EFFECTS OF MICROBIAL PHYTASE IN PIG DIETS ON CALCIUM REQUIREMENTS, POST-WEANING GROWTH PERFORMANCE, PHYTATE DEGRADATION, AND NUTRIENT DIGESTIBILITY

BY

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DISSERTATION

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Abstract

Seven experiments were conducted to test a series of formulated hypotheses regarding Ca and microbial phytase in pig diets. In experiment 1, the hypothesis was that analyzed values for Ca and P in commercial pig diets in the U.S. are not greater than calculated values. However, in 103 diet samples, an average of 0.19% (model; $P < 0.05$) more total Ca than expected was observed, whereas for total P, the average oversupply was only 0.06% (model; $P < 0.05$). It was, therefore, concluded that diets used in the U.S. swine industry contain more Ca than formulated, but this is not the case for P. In experiments 2 and 3, the hypothesis was that Ca requirements by pigs expressed as standardized total tract digestible (**STTD**) Ca can be used to formulate diets without or with phytase. In experiment 2, four diets were formulated with 2 formulation principles (total or STTD Ca) and 2 levels of phytase [0 or 500 phytase units/kg feed (**FTU**)] and fed to pigs from 11 to 130 kg in a 5-phase program. Results indicated that pigs fed non-phytase diets based on total Ca had greater bone ash than pigs fed STTD Ca diets, but if phytase was used, no differences were observed between formulation principles (interaction; $P < 0.05$). However, there was no effect of dietary treatment on growth performance of pigs. In experiment 3, the 4 diets used in phase 3 of experiment 2 were fed to 60-kg pigs housed in metabolism crates, and fecal and urine samples were collected for 4 d after a 5-d adaptation period. Results indicated that regardless of phytase, there were no differences in Ca retention between pigs fed STTD Ca and total Ca diets. It was concluded from experiments 2 and 3 that STTD Ca values can be used to formulate diets without affecting bone ash, growth performance, or Ca retention of pigs. In experiments 4 and 5, the hypothesis was that reducing dietary Ca and P reduces stomach pH and diarrhea incidence, and dietary phytase counters negative effects of weaning. In experiment 4, four diets were formulated with 2 levels of Ca and P (deficient and adequate) and 2 levels of

phytase (0 or 2,000 FTU). Diets were fed to newly weaned pigs in a 3-phase program with common diets used in phases 2 and 3. In phase 1, at deficient levels of Ca and P, inclusion of phytase resulted in greater average daily gain (**ADG**) and gain to feed (**G:F**), but at adequate levels, no effect of phytase inclusion was observed (interaction, $P < 0.05$). Gastric pH and fecal score were not influenced by dietary Ca and P. However, bone ash after phase 1 was greater ($P < 0.05$) if diets with adequate instead of deficient levels of Ca and P were used, but no effect of phytase was observed. In experiment 5, two control diets containing 100 or 50% of Ca and P relative to the requirement, and 6 diets in which 500, 2,000, or 16,000 FTU were added to each control diet were formulated, and a 3-phase program was used with common diets fed in phases 2 and 3. Results indicated that reducing Ca and P did not reduce gastric pH or fecal score, but pigs fed the 50% diets had reduced ($P < 0.05$) ADG in phase 1 and reduced ($P < 0.05$) bone ash at the end of phases 1 and 3 compared with pigs fed the 100% diets. In both 50 and 100% diets, phytase above 500 FTU increased ($P < 0.05$) plasma inositol and G:F of pigs. It was concluded from experiments 4 and 5 that reducing Ca and P in phase 1 diets did not influence gastric pH or fecal score, but compromised growth performance and bone ash. However, inclusion of phytase increased inositol absorption and G:F of pigs. In experiments 6 and 7, the hypothesis was that regardless of body weight (**BW**), increasing levels of phytase increases phytate degradation and nutrient digestibility. In both experiments, pigs with a T-cannula in the distal ileum and 6 diets with 0, 250, 500, 1,000, 2,000, or 4,000 FTU were used. In experiment 6, 11-kg pigs were used and samples were collected after 18 d of adaptation to the diets. Results indicated that apparent ileal digestibility (**AID**) of Trp (quadratic; $P < 0.05$), Lys and Thr (linear; $P < 0.05$) and apparent total tract digestibility (**ATTD**) of Ca and P (quadratic; $P < 0.05$) increased as phytase inclusion increased. Inositol in plasma and ileal digesta increased (linear; $P < 0.05$), but inositol phosphate

(**IP**) 6 and IP5 (quadratic; $P < 0.05$), as well as IP4 and IP3 decreased (linear; $P < 0.05$) in ileal digesta as dietary phytase increased. In experiment 7, phytase effects were evaluated in 4 phases in which the same pigs (from 25 to 130 kg) were used. Results indicated that regardless of BW, AID of most AA and ATTD of Ca and P increased (quadratic; $P < 0.05$) with increasing inclusion of phytase. However, AID of AA linearly increased ($P < 0.05$), whereas ATTD of Ca (linear; $P < 0.05$) and of P (quadratic; $P < 0.05$) decreased as BW of pigs increased. In all phases, concentrations of IP esters in ileal digesta decreased (quadratic; $P < 0.05$), whereas inositol in ileal digesta increased (quadratic; $P < 0.05$) with increasing dietary phytase. However, concentrations of IP esters increased (quadratic; $P < 0.05$) but inositol decreased (linear; $P < 0.05$) in ileal digesta as BW of pigs increased. It was, therefore, concluded from experiments 6 and 7 that regardless of BW, increasing dietary phytase increased phytate degradation and inositol release and absorption, and consequently, increased Ca, P, and AA digestibility. However, older pigs have reduced Ca and P digestibility, but increased AA digestibility compared with younger pigs and the efficiency of phytase decreased as pigs get older. Overall, commercial pig diets in the U.S. contain more Ca than expected. Diets for growing-finishing pigs can be formulated based on STTD Ca values. The use of high doses of phytase in phase 1 diets may provide benefits to weanling pigs in addition to increased release of Ca and P. The effect of phytase on phytate degradation and nutrient digestibility is independent of BW, but nutrient digestibility and phytase efficiency are influenced by BW of pigs.

Key words: Ca and P, gastric pH, phytase, nutrient digestibility, phytate degradation, pigs

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Chapter 1: Introduction

Phytate, a mixed salt of phytic acid (*myo*-inositol hexakis-dihydrogen-phosphate) is the major form of P storage in plants and a potential source of P for pigs. However, because pigs have scarce mucosal phytase activity, phytate reduces the digestibility of P (She et al., 2017). Phytate may also form complexes with starch, protein, and divalent cations, which negatively influences mineral, protein, and energy utilization by pigs (Woyengo and Nyachoti, 2013). Therefore, phytase is included at a standard level of 500 units per kg feed in swine diets to increase the release of P from phytate, reduce the inclusion of inorganic P in diets, and decrease P excretion in the manure (Selle and Ravindran, 2008). However, greater doses of phytase may improve growth performance of pigs due to complete degradation of phytate, which results in increased *myo*-inositol release and nutrient digestibility (Holloway et al., 2019; Moran et al., 2019).

Calcium tends to be oversupplied in swine diets (Walk, 2016), and excess dietary Ca is detrimental to growth performance of pigs because P digestibility is reduced (Stein et al., 2011). Requirements for Ca expressed as a ratio between digestible Ca and digestible P by growing pigs have been estimated in different body weight groups (González-Vega et al., 2016; Merriman et al., 2017; Lagos et al., 2019a; 2019b). These data were obtained from short-term experiments in which diets without phytase were used, but phytase may influence Ca requirements by increasing Ca digestibility (Selle et al., 2009). Therefore, a follow-up study was needed to validate those ratios when used to formulate diets for growing-finishing pigs without or with phytase.

Weanling pigs have limited secretion of HCl in the stomach, which results in reduced protein digestion (Kil et al., 2011). This situation may be exacerbated by the inclusion of limestone and monocalcium phosphate in weaning diets because of their high buffering capacity (Lawlor et al., 2005). Phytase reduces the need for inorganic sources of Ca and P in pig diets,

and in high doses (i.e., above 1,000 units/kg feed), phytase results in increased *myo*-inositol release. *Myo*-inositol is involved in several metabolic processes and has insulin-like effects for pigs (Lu et al., 2019). This indicates that reducing dietary Ca and P and/or including high doses of phytase in phase 1 diets may be alternatives to antibiotic growth promoters for weanling pigs.

Degradation of phytate results in increased concentrations of lower phytate esters that bind to cations and proteins to a lesser extent than phytate (Bedford and Walk, 2016). Thus, the use of high doses of phytase that degrade lower phytate esters may result in increased nutrient and energy digestibility. However, unlike poultry (Cowieson et al., 2017), the effect of high doses of phytase on protein and energy digestibility by pigs is not consistent (Liao et al., 2005; Mesina et al., 2019). In swine experiments, a maximum inclusion level of 3,000 phytase units/kg feed, a short adaptation period to the diet (i.e., 5 or 7 d), and growing pigs are usually used. Thus, it is possible that an increased protein and energy digestibility upon phytase supplementation is observed if a longer adaptation period and a greater phytase dosage are used. This also raises the question of a possible effect of pig body weight on phytase efficacy to hydrolyze phytate.

Therefore, 7 experiments were conducted to test a series of formulated hypotheses regarding Ca and microbial phytase in pig diets: 1) analyzed values for Ca in commercial diets from the U.S. swine industry are not greater than formulated values; 2) digestible Ca values can be used to formulate diets without or with phytase without affecting growth performance, bone ash, or Ca retention of growing-finishing pigs; 3) reducing limestone and monocalcium phosphate in phase 1 diets decreases gastric pH and diarrhea incidence, and high doses of phytase counter the negative effects of weaning; 4) increasing levels of phytase increases phytate degradation and nutrient and energy digestibility in diets fed to pigs regardless of body weight, but a long adaptation period and more than 3,000 phytase units/kg feed may be needed.

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Chapter 2: Phytate, phytase, and requirements for calcium and phosphorus by growing pigs: review of literature

Abbreviations

AA	amino acids
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
BEL	basal endogenous loss
DCP	dicalcium phosphate
DDGS	distillers dried grains with solubles
DMI	dry matter intake
FTU	phytase units per kilogram of feed
GIT	gastrointestinal tract
GLUT 4	glucose transporter type 4
IP	*myo*-inositol phosphate
IP6	phytate
MCP	monocalcium phosphate
PI	phosphatidylinositol
PIP_{32}	phosphatidylinositol-(4,5) bisphosphate
PIP_3	phosphatidylinositol-(3,4,5) trisphosphate
SBM	soybean meal
STTD	standardized total tract digestible/digestibility

Abbreviations (Cont.)

TEL	total endogenous loss
TTTD	true total tract digestibility

Introduction

Calcium and P are the 2 most abundant minerals in the body and are mainly stored in the skeletal tissue for development and maintenance (Veum, 2010). However, approximately 2% of Ca and 30% of P, are present in cellular fluids and cellular structures, and are involved in many physiological processes (Veum, 2010). These 2 macrominerals are supplemented in pig diets by mineral ingredients such as limestone and feed grade phosphates to overcome the low concentration of mainly Ca, but also digestible P, in plant feed ingredients where most P is stored in the form of phytate. The limited intestinal activity of phytase in pigs results in poor digestion of phytate, which can also form complexes with other nutrients, including Ca, and compromise its availability and utilization by pigs (Woyengo and Nyachoti, 2013). Exogenous phytase may increase the release of phytate-bound P in the gastrointestinal tract (**GIT**), increase P absorption, and decrease P excretion, providing economic, nutritional, and environmental benefits to the swine industry (Selle and Ravindran, 2008). Although 500 phytase units per kilogram of feed (**FTU**) is the standard phytase inclusion, greater concentrations of phytase may benefit growth, feed efficiency, and carcass yield of pigs, which is likely a result of the increased digestibility of non-P nutrients that are liberated as phytate is degraded into lower, more soluble, esters and *myo*-inositol (Cowieson et al., 2011). The release of P and Ca by phytase must be taken into account in diet formulation because of Ca and P interactions that may affect the availability of 1 mineral if the other is below or above the requirement (Crenshaw, 2001). Current requirements for Ca

and P are expressed as total Ca and standardized total tract digestible (**STTD**) P (NRC, 2012). However, an effort has been made to determine STTD of Ca in Ca-containing feed ingredients fed to pigs and to estimate Ca requirements expressed as a ratio between STTD Ca and STTD P (Stein et al., 2016; Lagos et al., 2019a). Thus, this review is focused on describing the anti-nutritional effects of phytate, the benefits of super-dosing of phytase on phytate destruction and nutrient digestibility, and the requirements for digestible Ca and P by growing pigs.

Sources of Dietary Calcium and Phosphorus

In swine diets, the use of mineral supplements is essential to provide the amount of Ca and P that animals need, although ingredients from animal and plant origin also contribute to meet the requirements for Ca and P. Cereal grains contain from 0.02 to 0.09% Ca and from 0.26 to 0.39% P, whereas oilseed meals contain from 0.33 to 0.69% Ca and from 0.71 to 1.08% P (NRC, 2012). Thus, corn and soybean meal (**SBM**) provide about 12% of the required total Ca and 47% of the required digestible P in a typical corn-SBM based diet fed to growing pigs. The amount of P bound to phytate ranges from 0.18 to 0.22% in cereal grains and from 0.38 to 0.84% in oilseed meals (NRC, 2012). This means that a corn-SBM diet contains around 0.22% phytate P. As a consequence, by inclusion of 500 FTU of phytase, dietary provisions of Ca and P can be reduced (Bedford and Cowieson, 2020), and corn and SBM will provide around 17% of total Ca and 83% of digestible P if phytase is included in the diet. Values for Ca, P, phytate P, phytate, and non-phytate P in plant feed ingredients used in pig diets are shown in Table 2.1.

Ingredients from animal origin are often included in weanling pig diets to provide sufficient quantities of highly digestible amino acids (**AA**; Rojas and Stein, 2012b). Most of these ingredients are also good sources of Ca and P, but limestone and feed grade phosphates are

also included to meet animal requirements. Values for the concentration of Ca and P in animal-based feed ingredients are listed in Table 2.2.

The main supplements of Ca in pig diets are limestone, dicalcium phosphate (**DCP**), and monocalcium phosphate (**MCP**), with the last 2 being mainly used as sources of P. Limestone mostly consist of calcium carbonate ($CaCO_3$), whereas DCP ($CaHPO_4$) and MCP [$Ca(H_2PO_4)$] are produced from defluorinated phosphoric acid and calcium carbonate (Leikam and Achorn, 2005). Based on the molecular weight, calcium carbonate, DCP, and MCP should contain 40.0, 29.5, and 17.1% Ca, and 0.0, 22.8, and 26.5% P, respectively. However, because of the presence of contaminating minerals in these ingredients, which may represent up to 5% of calcium carbonate and around 19% of DCP and MCP (Baker, 1989; Lima et al., 1995), the actual concentrations of Ca and P are less than calculated values. As a consequence, average values of 38.5, 24.8, and 16.9% for Ca and 0.02, 18.8, and 21.5% for P are often analyzed in calcium carbonate, DCP, and MCP, respectively (NRC, 2012; Table 2.3).

Anti-nutritional Effects of Phytate

Phytate

In cereals, legumes, and oilseeds, most P is stored as phytate (**IP6**), which is a mixed salt of phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate, $C_6H_{18}P_6O_{24}$) that involves Mg and K (Selle et al., 2009). Cereal grains accumulate phytate in the aleurone layer and germ, whereas legumes and oilseeds uniformly distribute phytate in the whole seed (Skoglund et al., 2009). Phytate P represents from 60 to 80% of the total P of cereals, legumes, and oilseeds, and is used as a source of P and other minerals during seed germination (Skoglund et al., 2009).

Phytate is a very stable compound that contains 12 proton replacement sites at acidic, neutral, and basic pH (Nolan et al., 1987; Maenz et al., 1999). Therefore, along the GIT, phytate is negatively charged and can chelate divalent cations such as Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+} and Fe^{2+} (in this order of stability; Cheryan, 1980), which results in a reduced digestibility of these minerals. The chelating capacity of phytate, however, is not limited to minerals. Phytate may also form complexes with starch and protein over a wide range of pH (Selle et al., 2000), reducing protein and energy utilization.

Phytate and minerals

Phytate is a potential source of P for pigs as it contains 282 g of P/kg (Selle et al., 2000), but because pigs have insufficient activity of mucosal phytase (Jongbloed et al., 1992), phytate negatively impacts P digestibility. Indeed, the apparent total tract digestibility (**ATTD**) of P in swine diets increased as the concentration of phytate decreased (Spencer et al., 2000; Bohlke et al., 2005; Htoo et al., 2007; Sheng et al., 2007; Hill et al., 2009; González-Vega et al., 2014).

Of the divalent cations that can form complexes with phytate, Ca^{2+} is the most abundant in pig diets. One molecule of phytate may bind up to 5 Ca atoms along the GIT of pigs, thus, 33% of dietary Ca may form complexes with phytate (Selle et al., 2009). However, despite the clear negative correlation between ATTD of P and dietary phytate (Letourneau-Montminy et al., 2012; Velayudhan et al., 2019), positive effects of low phytate diets on the ATTD and STTD of Ca in diets for pigs are less consistent (Kemme et al., 1999; Spencer et al., 2000; Bohlke et al., 2005; Sheng et al., 2007; González-Vega and Stein, 2014).

Regardless of mineral source, pigs fed diets with reduced phytate had increased ATTD of Zn and Cu compared with pigs fed diets with conventional levels of phytate (Liu et al., 2014). However, the ATTD of Mn and Fe was not affected by the concentration of phytate in the diet.

This concurs with data indicating less stable complexes between phytate and Zn or Cu than between phytate and Mn or Fe (Cheryan, 1980).

Phytate and protein

Phytate may bind protein in an acidic or a basic environment; however, with low pH, phytate directly binds to proteins in a binary complex, whereas ternary complexes mediated by divalent cations are formed under high pH (Reddy and Salunkhe, 1981). Under acidic conditions, the pH is below the isoelectric point of protein, which results in basic AA getting positively charged, which makes them susceptible to form complexes with phytate (Cheryan, 1980). If phytate-protein complexes precipitate, they may become less soluble and less digestible (Selle et al., 2000). Phytate may also compromise the activity of trypsin (Caldwell, 1992) and cause hypersecretion of pepsin, HCl, and mucin from the stomach, resulting in increased endogenous losses of AA (Selle et al., 2009).

The apparent ileal digestibility (**AID**) of His, Lys, Ile, Phe, Thr, and Val, and the standardized ileal digestibility of Lys, Phe, and Thr of corn-SBM based diets was greater in growing pigs fed low-phytate diets than in pigs fed diets with high phytate content (Bohlke et al., 2005; Liao et al., 2005). However, when synthetic diets were used, low phytate content did not improve the AID of AA in weanling pigs (Woyengo et al., 2009). Increased secretion of gastric pepsin was observed in weanling pigs as a result of phytate supplementation (Woyengo, 2010), however, no effect of phytate content was observed on the endogenous losses of AA (Woyengo et al., 2009).

Phytate and energy

The anti-nutritional effect of phytate on energy digestibility is associated with a decreased digestibility of protein, starch, and lipids. Phytate can also form complexes with starch or lipids,

compromising the digestibility of these nutrients (Thompson et al., 1987; Lee et al., 2007). Glucose absorption tended to be reduced in the jejunum of broilers as increasing concentrations of phytate were infused along with glucose (Onyango et al., 2008). In pigs, a reduction in the ATTD of gross energy from 88 to 83% was observed as the phytate content increased from 0.78 to 1.70% (Liao et al., 2005).

Role of Phytase in Swine Diets

Phytase

The stepwise hydrolysis of phytate to release inorganic phosphate from the *myo*-inositol ring is achieved by the activity of phytase (*myo*-inositol hexaphosphate phosphohydrolase), a phosphatase present in animal tissues, plants, and microorganisms (Selle et al., 2010). Phytases are divided into acidic and basic depending on their optimal pH (maximum P release at pH 5.0 and 8.0, respectively), and phytases are further classified as 3-phytases, 5-phytases, and 6-phytases based on the position on the *myo*-inositol ring of the P that is first released during hydrolysis (Greiner and Konietzny, 2010). Thus, these 3 phytases release P from C3, C5, and C6 of *myo*-inositol, respectively.

The activity of phytase in the stomach and small intestine of pigs is limited, and the microbial phytase activity in the hindgut (Selle et al., 2010) is irrelevant to pigs because minimal absorption of P takes place in the hindgut (Schlemmer et al., 2001; Rosenfelder-Kuon et al., 2020a). Cereals such as wheat, barley, and rye have intrinsic phytase activity and therefore, increased P digestibility compared with corn or sorghum (McGhee and Stein, 2019). However, intrinsic phytase in cereal grains is only 40% as effective as microbial phytase (Zimmermann et al., 2002). Inclusion of rye in corn-SBM-based diets results in increased P digestibility because

rye has substantial intrinsic phytase activity, but inclusion of microbial phytase further increased the digestibility of P in the diet (Archs Toledo et al., 2020). Therefore, inclusion of microbial phytase in pig diets has been a common practice for over 30 years (Simons et al., 1990). Current commercial microbial phytases are synthesized by bacteria (*Escherichia coli*, *Buttiauxella* sp., or *Citrobacter braakii*) or fungi (*Aspergillus niger* or *Peniophora lycii*), and with the exception of *A. niger* that is a 3-phytase, all the other phytases are 6-phytases (Menezes-Blackburn et al., 2015). The majority of microbial phytases are acidic, with a maximum dephosphorylation of phytate at pH 4.0 to 6.0 (Greiner and Konietzny, 2010), which indicates that gastric hydrolysis of phytate plays an important role in the removal of the anti-nutritional effects of phytate.

Commercially available microbial phytases have different enzymatic properties (i.e., pH optimum, resistance to digestive proteolytic activity, thermostability, kinetic constants, substrate specificity, starting point for phytate dephosphorylation, and specific activity) that altogether are responsible for phytase efficacy (Greiner and Konietzny, 2010; Menezes-Blackburn et al., 2015). Phytase activity, however, may be affected by metal cations such as Ca^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+} that bind phytate and form metal cation-phytate complexes in the gastrointestinal tract (Cheryan, 1980; Selle et al., 2009). A phytase is considered ideal if it has a low production cost, efficiently releases phosphate throughout the gastrointestinal tract, and remains active under heat during feed processing and storage (Greiner and Konietzny, 2010).

Nutrient digestibility

The main reason for including microbial phytase in swine diets is to increase the digestibility of P in plant ingredients, and to reduce the excretion of P in the manure (Jongbloed and Lenis, 1992). Therefore, diets for pigs usually contain between 500 and 1,000 FTU, with 1 FTU defined as the phytase activity needed to release 1 μmol of P from sodium phytate at 37 °C and 5.5 pH

(Jones et al., 2010). A phytase dosage above 1,000 FTU is known as super-dosing and is believed to provide extra-phosphoric benefits to pigs (Lu et al., 2019a).

Supplementation of 500 to 1,000 FTU increased the ATTD and STTD of P in cereal grains, corn and rice coproducts, and oilseed meals by an average of 21.6 percentage units (Akinmusire and Adeola, 2009; Almeida and Stein, 2010, 2012; Rodríguez et al., 2013; Rojas et al., 2013; Casas and Stein, 2015; Maison et al., 2015; McGhee and Stein, 2019). This concurs with a recent meta-analysis from 88 experiments reporting that supplementation of 1,000 FTU increases the digestibility of P by 21.1 percentage units in diets fed to pigs (Rosenfelder-Kuon et al., 2020b). From a practical point of view, this indicates that inclusion of 500 FTU reduces the required concentration of dietary P by 0.10 to 0.12% (Selle and Ravindran, 2008).

Values for ATTD and STTD of Ca in corn-based diets containing canola meal, limestone, or fish meal as the sole source of Ca were increased by the inclusion of 500 to 1,500 FTU of microbial phytase (González-Vega et al., 2013; 2015a, b; Lee et al., 2019). The same inclusion of phytase resulted in a reduction of the basal endogenous loss (**BEL**) of Ca in growing pigs (Lee et al., 2019), but did not affect the total endogenous loss (**TEL**; González-Vega et al., 2013). The ATTD of Ca, Mg, and Cu in diets fed to pigs was improved by the addition of 500 FTU in the diet, but Zn was not affected by phytase inclusion (Madrid et al., 2013). Likewise, inclusion of 500 to 1,000 FTU of microbial phytase in corn-SBM diets fed to pigs increased the ATTD of Ca and Mg, but this was not the case for Na or K (Zeng et al., 2016). However, increasing doses of phytase from 250 to 2,500 FTU linearly increased the ATTD of Ca, K, Na, Mg, and Zn, but not S, Cu, Fe, or Mn in corn-SBM diets fed to pigs (Arredondo et al., 2019).

Effects of phytase on the digestibility of AA in pigs are marginal as indicated by the increase of 2.8 percentage units as the overall mean from 342 observations (Selle and Ravindran,

2008). Inclusion of 1,000 FTU increased the AID of Ile, Thr, Asp, Ser, and Tyr, but 2,000 FTU were needed to increase the standardized ileal digestibility of Arg, Ile, Gly, Pro, Ser, and Tyr in corn-SBM diets fed to 25-kg pigs (Velayudhan et al., 2015). In 19-kg pigs fed corn-SBM diets, 1,000 FTU were not sufficient to improve the AID of AA in the diets, but 20,000 FTU increased the AID of Leu, Thr, Asp, Cys, and Ser (Zeng et al., 2016). However, more recent data indicated that inclusion of 3,000 or 4,000 FTU has no effect on the AID of AA in diets based on corn and SBM or corn, SBM, and canola meal fed to growing pigs (She et al., 2018a; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a).

Increasing levels of phytase from 0 to 1,000 FTU or 0 to 2,500 FTU linearly increased the concentration of digestible energy in corn, barley, and SBM or corn and SBM based diets fed to pigs, respectively (Brady et al., 2003; Arredondo et al., 2019). Likewise, phytase had a positive effect on the AID or ATTD of gross energy in diets and ingredients fed to pigs (Velayudhan et al., 2015; Kiarie et al., 2016). However, some data indicated negative or no effect of phytase inclusion on the AID or ATTD of gross energy (Cervantes et al., 2011; Zeng et al., 2016; She et al., 2018a; Mesina et al., 2019). Therefore, the effect of microbial phytase supplementation on the concentration of digestible energy in diets fed to pigs is limited and may be dependent on the phytase used or the ingredient composition of the diet.

Phytate degradation and *myo*-inositol production

As inorganic P is released by phytase, *myo*-inositol phosphate (**IP**) 5, IP4, IP3, IP2, IP1, and free *myo*-inositol are produced (Schlemmer et al., 2001), and depending on the degree of phosphorylation, this may provide beneficial effects to pigs (Cowieson et al., 2011). Mucosal phytase activity tends to increase if the number of phosphate groups decreases (Hu et al., 1996), thus, the lower the number of phosphates on the inositol ring, the more P will be released.

However, although lower IP esters such as IP4 or IP3 have less binding capacity for minerals than phytate, if those minerals are used as co-factors by digestive enzymes, nutrient digestibility may be compromised (Bedford and Walk, 2016). Likewise, in vitro data indicate that porcine pepsin activity is inhibited by IP4 and IP3 esters, although to a lesser extent than by IP6 or IP5 (Yu et al., 2012). This then indicates that complete destruction of phytate is needed for proper nutrient digestion by pigs. Inclusion of phytase in corn-SBM-canola meal-based diets results in reduced concentration of IP6 and IP5, but increased concentration of IP4 and IP3 in ileal content of growing pigs compared with diets without phytase; however, the concentration of IP6, IP5, IP4 and IP3 decreases in ileal content of pigs as phytase level increases from 750 to 3,000 FTU (Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a). However, no phytase is able to completely dephosphorylate phytate in plant feed ingredients because the phosphate located at the C2 position of phytate is resistant to phytase hydrolysis (Greiner and Konietzny, 2010). Therefore, the role of intestinal alkaline phosphatase in the release of phosphate from IP1 is crucial for the total breakdown of phytate and subsequent release of *myo*-inositol (Lu et al., 2019b).

The end product of phytate degradation is *myo*-inositol, a cyclic sugar that is absorbed in the small intestine via a Na^+ coupled transporter called $SMIT_2$ (Aouameur et al., 2007) and distributed to different tissues through the bloodstream. *Myo*-inositol can also be synthesized in the body from glucose and is primarily used as an osmolyte in brain, liver, and bone tissues, and for synthesis of phosphatidylinositol (**PI**; Huber, 2016). In the cell membrane, PI is phosphorylated at different carbons of the *myo*-inositol ring to produce phosphoinositides (i.e., PI-bisphosphates and -triphosphates), which are involved in several cell signaling pathways (Falkenburger et al., 2010). Upon stimulation, PI-(4,5) bisphosphate (**PIP_2**), the most important PI-bisphosphate, is irreversibly hydrolyzed by phospholipase C into PI-(1,4,5) triphosphate and

diacylglycerol, which are second messengers contributing to cell growth and differentiation (Gonzalez-Uarquin et al., 2020). Upon insulin-like growth-factor stimulation, phosphoinositide-3 kinase synthesizes PI-(3,4,5) trisphosphate (**PIP_3**) from PIP_2, which activates protein kinase B. Translocation of glucose transporter type 4 (**GLUT 4**) in muscle and adipose tissue to the plasma membrane is stimulated by protein kinase B, and results in increased glucose uptake (Lee and Bedford, 2016). Activated protein kinase B also participates in the activation of mTOR, a protein complex involved in the synthesis of protein in skeletal tissue (Lee and Bedford, 2016).

Dietary *myo*-inositol also plays a role in lipid metabolism as indicated by the increase in the hepatic concentration of triglycerides, the stimulation of low-density lipoproteins synthesis, the improvement in triglycerides synthesis and lipid storage capacity in adipocytes, and the stimulation of adipocytes differentiation, however, the mechanism of action is yet not well understood (Huber, 2016; Gonzalez-Uarquin et al., 2020). Data from rats and humans have also demonstrated that *myo*-inositol is involved in bone mineralization, brain and peripheral nerve function, and reproduction (Gonzalez-Uarquin et al., 2020).

In pigs, super-dosing of phytase had a positive effect on growth performance parameters, which may be a result of phytate destruction (Moran et al., 2017; Holloway et al., 2019; Lu et al., 2019a). Data indicated a positive linear effect of increasing levels of microbial phytase from 0 to 3,000 FTU on the concentration of *myo*-inositol in duodenal and ileal digesta, as well as in plasma of pigs (Guggenbuhl et al., 2016; Cowieson et al., 2017; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a). In weanling pigs, *myo*-inositol supplementation or inclusion of 2,000 to 2,500 FTU in diets with adequate levels of Ca and P resulted in increased feed efficiency, indicating that during the post-weaning period, where pigs undergo high levels of stress, *myo*-inositol may be a conditionally essential nutrient (Lu et al., 2019b; Moran et al.,

2019). Inclusion of 2,000 FTU of phytase in diets with reduced levels of Ca and P from d 21 to 49 post-weaning resulted in increased growth performance of pigs compared with pigs fed a positive control diet (Lu et al., 2019b). On d 49, pigs fed diets with 2,000 FTU had a greater concentration of inositol in plasma and greater GLUT 4 in muscle than pigs fed a negative control diet, indicating that high levels of phytase increases inositol release, which stimulates GLUT4 translocation and increases muscle glucose uptake (Lu et al., 2019b).

Digestibility of Calcium and Phosphorus

Values for the relative bioavailability of Ca and P used to be measured by the slope-ratio method using bone development as the main response variable. Monosodium phosphate or MCP was used as the standard for P availability and limestone as the standard for Ca availability (Soares, 1995a, b). The estimated relative bioavailability of P ranges from 14 to 78% in plant-based ingredients, from 73 to 94% in ingredients from animal origin, and from 53 to 97% in P supplements (Kiarie and Nyachoti, 2010). For Ca, the few available data indicate a relative bioavailability of 51 to 99% in Ca supplements (Ross et al., 1984). Nutrient bioavailability is the estimated fraction of an ingested nutrient that is absorbed and utilized by the animal relative to the amount absorbed from a standard source (Littell et al., 1995). However, the amount of a certain nutrient that is absorbed and excreted by the animal cannot be calculated from values for relative bioavailability and those values are not additive in mixed diets (NRC, 2012; Baker et al., 2013). Therefore, values for digestibility of nutrients are now used in diet formulation rather than values for relative bioavailability.

Digestibility, the disappearance of a nutrient from the GIT, is calculated after subtracting the amount of the nutrient present in ileal digesta or feces from the amount ingested by the

animal (Stein, 2017). Thus, digestibility is expressed as AID or ATTD depending on whether or not the nutrient is absorbed from or secreted into in the large intestine (Stein, 2017). The lack of differences between ileal and total tract digestibility values for Ca and P in corn, SBM, and calcium supplements, indicate that no net absorption or secretion of these 2 minerals takes place in the hindgut of pigs (Shen et al., 2002; Bohlke et al., 2005; González-Vega et al., 2014; Zhang et al., 2016; Liu et al., 2018; Mesina et al., 2019). Therefore, for economic and practical reasons, ATTD values are used to determine digestibility of Ca and P in diets for pigs.

The ATTD of Ca and P as a percentage is determined by total fecal collection following the marker to marker approach (Kong and Adeola, 2014) and can be calculated using Eq. 1 (Almeida and Stein, 2010).

$$\text{ATTD, \%} = \frac{\text{Ca/P intake - Ca/P fecal output}}{\text{Ca/P intake}} \times 100, \quad [1]$$

The ATTD of Ca and P in diets fed to pigs may not only be affected by dietary content of phytate and phytase, but also by the dietary concentration of Ca and P. The ATTD of Ca in diets is not affected by the concentration of dietary Ca, if this ranges from 50 to 150% the requirement (Stein et al., 2011; González-Vega et al., 2014) because when the transcellular absorption of Ca is hormonally downregulated by high dietary Ca, the paracellular Ca absorption increases (Crenshaw, 2001; Lagos et al., 2019a). However, if Ca is included by more than 150% of the requirement, the ATTD of Ca increases by increasing concentrations of Ca (González-Vega et al., 2013; 2016b). Increasing concentrations of DCP or MCP in diets increases the ATTD of P (Stein et al., 2008; Zhai and Adeola, 2012, 2013; González-Vega et al., 2016b; Liu et al., 2018) because P in feed phosphates is more digestible than P in plant ingredients (She et al., 2017).

The ATTD of Ca in diets fed to pigs is not affected by the concentration of dietary P (Stein et al., 2006; 2008; González-Vega et al., 2016b). However, increasing concentrations of

Ca reduces the ATTD of P (Stein et al., 2011; González-Vega et al., 2016b; Velayudhan et al., 2019) as a result of complexes between Ca and P being formed in the GIT of pigs.

The main concern with the use of ATTD values in diet formulation is that the endogenous loss of nutrients is not taken into account when ATTD values are calculated. It is assumed that the fecal output only contains nutrients from the diet that were not absorbed by the animal, although endogenous nutrients also are included in the output. Thus, ATTD values of feed ingredients may be underestimated in diets that have low concentration of the nutrient that is being analyzed (Fan et al., 2001), and the ATTD values will not be additive in mixed diets (Fan and Sauer, 2002). Endogenous losses are divided between BEL (non-diet dependent) and specific endogenous losses (diet dependent). Together, basal and specific endogenous losses add to TEL (González-Vega and Stein, 2016). Endogenous losses of Ca and P in pigs originate from sloughed off enterocytes, intestinal enzymes, and mucin secreted into the GIT and secretions of the pancreas, gallbladder, stomach, and salivary glands (Fan et al., 2001; González-Vega et al., 2013). The BEL of nutrients is often estimated using synthetic diets that do not contain the nutrient of interest and is expressed in milligrams per kilogram of dry matter intake (**DMI**). Values for BEL of Ca and P can be calculated using Eq. 2

$$\text{BEL, mg/kg DMI} = \frac{\text{Ca/P fecal output}}{\text{DMI}} \times 1{,}000, \quad [2]$$

The BEL of Ca ranges from 329 to 463 mg/kg DMI in growing pigs fed diets based on corn (González-Vega et al., 2015a; Merriman and Stein, 2016; Blavi et al., 2017; Lee et al., 2018; 2019), and from 123 to 220 mg/kg if cornstarch based diets are used (González-Vega et al., 2015a, b). The fiber content in corn that is absent in cornstarch may be the reason for the greater values for BEL of Ca in corn-based diets. For P, the BEL is estimated to be 190 mg/kg DMI (NRC, 2012), which is supported by studies that reported values from 187 to 212 mg/kg

DMI when P-free diets based on cornstarch were used (Rojas and Stein, 2012a; Kim et al., 2014; Adhikari et al., 2015).

Standardized total tract digestibility values are obtained after ATTD values are corrected for BEL and STTD values are additive in mixed diets (She et al., 2018b). The STTD of Ca and P as a percentage can be calculated using Eq. 3.

$$\text{STTD, \%} = \frac{[\text{Ca/P intake - (Ca/P fecal output - BEL of Ca/P)}]}{\text{Ca/P intake}} \times 100, \quad [3]$$

Values for P digestibility in feed ingredients fed to pigs have been extensively studied to decrease the use of feed grade phosphates and reduce the excretion of P in the manure. Limestone, in contrast, is a low-cost ingredient so less attention has been given to determination of digestibility values for Ca in feed ingredients. Table 2.4 includes values for the ATTD and STTD of Ca and P in Ca- and P-containing feed ingredients.

Total endogenous losses of nutrients may be determined by the regression of digested nutrients on ingested nutrients (Fan et al., 2001; González-Vega et al., 2013). Values for TEL of Ca by pigs fed diets based on corn and corn gluten meal were 207, 264, and 316 mg/kg DMI if the source of Ca was limestone, DCP, or limestone and DCP, respectively (Zhang and Adeola, 2017). The value for TEL of Ca and P obtained in pigs fed diets based on cornstarch and canola meal or SBM was 175 and 45 mg/kg DMI, respectively (Akinmusire and Adeola, 2009; González-Vega et al., 2013).

Values for ATTD corrected for TEL of nutrients result in true total tract digestibility (**TTTD**) values, which are also additive in mixed diets (Fang et al., 2007). The TTTD of Ca and P as a percentage can be calculated using Eq. 4.

$$\text{TTTD, \%} = \frac{[\text{Ca/P intake - (Ca/P fecal output - TEL of Ca/P)}]}{\text{mineral intake}} \times 100, \quad [4]$$

Values for TTTD of Ca and P were not affected by increasing levels of Ca and P when supplied by SBM and canola meal, respectively (González-Vega et al., 2013; Liu et al., 2016). The TTTD of Ca and P in calcium carbonate, DCP, canola meal, and SBM is shown in Table 2.5.

Retention

After absorption, nutrients are used for metabolic functions or stored in the body, but if this does not occur, nutrients will be excreted in the urine and will not be retained by the animal. Pigs retain Ca and P in the skeletal tissue only if the other mineral is also available (Crenshaw, 2001). Therefore, retention of Ca and P as a percentage can be used to estimate requirements for Ca and P by pigs (González-Vega et al., 2016a) and can be calculated using Eq. 5.

$$\text{Retention, \%} = \frac{[\text{Ca/P intake - (Ca/P fecal output - Ca/P urine output)}]}{\text{Ca/P intake}} \times 100, \quad [5]$$

Requirements for Calcium by Growing Pigs

The requirement for Ca varies depending on the age, sex, and genetics of pigs (Thomas and Kornegay, 1981; NRC, 2012). Likewise, the concentration of energy, Ca, P, and vitamin D in the diet, as well as the ratio between Ca and P, affect the amount of Ca that pigs need (Doige et al., 1975; Qian et al., 1996).

Total calcium requirements

Calcium requirements by pigs have been estimated for decades along with P due to the importance of the ratio between Ca and P for growth, bone development, digestion, and metabolism of pigs (Aubel and Hughes, 1936; Aubel et al., 1941). Empirical measurements using mainly growth performance, bone development, blood metabolites, and carcass characteristics as response variables, have been preferred over factorial calculations to determine

Ca requirements (Crenshaw, 2001). Studies were designed to have a fixed value for P and increasing levels of Ca (Stockland and Blaylock, 1973), or different levels of both Ca and P (Coalson et al., 1972). Different values and ratios for Ca and P were suggested for optimal animal performance, but a common observation was that requirements for Ca and P to maximize growth performance are not sufficient to maximize bone development. From these and other studies, recommendations for total Ca and total P were summarized and published (NRC, 1979).

Environmental concerns led to research aimed at reducing P excretion, which along with the role of phytase in the release of P from phytate in plant ingredients (Jongbloed and Lenis, 1992), resulted in P requirements being expressed as available P (NRC, 1998), but this was later changed to STTD P values (NRC, 2012). Calcium requirements, however, have always been expressed as total Ca, and are currently calculated by multiplying the STTD P requirement by 2.15 (NRC, 2012). The model approach used to estimate the STTD P requirement was based on the assumption that 1) 85% of P requirement to maximize P retention will maximize growth performance; 2) body P mass and body protein mass are linearly related; 3) P retention equals 77% of the STTD P ingested; 4) the daily BEL of P is 190 mg/kg DMI; and 5) there is a minimum loss of 7 mg/kg BW of P in urine per day. Body P mass (grams) and STTD P requirement as a percentage can be calculated using Eq. 6 and 7, respectively.

$$\text{Body P mass, g} = 1.1613 + 26.012 \times \text{body protein mass} + 0.2299 \times (\text{body protein mass})^2, \quad [6]$$

$$\text{STTD P requirement, \%} = 0.85 \times \left[\frac{(\text{maximum whole-body P retention})}{0.77} + 0.19 \times \text{DMI} + 0.007 \times \text{BW}\right], \quad [7]$$

The basis for the value of 2.15 to calculate total Ca requirements is not clear, and indeed, the NRC (2012) recognized that a ratio between digestible Ca and digestible P is preferred, but

because values for the STTD of Ca in feed ingredients were not available, recommended values for Ca and P by pigs from 11 to 25, 25 to 50, 50 to 75, 75 to 100, and 100 to 130 kg are expressed as total Ca and STTD P (Table 2.6).

Digestible calcium requirements

Values for STTD of Ca in different Ca-containing feed ingredients were determined, allowing for the formulation of diets based on STTD Ca (Stein et al., 2016). Therefore, 5 experiments were conducted to determine requirements for Ca by pigs from 11 to 130 kg expressed as a ratio between STTD Ca and STTD P. It was initially hypothesized that the STTD Ca requirement to maximize growth is greater than the requirement that maximizes bone development, but because 98% of Ca is stored in the skeleton, requirements to maximize bone ash and Ca retention are approximately the same (González-Vega, 2016).

In the first study conducted to determine the requirement for STTD Ca, 6 dietary treatments were fed for 22 d to 11- to-25 kg pigs (González-Vega et al., 2016a). The diets had a fixed concentration of STTD P (10% above the requirement; NRC, 2012) and 6 concentrations of STTD Ca. The response variables were growth performance, bone ash composition, and Ca retention. Results indicated that STTD Ca concentrations at or above 0.48% and 0.60%, maximize bone ash (grams per femur) and Ca retention, respectively. However, a reduction in ADG and G:F at STTD Ca concentrations above 0.50% prevented the estimation of the STTD Ca concentration that maximized growth performance. Therefore, a number of studies were subsequently designed to have 5 concentrations of STTD Ca (from 30 to 170% of the estimated requirement) and 3 (Merriman et al., 2017; Lagos et al., 2019b) or 4 (González-Vega et al., 2016c; Lagos et al., 2019a) concentrations of STTD P (from 50 to 150% of the estimated requirement). In these experiments, not only was the requirement for STTD Ca, but also the

optimal ratio between STTD Ca and STTD P estimated in pigs from 11 to 25 kg (Lagos et al., 2019a), 25 to 50 kg (González-Vega et al., 2016c), 50 to 85 kg (Lagos et al., 2019b), and 100 to 130 kg (Merriman et al., 2017).

Results of these studies indicated that 1) requirements for Ca and P should be expressed as a ratio between STTD Ca and STTD P instead of as individual values; 2) the production target should be established to decide if ratios that maximize growth performance (meat production) or bone development (reproduction) should be used to formulate diets; 3) excess Ca is detrimental to pig growth performance and should be avoided; 4) the ratio of STTD Ca to STTD P that maximizes growth performance decreases as pig body weight increases, whereas the ratios that maximize bone mineralization increase as pigs get heavier (Fig. 2.1); and 5) because all studies were short-term experiments, a follow up experiment using pigs from 11 to 130 kg is needed to validate the ratios between STTD Ca and STTD P that were reported (Table 2.7).

Conclusions

The existence of values for the digestibility of Ca in feed ingredients allowed for the estimation of Ca requirements by growing pigs expressed as a ratio between STTD Ca and STTD P, but a study that validates these ratios in diets without and with phytase needs to be conducted. Phytase increases the digestibility of P, Ca, and other minerals, but improvement in protein and energy digestibility is not always observed. This indicates that the positive effects of phytase on growth performance of pigs may be more related to the degradation of phytate and the subsequent production of *myo*-inositol. This compound is involved in many metabolic processes and has growth promoting potential, which can be particularly beneficial for weanling pigs. Therefore, the optimal inclusion of phytase to maximize phytate degradation needs to be estimated.

Figure

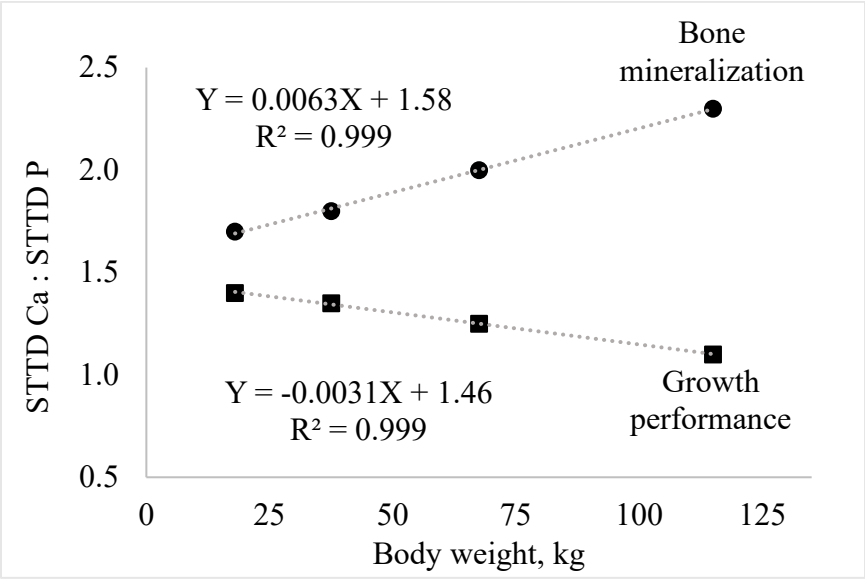

Figure 2.1. Linear correlation between body weight of pigs and the ratio between standardized total tract digestible (STTD) Ca and STTD P ratios to maximize growth performance (square) and bone mineralization (circle).

Tables

Table 2.1. Concentration of calcium, phosphorus, and phosphorus bound to phytate in cereal grains, corn co-products, and oilseed meals[1]

Source, %	Calcium	Phosphorus	Phytate P	Phytate[2]	Non phytate P[3]
Cereal grains					
Barley	0.06	0.35	0.22	0.78	0.13
Corn	0.02	0.26	0.21	0.74	0.05
Oats	0.03	0.35	0.19	0.67	0.16
Rice	0.09	0.34	0.18	0.64	0.16
Rye	0.08	0.30	0.20	0.71	0.10
Sorghum	0.02	0.27	0.18	0.64	0.09
Triticale	0.04	0.33	0.21	0.74	0.12
Wheat	0.06	0.39	0.22	0.78	0.17
Corn co-products					
Corn germ meal	0.02	1.27	1.07	3.79	0.20
Corn gluten meal	0.03	0.49	-	-	-
DDGS[4]	0.12	0.73	0.26	0.92	0.47
Oilseed meals					
Canola meal	0.69	1.08	0.65	2.30	0.43
Soybean meal	0.33	0.71	0.38	1.35	0.33
Sunflower meal	0.38	0.95	0.84	2.98	0.11

[1]Values from NRC (2012).

[2]Calculated by dividing the phytate P by 0.282 (Tran and Sauvant, 2004).

[3]Calculated as the difference between total P and phytate P.

[4]DDGS = Distillers dried grains with solubles.

Table 2.2. Concentration of calcium and phosphorus in blood, fish meal, milk products, meat and bone meal, and poultry products[1]

Product	Calcium (%)	Phosphorus (%)
Blood		
Cells	0.02	0.34
Meal	0.05	0.21
Plasma	0.13	1.28
Fish		
Meal	4.28	2.93
Milk		
Casein	0.20	0.68
Skim milk powder	1.27	1.06
Whey permeate	0.27	0.34
Whey powder	0.62	0.69
Meat and bone		
Meat meal	6.37	3.16
Meat and bone meal	10.94	5.26
Poultry		
Feather meal	0.41	0.28
Poultry byproduct	4.54	2.51
Poultry meal	2.82	1.94

[1]Values from NRC (2012).

Table 2.3. Concentration of calcium and phosphorus in Ca and P supplements[1]

Supplement	Calcium (%)	Phosphorus (%)
Calcium		
Calcium carbonate	38.5	0.02
Limestone	35.8	0.01
Phosphorus		
Dicalcium phosphate	24.8	18.8
Disodium phosphate	ND[2]	21.2
Magnesium phosphate	10.1	19.7
Monocalcium phosphate	16.9	21.5
Monosodium phosphate	0.1	24.7
Tricalcium phosphate	34.2	17.7

[1]Values from NRC (2012).

[2]ND = non-detectable.

Table 2.4. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of calcium and phosphorus in mineral supplements, plant feedstuffs, and animal feedstuffs without (-) or with 500 to 1,500 phytase units per kg of feed (+)[1]

Item, %	Calcium				Phosphorus			
	ATTD		STTD		ATTD		STTD	
Phytase	-	+	-	+	-	+	-	+
Mineral supplements								
Calcium carbonate[2,3,4,5,6,7,8,9]	67±6(26)	74±4(8)	72±5(13)	77±4(8)	-	-	-	-
Dicalcium phosphate[2,6,7,8,10]	72±3(11)	76(1)	81±4(4)	79(1)	80±2(2)	-	88±1(2)	-
Lithothamnium calcareum[2]	63(1)	66(1)	65(1)	69(1)	-	-	-	-
Monocalcium phosphate[2,6,10,11]	83(1)	83(1)	86(1)	86(1)	88±4(5)	89(1)	93±2(5)	90(1)
Plant feedstuffs								
Cereal grains								
Barley[12]	-	-	-	-	37(1)	60(1)	45(1)	68(1)
Corn[12,13,14,15,16]	-	-	-	-	27±9(5)	59±5(6)	34±9(4)	66±5(6)
Rice (brown)[17]	-	-	-	-	19(1)	50(1)	32(1)	65(1)
Rye[12]	-	-	-	-	44±4(3)	58±6(3)	51±5(3)	65±6(3)
Sorghum[12]	-	-	-	-	10(1)	46(1)	17(1)	54(1)
Wheat[12]	-	-	-	-	31(1)	52(1)	37(1)	58(1)
Corn co-products								
Corn germ meal[15]	-	-	-	-	49(1)	64(1)	53(1)	68(1)
Corn gluten meal[15]	-	-	-	-	71(1)	83(1)	75(1)	87(1)
DDGS[13,14,15,18]	-	-	-	-	70±3(2)	77±2(5)	74±4(2)	81±3(5)

Table 2.4. (Cont.)

Oilseed meals								
Canola meal[9,19,20,21,22,23]	39±5(6)	58±9(4)	42(1)	-	40±9(9)	63±4(9)	50±4(6)	69±4(6)
Soybean meal[9,16,20,21,22]	53±9(3)	-	78(1)	-	41±9(5)	71±2(4)	62(1)	78(1)
Sunflower meal[20,22]	22(1)	-	-	-	33(1)	55(1)	37(1)	60(1)
Animal feed ingredients								
Fish meal[24,25]	64±2(2)	71±1(2)	65(1)	73(1)	66(1)	69(1)	68(1)	71(1)
Meat and bone meal[26,27]	74±9(9)	80(1)	77(1)	82(1)	66±9(8)	-	70±9(8)	-
Meat meal[26]	75(1)	83(1)	77(1)	86(1)	-	-	-	-
Poultry byproduct[26]	85(1)	83(1)	88(1)	87(1)	-	-	-	-
Poultry meal[26]	81(1)	74(1)	82(1)	76(1)	-	-	-	-
Whey powder[9,28]	97(1)	-	99(1)	-	84(1)	-	91(1)	-
Whey permeate[9,28]	61(1)	-	63(1)	-	86(1)	-	93(1)	-

[1]Values indicate: mean ± standard deviation (n).

[2]González-Vega et al. (2015b).

[3]Merriman and Stein (2016).

[4]Merriman et al. (2016a).

[5]Blavi et al. (2017).

[6]Kwon and Kim (2017).

[7]Zhang and Adeola (2017).

[8]Lee et al. (2019).

[9]Unpublished data from the University of Illinois.

[10]Petersen and Stein (2006).

[11]Lopez (2020).

Table 2.4. (Cont.)

[12]McGhee and Stein (2019).

[13]Almeida and Stein (2010).

[14]Almeida and Stein (2012).

[15]Rojas et al. (2013).

[16]Bohlke et al. (2005).

[17]Casas and Stein (2015).

[18]DDGS = Distillers dried grains with solubles.

[19]González-Vega et al. (2013).

[20]Zhang et al. (2016).

[21]Akinmusire and Adeola (2009).

[22]Rodriguez et al. (2013).

[23]Maison et al. (2015).

[24]González-Vega et al. (2015a).

[25]Lagos and Stein (2020).

[26]Merriman et al. (2016b).

[27]Sulabo and Stein (2013).

[28]Kim et al. (2012).

Table 2.5. True total tract digestibility (TTTD) of calcium and phosphorus in mineral supplements and oilseed meals without (-) or with phytase (+)[1]

Item, %	TTTD	Calcium		Phosphorus	
	Phytase	-	+	-	+
Mineral supplements					
Calcium carbonate[1]		70	-	-	-
Dicalcium phosphate[1]		76	-	-	-
Oilseed meals					
Canola meal[2,3]		47	70	34	61
Soybean meal[3]		-	-	41	71

[1]Zhang and Adeola (2017).

[2]González-Vega et al. (2013).

[3]Akinmusire and Adeola (2009).

Table 2.6. Requirements for total Ca, total P, and standardized total tract digestible (STTD) P for growing and finishing pigs[1]

	Body weight range, kg				
Item, %	11-25	25-50	50-75	75-100	100-135
Total Ca	0.70	0.66	0.59	0.52	0.46
Total P	0.60	0.56	0.52	0.47	0.43
STTD P	0.33	0.31	0.27	0.24	0.21
Total Ca:total P	1.17:1	1.18:1	1.13:1	1.11:1	1.07:1
Total Ca:STTD P	1.82:1	1.81:1	1.93:1	1.96:1	2.05:1

[1]Modified from NRC (2012).

Table 2.7. Ratio between standardized total tract digestible (STTD) Ca and STTD Ca to maximize growth performance and bone mineralization of growing and finishing pigs

	Body weight range, kg			
Item	11-25[1]	25-50[2]	50-75[3]	100-130[4]
Growth performance	< 1.40:1	< 1.35:1	< 1.25:1	< 1.10:1
Bone mineralization	1.70:1	1.80:1	2.00:1	2.30:1

[1]Lagos et al. (2019a).

[2]González-Vega et al. (2016c).

[3]Lagos et al. (2019b).

[4]Merriman et al. (2017).

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Chapter 3: Comparison between calculated and analyzed values for total calcium and total phosphorus in commercial diets from the U.S. swine industry

Abstract

An experiment was conducted to test the hypothesis that the analyzed concentrations of total Ca and total P in commercial pig diets in the U.S. are not greater than calculated values. A total of 103 diet samples from the commercial swine industry in the U.S. were collected between 2019 and 2021. Diet samples were provided by feed mills, feed companies, or swine farms located in NC, TN, IA, IN, KS, MN, NE, or IL. Diets were formulated for nursery pigs, growing-finishing pigs, or gestating or lactating sows. Each company provided formulated values for Ca and P in all samples. Samples were ground at the University of Illinois and analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry. The formulated values for Ca and P were regressed against analyzed values using the PROC REG procedure of SAS, and the intercept was considered the estimated under- or oversupply of each mineral. Results indicated that there was an average of 0.19 percentage units more Ca (model; $P < 0.001$) in pig diets in the U.S., whereas for total P, the average oversupply was only 0.06 percentage units (model; $P < 0.001$). In conclusion, diets used in the U.S. swine industry contain more total Ca than expected, therefore, during diet formulation, more attention should be given to the inclusion of Ca in all Ca-containing feed ingredients to avoid Ca oversupply and its detrimental effect on P digestibility and growth performance of pigs.

Key words: calcium, commercial diets, pigs, phosphorus

Abbreviations

ADFI	average daily feed intake
ATTD	apparent total tract digestibility
DDGS	distiller dried grains with solubles

Introduction

Calcium and P are required in swine diets at concentrations above 100 ppm, however, the amount of Ca and P in plant-based feed ingredients is low compared with what the animals need (NRC, 2012). Therefore, calcium carbonate, limestone, and calcium phosphates are usually included in the diets to provide additional Ca and P. Special attention has been given to the amount of P supplied in diets for pigs due to economic and environmental impacts of oversupplying P in diets for pigs (Knowlton et al., 2004), but because Ca is an inexpensive nutrient, the supply of limestone or calcium carbonate in diets is given less attention. However, the metabolism of Ca is closely related to P, and unbalanced provisions of Ca and P may influence the availability of both minerals (Crenshaw, 2001). Indeed, there is a negative correlation between concentrations of dietary Ca and the apparent total tract digestibility (**ATTD**) of P in pigs (Velayudhan et al., 2019). Likewise, results of a number of experiments have demonstrated the negative impact of excess Ca on growth performance of pigs (González-Vega et al., 2016a; Merriman et al., 2017; Lagos et al., 2019a; 2019b). Therefore, although an adequate supply of Ca in diets for pigs is crucial for normal growth and reproductive performance, it is also crucial to avoid oversupply of Ca because this may result in reduced absorption of P and therefore, indirectly create a P deficiency.

Limestone is commonly used as a carrier in vitamin and mineral premixes and as a flow agent in soybean meal and other feed ingredients (Ibáñez et al., 2020). Therefore, it is possible that the concentration of Ca in commercial diets for pigs is underestimated. In fact, the amount of Ca in 795 diets produced by the swine and poultry industries in Europe was on average 0.22 percentage units greater than formulated compared with an oversupply of 0.08 percentage units for P (Walk, 2016). There are, however, no data demonstrating if Ca and P in commercial diets produced in the U.S. also are oversupplied. Therefore, the objective of this work was to test the hypothesis that the analyzed concentration of Ca in commercial pig diets in the U.S. is not greater than formulated values.

Materials and Methods

Sample collection

One hundred and three diet samples for pigs in different productive and physiological stages were collected from feed mills, feed companies, or swine farms in 8 states (NC, TN, IA, IN, KS, MN, NE, or IL). A minimum of 250 g of sample were stored and sample information was recorded. The information included origin, diet form, production phase, and formulated values for total Ca and P.

Sample analysis

All diet samples were finely ground, subsampled, and analyzed at the University of Illinois (Urbana, IL, USA) for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2019) and for ash by incineration at 600 °C for 2 h (Method 942.05; AOAC Int., 2019). Samples were also analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2019). Samples were prepared for analysis by

overnight dry ashing (Method 942.05; AOAC Int., 2019) followed by wet digestion with nitric acid (Method 3050 B; US-EPA, 2000).

Statistical analyses

Data were analyzed using a regression between analyzed and formulated values for Ca and P using the PROC REG procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The response variable, analyzed values for Ca or P, was regressed against the explanatory variable, which was the formulated concentration of Ca or P. Because there were linear relationships between analyzed and calculated values for dietary nutrients, the intercept of the equation represented the estimate for under- or oversupply of Ca or P in the diets.

Results and Discussion

Interest in digestibility and requirements for Ca by pigs has increased in the last decade because of the negative impact that excess dietary Ca has on the digestibility of P and growth performance of pigs (Stein et al., 2011; González-Vega et al., 2016a). Values for the digestibility of Ca in Ca-containing feed ingredients have been generated and digestible Ca requirements by growing pigs have been published (González-Vega and Stein, 2016; Lagos et al., 2021). However, limestone, the main source of Ca in pig diets, is an inexpensive feed ingredient that is often oversupplied in diets, as indicated by data from the European swine and poultry industries (Walk, 2016).

The average dry matter in the 103 diets used in the present study was 86.6 ± 7.1%, whereas samples on average contained 5.59 ± 1.39% ash (Table 3.1). Values for Ca and P ranged from 0.37 to 1.27% and 0.32 to 0.96% with an average of 0.75 ± 0.19% and 0.60 ± 0.13%,

respectively. Diet samples were targeted for nursery pigs, growing-finishing pigs, gilts, gestating sows, or lactating sows.

The regression analysis indicated that there is an average of 0.19% (model; $P < 0.001$) more total Ca in diets than formulated (Fig. 3.1). This value concurs with data from Walk (2016) indicating an excess of 0.22% total Ca in commercial diets from the European swine and poultry industries. The reason for this observation may be that limestone is often used as a carrier in vitamin-mineral premixes, in feed additive premixes, and as a flow enhancer in feed ingredients including soybean meal, which is the main source of protein in pig diets in the U.S. (Sotak-Peper et al., 2017). The average value for total Ca in soybean meal reported by the NRC (2012) is 0.33%, but values that ranged from 0.21 to 0.76% Ca in soybean meal from crushing plants in the U.S. have also been reported (Sotak-Peper et al., 2016; Lagos and Stein, 2017). Bakery meal and distillers dried grains with solubles (**DDGS**) are believed to have a low concentration of Ca (0.13 and 0.10, respectively; NRC, 2012), but bakery meal may contain up to 0.51% Ca (Liu et al., 2018) and DDGS may contain up to 0.28% Ca (Pedersen et al., 2007). Therefore, if Ca-containing feed ingredients are not analyzed for Ca before diet formulation, and an average value is used, the concentration of Ca in the diet will likely be underestimated. Likewise, if the concentration of Ca in premixes is not accounted for in formulation, Ca in the final diets will be greater than formulated.

Results from the regression analysis between analyzed and calculated values for total P indicated that on average, diets from the U.S. swine industry contain 0.06% (model; $P < 0.001$) more P than what was formulated (Fig. 3.2), which is in close agreement with the value of 0.08% excess P reported by Walk (2016). This indicates that both in the E.U. and the U.S., more attention is given to the concentration of P in swine diets compared with Ca. The reason for this

is the increased price of feed phosphates compared with limestone as well as the possible negative environmental consequences of rock phosphate depletion and P excretion in the manure as a result of excessive use of P in diets (Selle and Ravindran, 2008).

Increasing levels of Ca in diets with a fixed level of P decreased the ATTD of P, likely as a result of the formation of Ca-P complexes that make P unavailable for pigs (Stein et al., 2011). Based on the linear correlation between dietary Ca and ATTD of P observed by Stein et al. (2011), the ATTD of P (%) can be estimated as 65.0 – 18.8 × dietary Ca ($r^2 = 0.83$; $P < 0.05$). This indicates that if a diet is formulated to contain 0.66% Ca, but the actual concentration is 0.85%, the ATTD of P will decrease from 52.6% to 49.0%. This reduced P digestibility may result in deficiency of P and P deficiency is associated with depression of feed intake by pigs (Sørensen et al., 2018). Data from Ca requirement experiments indicated that regardless of the concentration of dietary P, there was a linear decrease in average daily feed intake (**ADFI**) of pigs as dietary Ca increased (Merriman et al., 2017; Lagos et al., 2019b). Using equations for the correlation between dietary Ca and ADFI reported in pigs from 50 to 85 kg (Lagos et al., 2019b) and from 100 to 130 kg (Merriman et al., 2017), the inclusion of 0.19% more total Ca compared with the requirement, will result in 77 and 241 g less ADFI, respectively. The negative impact of excess dietary Ca on growth performance of younger pigs is also well known (González-Vega et al., 2016b; Wu et al., 2017; Lagos et al., 2019a). Therefore, the fact that diets from the U.S. swine industry on average contain 0.19% more total Ca, but only 0.06% more total P than formulated, indicates that growth performance of pigs is likely being compromised by excess Ca in diets, and more attention should be given to the concentration of Ca in feed ingredients and complete diets.

Conclusions

Commercial diets from the swine industry in the U.S. on average contain 0.19% more total Ca and 0.06% more total P than formulated. The reason for this observation is likely the low cost of limestone and its use as a carrier in premixes and as a flow agent in feed ingredients. The consequence of the excess dietary Ca is reduced P digestibility and reduced growth performance of pigs. Therefore, all Ca-containing feed ingredients and premixes should be analyzed for Ca prior to diet formulation to avoid underestimation of Ca concentration and oversupply in diets.

Figures

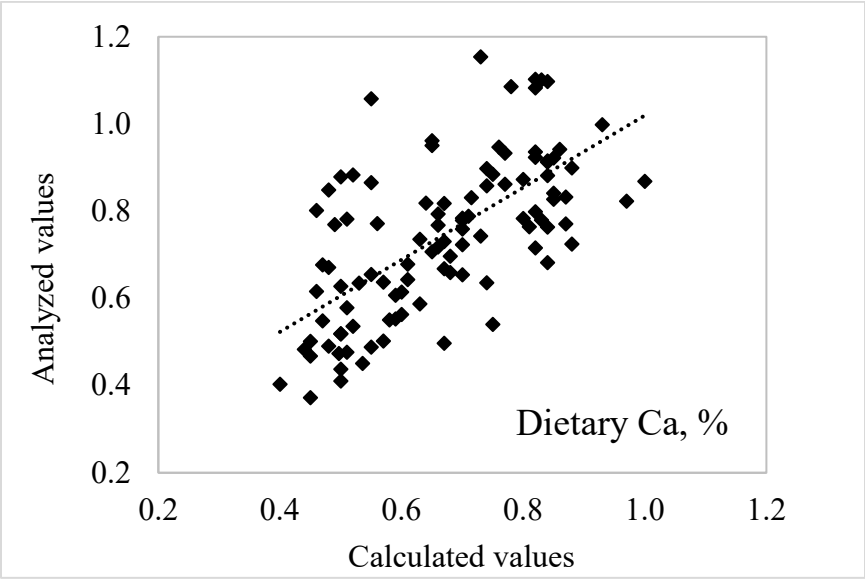

Figure 3.1. Regression of analyzed values for total Ca (%) on calculated values for total Ca (%) in swine diets from the U.S. [Y = (0.825 × X) + 0.194], with $r^2 = 0.42$ and $P < 0.001$.

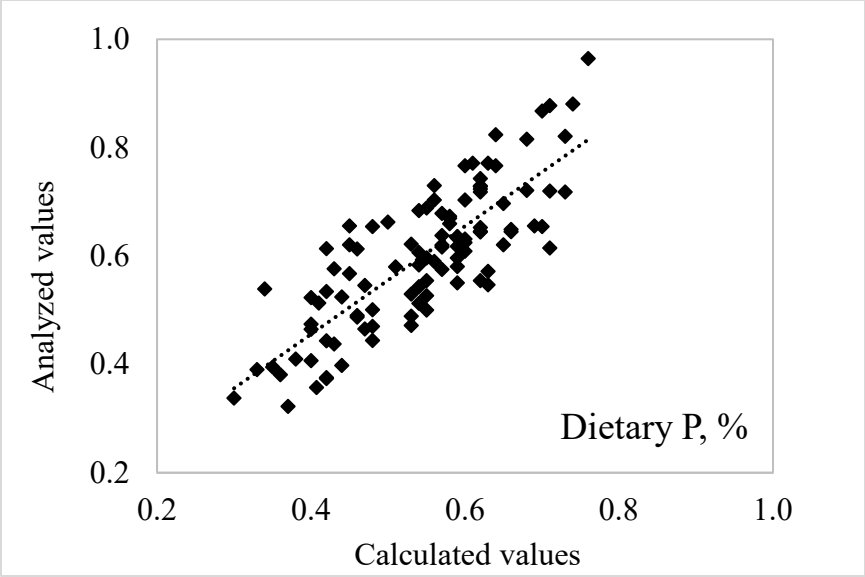

Figure 3.2. Regression of analyzed values for total P (%) on calculated values for total P (%) in swine diets from the U.S. [Y = (1.001 × X) + 0.055], with $r^2 = 0.66$ and $P < 0.001$.

Table

Table 3.1. Summary of diet samples collected and analyzed values for dry matter and ash[1,2]

Production phase	Number	Dry matter, %	Ash, %
Nursery pigs	22	87.0 ± 6.5	6.17 ± 0.73
Growing-finishing pigs	37	84.9 ± 8.8	5.25 ± 2.01
Sows[3]	36	88.1 ± 4.4	5.57 ± 0.98
Not reported	8	89.2 ± 4.9	5.67 ± 0.66
Total	103	86.6 ± 7.1	5.59 ± 1.39

[1]From the 103 diet samples, 68 were in mash form and 35 were pelleted.

[2]Values for dry matter and ash are expressed as mean ± standard deviation.

[3]Includes gilts, gestating, and lactating sows.

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Chapter 4: Formulating diets based on digestible calcium instead of total calcium does not affect growth performance or carcass characteristics, but microbial phytase ameliorates bone resorption caused by low calcium in diets fed to pigs from 11 to 130 kg[1]

Abstract

An experiment was conducted to test the hypothesis that the requirement for Ca expressed as a ratio between standardized total tract digestible (**STTD**) Ca and STTD P obtained in short-term experiments may be applied to pigs fed diets without or with microbial phytase from 11 to 130 kg. In a 5-phase program, 160 pigs (body weight: 11.2 ± 1.8 kg) were randomly allotted to 32 pens and 4 corn-soybean meal based diets in a 2 × 2 factorial design with 2 diet formulation principles (total Ca or STTD Ca), and 2 phytase inclusion levels (0 or 500 units/kg of feed) assuming phytase released 0.11% STTD P and 0.16% total Ca. The STTD Ca:STTD P ratios were 1.40:1, 1.35:1, 1.25:1, 1.18:1, and 1.10:1 for phases 1 to 5, and STTD P was at the requirement. Weights of pigs and feed left in feeders were recorded at the end of each phase. At the conclusion of phase 1 (d 24), 1 pig per pen was euthanized and a blood sample and the right femur were collected. At the end of phases 2 to 5, a blood sample was collected from the same pig in each pen. At the conclusion of the experiment (d 126), the right femur of 1 pig per pen was collected and carcass characteristics from this pig were measured. No interactions were observed between diet formulation principle and phytase inclusion for growth performance in any phase and no differences among treatments were observed for overall growth performance. Plasma Ca

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and P and bone ash at the end of phase 1 were also not influenced by dietary treatments. However, on d 126, pigs fed non-phytase diets formulated based on total Ca had greater bone ash than pigs fed STTD Ca based diets, but if phytase was used, no differences were observed between the 2 formulation principles (interaction $P < 0.05$). At the end of phases 2 and 3, pigs fed diets without phytase had greater ($P < 0.05$) plasma P than pigs fed diets with phytase, but no differences were observed at the end of phases 4 and 5. A negative quadratic effect ($P < 0.05$) of phase (2 to 5) on the concentration of plasma Ca was observed, whereas plasma P increased (quadratic; $P < 0.05$) from phase 2 to 5. However, there was no interaction or effect of diet formulation principle or phytase inclusion on any carcass characteristics measured. In conclusion, STTD Ca to STTD P ratios can be used in diet formulation for growing-finishing pigs without affecting growth performance or carcass characteristics and phytase inclusion ameliorates bone resorption caused by low dietary Ca and P.

Key words: Bone ash, calcium, growth performance, phytase, pigs, phosphorus

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AEE	Acid hydrolyzed ether extract
BW	body weight
dCa	digestible Ca
FTU	phytase units per kilogram of feed
G:F	gain to feed ratio
HCW	hot carcass weight

Abbreviations (Cont.)

LEA	loin eye area
LM	longissimus muscle
STTD	standardized total tract digestible
tCa	total Ca

Introduction

Values for standardized total tract digestible (**STTD**) Ca are believed to be additive in mixed diets for pigs (Stein et al., 2016). As a consequence, formulating diets based on values for STTD of Ca in each ingredient instead of total Ca may increase accuracy of diet formulation (NRC, 2012). Recent work has generated values for the digestibility of Ca in most Ca-containing feed ingredients (Stein et al., 2016). Most STTD values were determined without and with inclusion of microbial phytase, because supplementation of exogenous phytase increases not only the digestibility of P, but also the digestibility of Ca in some feed ingredients (González-Vega et al., 2013; 2015b).

The NRC (2012) indicated that requirements for Ca ideally would be expressed as a ratio between STTD Ca and STTD P, but because of a lack of data for the digestibility of Ca in commonly used feed ingredients at the time the NRC document was prepared, Ca requirements were expressed as requirements for total Ca (NRC, 2012). However, because data for the concentration of STTD Ca in feed ingredients are now available, the requirement for Ca can now be estimated based on STTD Ca:STTD P ratios. Data for Ca requirement of pigs from 11- to 22-kg (Lagos et al., 2019a), 25- to 50-kg (González-Vega et al., 2016), 50- to 85-kg (Lagos et al., 2019b), and 100- to 130-kg pigs (Merriman et al., 2017) indicate that a ratio between STTD Ca

and STTD P below 1.40:1, 1.35:1, 1.25:1, and 1.10:1, respectively, will maximize growth performance of pigs in these 4 weight groups. Results of these studies also demonstrated that the STTD Ca:STTD P ratios needed to maximize bone ash are greater than the ratios needed to maximize growth performance and ratios of 1.70:1, 1.80:1, 2.00:1, and 2.30:1 were determined to maximize bone ash in pigs from 11 to 22 kg, 25 to 50 kg, 50 to 85 kg, and 100 to 130 kg, respectively. These experiments were conducted over 3 to 5 weeks, and therefore, a follow-up study across all ranges of weights is needed to confirm that the ratios established to optimize growth performance within each weight group will also optimize growth performance throughout the growing-finishing phases. Therefore, the objective of this experiment was to test the hypothesis that the requirement for Ca expressed as a ratio between STTD Ca and STTD P by growing pigs obtained in short term experiments may be applied to pigs from 11 to 130 kg without detrimental effects on growth performance. The second hypothesis was that pigs fed diets based on values for STTD Ca will have growth performance that is not different from that of pigs fed diets formulated based on total Ca.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animal and housing

One hundred and sixty pigs with an initial average body weight (**BW**) of 11.2 ± 1.8 kg were allotted to 4 diets in a completely randomized design on d 18 post-weaning. There were 5 pigs per pen (3 gilts and 2 castrates) and 8 replicate pens per diet. The Experimental Animal

Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. During the nursery phase (Phase 1), pigs were housed in pens that had fully slatted floors, a feeder, and a nipple drinker. On d 24, pigs had an average BW of 26.8 ± 3.1 kg and were moved to a mechanically ventilated grower-finisher unit, where pens had partly slatted concrete floors and were equipped with a feeder and a nipple drinker. Feed and water were available at all times.

Diets and feeding

From weaning on d 20 to d 17 post-weaning, pigs were fed a common diet that met all nutrient requirements for pigs from 5 to 7 kg (NRC, 2012). From d 18 post-weaning (d 1), a 5-phase program was used (11 to 25 kg, 25 to 50 kg, 50 to 75 kg, 75 to 100 kg, and 100 to 135 kg). Phase changes were determined based on average pig weights and all pens changed phase on the same day. Thus, phases 1 to 4 were concluded at d 24, 52, 77, and 101, when pigs had a weight close to 25, 50, 75, and 100 kg, respectively. The experiment was terminated on d 126. In each phase, 4 diets based on corn and soybean meal (Table 4.1) were formulated for a total of 20 diets in the 5 phases (Tables 4.2 and 4.3). Within each phase, diets were formulated using a 2 × 2 factorial design with 2 requirement estimates for Ca (total Ca or STTD Ca), and 2 inclusion levels of microbial phytase [0 or 500 phytase units/kg of feed (**FTU**)]. In each phase, one diet was formulated based on the NRC (2012) requirement for total Ca (0.70, 0.66, 0.59, 0.52, and 0.46% for phases 1 to 5, respectively) and STTD P (0.33, 0.31, 0.27, 0.24, and 0.21% for phases 1 to 5, respectively). The second diet within each phase was formulated based on a ratio between STTD Ca and STTD P of 1.40:1, 1.35:1, 1.25:1, 1.18:1, and 1.10:1 for phases 1, 2, 3, 4, and 5, respectively (González-Vega et al., 2016; Merriman et al., 2017; Lagos et al., 2019a; 2019b). Concentrations of STTD P in these diets were based on NRC (2012) whereas concentrations of Ca corresponded to values of 0.62, 0.57, 0.45, 0.38, and 0.31% total Ca for phases 1 to 5,

respectively. Thus diets formulated to meet specific ratios between STTD Ca and STTD P contained less total Ca than diets formulated to meet the NRC (2012) requirement for total Ca. Values for STTD Ca used in the formulation of these diets were obtained for each Ca-containing ingredient in the absence of phytase (Stein et al., 2016). The third diet within each phase was formulated as the first diet with the exception that 500 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were included, and the provisions of total Ca and STTD P were reduced by 0.16 and 0.11 percentage units, respectively, compared with requirement estimates (NRC, 2012) to account for the expected release of Ca and P as a result of phytase inclusion. The last diet in each phase also contained microbial phytase (500 FTU) and the provision of STTD P was reduced by 0.11% compared with the NRC (2012) requirement. However, Ca was included to meet a ratio between STTD Ca and STTD P of 1.40:1, 1.35:1, 1.25:1, 1.18:1, and 1.10:1 for phases 1, 2, 3, 4, and 5, respectively, and STTD Ca values for each ingredient were based on values that were determined in the presence of phytase (Stein et al., 2016) to account for the increased STTD of Ca in some ingredients that is the result of phytase addition. Concentrations of Ca corresponded to values of 0.54, 0.52, 0.42, 0.35, and 0.29% total Ca for phases 1 to 5, respectively. In each phase, the 4 diets were formulated to contain the same quantities of net energy, Na, Cl, K, vitamin D, and all other nutrients.

Sample collection, carcass characteristics, and bone measurements

The amount of feed offered was recorded daily and the amount of feed in the feeders and the weight of the pigs was recorded at the conclusion of each phase. On the last day of phase 1, the gilt in each pen with a BW closest to the average BW of the pen was euthanized and a blood sample was collected in a lithium-heparin-containing tube and the right femur was also collected. Blood samples were immediately centrifuged and plasma was harvested and stored at -20 ºC

until analyzed. At the conclusion of phase 2, the gilt in each pen with a BW closest to the average BW of the pen was identified and a blood sample was collected by jugular venipuncture. Blood samples were collected from the same gilt on the last day of phases 3 and 4. On the morning of the last day of phase 5, the 32 gilts (1 per pen) that had been bled at the end of phases 2, 3, and 4 were transported to the Meat Sciences Laboratory at the University of Illinois (3 km) and kept in lairage overnight with free access to water. On the morning of the following day, pigs were weighed and humanely slaughtered as described by Overholt et al. (2016). A blood sample and the left femur were collected from each pig and standard carcass measurements were determined after slaughter.

Hot carcass weight (**HCW**) was recorded approximately 45 min post-mortem and carcass yield was calculated by dividing the HCW by the live weight obtained immediately before slaughter. Carcasses were split down the middle and stored at 4 °C for 24 h. Left half-carcasses were separated between the 10^{th} and 11^{th} rib to access the longissimus muscle (**LM**). Backfat thickness was measured at the 10th rib at 75% of the distance of the LM from the dorsal side of the vertebral column. The loin eye area (**LEA**) was determined by tracing the surface of the LM on acetate paper and measuring the tracings in duplicate on a digitizer tablet (Wacom, Vancouver, WA). Carcass lean percentage was calculated using the equation developed by Burson and Berg (2001): carcass lean % = ([8.588 + (0.465 × HCW, lb) – (21.896 × 10^{th} rib backfat, in) + (3.005 × 10^{th} LEA, in^2)] ÷ HCW, lb) × 100. Loin quality was determined by measuring ultimate pH, instrumental color, drip loss, and subjective marbling, visual color (NPPC, 1999), and firmness scores (NPPC, 1991) in 3 cuts of the LM with approximately 2.5 cm of thickness that were collected from the posterior portion of each half-carcass. Ultimate pH was measured 24 h post-mortem in one chop using a handheld pH meter and a glass electrode (Meat

Probes Inc., Topeka, KS). Subjective measurements (i.e., marbling, visual color, and firmness) were performed in the same chop by a single trained employee from the University of Illinois. Instrumental color was measured in another chop with a CR-400 chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using a D65 light source, 2º observer angle, and 8 mm aperture calibrated with a white tile. Drip loss was determined in the third chop as the weight difference after and before being suspended from a fish hook for 24 h at 4 ºC (Honikel, 1998).

Collected femurs were autoclaved at 125 ºC for 55 min and the remaining muscle and fat tissues attached to the bone were removed. Femurs were broken, dried overnight at 105 ºC, and soaked for 72 h in petroleum ether while placed in a chemical hood to remove marrow and remaining fat. Defatted femurs were left for 24 h in the chemical hood to allow the ether to fully evaporate. Femurs were then dried at 135 ºC for 2 h and ashed at 600 ºC for 16 h. The weight of the femurs was recorded before and after drying and ashing to obtain the amount of ash (grams per femur) and the percentage of ash of the defatted dried bone.

Sample analysis

Ingredient samples were collected at the feed mill immediately after mixing, whereas each diet sample was a composite of samples collected from eight randomly chosen 25 kg-bags. Samples were later ground and sub-sampled for nutrient analysis. All samples were analyzed in duplicate. Corn, soybean meal, calcium carbonate, monocalcium phosphate, diets, bone ash, and plasma samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007] at the University of Missouri, Columbus, MO. Phytate-bound P was analyzed in corn, soybean meal and all diets using a Foss near-infrared spectrometer with the phytate-P levels predicted using AUNIR calibration standards (AB Vista,

Plantation, FL). Phytase activity was analyzed in diets by the ELISA method using Quantiplate Kits for Quantum Blue (AB Vista, Plantation, FL). All other analyses were conducted at the Monogastric Nutrition Laboratory at the University of Illinois, Urbana-Champaign. Ingredients and diets were analyzed for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2007) and for ash by incineration at 600 °C for 2 h (Method 942.05; AOAC Int., 2007). Corn, soybean meal, and diets were also analyzed for N (Method 990.03; AOAC Int., 2007) using a LECO FP628 (LECO Corp., Saint Joseph, MI) and crude protein was calculated as N × 6.25. These samples were also analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL) and for acid hydrolyzed ether extract (Method 2003.06; AOAC Int., 2007) using an Ankom^{HCl} hydrolyser and an Ankom^{XT15} extractor (Ankom Technology, Macedon, NY).

Calculations and statistical analyses

The percentage of phytate in corn, soybean meal, and diets was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004), and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and average gain to feed ratio (**G:F**) were calculated for pigs fed each experimental diet. Concentrations of bone Ca and bone P in grams per femur were calculated by multiplying the total quantity of bone ash by the percentage of Ca and P in bone ash.

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Assumptions of the model and normality of residuals were tested using INFLUENCE, PROC GPLOT, and PROC UNIVARIATE procedures of SAS. Data for growth performance, concentration and percentage of bone ash, bone Ca, and bone P, concentrations of Ca and P in plasma, and carcass characteristics were analyzed by phase using the PROC MIXED of SAS with pen as the

experimental unit. The model included the main effects of diet formulation principle (total Ca or STTD Ca) and phytase inclusion (0 or 500 FTU), and the interaction between diet formulation principle and phytase inclusion. Least squares means were separated using the PDIFF option of SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Blood samples in phases 2, 3, 4, and 5 were collected from the same gilt, therefore, an additional analysis was conducted to analyze the effect of phase on plasma concentrations of Ca and P. These data were analyzed as repeated measures with unstructured variance using the PROC MIXED and REPEATED procedures of SAS. The model included diet formulation principle, phytase inclusion, and phase as the main effects, phase as the time effect, and pig as the subject. If the effect of phase was significant ($P < 0.05$), contrast statements were used to determine linear and quadratic effects of phase on the concentrations of Ca and P in plasma.

Results

All pigs consumed their diets without apparent problems, but 3 pigs did not complete the experiment. One pig fed the diet formulated based on STTD Ca:STTD P and no phytase was euthanized in phase 2 due to lameness. One pig was removed from the experiment in phase 4 due to bad condition, and 1 pig in apparent good condition died in phase 5; both of these pigs were fed the diet formulated based on total Ca and phytase. Values for growth performance parameters in the pens where the removed pigs were housed were adjusted as previously explained (Lindemann and Kim, 2007; Lee et al., 2016). The remaining pigs completed the experiment with no apparent health problems.

For ADG, ADFI, and G:F, there was no effect of inclusion of phytase or diet formulation principle at any phase or for the overall experimental period (Table 4.4). Likewise, no interactions between phytase inclusion and diet formulation principle on growth performance parameters were observed. However in phase 4, pigs fed diets formulated based on a ratio between STTD Ca and STTD P tended ($P < 0.10$) to have greater ADG and G:F than pigs fed diets formulated based on total Ca.

At the end of phase 1, none of the main effects or the interaction between main effects were significant for concentration (grams per femur) or percentage of bone ash, bone Ca, or bone P (Table 4.5). However, at the end of phase 5, if no phytase was used, pigs fed diets formulated based on total Ca had greater bone ash (concentration and percentage) and concentration of bone Ca and P than pigs fed diets formulated based on STTD Ca, but if phytase was used, no differences were observed in bone characteristics between the 2 diet formulation principles (interaction $P < 0.05$). However, values for percentage of bone ash and concentration of bone ash, bone Ca, and bone P from pigs fed diets formulated based on total Ca and no phytase were not different from values from pigs fed diets formulated based on STTD Ca and phytase (interaction $P < 0.05$). For percentage of bone Ca and bone P, the interaction between diet formulation principle and phytase inclusion was not significant, and no effect of diet formulation principle or phytase inclusion was observed.

The concentration of Ca and P in plasma of pigs was not affected by diet formulation principle at the end of phases 1, 2, 4, or 5 and no interaction between diet formulation principle and phytase inclusion was observed (Table 4.6). Likewise, there was no effect of diet formulation principle on plasma Ca at the end of phase 3, but for plasma P, an interaction ($P < 0.05$) between diet formulation principle and phytase inclusion was observed. There was no

effect of diet formulation principle on pigs fed diets without phytase, but pigs fed diets with phytase had a greater ($P < 0.05$) concentration of plasma P at the end of phase 3 if diets were formulated based on STTD Ca than if diets were formulated based on total Ca. No effect of phytase inclusion on plasma Ca and P at the end of phases 1 and 4 was observed. Likewise, at the end of phases 2 and 3, plasma Ca was not affected by the inclusion of phytase, but at the end of phase 2, plasma P was greater ($P < 0.05$) in pigs fed diets without phytase than in pigs fed diets with phytase. At the end of phase 5, there was a tendency ($P < 0.10$) for pigs fed diets with phytase to have reduced concentration of Ca in plasma compared with pigs fed diets without phytase, but no effect of phytase inclusion on the concentration of plasma P was observed. When the effect of phase (2 to 5) was included in the model, no effect of diet formulation principle or phytase inclusion on the concentration of Ca and P in plasma was observed (Table 4.7). However, there was a quadratic reduction ($P < 0.05$) in plasma Ca from phase 2 to phase 5. In contrast, a quadratic increase ($P < 0.05$) in plasma P was observed from phase 2 to 5.

There was no interaction between diet formulation principle and phytase inclusion for carcass characteristics and no effect of diet formulation principle or phytase inclusion was observed (Table 4.8). However, carcasses from pigs fed diets formulated based on STTD Ca tended ($P < 0.10$) to have less marbling than carcasses from pigs fed diets formulated based on total Ca.

Discussion

Calcium is mainly supplied in pig diets by limestone, which is an inexpensive ingredient that sometimes is oversupplied in diets because it is often used as a carrier in vitamin-mineral premixes or as a flow agent in feed ingredients (Walk, 2016). However, excess dietary Ca

reduces P digestibility in pigs (Stein et al., 2011; Velayudhan et al., 2019) and results in decreased feed intake and growth performance (Merriman et al., 2017; Lagos et al., 2019a). Therefore, Ca oversupply with limestone may have negative effects on pig production. Microbial phytase is commonly used in swine diets to reduce inclusion of feed phosphates, which also results in reduced excretion of P in the manure (Jongbloed and Lenis, 1992). However, phytase not only increases P digestibility in plant feed ingredients (She et al., 2017), but also increases the digestibility of Ca in plant and animal feed ingredients and in limestone (González-Vega et al., 2013; 2015a; Lee et al., 2019), which may exacerbate the negative effect of excess Ca if the Ca-releasing effect of phytase is not taken into account in diet formulation. Therefore, requirements for Ca should be expressed as digestible Ca, and because pigs excrete endogenous Ca (González-Vega et al., 2013), the use of STTD values that are additive in mixed diets is more accurate than the use of apparent total tract digestible values (She et al., 2018). The STTD Ca in most Ca sources used in pig diets have been reported (Stein et al., 2016), and experiments using pigs from 11 to 25 kg (Lagos et al., 2019a), 25 to 50 kg (González-Vega et al., 2016), 50 to 85 kg (Lagos et al., 2019b), and 100 to 130 kg (Merriman et al., 2017) have been conducted to determine the ratio between STTD Ca and STTD P that maximizes growth performance and bone mineralization. Therefore, in this experiment, the ratios that maximized growth performance of pigs in those 4 studies were used to formulate diets without and with the inclusion of microbial phytase and these diets were fed to pigs from 11 to 130 kg. In each of the four short-term experiments, different dietary levels of Ca and P were used to determine the optimal ratio between STTD Ca and STTD P. However, in each experiment, the optimum dietary concentration for total Ca was also determined and it was concluded, that as long as total Ca does not exceed NRC (2012) requirements, pig growth performance will not be reduced. It was

therefore, expected that pigs fed diets formulated based on STTD Ca would obtain growth performance that was not different from that of pigs fed diets formulated based on total Ca provided that the concentration of total Ca did not exceed NRC (2012) requirements and results of the experiment confirmed this hypothesis.

One of the consequences of formulating diets based on STTD Ca is that the total Ca in the diet reflects the digestibility of Ca in ingredients, and if ingredients with lower STTD of Ca are used, a greater quantity of total Ca is needed to reach a certain level of STTD Ca in the diet. This is illustrated in Phase 1 and Phase 2 diets where the total Ca in diets containing phytase increased if diets were formulated based on STTD Ca because use of phytases reduces the need for monocalcium phosphate in the diets and more calcium carbonate, therefore, needs to be added. However, because the STTD of Ca in calcium carbonate is less than in monocalcium phosphate (Gonzalez-Vega et al., 2015b), the concentration of total Ca in the diet will increase.

The lack of differences in growth performance of pigs among the 4 dietary treatments in each phase and for the entire experimental period indicates that regardless of the inclusion of phytase, both diet formulation principles can be used to formulate diets for growing-finishing pigs. These results also confirm that under the conditions of this experiment, the ratios between STTD Ca and STTD P obtained in short-term experiments can be used to optimize growth performance of pigs in the entire growing-finishing period. The observation that the growth performance of pigs fed diets with 500 FTU of phytase was not different between the 2 formulation principles was expected because the composition of these diets was almost identical. On the other hand, the observation that there are no differences in growth performance of pigs fed non-phytase diets based on total Ca or STTD Ca indicates that the reduced concentration of Ca in STTD Ca diets compared with diets based on total Ca does not compromise growth

performance of pigs. However, diets will be properly formulated only if the analyzed concentration of dietary Ca is consistent with the formulated value. Commercial diets from the swine and poultry industries in the European Union contain on average 0.22 percentage units more Ca than formulated (Walk, 2016), which negatively affects animal growth performance. Diets used in this experiment were formulated using a Ca-free vitamin-mineral premix and all Ca-containing ingredients contained Ca that was close to published values (NRC, 2012). The current data demonstrate that if Ca is not provided in excess of requirements, diets formulated based on total Ca (NRC, 2012) will result in the same growth performance of pigs as if diets are formulated based on a ratio between STTD Ca and STTD P. Thus, it is likely that avoiding excess Ca in the diets is at least as important as formulating diets based on STTD Ca and requirements for total Ca by NRC (2012) should be considered maximum values. In contrast, although not investigated in the present experiment, results of our previous work clearly indicate that NRC requirements for STTD P should be considered minimum requirements (Gonzalez-Vega et al., 2016; Merriman et al., 2017; Lagos et al., 2019a; 2019b).

Calcium and P requirements to maximize bone mineralization are greater than requirements to maximize growth performance of pigs (Crenshaw, 2001). Although this statement is true for all productive phases, the difference between requirements to optimize growth performance and bone ash is less in young pigs than in finishing pigs (Lagos et al., 2019a). This is likely the reason for the lack of differences in the concentration and percentage of Ca, P, and ash in bones from pigs fed non-phytase diets based on total Ca or STTD Ca during the first phase of the experiment. Without phytase, phase 1 diets based on STTD Ca were formulated to have 0.08 percentage units less Ca than diets formulated based on total Ca, and the observation that this did not affect bone mineralization of pigs indicates that the STTD Ca:STTD

P ratio that maximizes growth performance of 11 to 25 kg does not affect bone mineralization. The reason for this lack of differences is likely that these pigs had enough Ca stored from either the milk while nursing or from the common diet they were fed for 17 d after weaning. Around two-thirds of the defatted dry bone is composed of inorganic material, which mainly consists of calcium phosphate in the form of hydroxyapatite salts (Fails and Magee, 2018). The Ca to P ratio in the mineral portion of bones is maintained at 2.1:1 with concentrations of Ca and P in bone ash that range from 36 to 39% and from 17 to 19%, respectively (Crenshaw, 2001). The concentrations of Ca and P in bone ash obtained in this study are consistent with the values reported by Crenshaw (2001) and explain the lack of differences in the percentage of Ca and P in bone ash among treatments at the end of phase 5. However, because STTD Ca diets were formulated using ratios between STTD Ca and STTD P that maximize growth performance, the observation that without phytase, pigs fed diets based on STTD Ca had less bone ash than pigs fed total Ca diets was expected. The STTD Ca to STTD P ratio to optimize growth performance decreases from 1.40:1 to 1.10:1 as pigs get heavier (Lagos et al., 2019a), whereas the ratio between total Ca and STTD P recommended by NRC (2012) increases from 1.82:1 to 2.05:1 from phase 1 to 5. Thus, phase 2 diets based on STTD Ca were formulated to have 0.10 percentage units less Ca than total Ca diets, whereas for phase 5, diets were formulated to have a difference of 0.15 percentage units of Ca.

The interactions between diet formulation principle and phytase inclusion that were observed for bone characteristics at the end of phase 5 indicate that phytase ameliorates bone resorption caused by the low Ca and P in diets formulated to meet specific ratios for STTD Ca to STTD P because bone ash (concentration and percentage) from pigs fed STTD Ca diets with phytase was not different from that from pigs fed total Ca diets without phytase. This observation

also indicates that values for STTD Ca in feed ingredients with phytase that were used in diet formulation were accurate. The amount of ash in bone represents the bone size, whereas the percentage of bone ash represents the composition of the bone (Lagos et al., 2019b). Thus, the reason for the inconsistency in the results for growth performance (BW) and bone ash (grams) at the end of phase 5 is that only 1 pig per pen was used to analyze bone characteristics and this pig was chosen at the end of phase 2. Therefore, at the conclusion of the experiment, some of these pigs were above or below the average BW of the pen.

Finishing pigs have greater bone mineralization than young pigs as indicated by the difference in percentage of bone ash at the end of phases 1 and 5 (approximately 50 and 60%, respectively). This observation is in agreement with a linear increase in bone ash (52 to 59%) observed from day 46 to 173 of age in pigs fed diets with different concentrations of Ca and P (Crenshaw et al., 1981). Values for percentage of bone ash obtained in this study concur with data from 24-kg pigs (49%; Lagos et al., 2019a) and 86-kg pigs (58%; Lagos et al., 2019b) fed diets with adequate levels of Ca and P. Bone ash in femur of parity 3 sows fed diets with 3 different ratios between Ca and P was on average 68 and 70% for pregnant/lactating and non-pregnant sows, respectively (Mahan and Fetter, 1982). These data indicate that the skeleton of young pigs has a greater proportion of organic components than older pigs. Data from monkeys support this hypothesis as indicated by an increase in the mineral content and the ratio between mineral and organic matter in bones from age 0 to 13 yr with a peak at 8 yr of age (Cerroni et al., 2000; Boskey and Coleman, 2010). Bone data in this experiment was only obtained from gilts because there is no effect of sex on bone development of growing-finishing pigs (Ganelang et al., 2014).

The concentration of plasma Ca in pigs was not affected by diet formulation principle or by phytase inclusion, which is likely a result of the hormonal regulation of Ca homeostasis (Veum, 2010). The concentration of Ca in plasma ranged from 9.25 to 10.64 mg/dL, which is within the physiological range of serum Ca in pigs (8 to 12 mg/dL; Amundson et al., 2017). Because P is less tightly regulated, a few differences were observed in the concentration of plasma P across dietary treatments at the end of phases 2 and 3. These data may indicate that during the early phases, P release by phytase was slightly overestimated and to meet the P requirement for bone mineralization, more P was pulled from the bloodstream to the bones, resulting in lower P concentration in plasma from pigs fed diets with phytase than from pigs fed non-phytase diets. However, this difference was not observed in phases 4 and 5. The negative quadratic effect of phase on the concentration of plasma Ca may be a result of the greater mineral content in bones of finishing pigs compared with young pigs. However, besides bone mineralization, P is also required for soft tissue deposition, therefore, the observation that there was a positive quadratic effect of phase on the concentration of P in plasma, may also reflect that older pigs deposit less lean tissue than young pigs. These results concur with observations that the ratio between STTD Ca and STTD P that maximizes bone mineralization increases as pigs grow, whereas the ratio that maximizes growth performance decreases as pigs grow (Lagos et al., 2019a).

The observation that carcass characteristics were not affected by formulation principle or phytase inclusion is in agreement with observations that there are no differences in backfat thickness, LEA, or carcass yield among pigs fed diets with increasing ratios between Ca and P (Liu et al., 1998; Hanni et al., 2005). A reduction in slaughter BW and HCW of pigs fed diets with total Ca to total P ratios at or above 1.50:1, compared with pigs fed diets with a lower ratio

was observed (Liu et al., 1998; Hanni et al., 2005), but in the present study, ratios between total Ca and total P did not exceed 1.25:1, which likely contributed to the lack of differences among treatments in carcass characteristics. Data from Liu et al. (1998) and Hanni et al. (2005) also demonstrate the negative effect of excess Ca on growth performance of pigs. Increased concentration of Ca in plasma and muscle improves the oxidative metabolism in muscle and results in increased meat quality (Wilborn et al., 2004). High doses of vitamin D_3 increase tenderness of beef cuts as a result of increased Ca mobilization, but when supplemented to pigs, color and ultimate pH, but not tenderness of loin chops were improved (Wilborn et al., 2004). Diets used in this experiment had equal concentrations of vitamin D_3, which may have contributed to the lack of differences in loin quality measurements among dietary treatments. The observation that carcasses from pigs fed diets formulated on the basis of STTD of Ca tended to have reduced marbling compared with carcasses from pigs fed diets based on total Ca was not expected, and because the values for marbling obtained in this study are within a narrow range (1.31 to 1.88) it is difficult to hypothesize the reason behind this observation.

Conclusions

Ratios between STTD Ca and STTD P obtained in short-term experiments can be used to formulate diets without or with phytase for growing-finishing pigs without affecting growth performance parameters or carcass characteristics of pigs. However, results of the experiment demonstrated that as long as dietary Ca is not provided in excess of the requirement, diets may also be formulated based on STTD Ca or total Ca without negative impacts on pig growth performance. Inclusion of microbial phytase ameliorates bone resorption caused by low Ca and P in diets. The effect of formulation principle and phytase inclusion on plasma Ca and P was

limited, but the effect of phase on the concentration of Ca and P in plasma reflects the changing needs for Ca and P for deposition of bone and soft tissue as pigs become older.

Tables

Table 4.1. Analyzed composition of ingredients, as fed basis

Item	Corn	Soybean meal	Calcium carbonate	Monocalcium phosphate
Gross energy, Mcal /kg	3.78	4.32	-	-
Dry matter, %	84.50	88.36	99.96	92.15
Ash, %	1.34	6.94	92.65	81.23
Crude protein, %	7.25	49.84	-	-
AEE,[1] %	2.67	0.92	-	-
Ca, %	0.03	0.35	38.96	17.70
P, %	0.30	0.78	0.04	20.91
Phytate-bound P, %	0.13	0.45	-	-
Phytate,[2] %	0.46	1.60	-	-
Non-phytate P,[3] %	0.17	0.33	-	-

[1]AEE = acid hydrolyzed ether extract.

[2]Phytate was calculated by dividing phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 4.2. Ingredient composition and calculated and analyzed values of experimental diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 phytase units (FTU) per kg of feed (Phases 1, 2, and 3)[1]

Item	Phase 1				Phase 2				Phase 3			
Phytase inclusion:	0 FTU		500 FTU		0 FTU		500 FTU		0 FTU		500 FTU	
Ca requirement:	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa
Ingredient, %												
Ground corn	52.02	52.42	53.48	53.32	72.25	72.76	73.76	73.63	76.22	76.89	77.68	77.74
Soybean meal, 48% CP	32.00	32.00	32.00	32.00	22.00	22.00	22.00	22.00	18.50	18.50	18.50	18.50
Lactose	10.00	10.00	10.00	10.00	-	-	-	-	-	-	-	-
Choice white grease	2.40	2.20	1.68	1.75	2.58	2.32	1.82	1.90	2.50	2.18	1.78	1.75
Calcium carbonate	1.10	0.90	0.95	1.04	1.09	0.84	0.94	0.99	1.00	0.65	0.86	0.83
Monocalcium phosphate	0.94	0.94	0.34	0.34	0.93	0.93	0.32	0.32	0.76	0.76	0.15	0.15
Sodium bicarbonate	0.35	0.35	0.35	0.35	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl, 78% Lys	0.37	0.37	0.37	0.37	0.34	0.34	0.34	0.34	0.28	0.28	0.28	0.28
DL-Met	0.15	0.15	0.15	0.15	0.07	0.07	0.07	0.07	0.03	0.03	0.03	0.03
L-Thr	0.12	0.12	0.12	0.12	0.09	0.09	0.09	0.09	0.06	0.06	0.06	0.06
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[3]	-	-	0.01	0.01	-	-	0.01	0.01	-	-	0.01	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated values												
Ca, %	0.70	0.62	0.54	0.57	0.66	0.57	0.50	0.52	0.59	0.45	0.43	0.42

Table 4.2. (Cont.)

P, %	0.56	0.56	0.44	0.44	0.54	0.54	0.42	0.42	0.49	0.49	0.36	0.37
STTD Ca,[4] %	0.52	0.46	0.44	0.46	0.49	0.42	0.40	0.42	0.43	0.34	0.35	0.34
STTD P,[4] %	0.33	0.33	0.33	0.33	0.31	0.31	0.31	0.31	0.27	0.27	0.27	0.27
STTD Ca:STTD P	1.56	1.40	1.32	1.40	1.57	1.35	1.30	1.35	1.59	1.25	1.28	1.25
Analyzed values												
Gross energy, Mcal/kg	3.96	4.00	3.97	3.99	3.96	3.98	3.91	3.93	3.95	3.95	3.95	3.93
Dry matter, %	88.77	88.47	88.41	88.27	91.09	89.67	89.59	89.44	88.12	87.91	87.93	87.87
Ash,%	5.18	4.64	4.49	4.57	4.17	4.08	3.81	3.88	4.35	3.83	3.64	3.74
Crude protein, %	21.83	19.47	18.97	18.77	16.81	17.14	16.80	18.36	14.72	15.26	14.59	13.88
AEE,[5] %	4.39	3.73	3.19	3.19	4.65	4.19	3.64	3.91	5.29	4.52	4.37	4.40
Ca, %	0.73	0.64	0.53	0.59	0.65	0.64	0.57	0.57	0.61	0.48	0.46	0.47
Total P, %	0.62	0.59	0.44	0.44	0.59	0.60	0.44	0.45	0.52	0.53	0.38	0.39
Phytate-bound P, %	0.19	0.16	0.17	0.16	0.26	0.25	0.25	0.25	0.25	0.24	0.25	0.25
Phytate,[6] %	0.67	0.57	0.60	0.57	0.92	0.89	0.89	0.89	0.89	0.85	0.89	0.89
Non-phytate P,[7] %	0.43	0.43	0.27	0.28	0.33	0.35	0.19	0.20	0.27	0.29	0.13	0.14
Phytase activity, FTU	< 50	< 50	572	640	< 50	< 50	509	569	< 50	< 50	567	464

[1]Diets were formulated to have the following quantities of net energy and amino acids (standardized ileal digestible amino acids): Net energy: 2,479, 2,528, and 2,557 kcal per kg; Lys: 1.23, 0.98, and 0.85%; Met: 0.42, 0.31, and 0.25%; Thr: 0.74, 0.59, and 0.52%; and Trp: 0.22, 0.17, and 0.15% for phase 1, 2, and 3, respectively.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu,

Table 4.2. (Cont.)

20 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[3]The phytase concentrate contained 5,000 FTU per g (Quantum Blue, AB Vista, Marlborough, UK).

[4]Values for STTD Ca and STTD P in the phytase diets include expected release of Ca and P by phytase.

[5]AEE = acid hydrolyzed ether extract.

[6]Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[7]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 4.3. Ingredient composition and calculated and analyzed values of experimental diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 phytase units (FTU) per kg of feed (Phases 4 and 5)[1]

Item	Phase 4				Phase 5			
Phytase inclusion:	0 FTU		500 FTU		0 FTU		500 FTU	
Ca requirement:	tCa	dCa	tCa	dCa	tCa	dCa	tCa	dCa
Ingredient, %								
Ground corn	84.87	85.61	86.37	86.40	89.11	89.89	90.57	90.66
Soybean meal, 48% CP	11.00	11.00	11.00	11.00	7.00	7.00	7.00	7.00
Choice white grease	1.36	0.98	0.60	0.58	1.35	0.95	0.62	0.56
Calcium carbonate	0.91	0.55	0.76	0.75	0.83	0.45	0.68	0.65
Monocalcium phosphate	0.70	0.70	0.10	0.10	0.59	0.59	-	-
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys HCl, 78% Lys	0.36	0.36	0.36	0.36	0.33	0.33	0.33	0.33
DL-Met	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01
L-Thr	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Trp	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[3]	-	-	0.01	0.01	-	-	0.01	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated values								
Ca, %	0.52	0.38	0.36	0.35	0.46	0.31	0.30	0.29
P, %	0.45	0.45	0.32	0.32	0.41	0.41	0.29	0.29
STTD Ca,[4] %	0.38	0.28	0.29	0.28	0.34	0.23	0.24	0.23
STTD P,[4] %	0.24	0.24	0.24	0.24	0.21	0.21	0.21	0.21
STTD Ca:STTD P	1.58	1.18	1.21	1.18	1.59	1.10	1.14	1.10
Analyzed values								
Gross energy, Mcal/kg	3.82	3.82	3.84	3.84	3.82	3.84	3.81	3.82
Dry matter, %	87.54	87.57	87.85	86.91	87.32	87.04	86.95	87.04
Ash,%	4.02	3.19	3.12	2.82	3.31	3.14	3.00	2.70
Crude protein, %	11.21	10.99	11.39	11.14	9.77	9.12	10.19	9.23

Table 4.3. (Cont.)

AEE,[5] %	4.34	3.41	3.17	3.37	3.49	2.89	2.53	2.67
Ca, %	0.55	0.43	0.40	0.38	0.49	0.33	0.33	0.34
Total P, %	0.46	0.46	0.35	0.34	0.42	0.42	0.30	0.29
Phytate-bound P, %	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.23
Phytate,[6] %	0.85	0.85	0.85	0.85	0.78	0.78	0.78	0.82
Non-phytate P,[7] %	0.22	0.22	0.11	0.10	0.20	0.20	0.08	0.06
Phytase activity, FTU	< 50	< 50	429	469	< 50	< 50	575	659

[1]Diets were formulated to have the following quantities of net energy and amino acids (standardized ileal digestible amino acids): Net energy: 2,570 and 2,599 kcal per kg; Lys: 0.73 and 0.61 %; Met: 0.22 and 0.19%; Thr: 0.46 and 0.40%; and Trp: 0.13 and 0.11% for phase 4 and 5, respectively.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[3]The phytase concentrate contained 5,000 FTU per g (Quantum Blue, AB Vista, Marlborough, UK).

[4]Values for STTD Ca and STTD P in the phytase diets include expected release of Ca and P by phytase.

[5]AEE = ether hydrolyzed ether extract.

[6]Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[7]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 4.4. Growth performance of pigs fed diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 units of microbial phytase (FTU) per kg of feed[1]

	0 FTU		500 FTU			*P*-value		
Item,[2] kg	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
Phase 1, d 1 to 24								
Initial BW	11.17	11.18	11.17	11.19	0.646	0.982	0.998	0.993
ADG	0.656	0.661	0.660	0.626	0.023	0.545	0.500	0.405
ADFI	1.040	1.021	1.031	0.998	0.046	0.580	0.730	0.873
G:F	0.633	0.650	0.642	0.630	0.009	0.772	0.534	0.093
Final BW	26.92	27.05	27.00	26.21	1.157	0.780	0.745	0.694
Phase 2, d 24 to 52								
ADG	0.848	0.782	0.750	0.788	0.037	0.704	0.226	0.168
ADFI	1.643	1.541	1.500	1.553	0.086	0.780	0.455	0.375
G:F	0.517	0.509	0.505	0.510	0.009	0.901	0.596	0.484
Final BW	50.74	48.89	48.01	48.24	2.063	0.699	0.419	0.619
Phase 3, d 52 to 77								
ADG	1.095	1.086	1.051	1.038	0.038	0.783	0.230	0.953
ADFI	2.489	2.433	2.420	2.393	0.087	0.638	0.534	0.871
G:F	0.440	0.448	0.435	0.434	0.008	0.703	0.269	0.639
Final BW	78.10	76.20	74.44	74.18	2.73	0.697	0.308	0.766
Phase 4, d 77 to 101								
ADG	1.035	1.085	0.982	1.047	0.031	0.074	0.148	0.808
ADFI	2.978	3.103	2.935	2.983	0.096	0.373	0.401	0.694
G:F	0.348	0.350	0.335	0.351	0.005	0.094	0.242	0.170
Final BW	102.94	102.24	98.00	99.30	3.236	0.928	0.234	0.759
Phase 5, d 101 to 126								
ADG	1.047	1.057	1.065	1.115	0.030	0.315	0.203	0.510
ADFI	3.401	3.457	3.435	3.487	0.078	0.492	0.687	0.981

Table 4.4. (Cont.)

G:F	0.308	0.306	0.310	0.321	0.007	0.577	0.248	0.390
Final BW	129.10	128.34	125.29	127.18	3.490	0.873	0.482	0.708
Overall phase, d 1 to 126								
ADG	0.936	0.930	0.906	0.921	0.024	0.854	0.409	0.659
ADFI	2.299	2.276	2.250	2.270	0.067	0.978	0.684	0.750
G:F	0.408	0.409	0.403	0.406	0.004	0.640	0.432	0.815

[1]Data are least squares means of 8 observations.

[2]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 4.5. Data from bones collected at the end of phase 1 (d 24) and 5 (d 126) from pigs fed diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 units of microbial phytase (FTU) per kg of feed[1]

	0 FTU		500 FTU			*P*-value		
Item	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
End of phase 1 (d 24)								
Bone ash, g	13.25	13.20	12.01	12.51	0.693	0.748	0.175	0.698
Bone Ca, g	5.03	5.06	4.59	4.77	0.274	0.700	0.194	0.798
Bone P, g	2.40	2.45	2.20	2.27	0.124	0.630	0.140	0.921
Bone ash, %	50.28	50.75	49.39	49.46	0.823	0.743	0.197	0.810
Bone Ca, %	38.49	38.83	38.64	38.59	0.318	0.657	0.890	0.544
Bone P, %	18.13	18.52	18.29	18.14	0.138	0.392	0.427	0.058
End of phase 5 (d 126)								
Bone ash, g	81.07[a]	69.53[b]	70.62[b]	75.37[ab]	2.275	0.148	0.321	0.001
Bone Ca, g	30.47[a]	26.08[b]	26.35[b]	28.39[ab]	0.866	0.188	0.304	0.001
Bone P, g	14.40[a]	12.42[b]	12.53[b]	13.53[ab]	0.413	0.247	0.367	0.001
Bone ash, %	62.07[a]	60.15[b]	60.44[b]	60.99[ab]	0.394	0.092	0.327	0.004
Bone Ca, %	37.59	37.39	37.29	37.43	0.365	0.932	0.732	0.645
Bone P, %	17.77	17.80	17.74	17.87	0.161	0.594	0.911	0.762

[a-b]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least squares means of 7 or 8 observations.

Table 4.6. Concentration of Ca and P in plasma of pigs fed diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 units of microbial phytase (FTU) per kg of feed[1]

	0 FTU		500 FTU			*P*-value		
Item, mg/dL	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
End of phase 1, d 24								
Plasma Ca	10.55	9.95	10.15	9.92	0.267	0.131	0.428	0.489
Plasma P	10.66	11.71	10.77	10.22	0.492	0.615	0.169	0.117
End of phase 2, d 52								
Plasma Ca	10.50	10.56	10.64	10.54	0.176	0.922	0.735	0.664
Plasma P	12.01	12.54	10.66	10.84	0.458	0.449	0.002	0.707
End of phase 3, d 77								
Plasma Ca	10.34	9.93	10.23	10.36	0.206	0.509	0.451	0.208
Plasma P	12.23[a]	12.27[a]	10.70[b]	11.90[a]	0.209	0.006	< 0.001	0.011
End of phase 4, d 101								
Plasma Ca	10.64	10.11	10.36	10.28	0.217	0.172	0.810	0.295
Plasma P	12.38	11.81	12.25	11.83	0.371	0.194	0.881	0.854
End of phase 5, d 126								
Plasma Ca	9.81	9.40	9.28	9.25	0.168	0.207	0.059	0.274
Plasma P	10.76	11.26	11.32	11.08	0.338	0.703	0.569	0.282

[a-b]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least squares means of 7 or 8 observations.

Table 4.7. Concentration of Ca and P in plasma of pigs fed diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 units of microbial phytase (FTU) per kg of feed during phases 2 to 5[1]

Item, mg/dL	Diet		Phytase, FTU			*P*-value		Phase					*P*-value	
	tCa	dCa	0	500	SEM	Diet	Phytase	2	3	4	5	SEM	L	Q
Ca	10.22	10.06	10.19	10.09	0.076	0.161	0.336	10.56	10.23	10.35	9.43	0.096	< 0.001	0.006
P	11.55	11.66	11.71	11.50	0.161	0.640	0.354	10.75	11.36	12.04	11.20	0.182	0.217	< 0.001

[1]Data are least squares means of 60 or 61 observations for diet and phytase effects and 29 to 31 observations for phase effect.

Table 4.8. Carcass measurements of pigs fed diets formulated based on total Ca (tCa) or standardized total tract digestible Ca (dCa), without microbial phytase or with 500 units of microbial phytase (FTU) per kg of feed[1]

	0 FTU		500 FTU			*P*-value		
Item	tCa	dCa	tCa	dCa	SEM	Diet	Phytase	Diet × phytase
HCW,[2] kg	96.87	94.91	89.18	93.33	3.903	0.782	0.245	0.441
Carcass yield, %	77.35	76.71	77.11	77.56	0.568	0.870	0.599	0.340
Backfat thickness, cm	1.53	1.57	1.51	1.48	0.131	0.959	0.675	0.781
Loin eye area, cm^2	53.08	51.98	49.93	50.61	2.290	0.926	0.333	0.702
Carcass lean, %	56.13	55.93	56.19	55.90	0.732	0.739	0.987	0.947
Loin quality								
Ultimate pH	5.61	5.58	5.61	5.59	0.014	0.143	0.684	0.751
Visual color[3]	3.44	3.50	3.75	3.38	0.135	0.258	0.494	0.117
Marbling[4]	1.38	1.31	1.88	1.31	0.152	0.050	0.112	0.112
Firmness[5]	3.00	2.75	3.00	2.88	0.253	0.465	0.807	0.807
Instrumental color[6]								
L*	51.11	53.81	50.52	51.83	1.194	0.104	0.291	0.563
a*	8.69	8.82	8.49	9.16	0.658	0.550	0.919	0.686
b*	7.19	7.80	6.92	7.43	0.671	0.408	0.632	0.946
Drip loss, %	5.74	6.85	5.81	7.22	0.780	0.119	0.781	0.847

[1]Data are least squares means of 8 observations.

[2]HWC = hot carcass weight.

[3]Color score: 1 = pale pink to 6 = dark purplish red (NPPC, 1999).

[4]Marbling score: 1 = 1% intramuscular lipid to 10 = ≥ 10% intramuscular lipid (NPPC, 1999).

[5]Firmness score: 1 = very soft to 5 = very firm (NPPC, 1991).

[6]L* = lightness (the greater the value, the lighter the color), a* = redness (the greater the value, the redder the color), and b* = yellowness (the greater the value, the more yellow the color).

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Chapter 5: Formulation of diets for pigs based on a ratio between digestible calcium and digestible phosphorus results in reduced excretion of calcium in urine without affecting retention of calcium and phosphorus compared with formulation based on values for total calcium[2]

Abstract

An experiment was conducted to test the hypothesis that formulating diets for pigs based on a ratio between standardized total tract digestible (**STTD**) Ca and STTD P instead of total Ca and STTD P does not decrease Ca retention, but increases P utilization. Forty barrows (59.4 ± 3.8 kg) were individually housed in metabolism crates and allotted to 4 corn-soybean meal-based diets in a randomized complete block design with 2 blocks and 5 pigs per diet in each block. Diets were formulated using a 2 × 2 factorial design with 2 diet formulation principles (total Ca or STTD Ca) and 2 inclusion levels of microbial phytase (0 or 500 units per kg of feed). Phytase was assumed to release 0.11% STTD P and 0.16% total Ca. Diets were formulated based on requirements for total Ca and STTD P or a ratio between STTD Ca and STTD P of 1.25:1. Diets were fed for 11 d and fecal and urine samples were collected from feed provided on d 6 to 9. Interactions ($P < 0.05$) between diet formulation principle and phytase level were observed for Ca intake, Ca in feces, Ca absorbed, Ca retained, P digestibility, P absorbed, and P in urine. Phytase increased ($P < 0.05$) the digestibility of Ca in both total Ca and STTD Ca diets. Without

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phytase, Ca intake, Ca in feces, and Ca absorbed was greater ($P < 0.05$) from pigs fed total Ca diets than from pigs fed STTD Ca diets, but P absorbed, P digestibility, and P in urine was greater ($P < 0.05$) from pigs fed STTD Ca diets than from pigs fed total Ca diets. However, in the presence of phytase, no differences between diet formulation principles were observed in these variables. Regardless of phytase, Ca in urine was lower ($P < 0.05$) from pigs fed STTD Ca diets than from pigs fed total Ca diets. There were no differences in Ca retention between pigs fed STTD Ca diets and total Ca diets, but pigs fed total Ca diets retained less ($P < 0.05$) Ca if diets contained phytase. No differences in P retention were observed between diet formulation principles, but pigs fed non-phytase diets retained more ($P < 0.05$) P than pigs fed diets with phytase. In conclusion, because diets formulated based on STTD Ca contain less Ca than total Ca diets, pigs fed STTD Ca diets excreted less Ca in urine, but retention of Ca was not affected. Formulating non-phytase diets based on STTD Ca instead of total Ca increased P absorption, which confirms the detrimental effect of excess Ca on P digestibility. However, P retention was not improved if pigs were fed STTD Ca diets.

Key words: calcium, digestible calcium, phosphorus, phytase, pigs, retention

Abbreviations

ATTD	apparent total tract digestibility
BW	body weight
EPL	endogenous loss of P
FTU	phytase units per kilogram of feed
STTD	standardized total tract digestibility

Introduction

Requirements for P by pigs are expressed on the basis of standardized total tract digestibility (**STTD**) of P, but because of a lack of data for the digestibility of Ca in feed ingredients, Ca requirements are expressed on the basis of total Ca (NRC, 2012). However, it was recognized that a more accurate way to express Ca and P requirements may be to use a ratio between STTD Ca and STTD P (NRC, 2012). Therefore, research has been conducted to determine digestibility values in feed ingredients that contain Ca and to estimate the effect of phytase on the digestibility of Ca in ingredients fed to pigs (Stein et al., 2016). Data for the concentration of STTD Ca in feed ingredients have been used to formulate diets based on STTD Ca and to estimate Ca requirements expressed as a ratio between STTD Ca and STTD P by pigs from 11 to 130 kg (González-Vega et al., 2016c; Merriman et al., 2017; Lagos et al., 2019a, 2019b). Results from these studies indicated that ratios for STTD Ca to STTD P that maximize bone mineralization are greater than those that maximize growth performance, and it was suggested that to maximize Ca retention, a ratio greater than the ratio that maximizes bone mineralization is needed (González-Vega et al., 2016a). Therefore, it is important to not only evaluate the effect of using STTD Ca:STTD P ratios that maximize growth performance on bone mineralization, but also on Ca retention. Another observation from requirement studies was that excess dietary Ca has detrimental effects on growth performance of pigs, which is likely because excess Ca reduces P digestibility (Stein et al., 2011). Excess Ca may, therefore, induce a P deficiency and result in decreased feed intake (Sørensen et al., 2018). Thus, the use of STTD Ca to STTD P ratios in diet formulation may prevent oversupplying Ca in diets. Therefore, the objective of this experiment was to test the hypothesis that formulating diets for growing pigs based on a ratio between STTD

Ca and STTD P instead of values for total Ca and STTD P does not decrease Ca retention, but increases P utilization.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals, housing, and diets

Forty barrows [body weight (**BW**): 59.4 ± 3.8 kg] were randomly allotted to 4 diets and 2 blocks in a randomized complete block design with 20 pigs per block. Each block had 5 replicate pigs per diet for a total of 10 replicate pigs per diet in the 2 blocks. Pigs were individually housed in metabolism crates that were equipped with a feeder, a nipple drinker, and a slatted floor to allow for the total, but separate, collection of urine and fecal materials. Pigs had free access to water throughout the experiment.

Diet formulation followed a 2 × 2 factorial design with 2 diet formulation principles (based on requirements for total Ca or for STTD Ca) and 2 inclusion levels of microbial phytase [0 or 500 phytase units per kg of feed (**FTU**)]. Therefore, 4 corn-soybean meal based diets were formulated (Table 5.1). Requirements for total Ca and STTD P were based on requirements for 50- to 75-kg pigs (NRC, 2012) and requirements for STTD Ca were calculated by multiplying the requirement for STTD P by 1.25 according to Lagos et al. (2019b). As a consequence, diets 1 and 2 contained no phytase and were formulated based on either the requirement for total Ca (0.59%) or the calculated requirement for STTD Ca (0.34%). Both diets were formulated to contain 0.27% STTD P. Diets 3 and 4 both contained 500 FTU of microbial phytase (Quantum

Blue; AB Vista Feed Ingredients, Marlborough, UK). Diet 3 was formulated based on the same requirements for total Ca and STTD P as diet 1, with the exception that it was assumed that phytase would release 0.16% total Ca and 0.11% STTD P. This diet was, therefore, calculated to contain 0.43% total Ca and 0.16% STTD P. Diet 4 was formulated as diet 2 with the exception that the release of STTD P by phytase was taken into account so 0.16% STTD P was included in this diet as was the case for diet 3. The provision of STTD Ca in diet 4 was calculated as in diet 2, i.e., by multiplying STTD P by 1.25, and although the assumed release of STTD P by phytase was taken into account, the provision of STTD Ca was calculated by multiplying 0.27 by 1.25. Values for STTD Ca in the ingredients were from Stein et al. (2016). Values obtained in the absence of phytase were used to formulate diet 2 and values for STTD Ca in the presence of phytase were used to formulate diets for diet 4 (Table 5.2). Because phytase does not increase the STTD of Ca in monocalcium phosphate, values for STTD of Ca in monocalcium phosphate was the same without and with phytase, whereas the STTD values for corn, soybean meal, and calcium carbonate were greater in the presence of phytase than without phytase. Using these formulation principles, it turned out that concentrations of total and STTD Ca in diets 3 and 4 were practically identical, although calculated using different formulation principles. All diets were formulated to have identical concentrations of net energy, Na, Cl, K, and vitamin D.

Feeding and sample collection

Pigs were fed 3 times the daily maintenance energy requirement (i.e., 197 kcal of metabolizable energy per kg of $BW^{0.60}$; NRC, 2012). The daily allotments of feed were divided into 2 equal meals that were provided at 0800 and 1600 h. Pigs were fed each diet for 11 d. The initial 5 d were considered the adaptation period to the diets and fecal samples were collected quantitatively from the feed provided on d 6, 7, 8, and 9 using the marker-to-marker approach

(Kong and Adeola, 2014). The beginning of fecal collections was marked by adding a color marker (indigo carmine) to the morning meal on d 6, and the conclusion of fecal collection was marked by adding ferric oxide to the morning meal on d 10. Urine was collected in urine buckets that contained 50 mL of 6*N* HCl and urine collections started after feeding the morning meal on d 6 and ceased after feeding the morning meal on d 10. Fecal samples and 20% of the collected urine was stored at -20 °C. At the conclusion of the experiment, fecal samples and orts were dried at 65 °C in a forced air oven. Fecal samples were then finely ground through a 1-mm screen before analysis using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ).

Sample analysis

Ingredient, diet, urine, and fecal samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Diets and ingredients were analyzed for dry matter (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007). Diets were also analyzed for acid hydrolyzed ether extract (Method 2003.06; AOAC Int., 2007) using 3 *N* HCl in an Ankom^{HCl} hydrolyzer and petroleum ether in an Ankom^{XT15} extractor (Ankom Technology, Macedon, NY), gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL), and N (method 990.03; AOAC Int., 2007) using a LECO FP628 (LECO Corp., Saint Joseph, MI); crude protein was calculated as N × 6.25. Phytate-bound P in diets and ingredients was predicted by near infra-red reflectance spectroscopy using AUNIR calibration standards (AB Vista, Plantation, FL). Phytase activity was analyzed in diets by the ELISA method using Quantiplate Kits for Quantum Blue (Enzyme Services and Consultancy Ltda., Ystrad Mynach, UK).

Calculations and statistical analyses

Dietary concentration of phytate was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004) and non-phytate P was then calculated by subtracting the amount of phytate-bound P from the concentration of total P. Feed intake was calculated by subtracting the weight of dried orts from feed provisions. The apparent total tract digestibility (**ATTD**) of Ca and P in diets was calculated (Almeida and Stein, 2010) and values for STTD of P were calculated by correcting the ATTD values for the basal endogenous loss of P (**EPL**; i.e.,190 mg/kg dry matter intake; NRC, 2012). Values for retention of Ca and P were calculated as explained by González-Vega et al. (2013).

Normality of residuals and model assumptions were tested using INFLUENCE, PROC GPLOT, and PROC UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC). Data for Ca and P balance were analyzed using the PROC MIXED of SAS with the experimental unit being the pig. The model included diet formulation principle (total Ca or STTD Ca) and phytase inclusion (0 or 500 FTU), and the interaction between diet formulation principle and phytase inclusion. The model also included the random effects of block and replicate within block. Treatment means were calculated using the LSMEANS statement and means were separated using the PDIFF procedure in SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy throughout the experiment and consumed the diets without apparent problems. No interaction between diet formulation principle and phytase inclusion was observed for daily feed intake, ATTD of Ca, daily urine output, percentage and quantity (grams per day)

of Ca in urine, or Ca retention as a percentage of intake (Table 5.3). For daily feed intake and urine output, no effect of diet formulation principle or phytase inclusion was observed, but pigs fed diets formulated based on total Ca had an increased ($P < 0.05$) percentage and quantity (grams per day) of Ca excreted in the urine compared with pigs fed diets formulated based on a ratio between STTD Ca and STTD P. The ATTD of Ca and the retention of Ca as a percentage of intake were greater ($P < 0.05$) in diets with phytase than in diets without phytase, and the retention of Ca as a percentage of intake was greater ($P < 0.05$) in diets based on a ratio between STTD Ca and STTD P than in total Ca based diets. Pigs fed diets without phytase had greater Ca intake if diets were formulated based on total Ca compared with diets formulated based on a ratio between STTD Ca and STTD P, but there were no differences between the 2 diet formulation principles for Ca intake in pigs fed diets with phytase (interaction, $P < 0.01$). Pigs fed diets formulated based on total Ca had reduced fecal output and reduced Ca retention (grams per day) if phytase was included in the diet compared with pigs fed diets without phytase, but for pigs fed diets formulated based on a ratio between STTD Ca and STTD P, no differences in fecal output or Ca retention between the 2 inclusion levels of phytase were observed (interaction, $P < 0.05$). For pigs fed diets with phytase, there were no differences in the percentage and quantity (grams per day) of Ca in feces or the quantity of Ca absorbed between the 2 diet formulation principles, but if phytase was not used, pigs fed diets formulated based on a ratio between STTD Ca and STTD P had a reduced ($P < 0.05$) quantity of Ca absorbed, and reduced percentage and quantity (grams per day) of Ca in feces compared with pigs fed diets formulated based on total Ca (interaction, $P < 0.05$). Pigs fed diets formulated based on total Ca had lower Ca intake, Ca absorption (grams per day), and percentage and quantity (grams per day) of Ca in feces, if phytase was used, but there was no effect of inclusion of phytase on these variables if pigs were

fed diets formulated based on a ratio between STTD Ca and STTD P (interaction, $P < 0.05$).

Pigs fed diets without phytase had greater ($P < 0.05$) P intake and P in feces (percent and grams per day) than pigs fed diets with phytase (Table 5.4). Pigs fed diets formulated based on total Ca tended ($P < 0.10$) to ingest less P (grams) daily than pigs fed diets formulated based on a ratio between STTD Ca and STTD P. Pigs fed diets without phytase had greater ATTD of P, STTD of P, absorbed P (grams per day), and percentage and quantity (grams per day) of P in urine if diets were formulated based on a ratio between STTD Ca and STTD P compared with pigs fed diets formulated based on total Ca. However, no differences between the 2 diet formulation principles were observed for pigs fed diets containing phytase (interaction, $P < 0.05$). The ATTD of P was greater, but the quantity (grams per day) of P absorbed was lower when phytase was included in diets formulated based on total Ca, but if diets were formulated based on a ratio between STTD Ca and STTD P, there was no effect of phytase on the ATTD of P, and the quantity of P absorbed was lower if 500 FTU of phytase were included in the diet than if no phytase was used (interaction, $P < 0.05$). Pigs fed diets formulated based on a ratio between STTD Ca and STTD P had reduced percentage and quantity (grams per day) of P in urine if phytase was included in the diet compared with pigs fed no phytase, but no differences between the 2 inclusion levels of phytase were observed in pigs fed diets based on total Ca (interaction, $P < 0.05$). Pigs fed diets with phytase retained less ($P < 0.05$) P (grams per day) daily, but had a greater ($P < 0.05$) retention of P as a percentage of intake, than pigs fed diets without phytase.

Discussion

Diets for growing-finishing pigs may be formulated based on STTD Ca values because data for the digestibility of Ca in Ca-containing feed ingredients have been generated from diets without

or with microbial phytase (Stein et al., 2016). This also allowed for the estimation of requirements for a ratio between STTD Ca and STTD P by growing pigs, which is believed to be a more appropriate way to express Ca requirements (NRC, 2012). Therefore, STTD Ca:STTD P requirements to optimize growth performance of 11- to 25-kg (Lagos et al., 2019a), 25- to 50-kg (González-Vega et al., 2016c), 50- to 85-kg (Lagos et al., 2019b), and 100- to 130-kg pigs (Merriman et al., 2017) have been determined. These values were recently validated in diets without microbial phytase or with 500 FTU of microbial phytase when fed to pigs from 11 to 130 kg (Lagos et al., 2021).

The 4 diets used in this experiment were formulated using NRC (2012) values for Ca and P in corn, soybean meal, calcium carbonate, and monocalcium phosphate. The analyzed values for Ca and P in these feed ingredients were slightly greater than those used in diet formulation, which is likely the reason for the differences between calculated and analyzed values in diets. However, the expected differences or similarities in concentrations of Ca and P among the 4 diets were obtained. The reason the 2 diets with phytase turned out to be practically identical regardless of the diet formulation principle used, is that in formulation of the diet based on total Ca, it was assumed that phytase would release 0.16% total Ca, and the provision of Ca was, therefore, reduced by that amount (i.e., from 0.59 to 0.43). Taken the digestibility of Ca in the 4 ingredients into account (in the presence of phytase) the calculated STTD Ca in this diet was 0.35%. Using actual STTD values for the 4 ingredients in diet formulation, instead of using values for total Ca, and assuming the requirement for STTD Ca was 1.25 times the requirement for STTD P, resulted in a calculated requirement of 0.34% STTD Ca and a provision of 0.42% total Ca. As a consequence, the provisions of total Ca and STTD Ca in the 2 diets with phytase turned out to be almost the same although calculated in very different ways. This indicates that

the assumed release of total Ca by phytase likely was accurate. However, because of the similarities between the 2 diets with phytase, it was not surprising that no differences between these diets were observed.

The ability of microbial phytase to release P from phytate in ingredients of plant origin and to increase P digestibility in diets fed to pigs (She et al., 2017) was evidenced in this study by the increase in the ATTD and STTD of P in diets with 500 FTU of microbial phytase compared with diets without phytase. Values for the digestibility of P in diets obtained in this experiment concur with data from Zeng et al. (2016) and Archs Toledo et al. (2020) where corn-soybean meal-based diets with 500 FTU of microbial phytase were fed to 19 and 40 kg-pigs, respectively. The fact that phytase also releases Ca bound to phytate in plant feed ingredients (González-Vega et al., 2013) and from limestone (González-Vega et al., 2015; Lee et al., 2019) was demonstrated in this experiment by the increased ATTD of Ca in diets containing phytase compared with diets without phytase. Similar observations have been reported in the past (Almeida et al., 2013; Arredondo et al., 2019; Archs Toledo et al., 2020).

Maximizing bone mineralization requires more Ca than maximizing growth performance of pigs (Crenshaw, 2001), but the amount of dietary Ca needed to optimize Ca retention in pigs is greater than the amount needed to optimize bone mineralization (González-Vega et al., 2016a, b). Therefore, it was hypothesized that feeding diets based on a ratio between STTD Ca and STTD P that contained less Ca than diets based on total Ca, especially when microbial phytase was not included, would not affect the retention of Ca by pigs. As expected, regardless of the inclusion of phytase there were no differences in Ca retention between pigs fed diets based on total Ca and pigs fed diets based on a STTD Ca to STTD P ratio. The decreased concentration (percentage and grams per day) of Ca in urine from pigs fed diets formulated based on a ratio

between STTD Ca and STTD P compared with pigs fed diets based on total Ca is the reason there was no difference between the 2 diet formulation principles for retention of Ca despite the greater Ca concentration in diets formulated based on total Ca. This observation indicates that if diets are formulated on calculated ratios between STTD Ca and STTD P, less Ca is needed in the diet. The reason there was no difference in Ca retention between the 2 diets containing phytase likely is that although formulated based on different principles, the 2 diets contained similar quantities of Ca.

The observation that the ATTD of P, STTD of P, and the quantity of P absorbed by pigs fed diets formulated on a ratio between STTD Ca and STTD P was greater than by pigs fed diets based on total Ca if microbial phytase was not used, concurs with data indicating that increasing concentration of dietary Ca reduces P digestibility (Stein et al., 2011; González-Vega et al., 2016b; Velayudhan et al., 2019). This is likely a result of formation of indigestible complexes between Ca and P in the gastrointestinal tract of pigs. Therefore, by reducing the concentration of dietary Ca (0.35 percentage units less calcium carbonate), as is the case for the non-phytase diets based on a ratio between STTD Ca and STTD P compared with total Ca based diets, less P is bound by Ca in the digestive system of pigs, which results in increased ATTD and STTD of P. However, data from this experiment also indicate that pigs fed diets without phytase and based on a STTD Ca to STTD P ratio absorbed more P than needed because these pigs excreted more P in urine than pigs fed total Ca based diets. Because both Ca and P are needed in adequate concentrations in the diet for bone mineralization to occur (Crenshaw, 2001), pigs fed diets with reduced concentration of Ca absorbed more P, but did not synthesize more bone tissue, which is likely because they already met the requirement. As a consequence, there were no differences in P retention between pigs fed diets formulated on the 2 diet principles. This indicates that less P

may be needed in diets formulated on the basis of a STTD Ca to STTD P ratio, but research needs to be conducted to validate this hypothesis.

The negative effect of the inclusion of microbial phytase on retention of P regardless of the diet formulation principle indicates that the assumed value of 0.11% STTD P release by phytase may have been overestimated. However, the observation that there are no differences in Ca and P balance between pigs fed diets with phytase based on total Ca and a STTD Ca to STTD P ratio was expected because these diets only had 0.03 percentage units difference in calcium carbonate. This observation also indicates that the STTD values for Ca used in this experiment for diet formulation were accurate. The current data also indicate that ratios between STTD Ca and STTD P can be used to formulate diets without or with microbial phytase, as demonstrated by the lack of differences in the Ca balance of pigs.

Conclusions

Calcium retention was not affected by diet formulation principle because pigs fed diets based on a ratio between STTD Ca and STTD P ingest and absorb less Ca than pigs fed total Ca based diets, and also excrete less Ca in urine. In diets without microbial phytase, formulating diets based on a ratio between STTD Ca and STTD P improved the ATTD of P and the quantity of P absorbed by pigs, which confirm the negative effect of excess Ca on P digestibility. However, P in urine was increased and P retention was not improved in these pigs, which indicates that less P is needed in diets formulated on a ratio between STTD Ca and STTD P. A value of 0.16% total Ca released by phytase or STTD Ca values for each ingredient can be used for diet formulation to account for the Ca-releasing effect of phytase.

Tables

Table 5.1. Ingredient composition and calculated and analyzed values of experimental diets formulated based on total Ca or standardized total tract digestible (STTD) Ca, without microbial phytase or with 500 units of microbial phytase per kilogram of feed (FTU)

Item	Phytase inclusion:	0 FTU		500 FTU	
	Ca requirement:	Total Ca	STTD Ca	Total Ca	STTD Ca
Ingredients, %					
Ground corn		76.22	76.89	77.68	77.74
Soybean meal, 48% crude protein		18.50	18.50	18.50	18.50
Choice white grease		2.50	2.18	1.78	1.75
Calcium carbonate		1.00	0.65	0.86	0.83
Monocalcium phosphate		0.76	0.76	0.15	0.15
Sodium bicarbonate		0.10	0.10	0.10	0.10
L-Lys HCl, 78% Lys		0.28	0.28	0.28	0.28
DL-Met		0.03	0.03	0.03	0.03
L-Thr		0.06	0.06	0.06	0.06
Sodium chloride		0.40	0.40	0.40	0.40
Vitamin mineral premix[1]		0.15	0.15	0.15	0.15
Phytase concentrate[2]		-	-	0.01	0.01
Total		100.00	100.00	100.00	100.00
Calculated values					
Ca, %		0.59	0.45	0.43	0.42
P, %		0.49	0.49	0.36	0.37
Phytate, %		1.00	1.00	1.00	1.00
STTD Ca[3], %		0.43	0.34	0.35	0.34
STTD P[3], %		0.27	0.27	0.27	0.27
STTD Ca:STTD P		1.59	1.25	1.28	1.25
Analyzed values					
Gross energy, kcal/kg		3,988	3,977	3,969	3,970

Table 5.1. (Cont.)

Dry matter, %	89.42	88.10	87.83	87.82
Ash,%	3.77	3.76	3.43	3.64
Crude protein, %	13.93	14.22	14.19	13.62
Acid hydrolyzed ether extract, %	5.24	4.24	4.28	4.36
Ca, %	0.61	0.48	0.46	0.47
Total P, %	0.52	0.53	0.38	0.39
Phytate-bound P, %	0.25	0.24	0.25	0.25
Phytate,[4] %	0.89	0.85	0.89	0.89
Non-phytate P,[5] %	0.27	0.30	0.13	0.14
Phytase activity, FTU	< 50	< 50	567	464

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[2]The phytase concentrate contained 5,000 units of phytase/g (Quantum blue, AB Vista, Marlborough, UK).

[3]Values for STTD Ca and STTD P in the phytase diets include expected release of Ca and P by phytase.

[4]Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[5]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 5.2. Analyzed composition and calculated values for total and standardized total tract digestible (STTD) Ca and P in feed ingredients, as fed basis

Item	Corn	Soybean meal	Calcium carbonate	Monocalcium phosphate
Analyzed values				
Dry matter, %	84.50	88.36	99.96	92.15
Ash, %	1.34	6.94	92.65	81.23
Ca, %	0.03	0.35	38.96	17.70
P, %	0.30	0.78	0.04	20.91
Phytate-bound P, %	0.13	0.45	-	-
Calculated values				
Phytate,[1] %	0.46	1.60	-	-
Non-phytate P,[2] %	0.16	0.24	-	-
Total Ca, %	0.020	0.33	38.20	17.10
Total P, %	0.26	0.71	0.02	21.00
STTD without phytase				
Ca, %	0.013	0.23	26.74	14.71
P, %	0.09	0.34	0.012	18.48
STTD with phytase				
Ca, %	0.015	0.26	30.56	14.71

[1]Phytate was calculated by dividing phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[2]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 5.3. Calcium balance for pigs fed diets formulated based on requirements for total Ca or standardized total tract digestible (STTD) Ca, without or with 500 units of microbial phytase per kilogram of feed (FTU)[1,2]

Item	Phytase:	0 FTU		500 FTU			*P*-value		
	Requirement:	Total Ca	STTD Ca	Total Ca	STTD Ca	SEM	Req.	Phytase	Req. × phytase
Feed intake, g/d		2,134	2,162	2,168	2,169	31.2	0.632	0.523	0.672
Ca intake, g/d		12.91[a]	10.43[b]	9.86[c]	10.29[bc]	0.165	< 0.001	< 0.001	< 0.001
Dry fecal output, g/d		216.3[a]	196.2[ab]	195.4[b]	211.1[ab]	7.94	0.775	0.694	0.023
Ca in feces, %		2.36[a]	1.77[b]	1.58[b]	1.51[b]	0.126	0.013	< 0.001	0.042
Ca output in feces, g/d		5.07[a]	3.45[b]	3.06[b]	3.16[b]	0.249	0.004	< 0.001	0.001
ATTD of Ca, %		60.77	66.88	69.16	69.34	2.089	0.141	0.014	0.164
Ca absorbed, g/d		7.84[a]	6.97[b]	6.81[b]	7.13[b]	0.226	0.241	0.062	0.012
Urine output, g/d		3,983	4,180	4,469	4,456	819.2	0.909	0.638	0.896
Ca in urine, %		0.014	0.005	0.008	0.005	0.0030	0.035	0.262	0.352
Ca in urine, g/d		0.41	0.15	0.30	0.18	0.059	0.003	0.521	0.231
Ca retention, % of intake		57.58	65.46	66.14	67.58	1.997	0.025	0.011	0.116
Ca retained, g/d		7.43[a]	6.82[ab]	6.51[b]	6.95[ab]	0.224	0.707	0.084	0.025

[a-c]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Each least squares mean represents 10 observations.

[2]Values for daily intake and output are the average of a 4-d collection.

Table 5.4. Phosphorus balance for pigs fed diets formulated based on requirements for total Ca or standardized total tract digestible (STTD) Ca, without or with 500 units of microbial phytase per kilogram of feed (FTU)[1,2]

Item[3] Phytase:	0 FTU		500 FTU			*P*-value		
Requirement:	Total Ca	STTD Ca	Total Ca	STTD Ca	SEM	Req.	Phytase	Req. × phytase
Daily DMI, g/d	1,908	1,905	1,904	1,905	27.6	0.970	0.939	0.936
P intake, g/d	11.10	11.46	8.24	8.46	0.150	0.058	< 0.001	0.642
P in feces, %	2.33	2.34	1.49	1.47	0.075	0.910	< 0.001	0.837
P output in feces, g/d	4.99	4.56	2.91	3.09	0.176	0.426	< 0.001	0.101
ATTD of P, %	55.06[c]	60.17[b]	64.66[a]	63.51[ab]	1.616	0.170	< 0.001	0.034
EPL[4], mg/d	362.6	362.0	361.7	362.0	5.25	0.975	0.940	0.936
STTD of P, %	58.33[c]	63.33[b]	69.05[a]	67.79[a]	1.616	0.195	< 0.001	0.034
P absorbed, g/d	6.11[b]	6.90[a]	5.32[c]	5.37[c]	0.198	0.017	< 0.001	0.033
P in urine, %	0.006[b]	0.025[a]	0.006[b]	0.006[b]	0.0042	0.048	0.030	0.030
P in urine, g/d	0.16[b]	0.62[a]	0.17[b]	0.19[b]	0.070	< 0.001	< 0.001	< 0.001
P retention, % of intake	53.65	54.79	62.54	61.22	1.397	0.949	< 0.001	0.385
P retained, g/d	5.95	6.28	5.15	5.18	0.162	0.268	< 0.001	0.354

[a-c]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Each least squares mean represents 10 observations.

[2]Values for daily intake and output are the average of a 4-d collection.

[3]DMI = dry matter intake; EPL = endogenous loss of P.

[4]Calculated by multiplying the EPL (190 mg/kg DMI; NRC, 2012) by the daily DMI.

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Chapter 6: Reduced concentrations of limestone and monocalcium phosphate in diets without or with microbial phytase did not influence gastric pH, fecal score, or growth performance, but reduced bone ash and serum albumin in weanling pigs[3]

Abstract

An experiment was conducted to test the hypothesis that reducing limestone and monocalcium phosphate in diets for weanling pigs, both by lowering the concentration of Ca and P and by including microbial phytase in the diet, will reduce stomach pH and fecal score and will improve growth performance of pigs. A total of 160 weanling pigs (5.75 ± 1.04 kg) were allotted to 4 corn-soybean meal-based diets in a completely randomized design with 5 pigs per pen. Diets for phase 1 (d 1 to 15) were formulated using a 2 × 2 factorial design with 2 concentrations of Ca and P (adequate or deficient levels of total Ca and digestible P) and 2 inclusion levels of phytase (0 or 2,000 units/kg feed). Phytase was assumed to release 0.16% total Ca and 0.11% digestible P. Common diets were fed in phases 2 (d 16 to 21) and 3 (d 22 to 35). Fecal scores were recorded in phase 1 and on d 15, gastric pH was measured and a blood sample and the right femur were collected from 1 pig per pen. Growth performance data were recorded within each phase. In phase 1, at deficient levels of Ca and P, pigs fed the diet with phytase had greater ($P <$ 0.05) average daily gain (**ADG**) and gain to feed (**G:F**) compared with pigs fed the diet without phytase, but in the diets with adequate levels of Ca and P, no effect of phytase inclusion was

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observed (interaction, $P < 0.05$). Without phytase, pigs fed the diet with deficient levels of Ca and P had reduced ($P < 0.05$) G:F compared with pigs fed the diet with adequate Ca and P levels, but if phytase was included, there was no effect of Ca and P on G:F (interaction, $P < 0.05$). For phases 2 and 3, and from d 1 to 35, no differences among dietary treatments were observed for ADG or G:F of pigs. Bone ash was greater ($P < 0.05$) in pigs fed diets with adequate levels of Ca and P than in pigs fed diets with deficient levels, but no effect of phytase inclusion was observed. The concentrations of Ca and P did not affect stomach pH or fecal score, but pigs fed diets with phytase tended ($P < 0.10$) to have reduced stomach pH and fecal score compared with pigs fed diets without phytase. Pigs fed diets with adequate levels of Ca and P had greater ($P < 0.05$) albumin in serum than pigs fed the Ca- and P-deficient diets. In conclusion, phytase inclusion in phase 1 diets may reduce diarrhea, but lowering Ca and P does not reduce stomach pH or fecal score and decreases bone ash, although growth performance during the entire weanling period is not affected.

Key words: bone ash, dietary Ca and P, fecal score, gastric pH, pigs, phytase

Abbreviations

AEE	acid hydrolyzed ether extract
AGP	antibiotic growth promoters
ADFI	average daily feed intake
ADG	average daily gain
BUN	blood urea nitrogen
BW	body weight
FTU	phytase units per kilogram of feed

Abbreviations (Cont.)

G:F	gain to feed ratio
MCP	monocalcium phosphate
STTD	standardized total tract digestible

Introduction

Weaning is a critical period for pigs because they are exposed to a variety of stressors including removal from the sow, transportation, interaction with pigs from different litters, and the transition from sow milk to a less digestible diet (Pluske et al., 1997). The change in diet may result in disrupted intestinal barrier function and often causes diarrhea because young pigs have reduced digestive enzymatic activity compared with older pigs (Hedemann et al., 2006). To mitigate the negative effects of weaning on growth performance of pigs, antibiotic growth promoters (**AGP**) have been used in nursery diets, but restriction on the use of AGP has increased due to the risk of intestinal microbes acquiring resistance to antibiotics (Casewell et al., 2003). As a consequence, direct-fed microbials, prebiotics, phytogenic feed additives, and acidifiers have been investigated as alternatives to AGP (Liu et al., 2018). Dietary acidifiers are used to create an adequate gastric environment that favors pepsin activity (Liu et al., 2018) because weanling pigs lack the ability to secrete sufficient HCl in the stomach to reach a stable low pH for proper digestion of proteins (Suiryanrayna and Ramana, 2015). However, inclusion of limestone and monocalcium phosphate (**MCP**) in weaning diets may exacerbate problems caused by limited secretion of HCl because these ingredients have a high buffering capacity at pH 3 (Lawlor et al., 2005). Therefore, reducing limestone and MCP in phase 1 diets may result in decreased stomach pH. Inclusion of microbial phytase in diets increases the digestibility of Ca

and P and reduces the necessity for MCP and limestone in diets (Selle et al., 2009), which may further contribute to a reduced pH in the stomach. However, data to demonstrate these hypotheses are limited. Therefore, the objective of this experiment was to test the hypothesis that reducing the amount of limestone and MCP in diets for weanling pigs, by lowering the concentrations of dietary Ca and P, and (or) by including microbial phytase in the diet, will reduce stomach pH and fecal score and improve growth performance of pigs.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals, housing, and diets

One hundred and sixty weanling pigs with an initial body weight (**BW**) of 5.75 ± 1.04 kg were randomly allotted to 4 diets in a completely randomized design. There were 5 pigs per pen (3 gilts and 2 castrates) and 8 replicate pens per diet. Pens had fully slatted floors, a feeder, and a nipple drinker. Feed and water were available at all times. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

The experiment was conducted for 5 wk. A 3-phase feeding program was used with d 1 to 15 as phase 1, d 16 to 21 as phase 2, and d 22 to 35 as phase 3. Pigs were fed one of 4 diets during phase 1, whereas a common diet was fed in phases 2 and 3. Therefore, a total of 6 diets were formulated (Table 6.1).

The 4 diets in phase 1 were based on corn and soybean meal and were formulated using a 2 × 2 factorial design with 2 concentrations of Ca and P [adequate or deficient levels of total Ca

and standardized total tract digestible (**STTD**) P] and 2 inclusion levels of microbial phytase [0 or 2,000 phytase units per kilogram of feed (**FTU**)]. Diet 1 was formulated based on the NRC (2012) requirement for total Ca (0.83%) and STTD P (0.43%). Diet 2 was formulated with 50% of the NRC (2012) requirement for total Ca (0.42%) and STTD P (0.22%). Diet 3 was similar to diet 1 with the exception that 2,000 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were included, and the provisions of total Ca and STTD P were reduced by 0.16 and 0.11%, respectively, to account for the expected release of Ca and P by phytase. Diet 4 was formulated as diet 2, but with 2,000 FTU of phytase, and the provision of total Ca was reduced as explained for diet 3, thus the concentration of total Ca was 0.26%. However, STTD P was only reduced to 0.20%, which corresponds to 72% of the NRC (2012) requirement, because that was the value obtained after removal of all MCP in the diet and accounting for the expected release of P by phytase (Table 6.2). The 4 diets were formulated to contain identical quantities of net energy, Na, Cl, K, and vitamin D. Phase 2 and 3 diets were formulated to meet requirements for total Ca (0.80% and 0.70%, respectively) and STTD P (0.40% and 0.33%, respectively; NRC, 2012) and no phytase was used.

Sample collection and bone measurements

Pig weights were recorded at the beginning and at the conclusion of each phase. The amount of feed offered was recorded every day and the amount of feed in the feeders was recorded at the end of each phase. During the initial 15 d, fecal scores were assessed visually every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). On the last day of phase 1, the gilt in each pen with a BW closest to the average BW of the pen was euthanized via captive bolt stunning and a blood sample was collected in vacutainers that contained spray-coated silica to yield blood serum after

centrifugation at 1,500 × *g* at 4 ºC for 15 min. Serum samples were frozen at -20 ºC until used for analysis of blood urea nitrogen (**BUN**), total protein, and albumin. The abdominal cavity of the euthanized pig was opened and pH of gastric contents was measured twice *in-situ* by making a small incision for a pH electrode in the stomach cavity. All stomach content was then collected and mixed and *ex-situ* pH was measured twice. The right femur was collected and autoclaved at 125 ºC for 55 min and the muscles attached to the bone were removed. Femurs were broken, bone marrow was removed, and bones were dried overnight at 105 ºC. Femurs were soaked for 72 h in petroleum ether under a chemical hood to remove residual marrow and fat. Bones were then dried at 135 ºC for 2 h and ashed at 600 ºC for 16 h.

Sample analysis

Corn, soybean meal, soy protein concentrate, enzyme treated soybean meal, spray dried plasma protein, calcium carbonate, MCP, and diets were analyzed for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2019) and for ash by incineration at 600 °C for 2 h (Method 942.05; AOAC Int., 2019). These samples were also analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2019) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2019]. Corn, soybean meal, soy protein concentrate, enzyme treated soybean meal, and diets were analyzed for N (Method 990.03; AOAC Int., 2019) using a LECO FP628 (LECO Corp., Saint Joseph, MI, USA) and crude protein was calculated as N × 6.25. These samples were also analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA) and for acid hydrolyzed ether extract (**AEE**; Method 2003.06; AOAC Int., 2019) using 3 *N* HCl in an Ankom^{HCl} hydrolyzer followed by petroleum ether in an Ankom^{XT15} extractor (Ankom Technology, Macedon, NY, USA). Corn and soybean products were also analyzed for phytic

acid by analytical biochemistry (Ellis et al., 1977). Diet samples were analyzed for amino acids (Method 982.30 E (a, b, c); AOAC Int., 2019] using a Hitachi Analyzer [Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA), and phytase activity was analyzed by the colorimetric enzymatic method (Method AOAC 2000.12; AOAC Int., 2019). Serum samples were analyzed for BUN, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). The pH of stomach contents was measured using a Benchtop pH meter (Orion Star A111, Fisher Scientific, Waltham, MA, USA) with a pH electrode for semi-solid samples. The pH meter was calibrated using 3 buffer solutions (4.01, 7.01, and 10.01 pH; Fisher Scientific, Waltham, MA, USA).

Calculations and statistical analyses

The concentration of phytate-bound P in corn and soybean products was calculated by multiplying the analyzed concentration of phytate by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) were calculated for each phase and for the overall experimental period. Bone ash percentage was calculated by dividing the quantity of bone ash by the weight of the fat-free dried bone and multiplying by 100. Diarrhea frequency was calculated by dividing the number of days with fecal score ≥ 3 by the total number of scoring days and multiplied by 100.

Normality of residuals and homogeneity of variances were tested using the INFLUENCE, GPLOT, and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC, USA). Data were analyzed using the PROC MIXED procedure of SAS. Pen was the experimental unit for growth performance and fecal evaluation, whereas the sacrificed pig in each pen was the experimental unit for stomach pH, bone ash, and blood metabolites. The fixed effects of the model were

concentrations of Ca and P, phytase inclusion, and the interaction between concentrations of Ca and P and phytase inclusion. If the interaction was not significant, only main effects were included in the final model. The model also included the random effect of replicate. Outliers were determined by plotting the residuals in a quantile-quantile plot against the normal distribution and identifying values that were beyond ± 2.5 standard deviations. Treatment means were calculated using the LSMEANS statement and means were separated using the PDIFF statement of SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs remained healthy during the experiment and consumed their assigned diets without apparent health issues. However, 1 pig fed the diet formulated to have deficient levels of Ca and P and 2,000 units of phytase died at the end of phase 1. Values for ADFI in this pen were adjusted as previously described (Lindemann and Kim, 2007). No other pigs died during the experiment. For ADG and G:F in phase 1, an interaction ($P < 0.05$) between concentration of Ca and P and phytase inclusion was observed (Table 6.3). At adequate levels of Ca and P, no difference was observed for ADG or G:F of pigs fed diets without or with 2,000 FTU of microbial phytase. However, for pigs fed diets with deficient levels of Ca and P, ADG and G:F were greater ($P < 0.05$) if the diet containing phytase was fed rather than the diet without phytase. No difference between concentrations of Ca and P was observed for G:F of pigs fed diets with 2,000 FTU of phytase, but pigs fed non-phytase diets had reduced ($P < 0.05$) G:F if the diet was formulated with deficient levels of Ca and P compared with the diet with adequate levels of Ca and P. There was a tendency ($P < 0.10$) for an interaction between concentrations of Ca and P and phytase

inclusion for ADFI of pigs, with ADFI tending to be reduced for pigs fed the Ca- and P-deficient diet compared with pigs fed the diet with adequate levels of Ca and P if no phytase was used, whereas the opposite trend was observed if phytase was included in the diet. The BW of pigs at the end of phase 1 was not influenced by dietary treatments. For phases 2 and 3 and for the overall experimental period, no effect of Ca and P concentrations or phytase inclusion in phase 1 diets were observed for growth performance parameters.

There was no interaction between concentrations of Ca and P and phytase inclusion for bone ash, stomach pH, fecal scores, or blood metabolites (Table 6.4). The concentration and percentage of bone ash was greater ($P < 0.05$) in pigs fed diets formulated to have adequate levels of Ca and P than in pigs fed diets with deficient levels of Ca and P. However, no effect of phytase inclusion on the concentration or percentage of bone ash of pigs was observed. There was no effect of dietary concentration of Ca and P on stomach pH (*in-situ*, *ex-situ*, or the average). Likewise, there was no effect of phytase inclusion on *in-situ* stomach pH, but pigs fed diets with 2,000 FTU tended ($P < 0.10$) to have reduced e*x-situ* stomach pH and average stomach pH compared with pigs fed diets without phytase. Pigs fed diets with phytase also tended ($P < 0.10$) to have reduced fecal score and diarrhea frequency compared with pigs fed diets without phytase, but no effect of dietary Ca and P concentration on fecal score or diarrhea frequency was observed. There was no effect of phytase inclusion on serum concentration of BUN, total protein, or albumin of pigs, and the concentration of BUN in serum of pigs fed diets with deficient or adequate levels of Ca and P was not different. However, pigs fed diets with adequate levels of Ca and P tended ($P < 0.10$) to have greater total protein and had greater ($P < 0.05$) albumin concentration in serum than pigs fed diets with deficient levels of Ca and P.

Discussion

The interest in non-antibiotic feed additives for weaning diets has increased in the last 2 decades due to the risk of continued use of antibiotics resulting in development of resistance to antibiotics used for animal and human disease treatment (Wegener, 2003). Among the numerous alternatives to AGP, acidifiers have been widely used in weaning diets because young pigs are believed to have a low ability to produce gastric acid (Kil et al., 2011). Acidifiers have the potential to provide an adequate environment in the stomach through pH reduction for proper nutrient digestion (Kil et al., 2011). However, diet composition also plays an important role because some feed ingredients have a high capacity to bind acid in the stomach of pigs, and inclusion of these ingredients in weaning diets may result in increased gastric pH (Lawlor et al., 2005). The buffering capacity for an aqueous solution is defined as the amount of acid required to influence a pH unit (Urbansky and Schock, 2000). Thus, inorganic sources of Ca and P such as limestone and MCP have a greater buffering capacity at pH 3 and 4 than ingredients originating from animals or plants including milk products, cereal grains, and plant proteins (Jasaitis et al., 1987; Lawlor et al., 2005). Indeed, it was hypothesized that a reduced concentration of ash in diets for weanling pigs can reduce diarrhea occurrence, but this may also affect growth performance and bone development as a result of mineral deficiencies (Bolduan et al., 1988).

Diets were formulated using NRC (2012) values for Ca and P in corn, soybean products, spray-dried plasma protein, limestone, and MCP, and the analyzed values for these ingredients were close to those used in diet formulation. Therefore, the small differences observed between calculated and analyzed values in diets for Ca that are less evident for P, may be a consequence of the potential feed particle segregation within diets that results in wider analytical ranges for

Ca than for P, and the need for a greater number of samples analyzed for Ca per diet (Jones et al., 2018). Ingredient and diet samples were analyzed for Ca and P in triplicate. The reason phytase inclusion was greater than the standard level of 500 FTU, which is often used in the industry, was that previous data indicate beneficial effects on growth performance of newly weaned pigs of including phytase above 1,000 FTU in weanling diets (Moran et al., 2017; 2019).

The observation that regardless of inclusion of microbial phytase, final BW, ADG, or ADFI of pigs were not influenced by the concentration of Ca and P in diets during phase 1 concurs with data from Létourneau-Montminy et al. (2010) indicating that the level of Ca and P does not affect growth performance of pigs during the phase 1 period. Schlegel and Gutzwiller (2017) also reported that growth performance of weanling pigs was not affected by the concentration of Ca when 3 different levels of Ca were used in diets with similar concentration of P and fed for 2 wk. The lack of differences in growth performance parameters in phases 2 and 3 indicates that pigs are able to recover from Ca and P deficient diets, which concurs with data from weanling pigs evaluated for 25 d after a 10-d period with a low Ca and P diet (Létourneau-Montminy et al., 2010).

Although inclusion of 2,000 FTU of phytase did not influence growth performance of pigs fed diets formulated to have adequate levels of Ca and P, the observation that pigs fed diets with deficient levels of Ca and P had improved ADG and G:F if phytase was used, indicates that phytase ameliorates the negative effects of low Ca and P in diets. However, in the diet with phytase and deficient levels of Ca and P, P was at 72% of the NRC (2012) requirement, and the concentrations of limestone and MCP were the lowest among treatments. Under commercial conditions, inclusion of phytase above 1,000 FTU in diets with no reduction of Ca and P resulted

in improved growth performance of pigs compared with diets without phytase when fed to pigs for 10 d after weaning (Moran et al., 2017; 2019).

The observation that reducing concentrations of Ca and P in phase 1 diets results in decreased concentration and percentage of bone ash concurs with published data from weanling pigs (Létourneau-Montminy et al., 2010; Schlegel and Gutzwiller, 2017) and broiler chickens (Walk et al., 2012). The requirement for Ca and P to maximize bone ash of pigs is greater than the requirement needed to maximize growth performance (Lagos et al., 2019), which explains the response in bone ash even though little effect of reduced dietary Ca and P during the first 2 wk post-weaning was observed for growth performance parameters. Bone ash was not measured after an adequate diet was fed in phases 2 and 3, and it is, therefore, not known if pigs are able to recover bone ash after the depletion in phase 1. However, Létourneau-Montminy et al. (2010) reported that after a 25-d repletion period, pigs fed Ca and P deficient diets for 10 d post-weaning tended to have lower bone ash than pigs fed diets with adequate levels of Ca and P.

Results from this experiment reject the hypothesis that lowering the concentration of Ca and P in phase 1 diets reduces stomach pH of pigs. This conclusion is in contrast with results from Walk et al. (2012) in broiler chickens and González-Vega et al. (2016) in pigs from 25 to 50 kg. Data from pigs indicated that regardless of the level of P, reducing dietary Ca to 30% of the requirement reduces gastric pH compared with values for pigs fed diets containing Ca at or above the requirement (González-Vega et al., 2016). However, in the present experiment, Ca reduction was only at 50% of the NRC (2012) requirement, which may be the reason a reduction in gastric pH was not observed.

The lack of differences in fecal score or diarrhea frequency between pigs fed diets with adequate or deficient levels of Ca and P reflects the results from stomach pH and further indicate

that a reduction in dietary Ca and P failed to change gastric and intestinal conditions. It is possible that this observation is a result of the fact that phase 1 diets used in this experiment contained 15% lactose, which may have resulted in production of lactic acid from bacterial fermentation (Suiryanrayna and Ramana, 2015) and therefore, reduced stomach pH, as has been recently reported (Zhao et al., 2021). If that was the case, lactic acid production could have reduced the possibility for a further reduction in gastric pH by reducing Ca and P in the diet. However, it is also possible that the low buffering capacity of ingredients used in this experiment such as corn, soy proteins, and lactose (between 9 and 50 times lower than limestone and MCP; Lawlor et al., 2005), contributed to the lack of differences in gastric pH between pigs fed diets with adequate or deficient concentrations of Ca and P. The buffering capacity of a complete diet is calculated from the buffering capacity of each ingredient (Jasaitis et al., 1987; Lawlor et al., 2005), and as a consequence, the ingredient composition of the diet influences the ability of dietary Ca and P to reduce gastric pH.

Addition of microbial phytase in diets results in reduced need for limestone and MCP because phytase releases P and Ca bound to phytate in plant feed ingredients (Selle et al., 2009). In the diet containing phytase and deficient levels of Ca and P, no MCP was included and the concentration of limestone was the least among diets. However, due to the lack of differences in stomach pH between pigs fed diets with adequate or deficient levels of Ca and P, it is difficult to conclude that the reason phytase tended to reduce stomach pH is the additional reduction of limestone and MCP in diets. Nevertheless, the tendency for reduced gastric pH in pigs fed diets with 2,000 FTU of phytase concurs with data from Lee et al. (2018) indicating that inclusion of 2,500 FTU of phytase in diets for weanling pigs resulted in reduced stomach pH (1.81 vs. 2.81) compared with pigs fed a non-phytase diet.

The tendency for a decrease in fecal score and diarrhea frequency in pigs fed diets containing phytase concurs with data from weanling pigs in commercial conditions that had a tendency for increased stool firmness if 2,600 FTU of phytase was used compare with pigs fed diets with 600 FTU (Moran et al., 2017). Therefore, the beneficial effect of phytase on diarrhea occurrence is likely due to its role in reducing the anti-nutritional effects of phytate with a subsequent increase of inositol production (Moran et al., 2019). It has been suggested that gastric pH may increase upon phytase supplementation because phytate has an acidogenic effect in diets due to its ability to bind positively charged amino acids in pepsinogen, which reduces pepsin activity and results in compensatory secretions of HCl in the stomach (Woyengo, 2010). However, data from the current experiment do not support this suggestion, and more research is needed to understand the influence of phytase and phytate on gastric pH of pigs.

The lack of differences in serum BUN indicates that protein utilization of weanling pigs is not influenced by the concentrations of Ca and P or phytase inclusion in diets. The observed decrease in the concentration of albumin along with the tendency for a reduced concentration of total protein in serum of pigs fed diets with deficient levels of Ca and P compared with pigs fed diets with adequate levels of Ca and P is likely associated with the way Ca is distributed in the bloodstream. After absorption, Ca circulates in the extracellular fluid in 3 forms: free, protein-bound, and complexed. The free ionized form represents 50% of the total Ca, whereas the protein-bound Ca is around 40% of which 80% is Ca bound to albumin and 20% to globulins; and the remaining 10% is Ca complexed with small anions (Taylor and Bushinsky, 2009). Calcium concentration is maintained constant in the blood via hormonal regulation of Ca homeostasis. The kidney is the main regulatory organ, which filters free and complexed Ca, but does not filter protein-bound Ca (Taylor and Bushinsky, 2009). Therefore, even though the level

of dietary Ca has limited influence on the concentration of Ca in blood of pigs (González-Vega et al., 2016), it appears that the levels of Ca and P in diets have an impact on the concentration of albumin in blood. However, more research is needed to elucidate this hypothesis.

Results from this experiment provide information about formulation of weanling pig diets based on corn and soy proteins. However, because of interactions among dietary Ca, dietary phytate, and phytase inclusion level (Selle et al., 2009), the outcomes may be different if diets with greater Ca concentration, with ingredients different from corn and soy protein, or with different inclusion levels of phytase are used.

Conclusions

Reducing limestone and MCP in phase 1 diets that contain lactose does not reduce stomach pH or fecal score and has limited impact on growth performance of weanling pigs, but bone development is compromised by Ca and P deficiencies. Low Ca and P also results in reduced concentration of serum albumin. However, inclusion of 2,000 FTU of phytase tended to decrease stomach pH and the incidence of diarrhea. Phytase also had beneficial effects on growth performance of pigs fed Ca- and P-deficient diets, which may be a consequence of the destruction of phytate and elimination of its anti-nutritional effects in diets for young pigs.

Tables

Table 6.1. Composition and analyzed values of experimental diets[1]

Item[2]	Phase 1					
Phytase	0 FTU		2000 FTU			
Ca and P levels	Adequate	Deficient	Adequate	Deficient	Phase 2	Phase 3
Ingredient, %						
Ground corn	42.42	45.91	43.85	46.80	42.01	51.55
Soybean meal, 48% CP	22.00	22.00	22.00	22.00	28.00	32.00
Lactose	15.00	15.00	15.00	15.00	15.00	10.00
Soy protein concentrate	8.00	8.00	8.00	8.00	8.00	-
Enzyme treated soybean meal	2.50	2.50	2.50	2.50	-	-
Spray dried plasma protein	3.00	3.00	3.00	3.00	-	-
Soybean oil	3.00	1.26	2.29	0.82	3.00	3.00
Ground limestone	1.28	0.72	1.13	0.37	1.19	1.11
Monocalcium phosphate	1.29	0.13	0.69	-	1.27	0.94
L-Lys HCL	0.26	0.25	0.26	0.25	0.31	0.38
DL-Met	0.17	0.16	0.16	0.15	0.17	0.15
L-Thr	0.08	0.07	0.08	0.07	0.10	0.12
Sodium chloride	0.85	0.85	0.85	0.85	0.80	0.60
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[4]	-	-	0.04	0.04	-	-
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated values[5], %						
Total Ca	0.83	0.42	0.67	0.26	0.80	0.70
Total P	0.66	0.43	0.54	0.40	0.64	0.56
STTD P	0.43	0.22	0.32	0.20	0.40	0.33
Analyzed values						
Gross energy, kcal/kg	3,990	3,947	3,982	3,966	3,973	3,968
Dry matter, %	89.41	88.84	89.46	89.23	88.75	88.62
Ash, %	6.33	4.63	5.80	4.54	5.63	5.27
Crude protein, %	21.50	22.21	21.87	22.58	20.34	18.58
AEE, %	4.81	2.88	4.20	2.61	4.48	4.35

Table 6.1. (Cont.)

Amino acids, %						
Arg	1.49	1.42	1.50	1.39	1.38	1.30
His	0.59	0.58	0.60	0.58	0.54	0.50
Ile	1.02	0.98	1.03	0.98	0.95	0.89
Leu	1.87	1.83	1.90	1.83	1.72	1.58
Lys	1.60	1.53	1.60	1.63	1.50	1.40
Met	0.51	0.44	0.48	0.42	0.43	0.40
Phe	1.13	1.09	1.15	1.09	1.04	0.97
Thr	1.00	0.94	1.03	0.97	0.95	0.91
Trp	0.29	0.28	0.28	0.28	0.27	0.25
Val	1.18	1.14	1.19	1.13	1.06	0.96
Ca, %	0.91	0.47	0.65	0.22	0.90	0.79
P, %	0.64	0.46	0.56	0.38	0.69	0.61
Phytate[6], %	0.90	0.93	0.91	0.93	0.95	0.93
Phytate bound-P[7], %	0.25	0.26	0.26	0.26	0.27	0.26
Non-phytate P[8], %	0.39	0.20	0.30	0.12	0.42	0.35
Phytase activity, FTU/kg	< 70	< 70	1,400	1,400	< 70	< 70

[1]Phase 1, phase 2, and phase 3 diets were formulated to have the following quantities of net energy (kcal/kg) and amino acids (expressed as standardized ileal digestible; %): net energy, 2,518, 2,496, and 2,498; Lys, 1.41, 1.35, and 1.23; Met, 0.46, 0.45, and 0.41; Thr, 0.83, 0.80, and 0.74; Trp, 0.26, 0.24, and 0.22, respectively.

[2]AEE = acid hydrolyzed ether extract; FTU = phytase units per kilogram of feed; STTD = standardized total tract digestible.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[4]The phytase concentrate contained 5,000 units of phytase/g (Quantum Blue, AB Vista, Marlborough, UK).

Table 6.1. (Cont.)

[5]Phytase was assumed to release 0.16% total Ca and 0.11% STTD P.

[6]Phytate values in the diets were calculated from analyzed phytate in the ingredients.

[7]Phytate-bound P was calculated by multiplying the phytate by 0.282 (Tran and Sauvant, 2004).

[8]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 6.2. Analyzed composition of ingredients

Item	Corn	Soybean meal	Soy protein concentrate	Enzyme treated soybean meal	Spray-dried plasma protein	Calcium carbonate	Monocalcium phosphate
Gross energy, kcal /kg	3,843	4,162	4,265	4,468	4,813	-	-
Dry matter, %	86.87	88.00	88.80	92.46	91.21	99.95	95.72
Ash, %	1.21	7.17	6.24	8.22	8.46	90.03	81.25
Crude protein, %	6.47	45.84	61.57	54.92	79.13	-	-
AEE[1], %	3.64	2.43	1.65	2.53	1.05	-	-
Ca, %	0.01	0.31	0.34	0.30	0.10	39.40	16.89
P, %	0.27	0.68	0.76	0.74	1.18	0.07	21.73
Phytate, %	0.82	1.60	1.92	1.72	-	-	-
Phytate-bound P[2], %	0.23	0.45	0.54	0.49	-	-	-
Non-phytate P[3], %	0.04	0.23	0.22	0.25	-	-	-

[1]AEE = acid hydrolyzed ether extract

[2]Phytate-bound P was calculated by multiplying the phytate by 0.282 (Tran and Sauvant, 2004).

[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 6.3. Growth performance of pigs fed diets formulated with adequate or deficient levels of Ca and P (Ca-P) without microbial phytase or with 2,000 phytase units/kg of feed (FTU)[1]

	0 FTU		2,000 FTU			*P*-value		
Item, kg	Adequate	Deficient	Adequate	Deficient	SEM	Ca-P	Phytase	Ca-P × phytase
Phase 1, d 1 to 15								
Initial BW	5.73	5.77	5.75	5.75	0.379	0.960	0.999	0.947
ADG	0.150[ab]	0.112[b]	0.139[ab]	0.176[a]	0.014	0.969	0.077	0.014
ADFI	0.218	0.190	0.196	0.234	0.016	0.758	0.516	0.053
G:F	0.684[a]	0.565[b]	0.705[a]	0.752[a]	0.031	0.252	0.002	0.013
Final BW	7.98	7.45	7.83	8.38	0.556	0.982	0.485	0.343
Phase 2, d 16 to 21								
ADG	0.305	0.294	0.296	0.256	0.032	0.439	0.462	0.655
ADFI	0.421	0.428	0.424	0.430	0.030	0.840	0.933	0.985
G:F	0.733	0.698	0.686	0.589	0.058	0.264	0.190	0.597
Final BW	9.82	9.24	9.53	9.94	0.678	0.901	0.764	0.473
Phase 3, d 22 to 35								
ADG	0.513	0.507	0.488	0.542	0.029	0.420	0.868	0.323
ADFI	0.771	0.746	0.753	0.820	0.044	0.641	0.527	0.300
G:F	0.667	0.682	0.649	0.660	0.014	0.343	0.156	0.896
Final BW	17.00	16.34	16.37	17.53	1.050	0.812	0.795	0.395
Overall, d 1 to 35								
ADG	0.323	0.305	0.308	0.325	0.022	0.984	0.908	0.429
ADFI	0.470	0.455	0.458	0.495	0.028	0.706	0.623	0.354
G:F	0.690	0.669	0.668	0.653	0.019	0.356	0.321	0.896

[a-b]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least squares means of 8 observations.

Table 6.4. Bone mineralization, stomach pH, and serum metabolites at d 15 and feces evaluation from phase 1 of pigs fed diets formulated with adequate or deficient levels of Ca and P (Ca-P) without microbial phytase or with 2,000 phytase units/kg of feed (FTU)[1]

Item	Ca and P levels		Phytase, FTU		SEM	*P*-value	
	Adequate	Deficient	0	2,000		Ca-P	Phytase
Bone mineralization[2]							
Ash, g	4.92	3.92	4.36	4.48	0.92	0.017	0.771
Ash, %	53.0	49.9	51.9	51.0	0.43	< 0.001	0.147
Stomach pH[3]							
In-situ	2.73	2.67	2.86	2.53	0.26	0.868	0.377
Ex-situ	2.90	2.95	3.21	2.64	0.20	0.864	0.058
Average	2.88	2.81	3.10	2.59	0.18	0.788	0.053
Fecal evaluation[2]							
Fecal score	1.88	1.82	1.99	1.71	0.10	0.667	0.060
Diarrhea frequency,[4] %	25.8	22.7	30.5	18.0	5.04	0.664	0.090
Serum metabolites[5]							
BUN,[6] mg/dL	14.0	13.8	13.9	13.8	0.92	0.850	0.923
Total protein, g/dL	4.31	4.06	4.18	4.18	0.09	0.052	0.997
Albumin, g/dL	2.51	2.29	2.43	2.38	0.06	0.012	0.533

[1]Data are shown as main effects because the interaction between concentrations of Ca and P and phytase inclusion was not significant ($P > 0.05$).

[2]Data are least squares means of 16 observations.

[3]Data are least squares means of 13 to 16 observations.

[4]Diarrhea frequency = number of days with fecal score ≥ 3 ÷ total number of days × 100.

[5]Data are least squares means of 15 or 16 observations.

[6]BUN = blood urea nitrogen.

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Chapter 7: Increased microbial phytase increased phytate destruction, plasma inositol, and feed efficiency of weanling pigs, but reduced dietary calcium and phosphorus did not affect gastric pH or fecal score, and reduced growth performance and bone ash

Abstract

An experiment was conducted to test 2 hypotheses: 1) reducing dietary Ca and P reduces gastric pH and diarrhea in weanling pigs; 2) negative effects of low Ca and P on pig growth performance may be overcome if phytase is added to the diets. A total of 320 weanling pigs (6.35 ± 0.87 kg) were allotted to 8 corn-soybean meal-based diets in a randomized complete block design with 5 pigs per pen. Two phase 1 (d 1 to 14) control diets containing 100 or 50% of total Ca and digestible P relative to the requirement, and 6 diets in which 500, 2,000, or 16,000 units of phytase/kg feed (**FTU**) were added to each control diet were formulated. Phytase was assumed to release 0.16% total Ca and 0.11% digestible P. Common diets were fed in phases 2 (d 15 to 27) and 3 (d 28 to 42). Growth performance data were recorded within each phase. Data for fecal scores and gastrointestinal pH were recorded for phase 1. Colon content (d 14), the right femur (d 14 and 42), and blood samples (d -1, 14, 27, and 42) were collected from 1 pig per pen. In phase 1, reducing Ca and P did not reduce gastric pH or fecal score, but pigs fed the 50% diets had reduced ($P < 0.05$) average daily gain (**ADG**) and average daily feed intake (**ADFI**) compared with pigs fed the 100% diets. In both 50 and 100% diets, phytase above 500 FTU increased ($P < 0.05$) gain:feed ratio (**G:F**) and tended ($P < 0.10$) to reduce gastric pH of pigs. From d 1 to 42, pigs fed the 50% diets tended ($P < 0.10$) to have reduced ADG and ADFI

compared with pigs fed the 100% diets, but among the 100% diets, pigs tended ($P < 0.10$) to have a linear increase in G:F as phytase level increased. Pigs fed the 50% diets had reduced ($P < 0.05$) concentrations of inositol phosphate esters (**IP**) in the colon and reduced bone ash (d 14 and 42) compared with pigs fed the 100% diets. Phytase did not affect bone ash or most blood metabolites. Concentrations of IP in the colon decreased, whereas plasma inositol increased (d 14; $P < 0.05$) in pigs fed diets with phytase (≥ 500 FTU). In pigs fed the 100% diets, IP in the colon linearly decreased ($P < 0.05$), but plasma inositol linearly increased ($P < 0.05$) with increasing levels of phytase. In conclusion, reducing Ca and P in diets for weanling pigs did not influence gastric pH or fecal score, but compromised growth performance and bone ash. However, regardless of dietary Ca and P, high doses of phytase increased phytate degradation and inositol absorption, which consequently increased G:F of pigs.

Key words: bone ash, dietary Ca and P, gastrointestinal pH, inositol, phytase, weanling pigs

Abbreviations

AEE	acid hydrolyzed ether extract
ADFI	average daily feed intake
ADG	average daily gain
BUN	blood urea nitrogen
BW	body weight
ELISA	enzyme-linked immunosorbent assay
FTU	phytase units per kilogram of feed
G:F	gain to feed ratio
IgA	immunoglobulin A

Abbreviations (Cont.)

IP	inositol phosphate
IFNγ	interferon γ
IL	interleukin
STTD	standardized total tract digestible
TNF-α	tumor necrosis factor-α

Introduction

During the post-weaning period, pigs are stressed due to environmental, nutritional, physiological, and immunological changes that increase morbidity and mortality (Pluske et al., 1997). This situation is exacerbated with the restriction in the use of antibiotic growth promoters (Casewell et al., 2003). Thus, alternatives to antibiotic growth promoters such as direct-fed microbials, prebiotics, plant extracts, and acidifiers have been studied (Liu et al., 2018). The reason for the use of acidifiers is that it is believed that weanling pigs are unable to secrete enough HCl in the stomach to provide an appropriate pH for pepsin to efficiently digest plant and animal proteins (Kil et al., 2011). However, the inability to reach a low pH in the stomach can also be attributed to the inclusion of limestone and monocalcium phosphate in nursery diets, because these ingredients have high acid binding capacity at pH 3 and 4 (Lawlor et al., 2005). Therefore, it is possible that reducing limestone and monocalcium phosphate in weaning diets results in decreased stomach pH.

Inclusion of microbial phytase in diets for pigs also contribute to lowering Ca and P in the diets due to increased release of digestible Ca and P from phytate, which results in reduced need for dietary limestone and monocalcium phosphate (Zeng et al., 2016). Additionally, high

doses of phytase may partially or fully alleviate the negative effects of lowering Ca and P in diets because elevated levels of phytase results in further degradation of lower phytate esters and greater digestibility of Ca and P in diets fed to young pigs (Almeida et al., 2013). Increasing levels of phytase also result in increased concentration of inositol in plasma of pigs from the complete destruction of phytate (Cowieson et al., 2017). Inositol is a cyclic sugar that plays an important role in several cellular functions and is believed to have a growth promoting effect in broiler chickens (Lee and Bedford, 2016).

Data from broiler chickens indicate that reducing dietary Ca reduces tibia ash and gizzard pH, whereas inclusion of high levels of phytase results in increased tibia ash and gizzard pH (Walk et al., 2012). However, limited data evaluating the interaction between dietary concentrations of Ca and P and increasing levels of phytase on growth performance, bone ash, and gastric pH of weanling pigs are available. Therefore, the objective of this experiment was to test the hypothesis that reducing Ca and P in diets for weanling pigs reduces diarrhea because of lower stomach pH. The second hypothesis was that inclusion of high doses of phytase in diets for pigs fully or partly overcomes the negative effects of low Ca and P due to phytate degradation, increased plasma inositol, improved protein utilization, and increased immune response.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs that were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used.

Animals, housing, and diets

Three hundred and twenty newly weaned pigs with an initial average body weight (**BW**) of 6.35 ± 0.87 kg were randomly allotted to 8 diets and 2 blocks in a randomized complete block design. There were 5 pigs per pen (3 gilts and 2 castrates) and 4 replicate pens per diet in each block (weaning group). Pigs were housed in pens with fully slatted floors equipped with a feeder and a nipple drinker. Water was available at all times.

The experiment was conducted for 6 wk after weaning. A 3-phase feeding program was used with d 1 to 14 as phase 1, d 15 to 27 as phase 2, and d 28 to 42 as phase 3. Pigs were fed one of 8 diets in phase 1, whereas a common diet was fed in phases 2 and 3. Therefore, a total of 10 diets were formulated (Table 7.1). A representative sample of 2 kg of diets and ingredients was collected.

In phase 1, the 8 diets were based on corn and soybean meal (Table 7.2) and included 2 control diets that contained 100 or 50% of total Ca and standardized total tract digestible (**STTD**) P relative to the requirement (0.85% and 0.45%, respectively; NRC, 2012). The 2 control diets were formulated based on the requirement for total Ca and STTD P (NRC, 2012) without inclusion of microbial phytase. Three diets were formulated to be identical to the 100% control diet with the exception that 500, 2,000, or 16,000 phytase units per kilogram of feed (**FTU**; Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were included and provisions of total Ca and STTD P were reduced by 0.16 and 0.11 percentage units, respectively, to account for the amount of Ca and P that was assumed to be released by phytase. Three additional diets were formulated to be identical to the 50% control diet, with the exception that 500, 2,000, or 16,000 FTU of microbial phytase were included and provisions of total Ca and STTD P were reduced as explained for the diets formulated at the requirement for Ca and STTD P. All phase 1

diets were formulated to have identical concentrations of net energy, Na, Cl, K, and vitamin D. Phase 2 and 3 diets were formulated to meet requirements for total Ca (0.80% and 0.70%, respectively) and STTD P (0.40% and 0.33%, respectively; NRC, 2012).

Feeding, sample collection, and bone measurements

Pigs were allowed *ad libitum* access to feed and water throughout the experiment. The amount of feed offered was recorded daily and the amount of feed in the feeders was recorded at the end of each phase. On the day before weaning (d -1), and at the conclusion of each phase (d 14, 27, and 42), all pigs were weighed and 2 blood samples were collected from 1 pig per pen. Pigs from which blood was collected were selected in such a way that there were 2 gilts and 2 castrates per treatment in each block, and blood was collected from the same pigs throughout the experiment. Blood samples were collected in vacutainers that contained spray-coated silica or ethylenediaminetetraacetic acid to yield blood serum or blood plasma, respectively, after blood samples had been centrifuged at 1,500 × *g* at 4 ºC. Serum and plasma samples were frozen at -20 ºC until used for analysis. During the initial 14 d, fecal scores were assessed visually every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). On the last day of phase 1, one pig per pen was euthanized via captive bolt stunning. Pigs were chosen so that there were 4 gilts and 4 castrates per treatment. The abdominal cavity was opened and pH was measured twice *in-situ* by making a small incision for a pH electrode in the stomach, duodenum, and ileum. All stomach contents were then collected and mixed and the *ex-situ* pH was measured twice. The right femur and colon contents were also collected. At the conclusion of the experiment, the pig that was used for blood collection throughout the experiment was euthanized and blood samples and the right femur were collected. Femurs were autoclaved at 125 ºC for 55 min and the muscles attached to

the bones were removed. Femurs were broken, dried overnight at 105 ºC, and soaked for 72 h in petroleum ether under a chemical hood to remove marrow and fat. Bones were then dried at 135 ºC for 2 h and ashed at 600 ºC for 16 h.

Sample analysis

Samples of colon content were lyophilized and diet, ingredient, and colon content samples were finely ground prior to analysis. Diets and ingredients were analyzed for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2019) and for ash by heating to 600 °C in a muffle furnace for 2 h (Method 942.05; AOAC Int., 2019). Calcium and P were also analyzed in these samples by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2019) after dry ash preparation (Method 942.05; AOAC Int., 2019) followed by wet digestion with nitric acid (Method 3050 B; US-EPA, 2000). Corn, soybean meal, potato protein concentrate, spray-dried plasma protein, and diets were analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA) and for N (Method 990.03; AOAC Int., 2019) using a LECO FP628 (LECO Corp., Saint Joseph, MI, USA) and crude protein was calculated as N × 6.25. These samples were also analyzed for acid hydrolyzed ether extract (**AEE**; Method 2003.06; AOAC Int., 2019) using an Ankom^{HCl} followed by an Ankom^{XT15} (Ankom Technology, Macedon, NY, USA). Corn, soybean meal, potato protein concentrate, and diets were also analyzed for phytate-bound P by wet chemistry using the Megazyme kit (Megazyme Inc., Chicago, IL, USA). Diet samples were analyzed for amino acids (Method 982.30 E [a, b, c]; AOAC Int., 2019) using a Hitachi Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) and for phytase activity by the enzyme-linked immunosorbent assay (**ELISA**) method using Quantiplate Kits for Quantum Blue (AB Vista, Plantation, FL, USA). Serum samples were analyzed for blood urea

nitrogen (**BUN**), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA), and for Ca by inductively coupled plasma-optical emission spectrometry after wet ash preparation [Method 975.03 B(b); AOAC Int., 2019]. Plasma samples were analyzed for concentrations of immunoglobulin A (**IgA**) using an ELISA kit (Bethyl Laboratories, Inc., Montgomery, TX, USA) and for cytokines including tumor necrosis factor-α (**TNF-α**), interferon gamma (**IFNγ**), interleukin (**IL**) -6, IL-1β, IL-8, and IL-10 using the MILLIPLEX MAP Porcine Cytokine Magnet Bead panel (MilliporeSigma, Burlington, MA, USA). Plasma samples were also analyzed for inositol after deproteination (Mesina et al., 2019) by high-performance ion chromatography-based techniques described by Walk et al. (2018). Colon samples were also analyzed for inositol and inositol phosphate (**IP**) esters by high-performance ion chromatography. Deproteinated plasma samples and colon samples were analyzed at the University of East Anglia, School of Biological Sciences, UK. The pH of the gastrointestinal tract was measured using a portable cheese pH meter (HANNA instruments, Woonsocket, RI, USA) that was mounted with a FC2423 pH electrode for semi-solid samples. The pH meter was calibrated using 2 buffer solutions (4.01 and 7.01 pH).

Calculations and statistical analyses

The concentration of phytate in corn, soybean meal, potato protein concentrate, and diets was calculated by dividing the analyzed concentration of phytate-bound P by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) were calculated for each phase and for the overall experiment. Bone ash percentage was calculated by dividing the quantity of bone ash by the quantity of fat-free dried bone and multiplying by 100. Data for pH were transformed into H^+ concentration by raising the negative

pH value to the power of 10 before statistical analysis to reduce the relative error defined as the absolute value of the difference between pH mean and $-\log_{10}(H^+)$ mean (Murphy, 1982). Diarrhea frequency as a percentage was calculated by dividing the number of days with fecal score ≥ 3 by the total number of scoring days (i.e., 8) times 100.

Normality of residuals and homogeneity of variances were tested using the INFLUENCE, GPLOT, and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC, USA). However, because data for the H^+ concentration did not meet the assumptions of the model, data were analyzed as pH values. On the contrary, data for cytokines in plasma were Log10 transformed before analysis because the residuals from the data were not normally distributed.

Data for growth performance, gastrointestinal pH, fecal evaluation, concentration and percentage of bone ash, concentrations of phytate esters in the colon, and blood metabolites were analyzed using the PROC MIXED procedure of SAS with the experimental unit being the pen. The model included the fixed effect of treatment and the random effect of block. Contrast statements were used to determine effects of dietary concentration of Ca and P and inclusion level of phytase. Contrasts included 1) 50% Ca and P diets vs. 100% Ca and P diets; 2) 100% diets: control diet vs. 500 FTU diet; 3) 100% diets: control diet vs. 2,000 + 16,000 FTU diets; 4) 50% diets: control diet vs. 500 FTU diet; and 5) 50% diets: control diet vs. 2,000 + 16,000 FTU diets. Contrast statements were also used to determine linear effects of inclusion level of phytase (i.e., from 500 to 16,000 FTU) at each concentration of Ca and P. Coefficients for the unevenly spaced linear contrasts were obtained using the PROC IML procedure of SAS. Data for blood metabolites obtained on d -1 was used as covariate to analyze blood data from d 14. Repeated measures were used to analyze the effect of time on the concentration of blood metabolites using an unstructured variance based on the likelihood ratio test. The model included the main effects

of dietary treatment and day and the interaction between dietary treatment and day. The time effect was day, the random effect was block, and the experimental unit was the pig. If the interaction was not significant, only main effects were included in the final model. Treatment means were calculated using the LSMEANS statement in SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Four pigs died during the experiment and 3 pigs were removed from their pens due to bad condition. The 7 removed pigs were from pens fed 5 different diets and data for ADFI in these pens were adjusted (Lindemann and Kim, 2007). The remaining pigs consumed their diets without apparent problems and no health problems were observed.

During phase 1, there was a reduction ($P < 0.05$) in ADG and ADFI of pigs fed the 50% Ca and P diets compared with pigs fed the 100% Ca and P diets (Table 7.3). Among diets with 100% Ca and P, there was a tendency ($P < 0.10$) for pigs fed the diet with 500 FTU to have greater ADG and ADFI than pigs fed the control diet. Likewise, ADG was greater ($P < 0.05$) and ADFI tended ($P < 0.10$) to be greater for pigs fed diets with phytase doses above 500 FTU compared with pigs fed the control diet. A tendency ($P < 0.10$) for a positive linear effect of phytase on the G:F of pigs was also observed in the 100% Ca and P diets, but regardless of the dietary concentration of Ca and P, pigs fed diets with 2,000 or 16,000 FTU of phytase had greater ($P < 0.05$) G:F than pigs fed the control diet. During phase 2, pigs fed the 50% Ca and P diets in phase 1 tended ($P < 0.10$) to have reduced ADFI compared with pigs fed the 100% Ca and P diets, and pigs fed the diet with 50% Ca and P and 500 FTU of phytase had lower ($P < 0.05$) ADFI than pigs fed the 50% control diet. In phase 3, pigs fed the 100% Ca and P diets in

phase 1 tended ($P < 0.10$) to have a linear reduction in ADFI as dietary phytase increased from 500 to 16,000 FTU in phase 1. Pigs fed the 50% control diet during phase 1 had greater ($P < 0.05$) G:F in phase 3 than pigs fed the 50% Ca and P diet with 500 FTU of phytase in phase 1. For the overall experimental period, there was a tendency ($P < 0.10$) for pigs fed the 50% diets in phase 1 to have reduced ADG and ADFI compared with pigs fed the 100% diets. For the 100% Ca and P diets, a tendency ($P < 0.10$) for a positive linear effect of phytase in phase 1 on G:F of pigs was observed, and the G:F was greater ($P < 0.05$) for pigs fed the 50% control diet during phase 1 than for pigs fed the 50% Ca and P diet with 500 FTU of phytase.

At the end of phase 1, there was a reduced ($P < 0.05$) concentration and percentage of bone ash in pigs fed the 50% Ca and P diets compared with pigs fed the 100% Ca and P diets (Table 7.4). Among the 50% Ca and P diets, there was a tendency ($P < 0.10$) for pigs fed the control diet to have a greater percentage of bone ash than pigs fed diets with 2,000 or 16,000 FTU of phytase. Among the 100% Ca and P diets, there was a tendency ($P < 0.10$) for lower gastric pH (*in-situ*) in pigs fed diets containing phytase doses above 500 FTU than in pigs fed the control diet. Likewise, pigs fed diets containing 500 FTU of phytase had lower ($P < 0.05$) gastric pH measured *ex-situ* than pigs fed the control diet. Among the 50% Ca and P diets, a tendency ($P < 0.10$) for a reduced gastric pH (*ex-situ*) in pigs fed diets containing phytase above 500 FTU was observed compared with pigs fed the control diet. However, regardless of the dietary concentration of Ca and P, pigs fed diets with 2,000 or 16,000 FTU of phytase tended ($P < 0.10$) to have lower gastric pH (average) than pigs fed the control diet. A tendency ($P < 0.10$) for a reduced duodenal pH in pigs fed the 50% Ca and P diets as phytase inclusion increased was also observed. However, there was no effect of dietary treatment on ileal pH, fecal score, or diarrhea frequency of pigs. At the end of phase 3, the concentration of bone ash in pigs fed the 50% Ca

and P diets in phase 1 was reduced ($P < 0.05$) compared with pigs fed the 100% Ca and P diets, but no differences in percentage of bone ash were observed among treatments.

On the last day of phase 1, pigs fed diets with 100% Ca and P had increased concentrations of IP6, IP5, and IP4 in colon contents compared with pigs fed the 50% Ca and P diets (Table 7.5). Regardless of the concentration of dietary Ca and P, pigs fed diets with phytase had reduced ($P < 0.05$) concentrations of IP6, IP5, and IP4 in the colon compared with pigs fed the control diets. Concentrations of IP6, IP5, and IP4 in the colon content of pigs linearly decreased ($P < 0.05$) as the dietary level of phytase increased from 500 to 16,000 FTU in diets with 100% Ca and P. In all samples, the concentration of IP3 and inositol were undetectable.

At the end of phase 1 (d 14), pigs had reduced ($P < 0.05$) concentrations of Ca and albumin in serum if fed the 50% Ca and P diets compared with the 100% Ca and P diets (Table 7.6). The concentration of BUN in serum of pigs was not influenced by dietary treatments, but pigs fed diets containing 500 FTU of phytase and 50% Ca and P had greater ($P < 0.05$) concentration of total protein in serum than pigs fed the 50% control diet.

Regardless of the concentration of Ca and P in the diet, on d 14, pigs fed diets with phytase had increased ($P < 0.05$) concentration of inositol in plasma compared with pigs fed the control diet (Table 7.7). The concentration of inositol in plasma tended ($P < 0.10$) to linearly increase in pigs fed diets with 50% Ca and P, and linearly increased ($P < 0.05$) in pigs fed 100% Ca and P diets, as the level of phytase increased in the diet. Pigs fed diets with 100% Ca and P and 500 FTU of phytase tended ($P < 0.10$) to have reduced concentration of IgA compared with pigs fed the 100% control diet. There was a tendency ($P < 0.10$) for pigs fed the 100% Ca and P diets to have a linear increase in plasma concentration of INFγ as phytase increased from 500 to 16,000 FTU in diets. Pigs fed diets with 50% Ca and P tended ($P < 0.10$) to have reduced

concentration of IL-1β in plasma compared with pigs fed diets with 100% Ca and P. In pigs fed diets with 50% Ca and P, plasma IL-6 and IL-10 tended ($P < 0.10$) to be reduced, whereas plasma IL-8 was reduced ($P < 0.05$) if diets contained 500 FTU of phytase compared with the control diet. However, dietary treatments had no influence on the concentration of TNF-α in plasma of pigs.

There was no interaction between dietary treatment and day for serum Ca, BUN, total protein, or albumin or for IgA or cytokines in plasma of pigs (Table 7.8). No effect of dietary treatment on BUN, total protein, IgA, IL-8, and IL-10 was observed. However, pigs fed phase 1 diets with 100% Ca and P had greater ($P < 0.05$) concentrations of Ca and albumin in serum than pigs fed phase 1 diets with 50% Ca and P. Concentrations of INFγ and IL-6 in plasma of pigs fed the control diet with 50% Ca and P were greater ($P < 0.05$) than in plasma of pigs fed diets with 500 FTU of phytase. The concentration of plasma IL-1β, IL-6, and TNF-α were greater ($P < 0.05$) in pigs fed the 50% Ca and P control diet than in pigs fed diets with 50% Ca and P and 2,000 or 16,000 FTU of phytase. Concentrations of BUN and IgA linearly increased ($P < 0.05$) and the concentration of IgA also tended to increase (quadratic; $P < 0.10$) from d -1 to d 42. Likewise, there was an increase (quadratic; $P < 0.05$) in the concentration of Ca, total protein, and albumin in serum of pigs from d -1 to d 42. In contrast, the concentration of INFγ and IL-8 linearly decreased ($P < 0.05$) with time, and pigs had a reduction (quadratic; $P < 0.05$) in the concentration of IL-6, IL-10, and TNF-α in plasma from d -1 to d 42. The concentration of IL-1β also tended to decrease (quadratic; $P < 0.10$) from d -1 to d 42 post-weaning.

There was an interaction ($P < 0.05$) between dietary treatment and day for plasma inositol (Fig. 7.1). At d -1, 28, and 42, there were no differences in the concentration of inositol in

plasma of pigs among treatments, but on d 14, pigs fed the 2 control diets had reduced ($P < 0.05$) concentration of inositol in plasma compared with pigs fed diets with phytase.

Discussion

The observed reduction in ADG and ADFI of pigs fed the 50% Ca and P diets compared with pigs fed the 100% diets during 2 wk after weaning is in contrast with published data indicating that the level of Ca and P in phase 1 diets had no effect on these 2 variables (Létourneau-Montminy et al., 2010; Lagos et al., 2021). The observed reduction in ADG and ADFI in phase 2 and for the overall period for pigs fed diets with 50% Ca and P compared with pigs fed adequate diets in phase 1 indicates that pigs were not able to recover from reduced concentrations of Ca and P in phase 1 diets. However, it is possible that a different result would have been obtained if the period with restriction in dietary Ca and P had been less than 14 d, or if phase 2 and 3 diets had contained Ca and P above the requirement.

The observed increase in ADG and ADFI of pigs fed diets with 100% Ca and P by the inclusion of phytase concurs with published data indicating that ADG and ADFI of weanling pigs linearly increased as phytase increased in phase 1 diets (Moran et al., 2017). These data indicate that phytase, in addition to the release of Ca and P, provides a beneficial effect to pigs fed diets with adequate levels of Ca and P, likely as a result of a reduction in the anti-nutritional effects of phytate. The observed increase in G:F of pigs fed diets with 2,000 or 16,000 FTU of phytase compared with pigs fed the control diet regardless of the concentration of Ca and P is in agreement with results by Holloway et al. (2019) and Moran et al. (2019), who demonstrated that inclusion of 2,500 FTU of phytase increased feed efficiency of newly weaned pigs. These observations indicate that inclusion of phytase at ≥ 2,000 FTU not only releases the amount of

Ca and P that was assumed in diet formulations, but also provides additional beneficial effects on the performance of pigs undergoing the stress of weaning.

The observed reduction in bone ash as a result of Ca and P deficiencies in phase 1 diets is in agreement with previous data (Létourneau-Montminy et al., 2010; Lagos et al., 2021). This response was expected because diets were formulated below the requirement for optimal growth performance and requirements for Ca and P to maximize bone mineralization are greater than to maximize growth performance (NRC, 2012; Lagos et al., 2019). Data from d 42 indicate that early bone mineralization is important because pigs were not able to recover in terms of bone ash from diets deficient in Ca and P, which also concurs with previous data (Létourneau-Montminy et al., 2010). Aiyangar et al. (2010) indicated that pigs fed diets containing 70% of Ca requirement for 28 d were able to recover from reduced bone mineral content and density after a repletion period of 42 d during which pigs were fed diets formulated to have 150% of Ca requirement. Therefore, this may indicate that pigs can recover from low Ca and P diets only if the repletion diets contain Ca and P above the requirement. The lack of differences in bone ash between pigs fed the control diets and pigs fed diets with phytase, regardless of the level of Ca and P, indicates that the release values for Ca and P assumed for phytase in diet formulations were accurate and that the positive effect of phytase on growth performance is beyond Ca and P release.

Values for stomach pH obtained in this experiment concur with previous data (Radcliffe et al., 1998; Rice et al., 2002) and are within the range of gastric pH reported from pigs, which varies from 1.0 to 4.5 depending on feeding time and site of measure (Chesson, 1987; Lee et al., 2018). Although for this experiment the site was maintained constant, the time of pH measure related to feeding was much harder to standardize. The observation that reducing the

concentration of Ca and P in diets did not influence gastrointestinal pH or diarrhea occurrence is in agreement with data from Lagos et al. (2021), but in contrast with data from broiler chickens (Walk et al., 2012). Although it was hypothesized that reducing the concentration of limestone and monocalcium phosphate reduces the buffering capacity of the diet and consequently decreases stomach pH and diarrhea incidence (Jasaitis et al., 1987; Bolduan et al., 1988; Lawlor et al., 2005), data from Lagos et al. (2021) and from the present experiment reject this hypothesis. It is possible that this is because diets in this experiment and in the experiment by Lagos et al. (2021) contained lactose, which through bacterial fermentation, produces lactic acid and contributes to a reduced stomach pH (Zhao et al., 2021). Lactic acid is an acidifier that can be included in diets for weanling pigs (Suiryanrayna and Ramana, 2015) and appears to counter the high acid binding capacity of limestone and monocalcium phosphate in weaning diets.

The observed tendency for phytase to reduce stomach pH, regardless of dietary concentrations of Ca and P, concurs with data from weanling pigs (Lee et al., 2018; Lagos et al., 2021; Lee et al., 2021); however, the mode of action is not well understood. Inclusion of phytase in diets for broiler chickens (Walk et al., 2012) and pigs (Radcliffe et al., 1998; Rice et al., 2002) resulted in increased stomach pH as a consequence of the reduction of the acidogenic effect of phytate (Józefiak et al., 2016). Thus, additional research is needed to elucidate how phytase influences gastric pH.

The reduced concentrations of phytate and IP esters in colon contents of pigs fed diets with phytase demonstrates the role of phytase in removing phosphate from phytate and IP esters. However, these values do not solely reflect the effect of dietary phytase, but also the phytase synthesized by intestinal microbes in the large intestine of pigs (Mesina et al., 2019). Phytate esters were measured in colon contents instead of ileal digesta samples because of the difficulty

associated with collecting sufficient quantities of ileal digesta from small pigs. Nevertheless, the observed influence of dietary levels of Ca and P on concentrations of IP6, IP5, and IP4 in colon contents was not expected because the concentration of dietary phytate was constant among treatments. Therefore, these results indicate that reducing the concentration of dietary Ca and P from inorganic sources results in less phytate-Ca-P complexes and greater phytase efficacy in the large intestine. However, research is needed to confirm this hypothesis.

The analyzed concentrations of serum metabolites are in agreement with previous data (Lagos et al., 2021), and indicate that neither concentrations of dietary Ca and P nor phytase level influenced protein utilization as indicated by the lack of differences among treatments in serum BUN. However, dietary Ca influenced the concentrations of Ca and albumin in serum of pigs, which may be because after absorption, around 32% of total Ca that circulates in blood is bound to albumin (Bazydlo et al., 2014). Therefore, it appears that reduced dietary Ca reduced the concentration of Ca in serum and the need of albumin as carrier. However, the concentration of Ca in serum was maintained between the physiological range of 8 to 12 mg/dL (Amundson et al., 2017), which is likely because the ionized Ca, which comprises 50% of the total circulating Ca, remains unchanged because of hormonal regulation (Bazydlo et al., 2014). Results also indicate that the effect of dietary Ca and P on serum Ca and albumin observed in phase 1 is maintained after a nutritionally adequate diet is fed in phases 2 and 3.

The observed increase in the concentration of plasma inositol upon phytase supplementation supports the data for phytate and IP esters in colon contents, and indicates that some dietary phytate was fully degraded if at least 500 FTU of phytase was included in the diet. These results concur with data indicating that inclusion of phytase above 1,000 FTU results in increased concentration of plasma inositol in pigs (Guggenbuhl et al., 2016; Cowieson et al.,

2017). Inositol is the end product of phytate degradation and plays an important role in several metabolic processes through cell signaling (Huber, 2016). Inositol is believed to have an insulin-like effect due to its role in protein B kinase activation, and increased inositol release results in increased concentration of glucose transporter type 4 in muscle of weanling pigs (Lu et al., 2019). This result concurs with the observed linear increase in G:F of pigs with increasing levels of phytase in diets formulated to have Ca and P at the requirement, and indicates that the increased plasma inositol concentration may be the main reason for the observed positive effect of phytase on pig growth performance immediately after weaning. This hypothesis is supported by data indicating that supplementation of inositol or inclusion of high doses of phytase in phase 1 diets resulted in increased plasma inositol and improved G:F of pigs (Moran et al., 2019).

The observation that dietary Ca and P did not influence plasma inositol indicates that the observed reduction or elimination of phytate and IP esters in colon contents of pigs fed diets containing 50% Ca and P is related to the efficacy of phytase synthesized by microbes in the hindgut. Pigs are unable to absorb P beyond the ileum (Mesina et al., 2019), and inositol released in the colon is likely metabolized by the microbes; therefore, this effect is nutritionally irrelevant for pigs.

The observed interaction between dietary treatments and day for inositol in plasma illustrates the importance of phytase on inositol release. The observation that regardless of the diet provided in phase 1, the concentration of inositol in plasma decreased to 15 μM at d 27 and at d 42 increased back to around 45 μM, indicates that pigs are only able to increase plasma inositol back to the concentration observed before weaning after wk 6. Therefore, inositol may be a conditionally essential nutrient during the immediate post-weaning period because it appears

that pigs are not able to synthesize sufficient quantities of inositol during the initial weeks post-weaning.

Cytokines are small proteins secreted by immune cells that play an important role in the regulation of the immune and inflammatory response (Zhang and An, 2007). The lack of a pattern in the results for plasma IgA and cytokines due to dietary treatments indicates that the concentration of Ca and P or phytase did not influence blood indicators for the immune and inflammatory response of weanling pigs. Results from the analysis of blood metabolites over time indicate that as pigs grow, concentrations of IgA in plasma and Ca, BUN, total protein, and albumin in serum increase, but cytokine concentrations in plasma decrease.

Conclusions

Reducing the concentration of Ca and P in diets for 2 wk after weaning did not reduce gastric pH or diarrhea incidence of pigs, but decreased growth performance and bone ash. Pigs did not recover from the negative effects of low Ca and P in phase 1 diets during the following 4 weeks. A 50% reduction in dietary Ca and P during the initial 2 wk post-weaning is therefore, not recommended. Phytase inclusion had limited impact on growth performance of pigs fed Ca and P deficient diets, which is likely because diets with phytase were formulated with reduced Ca and P. However, inclusion of high doses of phytase in diets with adequate levels of Ca and P improved growth performance of pigs, which was likely a result of increased phytate degradation and inositol release and absorption. Phytase tended to reduce gastric pH, but did not affect incidence of diarrhea or blood indicators for protein utilization or inflammatory response. Therefore, it appears that the positive effect of phytase on weanling pigs is related to the reduced anti-nutritional effect of phytate and the role of inositol in the metabolism of newly weaned pigs.

Figure

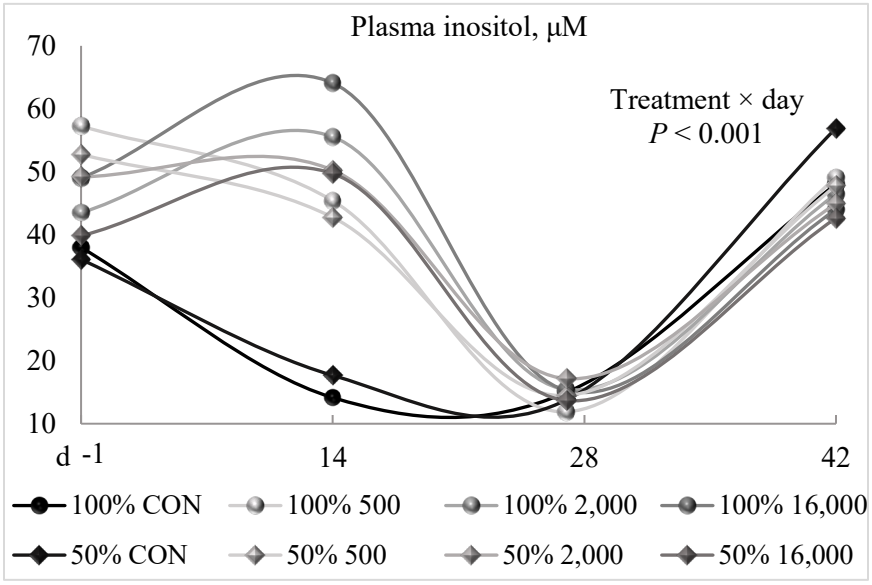

Figure 7.1. Concentration of inositol in plasma of pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (CON), 500, 2,000, or 16,000 units of microbial phytase during d -1 to 42.

Tables

Table 7.1. Ingredient composition, calculated values for Ca and P, and analyzed values of experimental diets[1,2]

Item	Phase 1								Phase 2	Phase 3
Ca and P requirement	100% of requirement				50% of requirement					
Phytase, FTU	0	500	2,000	16,000	0	500	2,000	16,000		
Ingredient, %										
Corn	52.20	53.45	53.40	52.92	55.19	56.38	56.34	55.87	49.78	54.89
Soybean meal, 48% crude protein	15.05	15.05	15.05	15.05	15.00	15.00	15.00	15.00	26.00	29.00
Lactose	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	-	-
Whey powder	-	-	-	-	-	-	-	-	11.50	10.00
Potato protein concentrate	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	-	-
Enzyme treated soybean meal	-	-	-	-	-	-	-	-	6.00	-
Spray dried plasma protein	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-
Soybean oil	2.89	2.38	2.40	2.60	1.70	1.22	1.23	1.42	3.00	3.00
Limestone	1.22	1.08	1.08	1.08	0.66	0.51	0.51	0.51	1.21	1.10
Monocalcium phosphate	1.75	1.14	1.14	1.14	0.57	-	-	-	0.89	0.67
L-Lys HCL	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.40	0.36
DL-Met	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.17	0.14
L-Thr	-	-	-	-	-	-	-	-	0.10	0.09
Sodium chloride	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.80	0.60
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[4]	-	0.01	0.04	0.32	-	0.01	0.04	0.32	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 7.1. (Cont.)

Calculated values, %										
Total Ca	0.83	0.68	0.68	0.68	0.42	0.26	0.26	0.26	0.80	0.70
Total P	0.64	0.52	0.52	0.51	0.40	0.28	0.28	0.28	0.63	0.56
STTD P	0.44	0.33	0.33	0.33	0.23	0.12	0.12	0.12	0.40	0.33
Analyzed values										
Gross energy, kcal/kg	4,084	3,991	4,029	4,120	4,098	4,089	4,080	4,109	4,030	4,010
Dry matter, %	88.43	88.39	88.25	88.34	88.09	88.00	87.80	88.14	89.14	88.85
Ash, %	5.46	4.57	4.66	4.41	3.66	3.03	3.15	2.95	6.16	6.22
Crude protein, %	21.37	21.28	21.46	21.49	21.83	21.00	21.23	21.55	19.63	18.30
AEE, %	4.11	3.35	3.25	3.50	3.20	2.93	2.71	2.61	4.43	4.81
Amino acids, %										
Arg	1.25	1.15	1.17	1.19	1.26	1.18	1.18	1.11	1.32	1.23
His	0.54	0.51	0.52	0.53	0.55	0.52	0.52	0.51	0.53	0.51
Ile	1.12	1.06	1.10	1.09	1.14	1.09	1.08	1.05	0.97	0.92
Leu	2.10	2.03	2.07	2.08	2.16	2.07	2.04	2.05	1.75	1.67
Lys	1.66	1.55	1.62	1.60	1.73	1.65	1.57	1.65	1.49	1.38
Met	0.52	0.54	0.53	0.55	0.52	0.54	0.57	0.51	0.44	0.43
Phe	1.27	1.22	1.25	1.25	1.32	1.24	1.22	1.23	1.03	0.97
Thr	1.03	0.97	1.03	1.03	1.07	1.04	1.02	1.05	0.93	0.83
Trp	0.28	0.27	0.28	0.30	0.29	0.27	0.28	0.28	0.26	0.24
Val	1.30	1.24	1.27	1.28	1.34	1.27	1.24	1.26	1.02	0.97
Ca, %	0.87	0.71	0.72	0.66	0.42	0.26	0.25	0.25	0.84	0.65
P, %	0.66	0.51	0.52	0.51	0.40	0.28	0.27	0.27	0.69	0.58

Table 7.1. (Cont.)

Phytate[5], %	0.61	0.59	0.58	0.59	0.60	0.59	0.58	0.58	0.80	0.75
Phytate bound-P, %	0.17	0.17	0.16	0.17	0.17	0.17	0.16	0.16	0.22	0.21
Non-phytate P[6], %	0.49	0.34	0.36	0.35	0.23	0.11	0.11	0.11	0.47	0.37
Phytase activity, FTU	< 50	700	2,660	19,100	< 50	609	2,560	20,200	< 50	< 50

[1]Phase 1, phase 2, and phase 3 diets were formulated to have the following quantities of net energy (NE; kcal/kg) and amino acids (expressed as standardized ileal digestible; %): NE, 2,696, 2,488, and 2,465; Lys, 1.41, 1.35, and 1.23; Met, 0.50, 0.46, and 0.41; Thr, 0.86, 0.80, and 0.73; Trp, 0.23, 0.24, and 0.22, respectively.

[2]AEE = acid hydrolyzed ether extract; FTU = phytase units per kilogram of feed; STTD = standardized total tract digestible.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[4]The phytase concentrate contained 5,000 units of phytase/g (Quantum Blue, AB Vista, Marlborough, UK).

[5]Phytate was calculated by dividing the concentration of phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[6]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 7.2. Analyzed composition of ingredients

Item	Corn	Soybean meal	Potato protein concentrate	Spray-dried plasma protein	Calcium carbonate	Monocalcium phosphate
Gross energy, kcal /kg	3,833	4,194	5,333	4,815	-	-
Dry matter, %	85.38	88.10	91.13	90.70	99.96	93.58
Ash, %	1.20	6.76	0.81	7.36	91.32	80.55
Crude protein, %	6.94	45.94	81.93	78.88	-	-
AEE[1], %	3.32	1.84	0.66	0.22	-	-
Ca, %	0.02	0.29	0.02	0.12	38.91	17.31
P, %	0.26	0.63	0.08	1.49	0.04	20.81
Phytate[2], %	0.61	1.40	0.18	-	-	-
Phytate-bound P, %	0.17	0.39	0.05	-	-	-
Non-phytate P[3], %	0.09	0.24	0.03	-	-	-

[1]AEE = acid hydrolyzed ether extract

[2]Phytate was calculated by dividing the concentration of phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 7.3. Growth performance of pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (control), 500, 2,000, or 16,000 units of microbial phytase per kilogram of feed (FTU)[1]

Item, kg	100% of Ca and P requirement				50% of Ca and P requirement					
		FTU				FTU				
	Control	500	2,000	16,000	Control	500	2,000	16,000	SEM	Contrasts[2,3]
Phase 1, d 1 to 14										
Initial BW	6.39	6.39	6.35	6.34	6.33	6.33	6.35	6.33	0.51	-
ADG	0.134	0.162	0.169	0.171	0.134	0.135	0.141	0.146	0.01	a**, b, c**
ADFI	0.181	0.205	0.210	0.200	0.188	0.179	0.178	0.184	0.01	a*, b, c
G:F	0.740	0.783	0.797	0.860	0.714	0.755	0.788	0.789	0.03	c*, d, f*
Final BW	8.27	8.65	8.72	8.73	8.20	8.23	8.32	8.37	0.63	-
Phase 2, d 15 to 27										
ADG	0.485	0.482	0.466	0.466	0.481	0.438	0.450	0.436	0.02	-
ADFI	0.635	0.621	0.623	0.605	0.636	0.551	0.591	0.584	0.03	a, e*
G:F	0.761	0.779	0.749	0.770	0.756	0.797	0.762	0.745	0.04	-
Final BW	14.63	14.81	14.83	14.80	14.63	13.79	14.17	14.00	0.70	-
Phase 3, d 28 to 42										
ADG	0.622	0.641	0.664	0.628	0.620	0.592	0.651	0.623	0.03	-
ADFI	0.982	1.030	1.036	0.967	0.967	0.970	1.019	0.977	0.03	d
G:F	0.633	0.622	0.642	0.650	0.641	0.609	0.641	0.638	0.02	e*
Final BW	23.96	24.43	24.79	24.13	24.07	22.66	24.06	23.34	1.04	-
Overall, d 1 to 42										
ADG	0.418	0.429	0.439	0.424	0.423	0.389	0.422	0.405	0.01	a

Table 7.3. (Cont.)

ADFI	0.608	0.628	0.633	0.599	0.605	0.577	0.606	0.591	0.02	a
G:F	0.688	0.683	0.694	0.707	0.698	0.673	0.694	0.685	0.01	d, e*

[1]Data are least squares means of 8 observations.

[2]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = 100% vs. 50%; b = 100%: control vs. 500 FTU; c = 100%: control vs. 2,000 + 16,000 FTU; d = 100%: linear effect of phytase; e = 50%: control vs. 500 FTU; f = 50%: control vs. 2,000 + 16,000 FTU; g = 50%: linear effect of phytase.

[3]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$.

Table 7.4. Bone mineralization, gastrointestinal pH, and fecal evaluation of pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (control), 500, 2,000, or 16,000 units of microbial phytase per kilogram of feed (FTU)

Item	100% of Ca and P requirement				50% of Ca and P requirement					
		FTU				FTU				
	Control	500	2,000	16,000	Control	500	2,000	16,000	SEM	Contrasts[1,2]
End of phase 1, d 14										
Bone mineralization[3]										
Ash, g	5.03	4.77	5.13	4.99	4.23	4.29	4.05	4.13	0.27	a***
Ash, %	54.5	53.4	53.7	53.0	51.6	50.9	50.4	50.3	0.65	a***, f
Gastrointestinal pH[4]										
Stomach *In-situ*	3.70	3.50	3.40	3.20	3.85	3.41	3.41	3.54	0.20	c
Stomach *Ex-situ*	3.68	3.19	3.48	3.33	3.60	3.50	3.37	3.22	0.16	b*, f
Stomach average	3.69	3.34	3.44	3.26	3.73	3.54	3.39	3.38	0.16	c, f
Duodenum *In-situ*	5.92	5.93	5.69	5.77	5.93	6.04	5.40	6.13	0.15	g
Ileum *In-situ*	6.75	6.66	6.48	6.63	6.68	6.72	6.77	6.62	0.13	-
Fecal evaluation[4]										
Fecal score	2.18	2.29	2.29	2.02	2.16	2.22	2.04	2.02	0.14	-
Diarrhea frequency, %	33.9	34.0	35.1	26.1	25.0	33.9	25.0	26.8	7.78	-
End of phase 3, d 42										
Bone mineralization[3]										
Ash, g	12.11	11.25	11.60	11.41	10.56	10.21	11.67	9.96	0.62	a*
Ash, %	50.7	49.9	49.4	49.8	48.9	49.8	49.8	48.8	1.09	-

[1]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = 100% vs. 50%; b = 100%: control vs. 500 FTU; c = 100%: control vs. 2,000 + 16,000 FTU; d = 100%: linear effect of phytase; e = 50%: control vs. 500 FTU; f = 50%: control vs.

Table 7.4. (Cont.)

2,000 + 16,000 FTU; g = 50%: linear effect of phytase.

[2]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[3]Data are least squares means of 8 observations.

[4]Data are least squares means of 7 or 8 observations.

Table 7.5. Concentration of inositol phosphate (IP) esters (nmol/g dry matter) in colon content from pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (control), 500, 2,000, or 16,000 units of microbial phytase per kilogram of feed (FTU)[1,2]

Item	100% of Ca and P requirement				50% of Ca and P requirement					
		FTU				FTU				
	Control	500	2,000	16,000	Control	500	2,000	16,000	SEM	Contrasts[3,4]
IP6	3,715	1,925	509	140	1,423	0	0	0	437.4	a***, b*, c***, d*, e*, f*
IP5	956	495	111	6	337	0	0	0	93.7	a***, b**, c***, d*, e*, f*
IP4	1,700	1,079	711	305	793	86	69	33	134.1	a***, b**, c***, d***, e***, f***

[1]Values for IP3 and inositol were undetectable in all samples.

[2]Data are least squares means of 7 or 8 observations.

[3]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = 100% vs. 50%; b = 100%: control vs. 500 FTU; c = 100%: control vs. 2,000 + 16,000 FTU; d = 100%: linear effect of phytase; e = 50%: control vs. 500 FTU; f = 50%: control vs. 2,000 + 16,000 FTU; g = 50%: linear effect of phytase.

[4]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 7.6. Calcium and indicators of protein utilization in serum from pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (control), 500, 2,000, or 16,000 units of microbial phytase per kilogram of feed (FTU)[1]

Item	100% of Ca and P requirement				50% of Ca and P requirement					
		FTU				FTU				
	Control	500	2,000	16,000	Control	500	2,000	16,000	SEM	Contrasts[2,3]
Ca, mg/dL[4]										
d -1	10.8	10.7	10.6	10.5	10.9	10.3	10.9	10.4	-	
d 14	9.7	10.1	9.1	10.0	9.4	9.2	9.0	9.2	0.27	a*
Blood urea nitrogen,[5] mg/dL										
d -1	6.75	5.75	7.13	6.50	6.38	6.75	5.46	5.63	-	
d 14	5.22	5.65	7.24	7.17	6.40	8.35	6.90	8.37	2.06	-
Total protein,[4] g/dL										
d -1	4.55	4.39	4.39	4.59	4.55	4.49	4.63	4.26	-	
d 14	4.39	4.48	4.34	4.38	4.13	4.50	4.22	4.41	0.14	e*
Albumin,[4] g/dL										
d -1	3.05	2.95	2.94	2.91	2.98	2.71	2.94	2.74	-	
d 14	2.51	2.54	2.43	2.48	2.34	2.33	2.24	2.30	0.11	a**

[1]Data from d -1 were used as a covariate for data obtained on d 14.

[2]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = 100% vs. 50%; b = 100%: control vs. 500 FTU; c = 100%: control vs. 2,000 + 16,000 FTU; d = 100%: linear effect of phytase; e = 50%: control vs. 500 FTU; f = 50%: control vs. 2,000 + 16,000 FTU; g = 50%: linear effect of phytase.

[3]* = $P < 0.05$; ** = $P < 0.01$.

[4]Data are least squares means of 8 observations.

[5]Data are least squares means of 7 or 8 observations.

Table 7.7. Plasma metabolites in pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (control), 500, 2,000, or 16,000 units of microbial phytase per kilogram of feed (FTU)[1]

Item	100% of Ca and P requirement				50% of Ca and P requirement					
		FTU				FTU				
	Control	500	2,000	16,000	Control	500	2,000	16,000	SEM	Contrasts[2,3]
Inositol,[4] μM										
d -1	38.0	57.3	43.5	49.0	36.1	52.8	49.2	39.8	-	
d 14	15.3	43.6	55.9	63.6	19.2	41.7	49.7	51.0	7.72	b**, c***, d* ,e**, f***, g
Immunoglobulin A,[4] mg/mL										
d -1	0.159	0.197	0.187	0.192	0.145	0.162	0.195	0.160	-	
d 14	0.316	0.226	0.308	0.254	0.316	0.276	0.269	0.291	0.0393	b
Interferon γ,[5,6] ng/mL										
d -1	15.8	26.5	5.2	10.4	40.9	7.2	27.9	7.3	-	
d 14	6.24	3.85	5.43	7.62	5.71	3.44	6.09	5.89	1.948	d
Interleukin 1β,[5,6] pg/mL										
d -1	234	546	93	222	596	179	491	99	-	
d 14	250	261	199	163	392	320	232	390	85.9	a
Interleukin 6,[5,6] pg/mL										
d -1	164	339	43	110	330	127	286	52	-	
d 14	97	89	98	67	186	65	92	134	36.4	e
Interleukin 8, [5,6] pg/mL										
d -1	48	58	34	56	91	39	91	44	-	
d 14	40	41	35	39	57	29	33	42	8.6	e*

Table 7.7. (Cont.)

Interleukin 10,[5,6] pg/mL										
d -1	875	1528	282	713	1,551	593	1,343	318	-	
d 14	446	508	506	296	744	345	472	490	152.5	e
Tumor necrosis factor-α, [5,6] pg/mL										
d -1	251	350	158	172	494	165	343	161	-	
d 14	151	176	141	150	162	111	91	159	43.0	-

[1]Data from d -1 were used as a covariate for data obtained on d 14.

[2]Contrasts that were significant ($P < 0.05$) or tended ($P < 0.10$) to be significant were expressed as follows: a = 100% vs. 50%; b = 100%: control vs. 500 FTU; c = 100%: control vs. 2,000 + 16,000 FTU; d = 100%: linear effect of phytase; e = 50%: control vs. 500 FTU; f = 50%: control vs. 2,000 + 16,000 FTU; g = 50%: linear effect of phytase.

[3]No * = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[4]Data are least squares means of 7 or 8 observations.

[5]Data are least squares means of 8 observations.

[6]Values were Log10 transformed before analysis to obtain a normal distribution, but data are shown as back-transformed least square means.

Table 7.8. Effect of treatment and day on blood metabolites from pigs fed diets formulated with 100 or 50% of the requirement for Ca and P and 0 (control), 500, 2,000, or 16,000 units of microbial phytase per kilogram of feed (FTU)[1,2]

Item	Treatment									Day					*P*-value for day	
	100%				50%											
		FTU				FTU										
	Control	500	2,000	16,000	Control	500	2,000	16,000	SEM	-1	14	27	42	SEM	L	Q
Serum																
Ca[3]	10.9	10.8	10.7	10.8	10.7	10.5	10.7	10.7	0.13	10.6	9.5	11.1	11.7	0.17	< 0.001	< 0.001
BUN[4]	7.26	7.06	8.42	8.18	7.78	8.37	7.37	7.72	0.70	6.30	6.86	8.52	9.41	1.01	< 0.001	0.704
Protein[4]	4.79	4.68	4.64	4.68	4.65	4.70	4.83	4.60	0.07	4.48	4.36	4.64	5.30	0.05	< 0.001	< 0.001
Albumin[3]	3.02	2.89	2.87	2.80	2.82	2.63	2.86	2.70	0.09	2.90	2.40	2.74	3.26	0.05	< 0.001	< 0.001
Plasma																
IgA[4]	0.48	0.49	0.52	0.50	0.46	0.48	0.50	0.49	0.028	0.17	0.28	0.64	0.86	0.024	< 0.001	0.065
INFγ[5,6]	3.44	5.86	1.25	2.18	8.52	1.68	5.81	1.68	0.196	13.86	5.38	1.65	0.72	0.096	< 0.001	0.470
IL-1β[5,7]	173	273	143	141	342	226	146	157	65.5	246	264	101	199	42.3	0.108	0.089
IL-6[5,6,7]	70	113	47	52	178	51	77	37	29.6	142	98	27	59	14.8	< 0.001	< 0.001
IL-8[4,5]	33	43	31	37	42	29	37	33	9.1	54	39	31	23	5.3	< 0.001	0.586
IL-10[4,5]	396	660	285	297	706	346	348	221	151.5	755	460	223	263	89.8	< 0.001	0.016
TNF-α[5,7]	127	145	142	125	163	156	114	123	21.8	350	121	66	55	23.3	< 0.001	0.004

[1]There was no interaction between treatment and day.

[2]BUN = blood urea nitrogen (mg/dL); Protein = total protein (g/dL); Albumin (g/dL); IgA = immunoglobulin A (mg/mL); TNF-α = tumor necrosis factor alpha (pg/mL); INF-γ = interferon gamma (ng/mL); IL = interleukin (pg/mL).

[3]Contrast 100% vs. 50% ($P < 0.05$).

[4]None of the contrasts analyzed were significant.

[5]Values were Log10 transformed before analysis to obtain a normal distribution, but data are shown as back-transformed least square means.

[6]Contrasts 50%: control vs. 500 ($P < 0.05$).

[7]Contrast 50%: control vs. 2,000 + 16,000 ($P < 0.05$).

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Chapter 8: Increasing levels of phytase increased phytate degradation, nutrient digestibility, plasma metabolites, and bone ash of young pigs fed corn-soybean meal-based diets for a long adaptation period

Abstract

An experiment was conducted to test the hypothesis that increasing dietary concentrations of microbial phytase increases plasma P and inositol, nutrient and energy digestibility, and bone ash of young pigs. The second hypothesis was that maximum phytate degradation requires more phytase than that needed for maximum digestible P release. A total of 36 pigs were equipped with a T-cannula in the distal ileum and allotted to 1 of 6 diets in a completely randomized design (body weight: 11.0 ± 0.6 kg). Diets based on corn and soybean meal were formulated by including 0, 250, 500, 1,000, 2,000, or 4,000 phytase units/kg feed (**FTU**). Phytase was assumed to release 0.16% total Ca and 0.11% digestible P. Blood (d -1 and 23), feces (d 19 and 20), ileal digesta (d 21 and 22), and the 3rd and 4th metatarsals (d 23) were collected. Results indicated that apparent ileal digestibility (**AID**) of Trp (quadratic; $P < 0.05$), and of Lys and Thr (linear; $P < 0.05$) increased, and AID of Met tended to increase (linear; $P < 0.10$) as phytase inclusion increased. Increasing dietary phytase also resulted in increased AID and apparent total tract digestibility (**ATTD**) of Ca and P (quadratic; $P < 0.05$) and increased ATTD of K and Na (linear; $P < 0.05$), but phytase did not influence the ATTD of Mg or gross energy. Concentrations of plasma P and bone ash increased (quadratic; $P < 0.05$), and plasma inositol also increased (linear; $P < 0.05$) with increasing inclusion of phytase. Reduced concentrations of inositol

phosphate (**IP**) 6 and IP5 (quadratic; $P < 0.05$), reduced IP4 and IP3 (linear; $P < 0.05$), and increased inositol (linear; $P < 0.05$) were observed in ileal digesta as inclusion of dietary phytase increased. The ATTD of P was maximized if at least 1,200 FTU of phytase were used, whereas more than 4,000 FTU of phytase were needed to maximize inositol release. In conclusion, increasing dietary levels of phytase after a prolonged adaptation period increased phytate and IP ester degradation and inositol release in the small intestine. Consequently, increasing dietary phytase resulted in improved digestibility of Ca, P, K, Na, and the first 4 limiting amino acids, and in increased concentrations of bone ash and P and inositol in plasma. In a typical corn-soybean meal-based diet, maximum inositol release requires approximately 3,200 FTU more phytase than that required for maximum P digestibility.

Key words: bone ash, inositol, nutrient digestibility, phytase, phytate degradation, pigs

Abbreviations

AEE	acid hydrolyzed ether extract
AA	amino acids
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
CP	crude protein
FTU	phytase units per kilogram of feed
GE	gross energy
IP	inositol phosphate
SBM	soybean meal

Introduction

Inclusion of microbial phytase in diets for swine and poultry has been practiced since the early 1990's (Simons et al., 1990). The standard level of inclusion ranges from 250 to 500 phytase units per kilogram of feed (**FTU**), which is expected to release phytate-bound P from plant based feed ingredients to reduce the amount of feed phosphate in the diet without compromising growth performance or bone mineralization (Walk, 2016). Calcium is also released by phytate as a result of interactions between Ca and phytate (Selle et al., 2009). Supplementation of phytase at doses above 1,000 FTU, also known as super-dosing, has resulted in improvements in growth performance of growing pigs (Holloway et al., 2019). This improvement in animal performance has been attributed to a reduction in the anti-nutritional effects of phytate, and a subsequent increase in nutrient digestibility and inositol release (Woyengo and Nyachoti, 2013). Degradation of phytate produces lower phytate esters that precipitate with available cations and interact with proteins to a lesser extent than phytate (Bedford and Walk, 2016). However, results of *in vitro* experiments indicate that inositol phosphate (**IP**) 4 and IP 3 also inhibit pepsin activity (Yu et al., 2012), and degradation of phytate and lower phytate esters, therefore, is important for proper mineral and protein digestion. Data from broiler chickens have demonstrated the beneficial effect of super-dosing of phytase on energy, amino acid (**AA**), and mineral digestibility (Cowieson et al., 2017b), but data from pigs have not consistently demonstrated similar effects (Selle and Ravindran, 2008; She et al., 2018).

Although the positive effect of phytase on mineral digestibility in pigs is often observed (Almeida et al., 2013; Arredondo et al., 2019; Archs Toledo et al., 2020), data indicate that increasing levels of phytase up to 3,000 FTU has limited effects on AA and energy digestibility if a 5 or 7 d adaptation period to the experimental diets is used (Liao et al., 2005; Mesina et al.,

2019). However, it is not known if providing greater phytase doses and allowing more time for pigs to adapt to their assigned diets will result in increased AA and energy digestibility. Additionally, data demonstrating the effect of increasing levels of phytase in a typical corn-soybean meal (**SBM**)-based diet on phytate and phytate ester degradation in pigs are scarce (Rosenfelder-Kuon et al., 2020). Therefore, the objective of this experiment was to test the hypothesis that increasing inclusion of dietary phytase from 0 to 4,000 FTU increases the apparent digestibility of minerals, AA, and energy, the concentration of P and inositol in plasma, and concentration and percentage of bone ash in growing pigs. The second hypothesis was that maximum phytate degradation in pigs fed corn-SBM-based diets requires more phytase than what is needed to maximize total tract digestible P release.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals, housing, and diets

A total of 36 pigs were equipped with a T-cannula (barrel length: 6 cm; inner diameter: 1.6 cm) in the distal ileum. After 3 to 5 days of recovery, pigs (initial body weight: 11.0 ± 0.6 kg) were allotted to 1 of 6 diets in a completely randomized design with 6 replicate pigs per diet. Pigs were housed individually in 0.9 × 1.8 m pens that had fully slatted concrete floors and were equipped with a feeder and a cup waterer in an environmentally controlled room. Water and feed were available at all times. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Six diets were formulated by including 0, 250, 500, 1,000, 2,000, or 4,000 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) in corn-SBM-based diets (Table 8.1). Provisions of total Ca and standardized total tract digestible P were reduced by 0.16 and 0.11%, respectively, compared with requirement estimates (11 to 25 kg; NRC, 2012) to account for the expected release of Ca and P by phytase. All diets contained 0.40% chromium oxide as an indigestible marker, and vitamins and minerals other than Ca and P were included to meet or exceed the requirement (Table 8.2). A representative sample of 2 kg of each diet and ingredient was collected immediately after diet mixing.

Sample collection and bone measurements

The day before the start of feeding experimental diets (d -1), pigs were weighed and a blood sample was collected by jugular venipuncture in lithium-heparin-containing tubes. Blood samples were centrifuged at 1,500 × *g* at 4 ºC immediately after collection and 2 samples of plasma were harvested from each pig and stored at -20 ºC. Pigs were fed experimental diets for 23 d. On the morning of d 19 and 20, fecal samples were collected via anal stimulation and on d 21 and 22, ileal digesta were collected for 9 h per d. Fecal and ileal digesta samples were stored at −20 °C immediately after collection.

On d 23, pigs were euthanized via captive bolt stunning and a blood sample and the right rear foot were collected. All collected feet were autoclaved at 125 ºC for 55 min and the 3rd and 4th metatarsals were identified, removed, and cleaned of soft tissue. Metatarsals were dried overnight at 105 ºC and soaked for 72 h in petroleum ether under a chemical hood to remove marrow and fat. Bones were then dried at 135 ºC for 2 h and ashed at 600 ºC for 16 h.

Sample analysis

Ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected. Ileal digesta and fecal samples were lyophilized (Lagos and Stein, 2019) and ingredient, diet, ileal digesta, and fecal samples were finely ground. Ingredients and diets were analyzed for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2019) and for ash at 600 °C at 2 h (Method 942.05; AOAC Int., 2019). Ingredient, diet, ileal digesta, and fecal samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2019) after dry ash preparation (Method 942.05; AOAC Int., 2019) followed by wet digestion with nitric acid (Method 3050 B; US-EPA, 2000). One of the plasma samples collected on d -1 and on d 23 was also analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry, but after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2019]. Diet and fecal samples were analyzed for K, Mg, and Na, and ileal digesta samples were also analyzed for K. Diets and ileal digesta samples were analyzed for AA (Method 982.30 E [a, b, c]; AOAC Int., 2019) using a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA). Corn, SBM, diet, and ileal digesta samples were analyzed for N by the combustion procedure (method 990.03; AOAC Int., 2019) using a LECO FP628 (LECO Corp., Saint Joseph, MI, USA) and crude protein (**CP**) was calculated as N × 6.25. Corn, SBM, diet, and fecal samples were analyzed for gross energy (**GE**) using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Corn, SBM, and diets were analyzed for acid hydrolyzed ether extract (**AEE**; Method 2003.06; AOAC Int., 2019) using an Ankom^{HCl} followed by an Ankom^{XT15} (Ankom Technology, Macedon, NY, USA) and for phytate-bound P by wet chemistry using the Megazyme kit (Megazyme Inc., Chicago, IL, USA). Diet, fecal, and ileal

digesta samples were also analyzed for Cr (Method 990.08; AOAC Int., 2019) and these samples were analyzed at the University of East Anglia, School of Biological Sciences (UK), for inositol and IP esters using high-performance ion chromatography-based techniques as described by Walk et al. (2018). Diets were analyzed for phytase activity by the enzyme-linked immunosorbent assay method using Quantiplate Kits for Quantum (AB Vista, Plantation, FL, USA). From the second sample of plasma collected on d -1 and on d 23, 0.5 mL were transferred to a 2-mL centrifuge tube that contained 1 mL perchloric acid (1 *N*). Samples were then stored at 4 ℃ for 30 min, centrifuged using a refrigerated centrifuge (Eppendorf Centrifuge 5427 R; Eppendorf AG, Hamburg, Germany) at 4 ℃ and 17,500 × g for 10 min. The supernatant was extracted using a 5-mL capacity sterile syringe (Fisherbrand™; Fisher Scientific, Waltham, MA, USA) with a needle attached, and the needle was then replaced with a syringe filter (Kinesis Polytetrafluoroethylene Syringe Filters; Cole-Parmer, Vernon Hills, IL, USA). The content was discharged into another tube, and this sample was analyzed for inositol as explained for diet, fecal, and ileal digesta samples.

Calculations and statistical analyses

The percentage of phytate in corn and SBM was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004), and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. The apparent ileal digestibility (**AID**) of CP, AA, Ca, P, and K and the apparent total tract digestibility (**ATTD**) of GE, Ca, P, K, Mg, and Na in experimental diets were calculated as previously outlined (Stein et al., 2007):

$$\text{Digestibility of nutrients, \%} = \left[1 - \left(\frac{\text{nutrient in sample}}{\text{nutrient in diet}}\right) \times \left(\frac{\text{marker in diet}}{\text{marker in sample}}\right)\right] \times 100$$

where digestibility is the AID or ATTD of nutrients or energy, nutrient in sample is the nutrient or energy concentration in ileal digesta or fecal samples and nutrient in diet is the concentration

of nutrient or energy in the diet. Marker in diet and marker in sample are the concentration of Cr in diets and ileal digesta or fecal samples, respectively. Bone ash percentage was calculated by dividing the quantity (grams) of bone ash by the quantity of fat-free dried bone and multiplying by 100.

Normality of residuals and homogeneity of variances were tested using the INFLUENCE, GPLOT, and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC). Data for phytate degradation, concentration and percentage of bone ash, plasma Ca, P, and inositol, and digestibility of energy and nutrients were analyzed using the PROC MIXED of SAS with the experimental unit being the pig. The experimental model included the main effect of phytase inclusion level and the random effect of replicate. For all parameters, contrast statements were used to determine linear and quadratic effects of phytase inclusion level. Coefficients for the unevenly spaced linear contrasts were obtained using the PROC IML procedure of SAS. Data for blood metabolites obtained on d -1 was used as covariate to analyze blood data from d 23. If a linear or quadratic effect of phytase on phytate degradation, bone ash, or Ca and P digestibility was observed, a broken line analysis was conducted using the analyzed values for phytase and the NLIN procedure of SAS. The single-slope model was $y = L + U \times (R - x)$, where $(R - x)$ is zero when $x > R$. The breakpoint value is R, the asymptote for the first segment is L, and the slope for the line segment is U (Robbins et al., 2006). A *t*-test was used to test the null hypothesis that the difference between AID and ATTD values for Ca, P, and K was equal to zero. Treatment means were calculated using the LSMEANS statement in SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

One pig fed the diet with no phytase died 5 d after the start of feeding experimental diets. This pig was replaced by an extra pig that was cannulated at the same time as the pig that died. The remaining pigs consumed their diets without apparent problems and no health problems were observed during the experiment.

No effect of inclusion level of dietary phytase on the AID of Arg, Ile, Leu, Phe, Val, Ala, Cys, and Glu in diets was observed, but increasing concentration of phytase resulted in increased (quadratic; $P < 0.05$) AID of CP, Trp, and Ser (Table 8.3). Likewise, the AID of Lys, Thr, and Asp linearly increased ($P < 0.05$) as phytase inclusion level increased in diets. There was also a tendency ($P < 0.10$) for an increase in the AID of Met, and Tyr (linear) and in the AID of His, Thr, Asp, and Gly (quadratic) as the dietary level of phytase increased.

There was an increase (quadratic; $P < 0.05$) in the AID of Ca and P and the ATTD of Ca, P, and K as the inclusion level of phytase increased in diets (Table 8.4). The broken-line analyses indicated that 853 and 1,215 FTU of phytase were needed to maximize the ATTD of Ca ($P < 0.05$; Fig. 8.1) and P ($P < 0.05$; Fig. 8.2), respectively. The AID of K increased (linear; $P < 0.05$) and tended to have a quadratic increase ($P < 0.10$) with increasing dietary concentrations of phytase. Likewise, there was a linear increase ($P < 0.05$) in the ATTD of Na in diets as phytase inclusion increased, but no effect of the level of phytase in diets was observed for the ATTD of GE or Mg. Differences between AID and ATTD of Ca differed ($P < 0.05$) from zero, but for P or K, no differences between AID and ATTD values were observed (Table 8.5).

There was no effect of dietary phytase on the concentration of Ca in plasma of pigs on d 23, but increased concentrations of plasma P (quadratic; $P < 0.05$) and plasma inositol (linear; $P < 0.05$) were observed as dietary phytase increased (Table 8.6). The concentration and

percentage of bone ash increased (quadratic; $P < 0.05$) with increasing dietary phytase. The broken-line analyses indicated that 1,222 FTU of phytase were needed to maximize the concentration of bone ash ($P < 0.05$; Fig. 8.3).

Concentrations of IP6 and IP5 in ileal digesta decreased (quadratic; $P < 0.05$) as the inclusion of dietary phytase increased from 0 to 4,000 FTU (Table 8.7). Increasing dietary phytase also resulted in reduced (linear; $P < 0.05$) concentrations of ileal IP4 and IP3, but increased (linear; $P < 0.05$) concentrations of ileal inositol. The broken-line analyses indicated that 801 and 4,464 FTU of phytase were needed to maximize IP6 degradation ($P < 0.05$; Fig. 8.4) and inositol release in ileal digesta ($P < 0.05$; Fig. 8.5), respectively. There was a linear decrease ($P < 0.05$) in the concentration of fecal IP5 and a tendency for an increase (quadratic; $P < 0.10$) in fecal IP4 with increasing inclusion of phytase, but no effect of dietary phytase on the concentration of IP6 in feces was observed.

Discussion

Phytate and lower phytate esters have anti-nutritional effects in swine diets because of binding of dietary nutrients including minerals and AA, which results in reduced nutrient digestibility (Woyengo and Nyachoti, 2013). Super-dosing of phytase is a strategy to reduce the anti-nutritional effects of phytate in diets by degradation of not only phytate, but also lower phytate esters in the small intestine of pigs (Mesina et al., 2019). The subsequent increase in inositol release is also considered a beneficial effect of super-dosing of phytase because of the role of inositol in metabolic processes (Huber, 2016). However, although positive effects of phytase on phytate degradation, mineral digestibility, and growth performance of pigs is usually observed

(Arredondo et al., 2019; Lu et al., 2019), effects of high levels of phytase on energy and AA digestibility are not consistent (Liao et al., 2005; Velayudhan et al., 2015; She et al., 2018).

The observed increase in the AID of CP, Lys, Trp, and Thr, as well as the tendency for an increased AID of Met, as dietary phytase increased from 0 to 4,000 FTU indicates that super-dosing of phytase increases the digestibility of the 4 first limiting AA in corn-soybean meal diets fed to pigs. The linear increase in the AID of Lys and Met indicates that more than 4,000 FTU may be needed to maximize AID of Lys and Met, whereas the observation that the increase in the AID of Thr and Trp was quadratic, indicates that 2,000 FTU of phytase was sufficient to maximize AID of these 2 AA. Nevertheless, besides a tendency for a quadratic increase in the AID of His as phytase increased in diets, no other indispensable AA nor the mean of all indispensable AA was influenced by phytase. Effects of phytase on AA digestibility may be influenced by the type and solubility of proteins, concentrations of minerals in the diet, gastrointestinal pH, and interactions among phytate, proteins, and proteolytic enzymes (Liao et al., 2005). The observation that increasing levels of phytase increased or tended to increase the AID of Asp, Ser, and Tyr is in agreement with results from experiments using dietary phytase from 0 to 2,000 FTU (Liao et al., 2005; Velayudhan et al., 2015). However, the AID of CP and AA was not affected by dietary phytase in multiple other experiments (Zeng et al., 2016; She et al., 2018; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020). It is not clear why results of the present experiment were different from some published data, but it is possible that the longer adaptation period used in this experiment compared with previous experiments influenced the results. However, more research is needed to verify this hypothesis. The numerical, but non-significant, increases in AID of 3.0, 4.4, and 3.7 percentage units for the average of indispensable, dispensable, and all AA that were calculated in this experiment as a result of using

2,000 FTU in diets are in agreement with data from Cowieson et al. (2017c) who reported an average increase of 2.8 percentage units in AID of all AA from a review of 28 publications. Thus, it is possible that more replications than typically used in digestibility experiments are needed to verify effects of phytase on AID of AA.

The observation that increasing levels of phytase did not increase the ATTD of GE is in agreement with published data (Liao et al., 2005; She et al., 2018; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020), but in contrast with data from Arredondo et al. (2019) who reported a positive effect of phytase on the ATTD of GE. The reason for the inconsistency in the effects of phytase on ATTD of GE may be related to difference in the phytases used.

The quadratic increase in the ATTD of P and Ca as phytase supplementation increased in diets is well documented (Almeida et al., 2013; She et al., 2018; Arredondo et al., 2019; Ren et al., 2020). The broken-line regression analysis indicated that inclusion of phytase above 853 and 1,215 FTU did not further improve the ATTD of Ca and P, respectively, which is in agreement with Almeida et al. (2013) and Arredondo et al. (2019). The observed increase in the ATTD of K and Na concurs with data from She et al. (2018) and Arredondo et al. (2019), but the lack of a phytase effect on the ATTD of Mg is in contrast with published data, and was not expected because phytate is present in plants as Ca, Mg, and K mixtures (Onyango and Adeola, 2009).

No differences have been observed between values for AID and ATTD of Ca and P in pigs, which indicates that there is no net absorption of these minerals in the hindgut (Bohlke et al., 2005; González-Vega et al., 2014). Data from this experiment concurs with the previous statement as indicated by the lack of differences between AID and ATTD values for P and K, although a 4 percentage units difference between AID and ATTD values was observed for Ca.

The observation that phytase level did not affect the concentration of Ca in plasma is in agreement with data from Cowieson et al. (2017a) and Mesina et al. (2019). The fact that Ca remained between 10.9 and 11.8 mg/dL regardless of ATTD of Ca is a result of the regulation of Ca homeostasis by systemic hormones (Crenshaw, 2001). The observed increase in the concentration of P and inositol in plasma as dietary phytase increased concurs with previous data from pigs (Cowieson et al., 2017a; Mesina et al., 2019). This indicates that as more phytase is included in the diet, more phosphate is removed from phytate and phytate esters, resulting in more P being absorbed and more phytate being fully degraded. The observed increase in plasma inositol from 81 μM when no phytase was used to 97 μM upon supplementation of 500 FTU of phytase indicates that even at a low inclusion of phytase, full degradation of some phytate in the gastrointestinal tract of pigs takes place. Inositol is involved in several metabolic processes as part of phosphatidylinositol phosphates, which are mediators in cellular signaling to activate protein B kinase and stimulate glucose uptake and protein synthesis (Lee and Bedford, 2016). Therefore, the increased release and absorption of inositol may be beneficial for pigs as indicated by the improved growth performance of newly weaned pigs upon inositol or phytase supplementation (Moran et al., 2019), and the increased abundance of glucose transporter type 4 in muscle of growing pigs fed diets with 2,000 FTU of phytase (Lu et al., 2019).

The quadratic increase in bone ash as phytase increased in diets is in agreement with previous data from pigs (Zeng et al., 2011; Cuyper et al., 2020), and is the result of the increased ATTD of Ca and P as phytase was added to the diets. The breakpoint in the linear-plateau analysis indicates that there is no improvement in the concentration of bone ash after addition of 1,222 FTU of phytase. This result concurs with the breakpoint at 1,215 FTU observed for the ATTD of P and indicates that P was the limiting nutrient for bone synthesis in these pigs.

The reduced concentrations of IP6 and IP5 in ileal digesta along with the increase and then decrease in the concentrations of IP4 and IP3 as phytase inclusion increased from 0 to 4,000 FTU is a result of the stepwise hydrolysis of phytate by phytase to release phosphate from inositol. These results are in agreement with data from Laird et al. (2018), Mesina et al. (2019), and Rosenfelder-Kuon et al. (2020) using increasing levels of phytase in barley-wheat-SBM-, corn-SBM-canola meal-, and corn-SBM-based diets, respectively. The concentrations of IP esters and inositol observed in this experiment are lower than those reported by Mesina et al. (2019), which is a result of a greater substrate supply in diets with canola meal. This statement is supported by data indicating that regardless of phytase, there is a greater concentration of ileal IP6, IP4, and inositol in pigs if rapeseed meal is included in diets (Rosenfelder-Kuon et al., 2020). Additionally, the observed linear increase in ileal inositol with increasing dietary phytase indicates that as more phytase is used, more phytate is fully degraded, and although this observation concurs with the results from plasma inositol, it also indicates that inositol absorption in the small intestine of pigs is not as efficient as in humans (Huber, 2016). The observation that after the inclusion of 800 FTU of phytase, there was no improvement in IP6 degradation, but the breakpoint where inositol release is no longer improved was 4,464 FTU, indicates that IP6 degradation and concentration does not represent the complete destruction of phytate. These results also demonstrate the progressive degradation of IP6 and IP5 into lower esters and inositol as dietary phytase increased, and validate the hypothesis that more phytase is required to maximize phytate degradation than to maximize digestible P release. However, the observation that the breakpoint for P digestibility and bone ash is at around 1,200 FTU indicates that the P released by phytase after this point is not absorbed, which is likely because of

formation of Ca-P complexes in the small intestine of pigs that makes P unavailable (Stein et al., 2011), but research is needed to validate this hypothesis.

The reduced concentrations of IP esters in feces compared with ileal digesta and the limited effect of dietary phytase on fecal IP esters is in agreement with Mesina et al. (2019) and indicates that phytate is degraded in the large intestine of pigs by phytase synthesized by intestinal microbes (Selle et al., 2010). However, as observed in this and many other experiments, phytate degradation beyond the ileum is not relevant for swine nutrition, because there is no net absorption of Ca and P in the hindgut of pigs (Bohlke et al., 2005; González-Vega et al., 2014; Rutherfurd et al., 2014). As a consequence, phytate and IP esters should be determined at the distal ileum rather than in feces.

Conclusions

Phytase supplementation increased phytate degradation, Ca and P digestibility, plasma P, and concentration of bone ash, indicating that degradation of phytate and lower phytate esters results in increased Ca and P release from phytate, and consequently increased absorption, mobilization, and retention of Ca and P. The observed increase in ileal and plasma inositol by phytase indicates that phytase also increased inositol release and absorption. Degradation of phytate by microbes in the hindgut of pigs was observed, but it is considered irrelevant because no net absorption of Ca and P in the large intestine takes place. The digestibility of energy was not influenced by dietary phytase, but increasing dietary phytase increased digestibility of minerals and some AA, which demonstrates that phytase reduces the anti-nutritional effects of phytate in pig diets. At least 1,200 FTU of phytase were needed to maximize P digestibility in corn-SBM-based diets, but to fully degrade phytate, more than 4,000 FTU of phytase were needed.

Figures

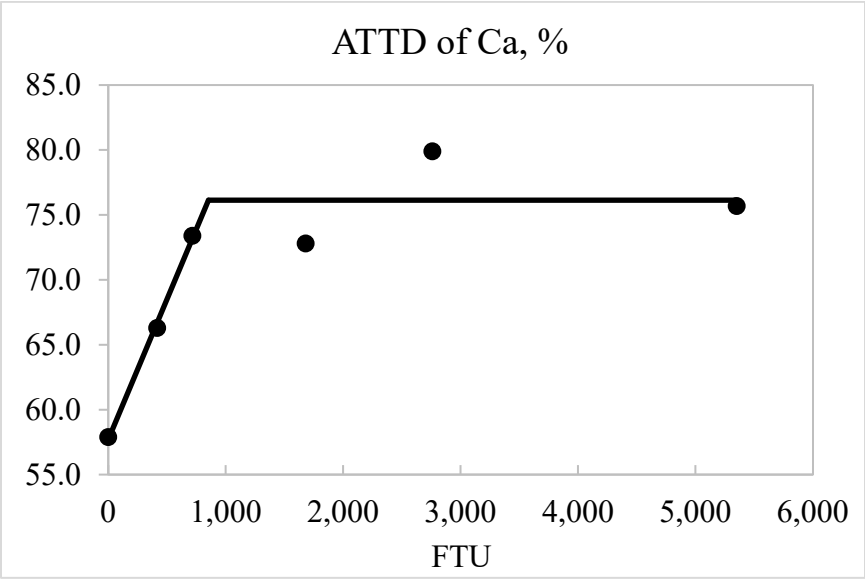

Figure 8.1. Fitted linear broken-line plots of the average apparent total tract digestibility (ATTD) of Ca in diets as a function of phytase inclusion level. The optimum concentration of phytase determined by linear broken-line model was 853 (SE = 168.7) units of microbial phytase per kg of feed (FTU); [Y = 76.1 - 0.022 × (853 – X)] where X is less than 853, with $r^2 = 0.915$ and $P < 0.05$. The SE for the estimates of the intercept and second parameter were 1.69 and 0.006, respectively.

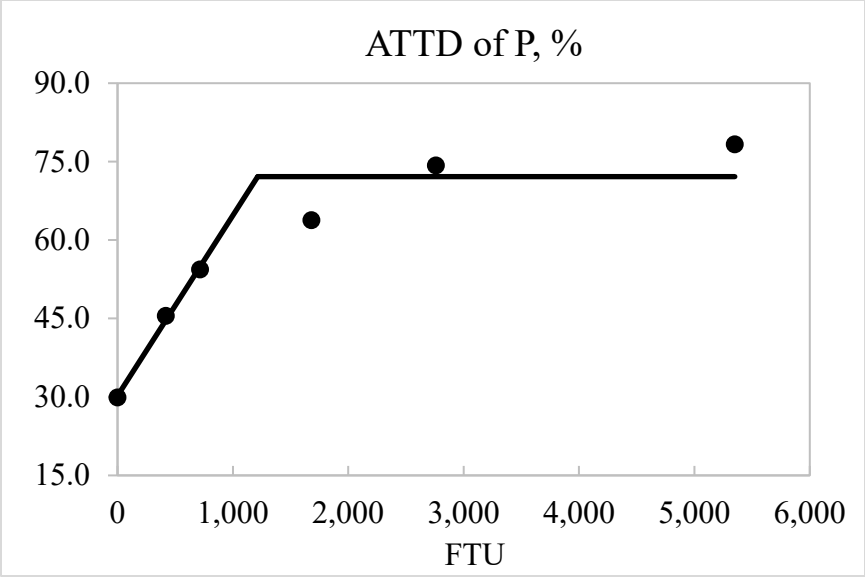

Figure 8.2. Fitted linear broken-line plots of the average apparent total tract digestibility (ATTD) of P in diets as a function of phytase inclusion level. The optimum concentration of phytase determined by linear broken-line model was 1,215 (SE = 328.1) units of microbial phytase per kg of feed (FTU); [Y = 72.1 - 0.035 × (1,215 – X)] where X is less than 1,215, with $r^2 = 0.932$ and $P < 0.05$. The SE for the estimates of the intercept and second parameter were 3.55 and 0.012, respectively.

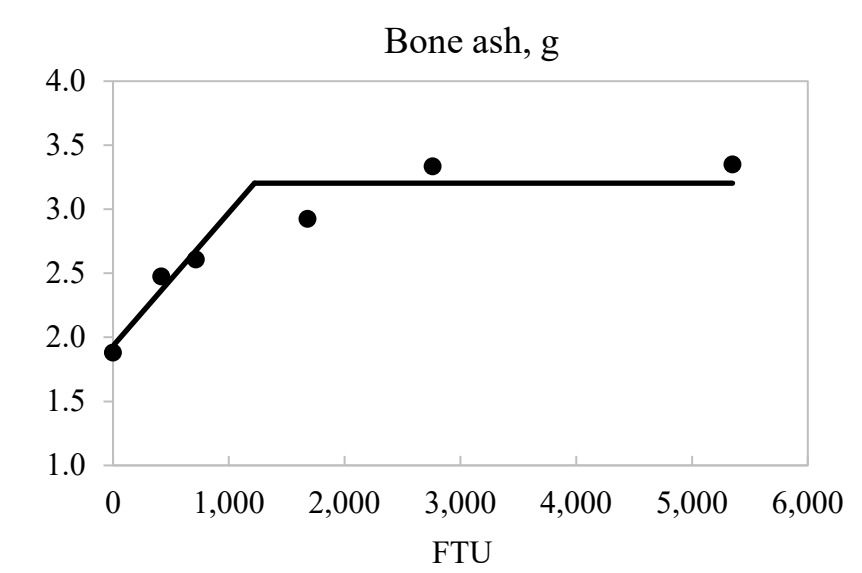

Figure 8.3. Fitted linear broken-line plots of the average concentration of bone ash (grams) as a function of phytase inclusion level. The optimum concentration of phytase determined by linear broken-line model was 1,222 (SE = 378.1) units of microbial phytase per kg of feed (FTU); [Y = 3.20 - 0.001 × (1,222 – X)] where X is less than 1,222, with $r^2 = 0.914$ and $P < 0.05$. The SE for the estimates of the intercept and second parameter were 0.123 and 0.0004, respectively.

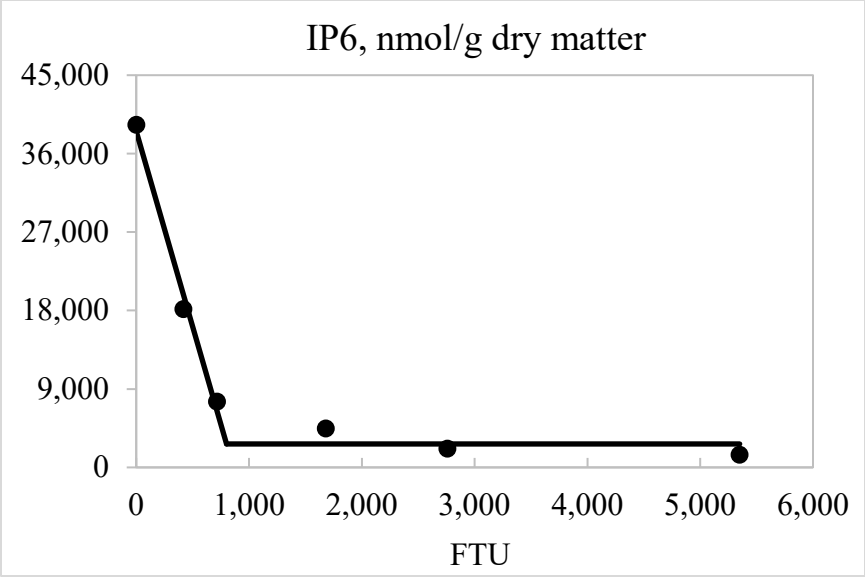

Figure 8.4. Fitted linear broken-line plots of the average concentration of ileal IP6 (nmol/g dry matter) as a function of phytase inclusion level. The optimum concentration of phytase determined by linear broken-line model was 801 (SE = 46.3) units of microbial phytase per kg of feed (FTU); [Y = 2,690 + 44.8 × (801 – X)] where X is less than 801, with $r^2 = 0.991$ and $P < 0.01$. The SE for the estimates of the intercept and second parameter were 1,027 and 3.50, respectively.

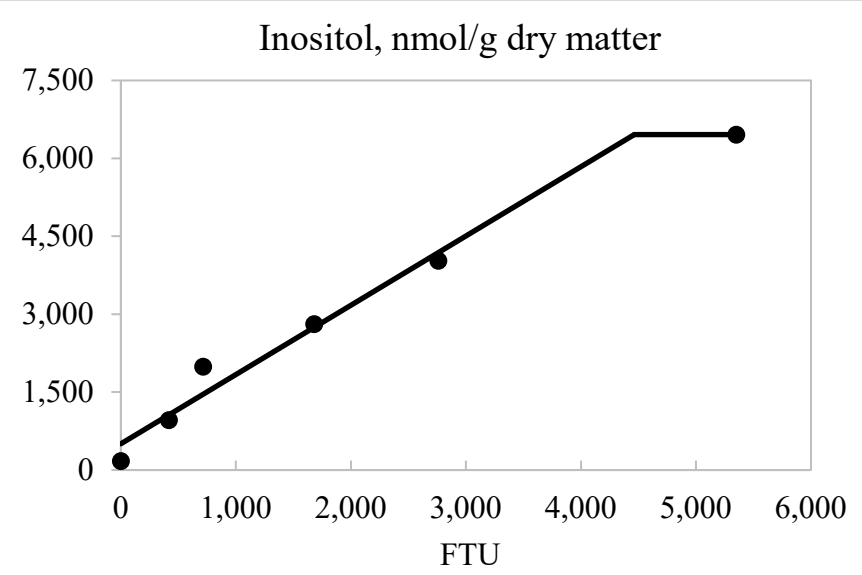

Figure 8.5. Fitted linear broken-line plots of the average concentration of ileal inositol (nmol/g dry matter) as a function of phytase inclusion level. The optimum concentration of phytase determined by linear broken-line model was 4,464 (SE = 531.0) units of microbial phytase per kg of feed (FTU); [Y = 6,458 – 1.33 × (4,464 – X)] where X is less than 4,464, with $r^2 = 0.983$ and $P < 0.01$. The SE for the estimates of the intercept and second parameter were 379.5 and 0.171, respectively.

Tables

Table 8.1. Analyzed composition of ingredients

Item	Corn	Soybean meal	Lactose	Calcium carbonate	Monocalcium phosphate
Gross energy, kcal /kg	3,829	4,193	3,591	-	-
Dry matter, %	85.53	88.27	94.96	99.99	93.88
Ash, %	1.70	7.47	0.30	89.68	81.35
Crude protein, %	5.91	45.60	-	-	-
AEE[1], %	3.86	1.63	-	-	-
Ca, %	0.04	0.28	0.02	38.93	17.26
P, %	0.25	0.59	0.01	0.03	20.46
Phytate[2], %	0.63	1.54	-	-	-
Phytate-bound P, %	0.18	0.43	-	-	-
Non-phytate P[3], %	0.07	0.16	-	-	-

[1]AEE = acid hydrolyzed ether extract.

[2]Phytate was calculated by dividing the concentration of phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 8.2. Ingredient composition and analyzed values of experimental diets[1]

Item	Phytase units per kilogram feed (FTU)					
	0	250	500	1,000	2,000	4,000
Ingredient, %						
Ground corn	49.70	49.695	49.69	49.68	49.66	49.62
Soybean meal, 48% CP	35.00	35.00	35.00	35.00	35.00	35.00
Lactose	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	2.30	2.30	2.30	2.30	2.30	2.30
Calcium carbonate	0.95	0.95	0.95	0.95	0.95	0.95
Monocalcium phosphate	0.30	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.32	0.32	0.32	0.32	0.32	0.32
L-Lys HCL, 78% Lys	0.28	0.28	0.28	0.28	0.28	0.28
DL-Met	0.12	0.12	0.12	0.12	0.12	0.12
L-Thr	0.08	0.08	0.08	0.08	0.08	0.08
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Chromium oxide	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[3]	-	0.005	0.01	0.02	0.04	0.08
Total	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed values						
Gross energy, kcal/kg	3,987	3,956	4,003	3,976	3,991	3,995
Dry matter, %	87.95	87.86	87.95	87.81	87.78	87.75
Ash, %	5.35	5.04	5.06	5.22	5.27	5.28
Crude protein, %	19.42	19.59	19.40	19.07	19.72	20.16
AEE,[4] %	3.72	3.77	3.96	3.74	3.83	3.93
Amino acids, %						
Arg	1.38	1.30	1.32	1.29	1.28	1.34
His	0.55	0.52	0.53	0.52	0.51	0.53
Ile	0.95	0.91	0.91	0.89	0.87	0.91
Leu	1.71	1.64	1.68	1.65	1.61	1.68
Lys	1.45	1.34	1.36	1.36	1.33	1.36
Met	0.44	0.37	0.40	0.41	0.37	0.44
Phe	1.02	1.01	0.99	0.97	0.95	1.03

Table 8.2. (Cont.)

Thr	0.86	0.81	0.79	0.81	0.80	0.83
Trp	0.28	0.26	0.27	0.26	0.27	0.27
Val	1.04	0.98	0.99	0.97	0.96	0.99
Ca, %	0.58	0.56	0.55	0.55	0.57	0.60
P, %	0.43	0.44	0.42	0.42	0.42	0.43
Phytate bound-P, %	0.26	0.25	0.24	0.24	0.23	0.21
Phytase activity, FTU	< 50	417	715	1,680	2,760	5,350
Phytate esters[5], nmol/g dry matter						
IP6	15,225	12,920	13,732	15,242	14,540	12,005
IP5	1,964	1,831	1,851	2,029	1,790	1,700
IP4	244	228	275	273	282	444

[1]Diets were formulated to contain the following quantities of net energy, amino acids (expressed as standardized ileal digestible), Ca, and P: net energy, 2,473 kcal/kg; Lys, 1.23%; Met, 0.40%; Thr, 0.74%; Trp, 0.23%; Ca, 0.54%; P, 0.44%.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganoussulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[3]The phytase concentrate contained 5,000 units of phytase/g (Quantum Blue, AB Vista, Marlborough, UK).

[4]AEE = acid hydrolyzed ether extract.

[5]IP = inositol phosphate. The concentrations of IP3 and inositol in the experimental diets were undetectable.

Table 8.3. Apparent ileal digestibility of crude protein (CP) and amino acids (AA) in diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase per kilogram of feed (FTU) fed to pigs[1]

Item, %	Phytase, FTU							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
CP	70.6	73.2	75.7	75.6	77.9	76.4	1.66	0.030	0.026
Indispensable AA									
Arg	89.5	89.5	90.2	90.1	90.3	90.4	0.61	0.287	0.505
His	79.4	80.3	82.7	82.4	82.6	81.2	1.32	0.544	0.087
Ile	80.5	80.4	82.2	82.6	83.1	82.5	1.18	0.190	0.161
Leu	80.3	80.1	82.3	82.6	82.5	81.7	1.30	0.463	0.174
Lys	82.7	84.7	86.2	85.8	85.7	88.1	1.10	0.008	0.593
Met	88.9	87.5	88.6	89.2	89.2	89.8	0.78	0.076	0.803
Phe	81.8	80.7	82.2	82.6	83.0	83.2	0.96	0.119	0.455
Thr	70.3	71.1	73.1	74.8	76.2	75.1	1.73	0.033	0.058
Trp	78.1	81.0	83.2	84.0	86.1	83.8	1.37	0.011	0.002
Val	76.4	76.7	79.0	79.3	80.1	78.8	1.50	0.241	0.103
Mean	80.8	81.3	83.2	83.4	83.8	83.1	1.19	0.196	0.116
Dispensable AA									
Ala	74.3	74.1	77.7	77.4	77.8	77.4	1.53	0.151	0.153
Asp	74.6	74.8	78.3	78.1	79.1	78.7	1.43	0.041	0.076
Cys	53.4	54.2	60.2	57.1	60.8	59.1	3.19	0.211	0.234
Glu	78.0	78.3	82.5	79.6	81.0	80.6	1.76	0.412	0.406
Gly	58.9	62.5	68.5	66.5	67.3	67.3	2.53	0.079	0.093
Ser	75.6	76.6	79.2	80.0	81.0	79.7	1.53	0.061	0.035
Tyr	81.3	80.4	82.2	82.6	83.1	83.6	1.07	0.050	0.405
Mean	74.1	74.9	78.7	77.4	78.5	78.1	1.61	0.124	0.141
All AA	77.4	78.1	81.0	80.4	81.1	80.6	1.36	0.143	0.121

[1]Data are least square means of 5 or 6 observations.

Table 8.4. Digestibility of gross energy (GE) and minerals in diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase per kilogram of feed (FTU) fed to pigs[1]

Item, %	Phytase, FTU							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Apparent ileal digestibility									
Ca	61.6	74.2	76.7	77.1	80.7	76.8	1.99	0.001	< 0.001
P	27.1	45.1	56.7	63.0	75.5	80.0	1.43	< 0.001	< 0.001
K	81.1	83.6	85.1	83.8	87.0	86.7	1.18	0.003	0.072
Apparent total tract digestibility									
GE	82.7	83.2	84.3	82.2	83.9	81.8	1.27	0.415	0.439
Ca	57.9	66.3	73.4	72.8	79.9	75.7	3.09	< 0.001	< 0.001
P	29.9	45.5	54.4	63.8	74.3	78.3	3.07	< 0.001	< 0.001
K	79.4	83.3	85.2	87.0	89.5	88.2	1.46	< 0.001	< 0.001
Mg	21.4	18.1	21.5	24.7	27.9	26.6	4.56	0.135	0.333
Na	83.3	82.5	83.5	84.3	85.7	89.7	2.54	0.035	0.840

[1]Data are least square means of 5 or 6 observations.

Table 8.5. Comparison between values for ileal and total tract digestibility of Ca, P, and K in diets fed to pigs[1]

Item	Digestibility, %		Difference	SEM	*P*-value
	Ileal	Total tract			
Ca	74.4	70.4	4.11	1.28	0.003
P	57.8	57.1	0.44	1.04	0.675
K	84.6	85.4	- 0.79	0.71	0.275

[1]Data are least square means of 33, 32, and 35 observations for Ca, P, and K, respectively.

Table 8.6. Concentration of Ca, P, and inositol in plasma and quantity and concentration of bone ash in pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase per kilogram of feed (FTU)[1]

Item	Phytase, FTU							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
d -1									
Plasma									
Ca, mg/dL	9.87	9.78	9.88	9.53	9.80	10.00	-	-	-
P, mg/dL	10.32	10.23	9.53	9.82	10.48	10.53	-	-	-
Inositol, μM	8.5	6.8	7.3	11.5	10.5	7.2	-	-	-
d 23									
Plasma[2]									
Ca, mg/dL	11.10	10.84	11.35	10.94	11.28	11.75	0.49	0.233	0.762
P, mg/dL	9.89	12.16	13.64	14.27	15.49	16.28	0.51	< 0.001	< 0.001
Inositol, μM	80.7	81.1	97.4	86.4	103.0	112.1	10.9	0.024	0.716
Bone									
Ash, g	1.88	2.48	2.61	2.92	3.34	3.35	0.09	< 0.001	< 0.001
Ash, %	48.3	50.4	53.1	53.9	55.2	55.5	0.60	< 0.001	< 0.001

[1]Data are least square means of 6 observations.

[2]Data from d -1 were used as a covariate for data obtained on d 23.

Table 8.7. Inositol phosphate (IP) esters and inositol (nmol/g dry matter) in ileal digesta and fecal samples from pigs fed diets containing 0, 250, 500, 1,000, 2,000, and 4,000 units of microbial phytase per kilogram of feed (FTU) fed to pigs[1]

Item	Phytase, FTU							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Ileal digesta samples									
IP6	39,298	18,147	7,546	4,460	2,136	1,474	1,597	< 0.001	< 0.001
IP5	5,223	3,822	1,703	1,006	334	225	522	< 0.001	< 0.001
IP4	1,253	7,312	5,749	5,700	2,580	822	1,182	0.003	0.111
IP3	727	3,569	2,541	2,618	1,109	543	583	0.005	0.292
Inositol	173	961	1,990	2,808	4,026	6,458	570	< 0.001	0.135
Fecal samples[2]									
IP6	775	509	606	968	670	741	136	0.683	0.641
IP5	149	73	59	97	58	48	24	0.041	0.295
IP4	24	33	58	62	31	26	10	0.258	0.076

[1]Data are least square means of 6 observations.

[2]The concentrations of IP3 and inositol in all samples were undetectable.

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Chapter 9: The apparent digestibility of energy and nutrients and the efficiency of dietary phytase to degrade phytate is influenced by body weight of pigs

Abstract

An experiment was conducted to test the hypothesis that regardless of pig body weight (**BW**), increasing dietary phytase results in increased phytate degradation and improved apparent digestibility of minerals, amino acid (**AA**), and energy. Eighteen pigs were equipped with a T-cannula in the distal ileum and allotted to a triplicated 6 × 3 Youden square design with 6 diets and 3 collection periods of 7 d, for a total of 9 replicate pigs per diet. This design was repeated 4 times to simulate 4 production phases, and there was a 7-d resting period before each collection phase started (BW at start of collection: 29.3, 53.6, 85.1, and 114.4 kg for phases 1 to 4, respectively). Corn and soybean-meal-based diets were formulated by including 0, 250, 500, 1,000, 2,000, or 4,000 phytase units/kg feed (**FTU**), and phytase was assumed to release 0.16% total Ca and 0.11% digestible P. The same 6 diets were used throughout the experiment. Fecal (d 5) and ileal digesta (d 6 and 7) samples were collected in each collection period. Results indicated that regardless of pig BW, increasing inclusion of phytase increased (quadratic; $P < 0.05$) apparent ileal digestibility (**AID**) of crude protein (**CP**) and most AA, increased apparent total tract digestibility (**ATTD**) of Ca, P, K, Mg (quadratic; $P < 0.05$), and Na (linear; $P < 0.05$), but decreased (quadratic; $P < 0.05$) ATTD of gross energy (**GE**). In all phases, ileal concentrations of inositol phosphate (**IP**) 6, IP5, IP4, and IP3 decreased (quadratic; $P < 0.05$), whereas ileal inositol increased (quadratic; $P < 0.05$) with increasing dietary phytase. However,

as pig BW increased, AID of CP and AA increased (linear or quadratic; $P < 0.05$), and ATTD of GE, K, and Mg also increased (quadratic; $P < 0.05$), but ATTD of Ca and Na (linear; $P < 0.05$) and of P (quadratic; $P < 0.05$) decreased. Ileal IP6 and IP3 (quadratic; $P < 0.05$) and ileal IP5 and IP4 (linear; $P < 0.05$) increased, whereas ileal inositol decreased (linear; $P < 0.05$) as pig BW increased. In conclusion, regardless of pig BW, increasing dietary phytase increased phytate degradation and inositol release in the small intestine, and consequently increased mineral and AA digestibility. Older pigs have reduced Ca, P, and Na digestibility, but increased K, Mg, AA and GE digestibility compared with younger pigs. The efficiency of dietary phytase to degrade phytate appears to decrease as pigs get older.

Key words: body weight, nutrient digestibility, energy, phytase, phytate degradation, pigs

Abbreviations

AEE	acid hydrolyzed ether extract
AA	amino acids
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
ADFI	average daily feed intake
ADG	average daily gain
CP	crude protein
FTU	phytase units per kilogram of feed
G:F	gain to feed ratio
GE	gross energy
IP	inositol phosphate

Introduction

Phytic acid (*myo*-inositol-hexakis dihydrogen phosphate) or its salt, phytate, is the main form of P storage in feed ingredients from plant origin and a potential P source for pigs. However, because pigs have limited phytase activity in the small intestine, use of microbial phytase at approximately 500 units per kilogram of feed (**FTU**) is common in commercial diets. Phytase releases some of the phytate-bound P in plant ingredients, reduces the need for inorganic P in the diet, and consequently decreases the amount of P excreted in the manure (Adeola and Cowieson, 2011). At intestinal pH, phytate carries a strong negative charge that allows for chelation of cations such as Ca and binding of protein, and the quantity of phytase needed to release all chelated nutrients is greater than 500 FTU (Wilcock and Walk, 2016). Inclusion of more than 500 FTU of phytase, also known as super-dosing, may result in extra-phosphoric effects as phytate is degraded to lower inositol phosphate (**IP**) esters with less capacity for interaction with minerals and amino acids (**AA**; Bedford and Walk, 2016). Indeed, in broiler chickens, inclusion of 1,000 to 2,000 FTU of phytase resulted in increased mineral and AA digestibility (Brenes et al., 2003; Cowieson et al., 2017a). In pigs, phytase increases digestibility of Ca and other minerals (She et al., 2015; 2018), but AA digestibility has not consistently been improved by phytase (Zeng et al., 2016; Mesina et al., 2019).

In pig experiments, the effect of increasing levels of phytase on phytate degradation and nutrient digestibility is usually evaluated using a maximum inclusion level of phytase of 3,000 FTU (Liao et al., 2005; Zeng et al., 2016; Mesina et al., 2019). Likewise, pig body weight (**BW**) usually ranges from 20 to 40 kg, and to our knowledge, no data for the effect of super-dosing of phytase on phytate degradation and nutrient digestibility over time are available. As a consequence, it is not known if results of super-dosing phytase in 20- to 40-kg pigs can be

extrapolated to the entire growing-finishing period. Therefore, the objective of this experiment was to test the hypothesis that increasing inclusion levels of phytase from 0 to 4,000 FTU results in increased phytate degradation and improved apparent digestibility of minerals, AA, and energy in diets regardless of pig BW.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Animals and housing

Eighteen growing pigs were equipped with a T-cannula (6 cm length and 2.24 cm inner diameter) in the distal ileum, and after 3 d of recovery, pigs (average BW: 24.0 ± 2.4 kg) were allotted to a triplicated 6 × 3 Youden square design with 6 diets and 3 collection periods of 7 d, for a total of 9 replicate pigs per diet. This design was repeated 4 times to simulate 4 production phases (24.0 to 46.8 kg, 46.8 to 76.1 kg, 76.1 to 106.3 kg, and 106.3 to 132.4 kg), and within each phase, there was a resting period of 7 d before sample collection started. Therefore, the experiment was conducted over 16 weeks and pigs had an average BW of 29.3, 53.6, 85.1, and 114.4 kg at the start of collection for phases 1, 2, 3, and 4, respectively. Pigs were housed individually in 1.2 × 1.5 m pens that were equipped with a feeder and a nipple drinker in an environmentally controlled room. Pens had smooth sides and fully slatted tri-bar floors. Water was available at all times throughout the experiment. A spreadsheet program for making a balanced Latin square design (Kim and Stein, 2009) was used to allot pigs to experimental diets.

Diets and feeding

Pigs were allowed *ad libitum* access to feed throughout the experiment. Six diets based on corn and soybean meal (Table 9.1) were formulated based on estimated nutrient requirements for 50- to 75-kg pigs (NRC, 2012). Diets included 0, 250, 500, 1,000, 2,000, or 4,000 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK), and no feed phosphate was included in the diets. Provisions of total Ca and standardized total tract digestible P were reduced by 0.16 and 0.11%, respectively, compared with the requirement (NRC, 2012) to account for the expected release of Ca and P by phytase. All diets also contained 0.40% titanium dioxide as an indigestible marker. For the 7-d resting period, a common diet without phytase was formulated to meet estimated requirements for pigs from 50 to 75 kg (NRC, 2012). Therefore, a total of 7 diets were formulated (Table 9.2).

Sample collection

Ingredient samples were collected at the feed mill after mixing, and each diet sample was a mix of samples collected from 10 randomly chosen feed bags of 25 kg. Samples were later ground and sub-sampled for nutrient analysis. Pigs were weighed weekly and the amount of feed offered was recorded daily. Within each production phase, the initial 4 d of each collection period were considered an adaptation period to the diet. Feces samples were collected in the morning of d 5 via anal stimulation, and ileal digesta samples were collected for 8 h on d 6 and 7. All samples were stored at −20 °C immediately after collection. At the end of each collection period, pigs were deprived of feed overnight, and a new experimental diet was offered the following morning. At the conclusion of each phase, fecal and ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized (Lagos and Stein, 2019) and finely ground.

Sample analysis

Ingredients and diets were analyzed for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2019) and for ash at 600 °C for 2 h (Method 942.05; AOAC Int., 2019). Corn, soybean meal, limestone, diets, and fecal samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2019) after dry ash preparation (Method 942.05; AOAC Int., 2019) followed by wet digestion with nitric acid (Method 3050 B; US-EPA, 2000). Diets and feces were also analyzed for K, Mg, and Na via inductively coupled plasma-optical emission spectrometry. The concentration of AA was analyzed in diet and ileal digesta samples (Method 982.30 E [a, b, c]; AOAC Int., 2019) using a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA). Corn, soybean meal, diets, and ileal digesta samples were also analyzed for N using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA) and crude protein (**CP**) was then calculated as N × 6.25. Corn, soybean meal, diets, and fecal samples were analyzed for gross energy (**GE**) using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Corn, soybean meal, and diets were also analyzed for acid hydrolyzed ether extract (**AEE**; Method 2003.06; AOAC Int., 2019) using an Ankom^{HCl} followed by an Ankom^{XT15} (Ankom Technology, Macedon, NY, USA). Diets, fecal, and ileal digesta samples were analyzed for IP esters and inositol using high-performance ion chromatography-based techniques as described by Walk et al. (2018). These samples were also analyzed for Ti following the procedure by Myers et al. (2004). Corn, soybean meal, and diets were also analyzed for phytate-bound P by wet chemistry using the Megazyme kit (Megazyme Inc., Chicago, IL, USA). Phytase activity was analyzed in

diets by the enzyme-linked immunosorbent assay method using Quantiplate Kits for Quantum Blue (AB Vista, Plantation, FL, USA).

Calculations and statistical analyses

Average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) were calculated for each productive phase. The percentage of phytate in corn and soybean meal was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. The apparent ileal digestibility (**AID**) of CP, AA, and IP6 and the apparent total tract digestibility (**ATTD**) of energy and macro minerals (Ca, P, K, Mg, and Na) in experimental diets were calculated as described by Stein et al. (2007):

$$\text{Digestibility of nutrients, \%} = \left[1 - \left(\frac{\text{nutrient in sample}}{\text{nutrient in diet}}\right) \times \left(\frac{\text{marker in diet}}{\text{marker in sample}}\right)\right] \times 100$$

where digestibility is the AID or ATTD of nutrients or energy, nutrient in sample is the nutrient or energy concentration in ileal digesta or fecal samples and nutrient in diet is the concentration of nutrient or energy in the diet. Marker in diet and marker in sample are the concentrations of Ti in diets and ileal digesta or feces samples, respectively.

Normality of residuals and homogeneity of variances were tested using the INFLUENCE, GPLOT, and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC, USA). Data for phytate degradation and digestibility of minerals, phytate, AA, and energy were analyzed using the PROC MIXED procedure of SAS with pig as the experimental unit. The initial model included the main effects of phytase level and phase and the interaction between phytase level and phase, and the random effects of period within phase and pig. If the interaction was significant, contrast statements were used to determine linear and quadratic effects of phytase level at each phase. Coefficients for unevenly spaced linear contrasts were obtained using

the PROC IML procedure of SAS. Likewise, if the effect of phase was significant, contrast statements were used to determine linear and quadratic effects of phase. However, if the interaction between phytase level and phase was not significant, only main effects were included in the final model, and contrast statements were used to determine linear and quadratic effects of phytase level and phase. Outliers were determined by plotting the residuals in a quantile-quantile plot against the normal distribution and identifying values that were beyond ± 3.0 standard deviations. Treatment means were calculated using the LSMEANS statement in SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

During the 3rd phase, 1 pig was removed from the experiment by the end of the second collection period due to poor condition. This pig was replaced by the extra pig that was assigned to the experiment. This new animal and the other 17 pigs consumed their assigned diets without apparent problems and no severe health problems were observed.

During phase 1, there was a linear increase ($P < 0.05$) in ADG and G:F of pigs as phytase inclusion increased in the diets (Table 9.3). Likewise, during phase 2, ADG of pigs linearly increased ($P < 0.05$) with increasing levels of dietary phytase. A tendency ($P < 0.10$) for a linear increase in G:F of pigs as the inclusion of phytase in diets increased was also observed during phase 3. In phase 4, there was a tendency for a decrease (quadratic; $P < 0.10$) in ADFI of pigs as the inclusion of dietary phytase increased.

There was no interaction between phytase inclusion level and phase for the AID of CP and AA in diets, therefore, the final model only included the main effects of phytase inclusion level and phase (Table 9.4). The AID of CP and all AA except Gly and His increased (quadratic;

$P < 0.05$) as the inclusion of phytase in diets increased. The AID of Gly tended to increase (quadratic; $P < 0.05$) with increasing dietary phytase, but there was a linear reduction ($P < 0.05$) in the AID of His as phytase inclusion in diets increased.

The AID of CP linearly increased ($P < 0.05$) and tended ($P < 0.10$) to have a quadratic increase with increasing BW of pigs. Similarly, there was an increase (quadratic; $P < 0.05$) in the AID of Met, Phe, Thr, Trp, Ala, Asp, Gly, and Ser as pig BW increased and increasing BW resulted in a linear increase ($P < 0.05$) in the AID of all other AA.

The final model for the ATTD of GE and macro minerals only included the main effects of phytase inclusion level and phase because no interactions between main effects were observed (Table 9.5). The ATTD of GE decreased (quadratic; $P < 0.05$), whereas the ATTD of Ca, P, K, and Mg increased (quadratic; $P < 0.05$) with increasing dietary phytase. Likewise, there was a positive linear effect ($P < 0.05$) of phytase level on the ATTD of Na in diets. The ATTD of GE, K, and Mg increased (quadratic; $P < 0.05$) as BW of pigs increased. In contrast, the ATTD of Ca and Na decreased (linear; $P < 0.05$) as pig BW increased, and there was a reduction (quadratic; $P < 0.05$) in the ATTD of P with increasing BW of pigs.

Regardless of phase, the AID of IP6 increased as increasing levels of phytase were added to the diets, but the increase was greater in phase 1 than in subsequent phases (interaction, $P < 0.05$; Table 9.6). Reductions in concentrations of IP6, IP5, IP4, and IP3 in ileal digesta were observed as levels of dietary phytase increased, but the magnitude of the responses diminished from phase 1 to phase 4 (interaction, $P < 0.05$; Table 9.7). Likewise, ileal concentrations of inositol increased more with increasing dietary phytase in phase 1 than in subsequent phases (interaction, $P < 0.05$). In phase 1, concentrations of IP6 and IP5 in ileal digesta decreased (quadratic; $P < 0.05$) as the inclusion of phytase increased from 0 to 4,000 FTU. There was also

a decrease in the concentration of IP4 (linear; $P < 0.05$), but an increase and then a decrease in the concentration of IP3 (quadratic; $P < 0.05$) in ileal digesta as phytase inclusion increased in the diet. The concentration of inositol in ileal digesta increased (quadratic; $P < 0.05$) in phase 1 with increasing inclusion of dietary phytase. In phase 2, concentrations of IP6 and IP5 decreased (quadratic; $P < 0.05$), whereas concentrations of IP4 and IP3 increased and then decreased (quadratic; $P < 0.05$) in ileal digesta as phytase inclusion increased from 0 to 4,000 FTU. The concentration of inositol in ileal digesta in phase 2 increased (quadratic; $P < 0.05$) as the inclusion of phytase increased. In phases 3 and 4, the concentration of IP6 decreased (quadratic; $P < 0.05$) and the concentrations of IP5, IP4, and IP3 increased and then decreased (quadratic; $P < 0.05$) in ileal digesta as the inclusion of dietary phytase increased. The ileal digesta concentration of inositol also increased (quadratic; $P < 0.05$) in phases 3 and 4 with increasing inclusion levels of phytase.

Fecal concentrations of IP6 were reduced by increasing concentrations of dietary phytase, but the effect of low doses of phytase on fecal IP6 concentration was more severe in phases 3 and 4 than in phases 1 and 2 (interaction, $P < 0.05$; Table 9.8). Fecal concentration of IP5 was not affected by dietary phytase in phase 1, but decreased with dietary phytase in phases 2, 3, and 4 (interaction, $P < 0.05$). In contrast, IP4 in feces increased with dietary phytase in phases 1, 3, and 4, but that was not the case in phase 2 (interaction, $P < 0.05$). In phase 1, the concentration of IP6 in feces linearly decreased ($P < 0.05$), whereas the concentration of fecal IP4 increased and then decreased (linear: $P < 0.05$; quadratic: $P < 0.10$) as phytase inclusion increased. In phase 2, there was a reduction in fecal concentrations of IP6 (linear; $P < 0.05$) and IP5 (linear: $P < 0.05$; quadratic: $P < 0.10$) as the inclusion of phytase increased. However, phytase did not influence fecal concentrations of IP5 in phase 1 or IP4 in phase 2. In phase 3, the concentration

of IP6 in fecal samples tended to decrease (quadratic; $P < 0.10$) with increasing dietary phytase, and there was a reduction in the concentration of IP5 (linear: $P < 0.05$; quadratic: $P < 0.10$) and IP4 (linear; $P < 0.05$) in fecal samples as the inclusion of phytase increased. In phase 4, fecal concentrations of IP6 and IP5 decreased (quadratic; $P < 0.05$), whereas fecal concentrations of IP4 increased (quadratic; $P < 0.05$) as phytase inclusion increased. Concentrations of IP3 and inositol in all fecal samples were below detection limits.

The AID of IP6 decreased (quadratic; $P < 0.05$) with increasing BW of pigs (Table 9). There was an increase in concentrations of IP6 (quadratic; $P < 0.05$), IP5 (linear; $P < 0.05$), IP4 (linear: $P < 0.05$; quadratic: $P < 0.10$), and IP3 (quadratic; $P < 0.05$) in ileal digesta as BW of pigs increased. However, the concentration of inositol in ileal digesta linearly decreased ($P < 0.05$) with increasing BW of pigs. In fecal samples, concentrations of IP6 (quadratic; $P < 0.05$), IP5 (linear; $P < 0.05$), and IP4 (quadratic; $P < 0.05$) also increased as pigs BW increased.

Discussion

The analyzed values for Ca, P, and phytate in corn, soybean meal, and limestone were close to those used in diet formulation (NRC, 2012), thus, the reason Ca concentration in diets was slightly greater than expected is likely the feed particle segregation that results in increased variability in analytical values for Ca compared with P (Jones et al., 2018). Because diets were formulated to meet NRC (2012) requirements for pigs between 50 and 75 kg, and the same diets were used throughout the experiment, pigs were fed nutrient-deficient diets in phase 1, but during phases 3 and 4, nutrient concentrations in diets were above the requirement. The observation that increasing dietary phytase resulted in improved growth performance of pigs during phases 1 and 2, but not in phases 3 and 4 is in agreement with Holloway et al. (2019) who

reported that super-dosing of phytase provided smaller benefits in growing-finishing pigs than in nursery pigs. However, this observation may also be a consequence of diets being formulated below or at the requirement for Ca and P in phases 1 and 2, but not in phases 3 and 4. The increased release of Ca and P from elevated levels of phytase may, therefore, have contributed to pigs absorbing Ca and P closer to the requirement as dietary phytase increased. In contrast, in phases 3 and 4, diets contained more Ca and P than required and the increased release of Ca and P from the greater levels of phytase did not provide additional benefits to the pigs.

The lack of an interaction between phytase inclusion level and phase for AA, mineral, and energy digestibility, indicates that the effect of phytase on nutrient digestibility is independent of pig BW. The quadratic increase in AID of most AA as the inclusion of dietary phytase increased is in agreement with Cowieson et al. (2017b) and Zouaoui et al. (2018) who reported a positive effect of microbial phytase on AA digestibility from a review of approximately 30 publications. The observation that phytase inclusion at 2,000 FTU resulted in a 2.0 percentage unit increase in AID of all AA is within the range of 1.7 to 2.8 percentage unit increase observed upon phytase supplementation (Selle and Ravindran, 2008; Cowieson et al., 2017b; Zouaoui et al., 2018). The linear reduction in the AID of His as dietary phytase increased was not expected, but a reduced median response in His digestibility to phytase compared with other AA, has been previously reported (Selle and Ravindran, 2008). Nevertheless, results from this experiment are in contrast with several experiments indicating a lack of phytase effect on AID of most or all AA (Liao et al., 2005; She et al., 2018; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a). The reason for this observation is likely the low number of replicates (i.e., 6 to 8) used in AA experiments, which results in a high standard error and a reduced capacity to detect

statistical differences. There were between 34 and 36 replicate pigs per diet in this experiment; therefore, a greater sample size may be necessary to validate effects of phytase on AID of AA.

The observed increase in AID of AA as BW of pigs increased is in contrast with data from Pedersen et al. (2016) who reported no differences in the standardized ileal digestibility of AA in soybean meal between 20- to 50-kg pigs and pigs above 50-kg pigs. However, gestating sows had increased AID of most AA in corn and soybean meal compared with growing pigs (Stein et al., 1999). Thus, the 3.2 percentage unit increase in the AID of indispensable AA in finishing pigs compared with growing pigs indicates that in diets for finishing pigs, less soybean meal is needed, which may result in reduced production costs. However, it is not known if the reason for the increased AID of AA in finishing pigs is related to reduced endogenous secretion of AA or increased efficiency of AA absorption.

The observation that increasing dietary phytase resulted in reduced ATTD of GE is in agreement with Mesina et al. (2019), but in contrast with Velayudhan et al. (2015) and Arredondo et al. (2019) who reported an increase in ATTD of GE with increasing inclusion of phytase. Results of other experiments indicated that phytase supplementation did not influence the ATTD of GE (Liao et al., 2005; She et al., 2018; Rosenfelder-Kuon et al., 2020a). The ability of phytate to bind glucose, starch, and amylase is the reason phytase supplementation may result in increased energy digestibility (Woyengo and Nyachoti, 2013). Therefore, the reason for the discrepancy in results among experiments is not clear.

The increased ATTD of Ca and P upon phytase supplementation was anticipated as the positive correlation between phytase inclusion and Ca and P digestibility is well documented (Velayudhan et al., 2019; Rosenfelder-Kuon et al., 2020b). Likewise, the positive effect of phytase on the ATTD of K and Mg concurs with previous data from pigs (Velayudhan et al.,

2015; She et al., 2018; Arredondo et al., 2019), and is a result of phytate in plants being complexed with Ca, K, and Mg (Angel et al., 2002). As in this experiment, a linear increase in ATTD of Na by increasing dietary phytase has previously been observed (Arredondo et al., 2019) and is likely a result of reduced endogenous secretion of Na into the small intestine in response to reduced presence of phytate in the lumen of the small intestine as dietary phytase increased (Woyengo et al., 2009). However, an increased ATTD of Na in response to phytase has not always been observed (Velayudhan et al., 2015; She et al., 2018).

The observation that the ATTD of GE increased as BW of pigs increased concurs with data indicating that the concentration of digestible energy and the ATTD of GE in sows is greater than in growing pigs, regardless of the level of feed intake (Le Goff and Noblet, 2001; Lowell et al., 2015; Casas and Stein, 2017). The reason for this observation needs to be elucidated because it is not a result of a greater fermentation capacity in the hindgut of sows than of growing pigs (Lowell et al., 2015; Casas and Stein, 2017). Nevertheless, data from this experiment indicate that BW of pigs influences the concentration of digestible energy in diets.

The reduced ATTD of Ca and P observed with increasing BW of pigs is in contrast with Kemme et al. (1997), but in agreement with Sulabo et al. (2004) who indicated a linear decrease in AID and ATTD of P as BW of pigs increased from 40 to 130 kg. This observation may be a consequence of providing Ca and P in excess of the requirement in phases 3 and 4, because the transcellular absorption of these minerals is activated by hormonal regulation as a result of low concentrations in plasma, and thus, high concentrations of dietary and plasma Ca and P results in negative feedback (Schröder et al., 1996; Crenshaw, 2001). However, high concentrations of Ca in diets also increases paracellular absorption of Ca (Lagos et al., 2019), which usually results in a lack of an effect of dietary Ca on the ATTD of Ca (Stein et al., 2011; González-Vega et al.,

2014). Excess dietary Ca and P may also result in increased ATTD of Ca and P, respectively (González-Vega et al., 2013; 2016; Liu et al., 2018). Therefore, it is not possible to conclude that the reason ATTD of Ca and P decreased with increasing BW is a consequence of using the same diets throughout the experiment. Although the reason remains to be elucidated, it is possible that the digestibility of Ca and P decreases as pigs get older as indicated by the reduced digestibility of these 2 minerals in gestating sows compared with growing pigs (Kemme et al., 1997; Lee et al., 2018a; 2018b). Therefore, the use of average digestibility values for Ca and P in diets during the growing-finishing phase may result in Ca- and P-deficient diets being fed to finishing pigs. To our knowledge, there are no available data about the effect of BW or physiological status on the ATTD of K, Mg, and Na. Therefore, more research is needed to validate the results observed in the present experiment and to elucidate the reason for the increased digestibility of K and Mg, but reduced digestibility of Na, with increasing BW of pigs.

The observation that regardless of phase, increasing dietary phytase resulted in increased AID of IP6, reduced concentrations of IP esters in ileal digesta, and increased inositol concentration in ileal digesta, indicates that regardless of BW, phytase degrades phytate and IP esters in the stomach and (or) small intestine of pigs. The decrease in IP6 and IP5, but increase and then decrease in IP4 and IP3 observed in phase 1 as phytase in diets increased is in agreement with data from pigs between 17 and 38 kg (Laird et al., 2018; Lu et al., 2019; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a). This observation reflects the progressive degradation of phytate into lower esters and inositol, and the further degradation of lower esters into inositol as inclusion of dietary phytase increased. Thus, the observed interaction between phytase inclusion level and phase for ileal degradation of phytate indicates that the efficiency of phytase to degrade phytate is dependent on BW of pigs. The efficiency of phytase appears to be

reduced in older pigs compared with young pigs as indicated by the AID of IP6 that surpassed 90% upon supplementation of 250 FTU of phytase in phase 1, whereas for phases 2 to 4, more than 1,000 FTU were needed to degrade 90% of IP6.

A less efficient IP ester degradation by phytase was also observed in finishing pigs compared with growing pigs as indicated by the lack of a reduction in ileal IP5 at 250 FTU of phytase in phase 2, and a built-up of IP5 at the same phytase dosage in phases 3 and 4, compared with the control diet. The reduced phytase efficiency by increasing BW of pigs is also indicated by the increased accumulation of IP4 and IP3 in ileal digesta at 250 and 500 FTU of phytase in the finishing phases compared with the growing phases. The implication of this observation is that a greater amount of phytase is needed to decrease the concentration of ileal IP4 and IP3 in the later phases than in the earlier phases. Results for ileal inositol concentration are aligned with the response of IP ester concentrations in ileal digesta to dietary phytase at each phase, and indicate that in older pigs, the amount of dietary phytase required to fully degrade phytate is greater than in young pigs. These data concur with results for growth performance from this experiment and from Holloway et al. (2019) indicating a limited effect of phytase on growth performance of finishing pigs compared with growing pigs.

The lack of a clear pattern within and among phases for the response of fecal concentrations of IP6, IP5, and IP4 to phytase supplementation indicates that dietary phytase has a limited impact on phytate degradation in the hindgut. This observation and the reduced concentration of IP esters in feces compared with ileal digesta is in agreement with Mesina et al. (2019) and indicates that there is phytate degradation in the large intestine of pigs by phytase synthesized by hindgut microbes (Selle et al., 2010). This, however, has no impact on the nutritional status of pigs because there is no net absorption of Ca or P in the large intestine of

pigs (González-Vega et al., 2014). Likewise, the absence of inositol in feces is likely due to microbial metabolism of inositol in the hindgut.

The observation that regardless of phytase dosage, there was a reduced AID of IP6 and an increased concentration of IP esters in ileal digesta as pigs grow, concurs with the reduced ATTD of Ca and P observed with increasing BW of pigs. Therefore, it appears that as pigs get older, the digestibility of Ca and P decreases and the efficiency of phytase to degrade phytate and release Ca and P also decreases. This hypothesis is supported by data from gestating sows indicating that inclusion of phytase at 500 FTU did not improve the digestibility of Ca or P (Lee et al., 2019). The reduction in ileal digesta concentration of inositol as BW of pigs increased may be a consequence of the reduced phytase efficiency in degrading phytate as pig BW increased, but it is also possible that older pigs have increased inositol absorption compared with young pigs. Humans are believed to absorb inositol with high efficiency because 99.8% of free inositol is absorbed in the intestinal tract (Huber, 2016). Likewise, the increased concentration of IP6 and IP5 in feces with increasing BW of pigs may indicate a reduced capacity for phytate degradation in the hindgut as pigs grow, or it may be the result of a greater amount of IP esters entering the hindgut from the small intestine. To our knowledge, no research has been conducted to evaluate the effect of phytase supplementation on phytate degradation at different production phases. Therefore, research is needed to verify these results and to elucidate the reason BW of pigs influences the efficiency of phytase to degrade phytate.

Conclusions

Increasing inclusion of phytase resulted in increased AA and macro-mineral digestibility, but reduced energy digestibility, regardless of BW of pigs. However, the efficiency of phytase to

degrade phytate and phytate esters into inositol in the small intestine of pigs decreased as pig BW increased. Considerable phytate degradation takes place in the large intestine of pigs due to phytase synthesized by hindgut microbes, but independently of dietary phytase. Older pigs had increased energy, AA, K, and Mg digestibility and reduced Ca, P, and Na digestibility compared with younger pigs. Finishing pigs also had increased concentrations of IP esters in ileal digesta and feces, but reduced inositol concentration in ileal digesta compared with growing pigs. Therefore, more research needs to be conducted to elucidate the influence of BW on nutrient digestibility and phytase efficiency to degrade phytate.

Tables

Table 9.1. Analyzed composition of ingredients

Item	Corn	Soybean meal	Calcium carbonate
Gross energy, kcal /kg	3,828	4,172	-
Dry matter, %	85.46	88.18	99.98
Ash, %	1.48	7.89	90.64
Crude protein, %	6.42	46.77	-
AEE[1], %	3.58	1.69	-
Ca, %	0.04	0.29	38.93
P, %	0.25	0.68	0.03
Phytate[2], %	0.63	1.54	-
Phytate-bound P, %	0.18	0.43	-
Non-phytate P[3], %	0.07	0.16	-

[1]AEE = acid hydrolyzed ether extract.

[2]Phytate was calculated by dividing the concentration of phytate-bound P by 0.282 (Tran and Sauvant, 2004).

[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Table 9.2. Ingredient composition and analyzed values of experimental diets[1,2]

Item[3]	Phytase units (FTU)						Common
	0	250	500	1,000	2,000	4,000	diet
Ingredient, %							
Ground corn	67.69	67.685	67.68	67.67	67.65	67.61	67.35
Soybean meal, 48% CP	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Calcium carbonate	0.86	0.86	0.86	0.86	0.86	0.86	1.00
Monocalcium phosphate	-	-	-	-	-	-	0.60
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Chromium oxide	0.40	0.40	0.40	0.40	0.40	0.40	-
Vitamin mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[5]	-	0.005	0.01	0.02	0.04	0.08	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed values							
GE, kcal/kg	3,966	3,957	3,957	3,935	3,937	3,938	4,010
Dry matter, %	86.94	87.28	86.77	86.72	86.65	86.80	88.11
Ash, %	4.19	4.32	4.19	4.30	4.38	4.26	4.61
CP, %	16.39	16.54	16.39	16.33	16.26	16.30	16.31
AEE, %	3.37	3.63	3.62	3.65	3.41	3.65	3.82
Amino acids, %							
Arg	1.07	1.17	1.14	1.09	1.11	1.12	1.13
His	0.45	0.48	0.48	0.46	0.46	0.46	0.47
Ile	0.77	0.82	0.80	0.77	0.78	0.79	0.79
Leu	1.47	1.54	1.55	1.48	1.52	1.51	1.53
Lys	0.93	1.01	0.98	0.94	0.95	0.97	0.98
Met	0.27	0.26	0.27	0.27	0.27	0.27	0.28
Phe	0.85	0.91	0.90	0.86	0.88	0.89	0.89
Thr	0.62	0.67	0.67	0.64	0.65	0.65	0.67
Trp	0.20	0.20	0.20	0.21	0.21	0.20	0.21

Table 9.2. (Cont.)

Val	0.83	0.89	0.88	0.84	0.85	0.86	0.86
Ca, %	0.48	0.49	0.44	0.45	0.48	0.47	0.62
P, %	0.37	0.37	0.39	0.38	0.36	0.38	0.48
Phytate bound-P, %	0.26	0.22	0.24	0.24	0.18	0.18	0.24
Phytase activity, FTU	< 50	327	534	1,050	2,260	4,280	< 50
Phytate esters[6], nmol/g dry matter							
IP6	16,057	14,650	14,176	15,559	14,828	17,239	-
IP5	1,889	1,699	1,833	2,199	1,902	2,233	-
IP4	213	191	181	361	259	549	-

[1]Diets were formulated to contain 2,526 kcal/kg of net energy and the following quantities of amino acids (expressed as standardized ileal digestible) Lys, 0.86%; Met, 0.27%; Thr, 0.59%; Trp, 0.20%.

[2]Diets were formulated to contain 0.43 and 0.59% Ca and 0.37 and 0.50% P, for the experimental diets and the common diet, respectively.

[3]AEE = acid hydrolyzed ether extract; CP = crude protein; GE = gross energy; IP = inositol phosphate.

[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganoussulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[5]The phytase concentrate contained 5,000 units of phytase/g (Quantum Blue, AB Vista, Marlborough, UK).

[6]The concentrations of IP3 and inositol in the diets were below detection limits.

Table 9.3. Growth performance of pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase during phases1, 2, 3, and 4[1]

Item, kg[2]	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Phase 1 (29.7 to 46.8 kg)									
ADG	0.759	0.786	0.757	0.758	0.876	0.894	0.065	0.020	0.766
ADFI	1.915	1.730	1.712	1.767	1.871	1.803	0.115	0.752	0.894
G:F	0.404	0.447	0.441	0.428	0.493	0.496	0.028	0.012	0.397
Phase 2 (53.6 to 76.1 kg)									
ADG	0.951	1.046	1.137	1.049	1.133	1.187	0.084	0.019	0.456
ADFI	2.391	2.568	2.580	2.426	2.634	2.564	0.155	0.147	0.260
G:F	0.391	0.420	0.452	0.432	0.428	0.446	0.021	0.222	0.539
Phase 3 (85.1 to 106.3 kg)									
ADG	1.060	0.956	0.930	1.098	1.006	1.121	0.105	0.313	0.757
ADFI	3.193	3.157	3.317	3.347	3.095	3.201	0.092	0.474	0.996
G:F	0.324	0.297	0.279	0.337	0.323	0.358	0.030	0.097	0.818
Phase 4 (114.4 to 132.4 kg)									
ADG	0.703	0.754	0.991	0.871	0.866	0.901	0.118	0.325	0.370
ADFI	3.516	3.520	3.531	3.450	3.382	3.664	0.106	0.303	0.050
G:F	0.199	0.216	0.277	0.247	0.253	0.247	0.032	0.376	0.188

[1]Data are least square means of 8 or 9 observations.

[2]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 9.4. Effect of inclusion level of microbial phytase and phase on the apparent ileal digestibility (%) of crude protein (CP) and amino acids (AA) in diets fed to growing pigs[1]

Item	Phytase units (FTU)							*P*-value		Phase[2]					*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic	1	2	3	4	SEM	Linear	Quadratic
CP	77.6	78.2	77.8	78.3	78.8	77.1	0.66	0.337	0.010	75.5	78.2	79.4	78.7	0.91	0.021	0.084
Indispensable AA																
Arg	89.5	90.5	90.2	90.3	90.6	89.7	0.24	0.503	< 0.001	89.1	90.1	90.5	90.8	0.26	0.001	0.183
His	83.6	85.0	84.5	84.2	83.4	82.1	0.38	< 0.001	0.138	81.8	84.1	83.6	85.6	0.40	< 0.001	0.673
Ile	82.3	84.1	83.5	83.7	84.0	82.8	0.35	0.505	< 0.001	82.4	83.6	83.1	84.5	0.33	< 0.001	0.723
Leu	82.7	84.1	83.9	84.2	84.3	82.6	0.37	0.037	< 0.001	82.1	83.4	84.1	85.0	0.33	< 0.001	0.388
Lys	80.9	83.0	82.5	82.8	83.2	82.5	0.44	0.108	< 0.001	80.2	82.6	82.6	84.5	0.47	< 0.001	0.572
Met	86.3	85.9	86.4	87.2	87.3	85.8	0.36	0.647	< 0.001	85.6	86.4	85.9	88.1	0.32	< 0.001	0.007
Phe	82.6	84.5	84.2	84.4	84.8	83.6	0.34	0.530	< 0.001	82.3	83.8	84.9	85.1	0.31	< 0.001	0.036
Thr	73.2	75.6	75.4	75.1	75.2	73.5	0.54	0.066	0.001	70.9	74.9	76.2	76.8	0.53	< 0.001	0.006
Trp	81.1	81.3	81.5	83.3	82.9	81.5	0.46	0.302	< 0.001	80.6	80.4	81.4	85.4	0.44	< 0.001	< 0.001
Val	78.7	80.8	80.3	80.2	80.4	78.9	0.42	0.062	0.001	78.2	79.5	80.7	81.2	0.37	< 0.001	0.184
Mean	82.3	83.9	83.6	83.7	83.9	82.6	0.36	0.256	< 0.001	81.6	83.2	83.7	84.8	0.34	< 0.001	0.339
Dispensable AA																
Ala	78.0	79.6	79.4	79.4	79.3	77.7	0.48	0.030	0.002	76.2	79.3	79.6	80.5	0.42	< 0.001	0.001
Asp	79.5	81.7	81.6	81.6	82.1	80.8	0.39	0.456	< 0.001	79.3	81.6	81.5	82.3	0.40	0.001	0.049
Cys	69.6	69.1	69.0	69.9	68.5	64.0	0.83	< 0.001	0.014	63.3	68.2	69.3	72.6	0.81	< 0.001	0.274
Glu	83.6	85.2	85.1	85.1	85.9	84.4	0.55	0.740	< 0.001	83.6	85.4	84.7	85.7	0.51	< 0.001	0.187
Gly	68.4	71.9	70.8	70.1	70.2	68.8	0.75	0.055	0.088	66.0	70.4	71.4	72.4	0.78	< 0.001	0.034

Table 9.4. (Cont.)

Ser	80.0	81.2	81.1	81.7	81.5	80.2	0.43	0.439	< 0.001	78.7	80.9	82.1	82.1	0.43	< 0.001	0.018
Tyr	83.1	84.5	84.0	84.2	84.7	83.3	0.36	0.468	< 0.001	82.5	83.7	84.1	85.5	0.38	< 0.001	0.715
Mean	79.8	81.6	81.4	81.3	81.8	80.3	0.47	0.492	< 0.001	79.0	81.4	81.4	82.3	0.44	< 0.001	0.056
All AA	81.0	82.7	82.5	82.5	82.8	81.4	0.40	0.364	< 0.001	80.3	82.3	82.5	83.5	0.38	< 0.001	0.127

[1]Data are least squares means of 34 to 36 observations for phytase and 52 to 54 observations for phase.

[2]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

Table 9.5. Effect of inclusion level of microbial phytase and phase on the apparent total tract digestibility (%) of gross energy (GE) and macro minerals in diets fed to growing pigs[1]

Item	Phytase units (FTU)							*P*-value		Phase[2]					*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic	1	2	3	4	SEM	Linear	Quadratic
GE	84.8	83.7	84.5	84.4	85.0	83.0	0.44	0.003	0.003	82.1	83.5	86.0	85.4	0.45	< 0.001	0.018
Ca	48.6	64.8	70.3	71.9	73.9	75.9	1.46	< 0.001	< 0.001	76.8	71.4	66.1	55.9	1.60	< 0.001	0.111
P	23.1	45.7	57.4	68.1	77.1	82.2	1.56	< 0.001	< 0.001	63.6	58.8	61.5	51.8	1.30	< 0.001	0.046
K	82.8	82.1	84.4	83.8	86.4	84.2	0.96	0.028	0.001	78.6	83.0	87.6	86.6	1.23	0.001	0.049
Mg	16.9	19.8	23.9	26.5	31.0	26.8	2.03	< 0.001	< 0.001	14.5	25.2	30.6	26.3	2.06	0.002	0.005
Na	82.0	82.4	82.6	84.1	86.9	88.2	1.79	< 0.001	0.295	87.5	86.4	83.9	79.7	1.98	0.011	0.389

[1]Data are least squares means of 34 to 36 observations for phytase and 52 to 54 observations for phase.

[2]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

Table 9.6. Apparent ileal digestibility (AID, %) of inositol phosphate (IP) 6 in diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase and fed to pigs during phases 1, 2, 3, and 4[1,2]

Phase[3]	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
1	28.1	92.1	95.6	96.5	98.7	98.6	2.07	< 0.001	< 0.001
2	16.3	53.8	77.9	88.5	94.8	96.5	2.76	< 0.001	< 0.001
3	14.9	53.7	74.6	86.2	93.8	94.9	2.42	< 0.001	< 0.001
4	13.0	60.6	70.9	86.4	89.3	91.8	2.37	< 0.001	< 0.001

[1]Data are least square means of 8 or 9 observations.

[2]The AID of IP6 increased faster in response to phytase inclusion in phase 1 than in subsequent phases (interaction, $P < 0.05$).

[3]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

Table 9.7. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g dry matter) in ileal digesta samples from pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase during phases 1, 2, 3, and 4[1,2]

Item	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Phase 1 (29.7 to 46.8 kg)									
IP6	36,863	6,279	2,309	2,157	665	720	1,989	< 0.001	< 0.001
IP5	6,107	1,144	474	424	95	80	360	< 0.001	< 0.001
IP4	2,806	5,176	3,262	1,007	1,133	151	821	< 0.001	0.121
IP3	1,213	2,067	1,248	582	452	203	269	< 0.001	0.046
Inositol	77	755	2,286	4,157	7,788	8,659	660	< 0.001	< 0.001
Phase 2 (53.6 to 76.1 kg)									
IP6	48,433	25,342	12,365	6,496	2,617	1,263	1,695	< 0.001	< 0.001
IP5	5,855	5,472	2,611	1,617	305	304	313	< 0.001	< 0.001
IP4	1,297	8,597	10,639	9,646	3,258	826	854	< 0.001	< 0.001
IP3	876	2933	3631	3139	1420	655	253	< 0.001	< 0.001
Inositol	57	357	908	2,209	5,146	7,245	477	< 0.001	0.004
Phase 3 (85.1 to 106.3 kg)									
IP6	48,716	26,444	14,383	7,996	4,271	2,993	1,751	< 0.001	< 0.001
IP5	5,348	7,393	4,158	1,493	534	187	456	< 0.001	< 0.001
IP4	2,097	9,390	10,807	10,755	2,458	1,289	867	< 0.001	0.003
IP3	747	3,033	3,710	3,975	1,442	793	169	< 0.001	< 0.001
Inositol	109	883	1,162	2,515	5,432	5,671	548	< 0.001	< 0.001
Phase 4 (114.4 to 132.4 kg)									
IP6	50,737	26,505	18,351	9,774	4,855	3,774	1,485	< 0.001	< 0.001
IP5	5,522	8,863	5,743	1,842	697	467	355	< 0.001	< 0.001
IP4	1,061	11,765	15,927	11,097	5,966	1,867	916	< 0.001	< 0.001
IP3	643	3,104	4,104	4,409	3,005	1,195	310	0.002	< 0.001
Inositol	35	55	5	373	417	1,804	139	< 0.001	0.007

[1]Data are least square means of 8 or 9 observations.

[2]The reduction in IP6, IP5, IP4, and IP3, and the increase in inositol in response to phytase inclusion was greater in phase 1 than in subsequent phases (interaction, $P < 0.05$).

Table 9.8. Concentration of inositol phosphate (IP) esters (nmol/g dry matter) in fecal samples from pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase during phases 1, 2, 3, and 4[1,2,3]

Item	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Phase 1 (29.7 to 46.8 kg)									
IP6	329	291	194	223	257	161	46.9	0.021	0.747
IP5	23	18	21	24	19	18	9.7	0.715	0.924
IP4	4	33	29	36	10	5	6.7	0.014	0.090
Phase 2 (53.6 to 76.1 kg)									
IP6	392	447	263	357	275	247	47.4	0.001	0.220
IP5	99	57	21	59	34	19	10.6	< 0.001	0.059
IP4	34	82	65	73	43	51	8.4	0.210	0.508
Phase 3 (85.1 to 106.3 kg)									
IP6	519	246	152	229	266	201	67.1	0.050	0.071
IP5	164	72	77	47	47	23	23.5	0.002	0.052
IP4	293	224	151	172	204	149	32.3	0.021	0.233
Phase 4 (114.4 to 132.4 kg)									
IP6	1,189	608	578	562	654	668	132.7	0.133	0.008
IP5	190	107	97	104	71	88	22.8	0.006	0.004
IP4	50	60	79	83	106	41	20.4	0.664	0.008

[1]Data are least square means of 8 or 9 observations.

[2]Concentrations of IP3 and inositol in fecal samples were below detection limits.

[3]The effect of low phytase doses of on fecal IP6 was more severe in phases 3 and 4 than in phases 1 and 2 (interaction, $P < 0.05$). Fecal IP5 decreased with increasing dietary phytase in phases 2, 3, and 4, but not in phase 1 (interaction, $P < 0.05$). Fecal IP4 increased with dietary phytase in phases 1, 3, and 4, but not in phase 2 (interaction, $P < 0.05$).

Table 9.9. Effect of phase on the apparent ileal digestibility (AID) of inositol phosphate (IP) 6 and concentrations of IP6, IP5, IP4, IP3 and inositol in ileal digesta and fecal samples from growing-finishing pigs[1]

Item	Phase[2]					*P*-value	
	1	2	3	4	SEM	Linear	Quadratic
AID of IP6, %	84.9	71.3	69.9	68.8	1.31	< 0.001	< 0.001
IP and inositol concentration, nmol/g dry matter							
Ileal digesta samples							
IP6	8,165	16,086	17,401	18,956	1,092	< 0.001	0.007
IP5	1,361	2,708	3,181	3,825	230	< 0.001	0.100
IP4	2,312	5,695	6,087	7,947	654	< 0.001	0.062
IP3	984	2,100	2,268	2,728	131	< 0.001	0.009
Inositol	3,938	2,613	2,629	717	291	< 0.001	0.275
Fecal samples[3]							
IP6	239	333	268	710	50.2	< 0.001	0.003
IP5	20	50	74	108	9.3	< 0.001	0.743
IP4	20	58	196	70	8.9	< 0.001	< 0.001

[1]Data are least square means of 51 to 53 observations.

[2]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

[3]Concentrations of IP3 and inositol in fecal samples were below detection limits.

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Chapter 10: Conclusions

Little attention is given to the supply of limestone because of its low cost, which may result in oversupply of Ca in diets and reduced growth performance of pigs. It was hypothesized that analyzed values for Ca in commercial diets from the U.S. swine industry are not greater than formulated values. However, based on analyzed values for Ca and P of over 100 commercial diets, it was concluded that diets used in the U.S. swine industry contain 0.19% more Ca and 0.06% more P than formulated. Therefore, more attention is required to the inclusion of Ca in diets to avoid Ca oversupply and its detrimental effect on pigs.

The interest in Ca nutrition by pigs has increased in recent years because of the negative effect of excess Ca on P digestibility and growth performance of pigs. Values for the digestibility of Ca in different feed ingredients without and with phytase have been determined in the last decade, which allowed for estimation of Ca requirements expressed as a ratio between digestible Ca and digestible P in pigs with different body weight (**BW**). Because these values were obtained in short-term experiments using diets without phytase, and because phytase increases Ca digestibility, 2 follow-up experiments were conducted to validate the reported ratios in diets without or with microbial phytase. Results indicated that diets for growing-finishing pigs can be formulated based on digestible Ca values without affecting growth performance, bone development, or Ca retention.

Acidifiers are used in weaning diets as alternatives to antibiotic growth promoters because weanling pigs may have secretion of HCl in the stomach that is insufficient to maximize pepsin activity. Therefore, it was hypothesized that reducing the dietary concentration of limestone and monocalcium phosphate, which have a high acid binding capacity, may create a more favorable gastric environment for proper protein digestion. Another hypothesis was that

inclusion of phytase in high doses may counter the negative effects of weaning by increasing phytate degradation and inositol release. Results indicated that reducing the concentration of Ca and P in phase 1 diets that contain lactose does not decrease gastric pH or diarrhea incidence, but results in reduced growth performance and bone mineralization, with pigs not being able to recover after a 4-wk repletion period. However, inclusion of high doses of phytase results in increased average daily gain and feed efficiency, possibly because of an increased release and absorption of inositol, which plays an important role in several metabolic processes. Therefore, super-dosing of phytase may be beneficial for newly weaned pigs undergoing post-weaning stress.

In pigs, the positive correlation between phytase level and digestibility of Ca and P is well documented, but the positive effect of increasing levels of phytase on amino acid (**AA**) and energy digestibility is not consistently observed. It was hypothesized that greater phytase doses than usually used [i.e. 3,000-phytase units/kg feed (**FTU**)] are needed to verify effects of phytase on nutrient digestibility in pigs. It was also hypothesized that effects of increasing dietary phytase on phytate degradation and nutrient digestibility observed in growing pigs can be extrapolated to the entire growing-finishing period. Data indicated that regardless of BW, increasing dietary phytase results in increased phytate and lower phytate ester degradation in the small intestine, and consequently increased digestibility of AA, Ca, P, K, Mg, and Na, increased inositol and P in plasma, and increased concentration of bone ash. However, to fully degrade phytate, more than 4,000 FTU are needed. Results also indicated that as pigs get older, the digestibility of energy, AA, K, and Mg increases, but the digestibility of Ca, P, and Na, and the efficacy of phytase to degrade phytate decreases. It was, therefore, concluded that effects of phytase on phytate degradation and digestibility of nutrients are independent of BW of pigs, but

phytase has a limited effect on energy digestibility and more replications may be needed to verify effects of phytase on AA digestibility. Additionally, BW of pigs influences the digestibility of nutrients and the efficacy of phytase to degrade phytate.