y = -0.27x + 6.4 \]

Trt \( P \)-value = 0.270
Linear \( P \)-value = 0.015