DIGESTIBILITY OF CALCIUM AND DIGESTIBLE CALCIUM REQUIREMENTS IN PIGS

BY

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DISSERTATION

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ABSTRACT: Seven experiments were conducted towards developing a system for determining digestible Ca requirements in growing pigs. Experiments 1 and 2 were conducted to establish standard total tract digestibility (STTD) values of Ca in a number of feed ingredients without and with microbial phytase. Results of Exp. 1 indicated that regardless of inclusion of microbial phytase, monocalcium phosphate had the greatest ($P < 0.05$) STTD of Ca. The STTD of Ca in dicalcium phosphate was greater ($P < 0.05$) than in calcium carbonate, *Lithothamnium calcareum* Ca, or in a sugar beet co-product, but no differences were observed among the STTD of Ca in calcium carbonate, *Lithothamnium calcareum* Ca, or sugar beet co-product. Inclusion of microbial phytase increased ($P < 0.05$) the STTD of Ca in the diets, but this was not the case in the Ca supplements. Results of Exp. 2 indicated that the STTD of Ca in fish meal increased ($P < 0.001$) if microbial phytase was used. Experiment 3 was conducted to determine the effect of fiber and soybean oil on the STTD of Ca, and to determine the effect of using a corn-based diet or a cornstarch-based diet on the STTD of Ca in fish meal. Results indicated that fiber increased ($P < 0.001$) the STTD of Ca, but the STTD of Ca was not affected by soybean oil. The STTD of Ca in the corn-based diet was greater ($P < 0.05$) than in the cornstarch-based diet, which indicates that corn-based diets need to be used to determine STTD of Ca in feed ingredient. Experiments 4 and 5 were conducted to determine the requirement of STTD Ca by 11 to 25 kg pigs. Six diets were formulated to contain 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca and 0.36% STTD P. Results indicated that the concentration of STTD Ca in the diets needed to maximize bone ash was 1.33 times the concentration of STTD P. Experiments 6 and 7 were conducted to determine the requirement for STTD Ca and STTD P by 25 to 50 kg pigs. A total of 20 diets were formulated to contain 0.13, 0.27, 0.42, 0.57, or 0.72% STTD Ca and 0.15, 0.31, 0.39, or 0.47% STTD P. Results indicated that the concentration of dietary STTD Ca needed to

maximize ADG and G:F was between 1.16 and 1.43 times the concentration of STTD P, but to maximize bone ash, dietary STTD Ca needs to be between 1.53 and 1.81 times the concentration of STTD P. In conclusion, diets for growing pigs may be formulated using values for STTD of Ca in feed ingredients, but, it is recommended that Ca digestibility of feed ingredients are determined in corn-based diets. If diets are formulated to meet STTD Ca and STTD P requirements, the utilization of both minerals is maximized. Additional research is needed to determine the STTD Ca requirements by pigs above 50 kg BW.

Key words: digestible calcium, digestible phosphorus, microbial phytase, phytate, pig, requirements

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CHAPTER 1

INTRODUCTION

Calcium and P are minerals that are important for bone health. Although, 90% of Ca and 80% of P in the body are present in bone tissue, the Ca and P in soft tissues and some fluids have other important physiological functions as well (Crenshaw, 2001; Ewing and Charlton, 2007; Vitti et al., 2010). Thus, both Ca and P must be present in diets fed to pigs. Due to the relatively low concentration of Ca in plant ingredients, Ca needs to be supplemented in swine diets. Calcium can be supplemented with animal sources or inorganic sources. The concentration of Ca in these ingredients has been reported (NRC, 2012). However, for most sources of Ca, no digestibility values have been reported.

Calcium is mainly absorbed in the small intestine by 2 mechanisms: paracellular and transcellular (Bronner, 1987). Paracellular absorption of Ca is nonsaturable and does not require energy, however, transcellular absorption of Ca is saturable and not only requires energy, but also Ca channels and Ca transporters (Christakos, 2012). Absorption of Ca is influenced by intrinsic factors such as vitamin D (Bouillon et al., 2003) and extrinsic factors such as phytate (Selle et al., 2009). Phytate binds Ca, which reduces the amount of Ca that can be absorbed. Because most plant ingredients contain phytate, phytate is an important factor that needs to be considered when evaluating the digestibility of Ca. During the last decades, microbial phytase has been used in swine diets to increase not only P digestibility (Selle and Ravindran, 2008; Akinmusire and Adeola, 2009; Rodríguez et al., 2013), but also Ca digestibility (González-Vega et al., 2013). However, elevated levels of Ca may reduce phytase efficacy (Lei et al., 1994; Selle et al., 2009) and the digestibility of P (Stein et al., 2011).

Standardized total tract digestibility (**STTD**) values of Ca and P represent the proportion of Ca and P that is absorbed and potentially used by the animal. Values for STTD of Ca and P are expected to be additive in mixed diets, therefore, diets can be formulated to meet the STTD Ca and STTD P requirements. However, only STTD P requirements have been reported (NRC, 2012). To increase accuracy of formulation, diets should be formulated based on STTD of not only P but also Ca. Thus, to maximize the utilization of both Ca and P by the animal, requirements of STTD Ca needs to be determined.

The objectives of this dissertation are to determine the STTD of Ca in different Ca sources, determine the effect of different factors such as phytase, phytate, fiber, oil, and Ca level on the STTD of Ca, determine the effect of Ca level on the gene expression of Ca transporters, and to determine the STTD Ca requirements for 11 to 25 and 25 to 50 kg pigs.

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CHAPTER 2

PHYTATE, PHYTASE, AND CALCIUM IN PIGS: LITERATURE REVIEW

Digestion is the process that takes place in the gastrointestinal tract to break down ingested nutrients into smaller molecules that are able to be absorbed by the animal. The gastrointestinal tract has a complex environment where secretions, pH, enzymes, cells, motility, bacteria, and others factors play a role in the digestion of nutrients. However, digestibility of a nutrient is not only influenced by these factors, but may also be influenced by extrinsic factors such as the level of the nutrient in the diet and interactions with other nutrients. All these factors may enhance or reduce the digestibility of the nutrient. As an example, phytate reduces the digestibility of Ca (Selle et al., 2009), whereas, phytase increases the digestibility of Ca (González-Vega et al., 2013). If the digestibility of Ca in feed ingredients is determined, diets can be formulated to meet the requirements for digestible Ca and digestible P, which likely will result in more accurate diet formulations.

PHYTATE

Phytate is a salt of phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate; **IP6**) and is present in most plant seeds. Phytic acid has a molecular weight of 660 g/mol and has 12 negative charges that may chelate different cations such as Ca, Mg, K, Cu, Zn, Fe, and phytate also may bind protein, starch, and lipids (Onyango et al., 2009; Selle et al., 2009). The functions of phytic acid are to protect the seed from oxidative stress (Graf, 1983) and to retain some P, inositol, and other minerals in the seed for the germination process, but some enzymes are required to release these nutrients during germination (Kies, 2005).

Phytate is mainly present in plants as Mg and K salt mixtures (Onyango et al., 2009) and the concentration of phytate in plant seeds is variable, but is mostly between 1 and 3% (Graf, 1983). Likewise, the location of phytate in the seed may vary (Kies, 2005). In wheat, barley, and rice, phytate is mainly located in the aleurone layer, in corn, most phytate is located in the germ, and in dicotyledonous seeds, phytate is located in the cotyledons (Kies, 2005; Bohn et al., 2008).

The process of biosynthesis and storage of phytic acid is different among plants and is influenced by weather conditions, soil conditions, and enzymes that are present in the plant (Bohn et al., 2008). Inositol phosphate kinase is one of the kinases responsible for phytic acid biosynthesis, but its functions may vary among plant species (Shi et al., 2003). For most seeds, the biosynthesis of phytate starts after flowering, and phytate is stored during maturation of the seed (Kies, 2005; Bohn et al., 2008). Research is ongoing to elucidate the process of biosynthesis and accumulation of phytic acid in seeds (Shi et al., 2003; Bohn et al., 2008).

Phytate and Minerals

Phytate contains 282 g P per kg (Selle et al., 2009) and most of the P in plant ingredients is bound to phytate, which is mostly unavailable for absorption by pigs and poultry. The majority of the phytate-bound P is, therefore, excreted in the feces and may contribute to increased P-pollution. Consequently, diets for pigs are most correctly formulated based on standardized total tract digestibility (**STTD**) of P (NRC, 2012). Inclusion of inorganic P in the diets is needed to compensate for the low STTD of the phytate-bound P, but this is expensive (Kies, 2005). During recent decades, research has, therefore, been conducted to identify solutions that may contribute to a reduction in the amount of P excreted in the feces and also to make more phytate bound P available to pigs or poultry.

Phytate is negatively charged and as phytate moves from a low pH in the stomach to a greater pH in the small intestine, phytate becomes more negative (Santos, 2012). This enhances the reaction between phytate and cations or proteins resulting in the precipitation of these salts (Santos, 2012). Phytate has more affinity for cations such as Cu and Zn, but because Ca is the most abundant cation in swine and poultry diets, Ca is more likely than other cations to form such salts (Selle et al., 2009). However, addition of Cu and Zn in diets for weanling pigs is often elevated because these minerals act as growth promoters (Jay et al., 2010). Therefore, Cu and Zn may also bind to phytate in the intestinal tract, and thereby, reduce the availability of these minerals for absorption (Santos, 2012).

Most of the Ca in diets for swine and poultry is supplemented as inorganic Ca because of the low concentration of Ca in most plant ingredients (NRC, 2012). The apparent total tract digestibility (**ATTD**) of Ca in diets containing both inorganic Ca and intrinsic Ca increases if microbial phytase is used (Poulsen et al., 2010; Rodríguez et al., 2013; Almaguer et al., 2014). Likewise, ATTD and true total tract digestibility (**TTTD**) of Ca from intrinsic Ca in canola meal also increase if microbial phytase is included in the diet (González-Vega et al., 2013). The reason for the increase in ATTD and TTTD of Ca if phytase is added to the diets is that phytase hydrolyzes the phytate, reducing the chance to bind Ca, because phytate is able to chelate up to 5.1 atoms of Ca (Selle et al., 2009).

Phytate and Protein

Phytate may react with proteins containing basic AA (i. e., Arg, His, and Lys) in an environment where the pH is below the isoelectric point because they get positively charged (Cowieson et al., 2009; Selle et al., 2009; Santos, 2012; Selle et al., 2012). The resulting phytate-protein complexes may precipitate and protein may become less soluble, which will result in a

reduced digestibility of protein (Cowieson et al., 2009; Selle et al., 2009; Santos, 2012). Phytate may also negatively affect the activation of endogenous enzymes such as pepsin, which is involved in protein digestion (Selle et al., 2009; Santos, 2012). In addition, phytate may bind to water, which will result in less water around the proteins, which reduces protein solubility (Santos, 2012). The negative effect of phytate on protein digestibility may also result in the gastrointestinal tract hypersecreting pepsin, HCl, mucus, and sodium bicarbonate (Selle et al., 2009), and endogenous losses of AA and minerals may, therefore, increase (Cowieson et al., 2004). Active absorption of AA may also be reduced because of reduced effectiveness of the Na-K pump (Onyango et al., 2009; Selle et al., 2009; Santos, 2012). One g of phytate may bind 10 g of protein (Cowieson et al., 2009), but effects of phytate on protein digestibility has sometimes been difficult to demonstrate in pigs.

Phytate and Energy

Phytate may have a negative impact on energy utilization because of a reduction in lipid and glucose uptake (Selle et al., 2009). Digestibility of lipids is affected because phytate decreases the activity of lipase (Knuckles, 1988), and also may form phytate-lipid-peptide complexes (Cosgrove, 1966). Likewise, starch digestibility and glucose absorption may be negatively affected (Selle et al., 2009) because phytate decreases the activity of α-amylase (Deshpande and Cheryan, 1984) and also reduces the activity of the Na-K pump, which is needed for glucose uptake. Phytate-starch complexes or protein-starch-phytate complexes may also be formed (Thompson et al., 1987), but it is believed that phytate has a greater impact on glucose absorption than on the digestibility of starch (Selle et al., 2009).

Release of Nutrients that are Bound to Phytate

Phytate is considered an anti-nutritional factor for pigs and poultry (Santos, 2012), but germination of seeds, soaking, dehulling, fermentation, or use of enzymes are techniques that may be applied to reduce the negative effects of phytate. Germination has been used to reduce the phytate concentration in seeds, due to an increase in activity of enzymes in the seed during germination (Camacho et al., 1992). During germination, the nutrient composition in the seed changes, while protein, starch, and phytate concentrations decrease, and concentrations of free AA, free sugars, reducing sugars, and phenolic compounds increase (Tian et al., 2010). Germination is very effective in decreasing the concentration of phytate compared with other techniques such as soaking, de-hulling, or cooking (Duhan et al., 2002). In vitro digestibility of protein and starch in cowpeas increases more by germination than by soaking the seeds in distilled water or dehulling soaked seeds (Preet and Punia, 2000). Increasing germination time of soaked rye and barley seeds reduces the concentration of phytate P and increases the activity of phytase and acid phosphatase enzymes compared with ungerminated seeds (Centeno et al., 2001). Thus, the reduction of phytate P and increase in endogenous phosphatases in the seeds that are a result of germination may contribute to an increased digestibility of P by pigs and poultry. However, feeding pigs and poultry with germinated seeds is not very practical in a commercial setting.

Fermentation may also be used to reduce the phytate content in seeds due to an increase in phytase activity. This technique was used in wheat germ, which increased not only phytase activity, but also the availability of Ca, Fe, K, Mn, Na, and Zn (Rizzello et al., 2010). Reduction of the pH by fermentation may enhance the activation of endogenous phytases and also phytases from lactic acid bacteria, which increase the availability of P bound to phytate (Rizzello et al.,

2010). Fermentation of feed ingredients such as soybean meal also may enhance the digestibility of P by hydrolyzing some of the phytate-bound P (Rojas and Stein, 2012).

PHYTASE

Inclusion of microbial phytase in diets fed to pigs and poultry has become more common during recent decades to make phytate-bound P available for absorption. Although some cereals such as wheat and rye contain phytase, the efficacy of this phytase is only 40% of the efficacy of microbial phytase (Zimmermann et al., 2002), therefore, inclusion of microbial phytase is common in poultry and pig diets.

Phytase (*myo*-inositol hexakisphosphate phosphohydrolase) hydrolyzes the ester bonds in phytate resulting in inositol phosphate (**IP**) IP5, IP4, IP3, IP2, IP1, inositol, and inorganic P (Wyss et al., 1999). Phytases can be divided mainly into 3 categories by the position of the inositol ring where they initiate the hydrolysis, which are the 3-, 5-, or 6- positions. Because only one type of phytase is not able to hydrolyze all the ester bonds, the myo-inositol intermediates have to be hydrolyzed by other types of phytases (Greiner and Konietzny, 2010). Inclusion of microbial phytase in pig diets may lead to generation of IP3, IP4, or IP5 and not complete hydrolysis of phytate, but this may not represent a problem because pigs may hydrolyze these components in the small intestine (Hu et al., 1996).

Phytases are known as 3-phytases (e.g., *Aspergillus niger* based phytase), 5-phytases (e.g., *Pisium sativum* based phytase), or 6-phytases (e.g., *Escherichia coli* based phytase; Greiner and Konietzny, 2010). Most diets fed to pigs and poultry contain 3-phytases or 6-phytases. In general, 3-phytases are from microbial origin, whereas 6-phytases are from plant origin, but there are some exceptions. As an example of these exceptions, lupin has 3-phytase activity and *E. coli*

and *Periophora lycii* have 6-phytase activity (Greiner and Konietzny, 2010). Although, microbial phytases are more effective than phytases from plant origin, a combination may be used. The pH in the gastrointestinal tract plays an important role in the efficacy of the enzyme. Low pH in the stomach enhances the activity of microbial phytase (60%) and plant origin phytase (40%), but a greater pH in the small intestine and secretion of HCl, pepsin, and proteases may reduce the activity of the enzyme (Rapp et al., 2001). Therefore, the hydrolysis of phytate in the stomach plays an important role for the digestibility of P (Jongbloed et al., 1992).

Microbial phytases may originate from either fungi or bacteria, but both have similar effects on Ca and P digestibility (Guggenbuhl et al., 2007; Selle and Ravindran, 2008). The fungi and bacterial phytases may differ in the place of enzyme production. The fungal phytase is mostly extracellularly located, whereas bacterial phytase is either extracellularly (e.g., *Bacillus subtilis, Enterobacter sp.*) or intracellularly (e.g., *E. coli, Klebsiella aerogenes*) located (Kerovuo, 2000). Fungal and bacterial phytases also differ in substrate specificity, thermostability, and pH optima (Kerovuo, 2000; Rao et al., 2009). Research on other sources of production of phytase such as phytases produced by genetically modified plants (e.g., wheat and corn) and production of phytase by pigs are still being conducted.

The advantage of adding microbial phytase to the diets is the increase in digestibility of P (Akinmusire and Adeola, 2009; Almeida and Stein, 2010; Rodríguez et al., 2013), Ca (Rodríguez et al., 2013; González-Vega et al., 2013; 2014b), and sometimes also protein (Ravindran et al., 1999; Kies et al., 2001) and energy (Kies et al., 2001). However, the positive effect of phytase on protein and energy digestibility is mostly observed if diets are deficient in P compared with diets containing adequate levels of P (Almeida et al., 2013a). Although, the use of microbial phytase in the diet is very common, phytase from genetically modified plants can also be used to feed

pigs. Phytase from transgenic corn increases energy and P digestibility, but not AA digestibility when fed to pigs (Li et al., 2013).

The ideal phytase is defined as being able to remain active after heat has been applied in feed processing or storage conditions, remain stable in the gastrointestinal tract, and have a low inclusion cost in the diet (Greiner and Konietzny, 2010). The efficacy of phytase is influenced by many factors such as stability under pH conditions, resistance of pepsin or proteases in the gastrointestinal tract (Greiner and Konietzny, 2010), feeding management (e.g., feeding level and feeding frequency), age or physiological status of the animal (Mroz et al., 1994; Kemme et al., 1997a), and others. The level of phytase also determines the effect of phytase on the digestibility of nutrients (Kies et al., 2006; Veum and Ellersieck, 2008). Increasing levels of microbial phytase up to 1,500 phytase units (**FTU**)/kg linearly and quadratically increased the digestibility of P (Almeida and Stein, 2012). Likewise, super-dosing of microbial phytase up to 15,000 FTU resulted in an increase in mineral digestibility (Kies et al., 2006), however, depending on the mineral, digestibility increased only between 4 and 12 percentage units from pigs fed 1,500 and 15,000 FTU (Kies et al., 2006). Thus, super-dosing of phytase is not always necessary to get the best Ca and P digestibility. Inclusion levels of phytase between 800 and 1,000 FTU/kg were reported to be optimum for weanling and growing pigs (Almeida et al., 2013b).

The activity of phytase may be affected by metal ions, however, it is not clear the cause of this negative effect. Binding of metal ions to the enzyme, formation of metal ion-phytic acid complexes, or increased intestinal pH may be some of the possible reasons for the decrease in the efficacy of the enzyme (Liu et al., 1998; Kerovuo, 2000). The most abundant metal ion in diets fed to pigs is Ca. The amount of Ca that is included in diets fed to pigs sometimes exceeds the requirement established by NRC (2012) because Ca supplements such as calcium carbonate are

inexpensive, therefore, excess of Ca has not been a concern from an economic stand point. However, it has been reported that increased levels of dietary Ca may decrease phytase activity (Lei et al., 1994; Lantzsch et al., 1995; Brady et al., 2002; Selle et al., 2009) and reduce P digestibility (Clark, 1969; Stein et al., 2011). Therefore, the interactions among Ca, P, phytate, and phytase in pigs need to be elucidated.

CALCIUM

Calcium and P are 2 important minerals in the body that are interrelated because the absorption and utilization of one mineral may influence the absorption and utilization of the other. Approximately 96 to 99% of Ca and 60 to 80% of the P in the body is present in the skeletal tissue, and only 20 to 40% of P is present in soft tissue or fluids (Crenshaw, 2001; Vitti et al., 2010). The greater variation in P in skeletal tissue compared with Ca is due to variation in the proportion of soft tissue to skeletal tissue during the growing phases of pigs (Crenshaw, 2001). Calcium and P have important functions in the body such as formation and maintenance of bones, transmission of nerve impulses, cofactors of enzymes, muscle contraction, synthesis of protein and phospholipids, components of nucleic acids, and other functions (Crenshaw, 2001; Ewing and Charlton, 2007; Vitti et al., 2010).

Absorption of Ca

Calcium needs to be in a soluble or ionic form to be absorbed. Therefore, Ca needs to be released from the other components in the diet, which is possible through enzymes and gastric acid secretions (Allen, 1982). Calcium is mainly absorbed in the small intestine (Partridge, 1978; Liu et al., 2000), mostly in the duodenum, but the place where Ca is absorbed may vary among Ca sources and types of diets (Partridge, 1978; González-Vega et al., 2014a). Although result of

some studies indicated absorption of Ca in the colon (Liu et al., 2000), results of recent studies have indicated that no absorption of Ca takes place in the large intestine (González-Vega et al., 2014a).

Calcium can be absorbed by nonsaturable paracellular (diffusion) absorption or by saturable transcellular absorption (active transport; Bronner, 1987). The amount of Ca transported by each mechanism is influenced by the level of Ca in the diet relatively to the requirement. At high levels of dietary Ca, the nonsaturable mechanism transports more Ca, whereas the saturable mechanism transports more Ca at lower Ca levels (Bronner, 1987). The nonsaturable mechanism takes place using paracellular routes, and the saturable mechanism takes place using the cellular routes. The saturable mechanism needs to transport Ca through Ca channels that are located in the apical side of the cell. These channels are called transient receptor potential vanilloid (**TRPV**), and designated as TRPV6 and TRPV5 for the intestines and the kidneys, respectively (Christakos, 2012). Once Ca is inside the cell, the Ca binding proteins (**CaBP**), also known as calbindin, diffuses the Ca across the cell. The CaBP9K is principally found in the intestines and CaBP28k in kidneys. Calcium exits the cell from the basolateral side through a Na/Ca exchanger with the plasma membrane Ca-ATPase. The saturable mechanism is vitamin D (1,25 dihydroxycholecalciferol (1,25-$(OH)_2D_3$)) –dependent because 1,25-$(OH)_2D_3$ influences the expression of CaBP9k and TRPV6 (Christakos, 2012), and the expression of Ca-ATPase (Kutuzova and DeLuca, 2004). Several experiments have been conducted to determine the influence of vitamin D on the expression of genes involved in Ca absorption with wild type mice or vitamin D receptor-knock out mice (Bouillon et al., 2003; van Abel et al., 2003; Kutuzova and DeLuca, 2004). By using real-time quantitative PCR the mRNA of the genes involved in Ca absorption can be quantified (van Abel et al., 2003). Although results of some

studies have demonstrated that vitamin D up-regulated TRPV5, TRPV6, CaBP, and Ca-ATPase (van Abel et al., 2003; Kutuzova and DeLuca, 2004) and dietary Ca up-regulates only TRPV6 and CaBP (van Abel et al., 2003), results of other studies have indicated up-regulation only in some genes (Bouillon et al., 2003). Therefore, it is concluded that because vitamin D up-regulates the synthesis of genes involved in Ca absorption, Ca absorption in intestine and kidney reabsorption increases. It has been believed that 1,25-$(OH)_2D_3$ influences only the saturable mechanism, but results of recent studies indicated that 1,25-$(OH)_2D_3$ may also play a role in the nonsaturable paracellular mechanism (Kutuzova and DeLuca, 2004; Christakos, 2012). Activation of vitamin D starts in the liver where a hydroxyl group is added at the C-25 position of cholecalciferol to form 25-hydroxycholecalciferol (25-$(OH)_2D_3$). The C at the 1 position is then hydroxylated in the kidney to form 1,25-$(OH)_2D_3$, which is the active form of vitamin D. This form is released in the circulation to regulate directly or indirectly the expression of genes involved in homeostasis of Ca, as well as the proliferation and differentiation of bone cells, epithelial cells, and the immune system (Omdahl et al., 2002; Bouillon et al., 2003; Kutuzova and DeLuca, 2004; Veum, 2010).

Fiber may influence the absorption of minerals due to its physical or chemical properties (Woldeghebriel et al., 2013). Some properties such as formation of complexes, viscosity, and physical entrapment may reduce the availability of minerals (Torre et al., 1995; Van der Klis et al., 1995; Fly et al., 1996; Guillon and Champ, 2000; Debon and Tester, 2001; Miyada et al. 2011). However, fermentation of fiber may increase absorption of minerals in large intestine (Miyada et al., 2012). It is possible that reduction in intestinal pH caused by the production of short chain fatty acids may enhance solubility of minerals (Wong et al., 2006; Rose et al., 2007;

Bird et al., 2000). Additionally, butyrate may increase the proliferation of epithelial cells increasing surface area for absorption (Montagne et al., 2003).

The effect of fat in digestibility of nutrients may vary. The reduction of rate of passage caused by fat may enhance digestibility of AA (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). However, formation of Ca soaps reduces digestibility of Ca in rats (Frommelt et al., 2014). Limited information is available about the effect of different dietary factors on the digestibility of Ca in pigs.

Digestibility of Ca

Among Ca sources, the concentration and digestibility of Ca differ. Plant sources have relatively low concentrations of Ca, whereas animal and inorganic sources have relatively high concentrations of Ca. As a consequence, Ca originating from animal proteins or inorganic sources are used to supply Ca in swine and poultry diets (NRC, 2012). Although the concentration of Ca in most feed ingredients has been reported, only few digestibility values are available. Digestibility can be defined as apparent, standardized, or true digestibility (Stein et al., 2007). Apparent digestibility values of a nutrient may vary with the concentration of the nutrient in the diet if there are endogenous losses of the nutrient. Therefore, apparent digestibility values are not expected to be always additive in mixed diets, whereas values for standardized or true digestibility are additive in mixed diets (Stein et al., 2005) because these values are corrected for endogenous losses. Thus, if basal or total endogenous losses are subtracted from the output in the digestibility calculations, values are not influenced by the nutrient concentration. It is, therefore, recommended to formulate diets using standardized or true digestibility values (NRC, 2012). Due to measurable endogenous losses of Ca, ATTD values need to be corrected to determine STTD or TTTD **(**Fan and Archbold, 2012; González-Vega et al., 2013). Some studies have

reported ATTD of Ca in diets (Kemme et al., 1997b; Stein et al., 2006, 2008; Malde et al., 2010), or in corn, soybean meal, or calcium carbonate (Bohlke et al., 2005; Stein et al., 2011). Recent STTD and TTTD values of Ca have been reported in inorganic Ca sources and in canola meal (González-Vega et al., 2013; 2014a; 2014b), and in corn-SBM diets (Fan and Archbold, 2012), but further research needs to be conducted to determine STTD or TTTD of Ca in animal sources and other feed ingredients.

Modeling is a mathematical tool that can be used to predict the digestibility of a nutrient by taking into account parameters that influence digestibility to obtain a good predictor value, but a good correlation between predicted and observed values needs to be demonstrated. Recent models for ATTD of Ca and P were reported by Létourneau-Montminy et al. (2011), which integrate different physiological aspects along the gastrointestinal tract of pigs. Although many factors were considered such us pH, retention time, endogenous losses, solubilization, insoluble complexes, and others, the model only had 0.78 R^2 and 20.4% disturbance error for ATTD of Ca, but 0.90 R^2 and 96.5% disturbance error for ATTD of P between the observed and the predicted value. Modeling for P digestibility has been easier than for Ca digestibility because there are more available data for P than for Ca. While the model reported by Symeou et al. (2012) overestimates P digestibility by 10%, the model predicted the negative effect of increasing levels of Ca and the positive effect of microbial phytase on P digestibility. A more sophisticated model that includes the interactions between genotype of pigs and P retention and excretion was recently proposed (Symeou et al., 2014). This model predicts P digested, P retained, and P excreted, but if canola meal or DDGS were used in the diets, the prediction of these parameters was poor. Some inconsistencies in the model may have been a result of Ca and P interactions that

are still not well understood. This model also was not able to predict Ca digestibility. Therefore, more research is needed to elucidate factors that influence Ca and P digestibility in pigs.

CALCIUM REQUIREMENTS

Nutrient requirements can be determined by factorial calculations and empirical measurements. Because the factorial technique accounts for several factors such as availability, obligatory losses of the nutrient in the body, and retention is believed that requirements obtained using this approach is more precise (Weremko et al., 1997). However, empirical measurements, which uses one or several response criteria to determine the nutrient requirement, have been most commonly used (Weremko et al., 1997; NRC, 2012). However, several factors such as sex and age may influence the requirement values (NRC, 2012).

Diets formulated to contain energy and nutrients that are at or close to the requirements of the animal allow the most economical and sustainable production. Experiments have been conducted to determine the requirement of Ca and P by pigs (Rutledge et al., 1961; Coalson et al., 1972, 1974; Thomas and Kornegay, 1981; Partanen et al., 2010; Saraiva et al., 2011). However, in most of these experiments the requirements of total Ca and total P have been determined. The requirement of digestible P has been reported (NRC, 2012), but no values for the requirement of digestible Ca are available. Most of the studies conducted to determine Ca and P requirements consider the levels of Ca and P that result in the maximum growth performance of the pigs rather than the maximum bone strength or bone ash. This is because requirements of Ca and P are greater for maximum bone ash than for maximum ADG (NRC, 2012).

Muscle and bones growth independently, therefore, deposition of Ca and P in lean tissue and skeletal tissue are not directly proportional (Crenshaw, 2001). Thus, the ratio between Ca and P in the body range between 1.20:1 and 1.60:1 for a 25 kg pig, and between 1.25:1 and 1.70:1 for a 50 kg pig (Rymarz et al., 1982; Hendriks and Moughan, 1993; Mahan and Shields, 1998; Wiseman et al., 2009; Pettey et al., 2015). However, because 96 to 99% of Ca is present in skeletal tissue (Crenshaw, 2001), it is expected that requirements of STTD Ca to maximize bone ash are close to requirements to maximize Ca retention in the body.

The importance of the Ca:P ratio in formulation of diets fed to pigs has been addressed for several decades. This is because excess or deficiency of one mineral may affect the utilization of the other (Crenshaw, 2001). The NRC (2012) recommends to formulate diets using a total Ca: total P ratio of 1:1 or 1:25:1 or a total Ca:digestible P ratio of 2.15:1. The requirements suggested by NRC (2012) are expressed as total Ca and STTD P. The reason requirements are not expressed as STTD Ca is that no data for the requirement of STTD of Ca have been reported. However, it is believed that if diets are formulated using STTD Ca:STTD P, it will give an estimate of the amount of Ca and P that will be absorbed by the pig, theoretically should result in the most economic and environmentally friendly diet formulation.

Recent studies that determined digestible P requirements in pigs had at least 5 levels of digestible P with a constant level of total Ca (Saraiva et al., 2009; 2012), or with a constant total Ca:digestible P ratio (Ruan et al., 2007; Partanen et al., 2010; Zhai and Adeola, 2013a; 2013b). However, because the requirements for digestible Ca are unknown, it is difficult to design these types of experiments. Therefore, having only one level of Ca may limit the absorption and retention of P if the Ca level is less than the Ca requirement. Likewise, because it is not known what is the ideal total Ca:digestible P ratio or digestible Ca:digestible P ratio, absorption and

retention of Ca and P may vary, depending on the Ca:P ratio used. In a recent study, results indicated an optimum true digestible Ca:true digestible P ratio of 0.9:1 and 1.0:1 to maximize G:F in growing pigs (Fan and Archbold, 2012), the question of which levels of Ca and P should be used is still unanswered.

The requirement of STTD P suggested by NRC (2012) was obtained from a model based on maximum whole-body P retention using Eq. [1]:

$$\text{STTD P requirements (g/d)} = 0.85 \times [(\text{maximum whole-body P retention})/0.77 + 0.19 \times \text{feed dry matter intake} + 0.007 \times \text{BW}], \quad [1]$$

where 85% of P requirement was assumed to achieve the maximum growth performance, 77% of STTD P was assumed to be retained in the body, 190 mg/kg DMI was estimated as basal endogenous losses of P, and 7 mg/kg BW per day was assumed as minimum urinary losses. It was also assumed that whole-body P mass has a direct relationship with body protein, Eq. [2]:

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

After the requirements of STTD P were established, the requirements for total Ca suggested by NRC (2012) were derived from STTD P requirements by using a 2.15:1 Ca: STTD P ratio (Table 2.1). However, the basis for using this ratio is not clear and more research in this area is needed.

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TABLE

Table 2.1. Total Ca and standardized total tract digestible (STTD) P requirements for growing and finishing pigs, (NRC, 2012)

	Body weight range, kg						
Item	5-7	7-11	11-25	25-50	50-75	75-100	100-135
Total Ca, %	0.85	0.80	0.70	0.66	0.59	0.52	0.46
STTD P, %	0.45	0.40	0.33	0.31	0.27	0.24	0.21
Ca:STTD P	1.89:1	2.00:1	2.12:1	2.13:1	2.19:1	2.16:1	2.19:1

CHAPTER 3

EFFECTS OF MICROBIAL PHYTASE ON APPARENT AND STANDARDIZED TOTAL TRACT DIGESTIBILITY OF CALCIUM IN CALCIUM SUPPLEMENTS FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to test the hypothesis that differences in the apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca exist among Ca supplements and that inclusion of microbial phytase increases the ATTD and STTD of Ca. One hundred and four growing barrows (average initial BW of 17.73 ± 2.53 kg) were allotted to a randomized complete block design with 13 dietary treatments and 8 pigs per treatment. A basal diet containing corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate, vitamins, and minerals was formulated. Five additional diets were formulated by adding monocalcium phosphate (MCP), dicalcium phosphate (DCP), calcium carbonate, *L. calcareum* Ca, or a high-Ca sugar beet co-product to the basal diet at the expense of cornstarch. Six additional diets with 500 units per kilogram of microbial phytase were also formulated. A Ca-free diet was used to determine basal endogenous losses of Ca. Feces were collected using the marker-to-marker approach. Results indicated that regardless of inclusion of microbial phytase, MCP had the greatest ($P < 0.05$) ATTD and STTD of Ca. The ATTD and STTD of Ca in DCP were greater ($P < 0.05$) than in calcium carbonate, *L. calcareum* Ca, or in the sugar beet co-product, but no differences were observed among the ATTD and STTD of Ca in calcium carbonate, *L. calcareum* Ca, or sugar beet co-product. Inclusion of microbial phytase increased ($P < 0.05$) the ATTD and STTD of Ca in the diets, but this was not the case in the Ca supplements. Regardless of inclusion of microbial phytase, the ATTD of P was

greater ($P < 0.05$) in pigs fed basal, MCP, or DCP diets than in pigs fed calcium carbonate, *L. calcareum* Ca, or the sugar beet co-product, but pigs fed calcium carbonate diets had greater ($P < 0.05$) ATTD of P than pigs fed *L. calcareum* Ca or the sugar beet co-product. Regardless of Ca source, inclusion of microbial phytase increased ($P < 0.001$) the ATTD of P. In conclusion, MCP has the greatest ATTD and STTD of Ca among the calcium supplements used in this experiment, followed by DCP. Basal, MCP, and DCP diets had greater ATTD of P than the other diets, and inclusion of microbial phytase increased the ATTD and STTD of Ca and ATTD of P in the diets.

Key words: apparent digestibility, calcium, calcium supplements, phytase, pigs, standardized digestibility

INTRODUCTION

Most Ca in diets fed to pigs is supplemented as inorganic Ca due to the low concentration of Ca in most plant-based feed ingredients. In a typical corn-soybean meal diet for a 40-kg pig, the Ca contribution from corn and soybean meal is around 1 g per kg of diet, whereas approximately 5 g of Ca per kg of diet is supplied by limestone and calcium phosphates (NRC, 1998). Apparent total tract digestibility (**ATTD**) values for Ca in some ingredients have been reported (Bohlke et al., 2005; Stein et al., 2011; González-Vega et al., 2013), but for most commonly used Ca sources, digestibility values are not available. Values for ATTD of Ca may be influenced by the concentration of Ca in the diet, which is a result of the endogenous loss of Ca from the intestinal tract of pigs (González-Vega et al., 2013). Therefore, it is likely that values for standardized total tract digestibility (**STTD**) of Ca are more accurate to use in diet formulations than values for ATTD because STTD values are additive in mixed diets (NRC, 2012). However, for most feed ingredients, values for the STTD of Ca are not available, but the

relative bioavailability of Ca may vary among Ca supplements (Ross et al., 1984). It is, therefore, expected that STTD of Ca also differs among Ca sources.

Inclusion of microbial phytase in swine diets often increases the digestibility of Ca and P (Brady et al., 2002; Liao et al., 2006; Poulsen et al., 2010), but effects of phytase on the STTD of Ca in individual ingredients have not been reported. Therefore, the objectives of this experiment were to test the hypothesis that 1) differences in the ATTD and STTD of Ca exist among Ca supplements; and 2) that inclusion of microbial phytase to the diets increases the ATTD and STTD of Ca. If these hypotheses are confirmed, it will be concluded that diets fed to pigs are most accurately formulated if values for the STTD of Ca in all feed ingredients are used.

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 G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN).

Animals and Housing

One hundred and four growing barrows with an average initial BW of 17.73 ± 2.53 kg were randomly allotted to 13 diets with 8 replicate pigs per treatment using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). The weight of the pigs was used as the block for the allotment. Pigs were housed individually in metabolism cages that were equipped with a feeder, a nipple drinker, a slatted floor, and a screen floor for total fecal collection. This experiment was conducted in 5 blocks, 3 blocks with 2 replicate pigs per diet and 2 blocks with 1 pig per diet.

Diets and Feeding

The sources of Ca that were used in this experiment were monocalcium phosphate

(**MCP**), dicalcium phosphate (**DCP**), calcium carbonate, *L. calcareum* Ca (Vistacal; AB Vista Feed Ingredients, Marlborough, UK), and a sugar beet co-product (Limex; British Sugar PLC, Peterborough, UK; Table 3.1). Monocalcium phosphate and DCP were obtained from PCS Sales, Northbrook, IL. Calcium carbonate was obtained from ILC Resources, Urbandale, IA. *Lithothamnium calcareum* Ca is a source of Ca produced by calcified seaweeds, *Lithothamnium calcareum*, and was obtained from Celtic Sea Minerals, Currabinny, Co. Cork, Ireland. The sugar beet co-product was obtained during sugar juice purification, and is a source of Ca in the form of calcium dihydroxide precipitated with carbon dioxide (Associated British Foods Annual Report and Accounts, 2008).

A basal diet containing corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate (**MSP**), vitamins, and minerals was formulated (Table 3.2). This diet contained 0.33% Ca and 0.42% total P (Table 3.3). Two diets containing 0.70 and 0.79% Ca and 0.64 and 0.67% total P were formulated by adding MCP or DCP, respectively, to the basal diet and the inclusion of MSP was eliminated. Three additional diets that all contained between 0.75 and 0.86% Ca and between 0.40 and 0.42% total P were formulated by adding calcium carbonate, *L. calcareum* Ca, or the sugar beet co-product to the basal diet at the expense of cornstarch.

Six additional diets that were similar to the previous 6 diets, with the exception that they also contained 500 units per kg of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK), were also formulated. Microbial phytase was included in these diets at the expense of cornstarch. A Ca-free diet that was used to measure basal endogenous losses of Ca was also formulated. This diet contained cornstarch, potato protein isolate, sucrose, soybean oil, Solka floc, MSP, crystalline AA, vitamins, and minerals.

Pigs were fed each diet for 13 d and the feed allotment was calculated as 3 times the daily maintenance energy requirement (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). The allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h daily. Pigs had free access to water throughout the experiment. The initial 5 d was an adaptation period to the diets and fecal samples were collected quantitatively from d 6 to 11 using the marker-to-marker approach (Adeola, 2001). Indigo carmine was the indigestible marker added to the morning meal on d 6 to mark the beginning of fecal collection. Ferric oxide was the marker added to the morning meal on d 11 to mark the end of fecal collection. Fecal samples were stored at -20°C immediately after collection and samples were dried in a forced-air oven at 65°C and finely ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) before laboratory analysis.

Sample Analysis

Corn, potato protein isolate, MCP, DCP, calcium carbonate, *L. calcareum* Ca, the sugar beet co-product, MSP, all diets, and all fecal samples were analyzed for DM (Method 930.15; AOAC Int., 2007) and for Ca and P by inductively coupled plasma spectroscopy-optical emission spectroscopy (Method 985.01 A, B, and D; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC Int., 2007). All ingredients and diets were also analyzed for ash (Method 942.05; AOAC Int., 2007). Corn, potato protein isolate, and diets were analyzed for GE using an adiabatic bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) and for CP using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). These samples were also analyzed for ADF (Method 973.18; AOAC Int., 2007), NDF (Holst, 1973), phytic acid (Ellis et al., 1977), and for acid-hydrolyzed ether extract using 3*N* HCl

(Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Diets were also analyzed for phytase activity (Engelen et al., 2001).

Calculations and Statistical Analysis

The amount of phytate-bound P in corn, potato protein isolate, and diets was calculated as 28.2% of the concentration of phytate (Tran and Sauvant, 2004) and the amount of non-phytate P was calculated as the difference between phytate-bound P and total P. Values for ATTD and STTD of Ca were calculated for each diet according to standard procedures (NRC, 2012). Samples from pigs fed the Ca-free diet were used to determine basal endogenous losses of Ca. The ATTD of Ca in each of the Ca-containing ingredients without or with microbial phytase was calculated using the difference procedure (Adeola, 2001). The values for ATTD of Ca were corrected for endogenous losses of Ca to obtain the STTD of Ca using the same principles as those outlined to calculated standardized ileal digestibility of AA (Stein et al., 2007).

Normality of residuals and outliers were tested using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data of the diets were analyzed as a 6 × 2 factorial and the ATTD and STTD of Ca in the Ca sources were analyzed as a 5× 2 factorial using the MIXED procedure of SAS. The model included the fixed effects of Ca source, the level of phytase, and the interaction between Ca source and level of phytase, and the random effect of block. Least square means were calculated for each treatment using the LSMeans procedure in SAS and differences among means were separated using the PDIFF option of SAS. The pig was the experimental unit and an alpha level of 0.05 was used to determine significance among means.

RESULTS

Nutrient Composition of Ingredients and Diets

The concentration of Ca in corn, potato protein isolate, MCP, DCP, Ca carbonate, *L. calcareum* Ca, and sugar beet co-product was between 0.01 and 41.82%, and the concentration of P was between 0.15 and 21.60% (as-fed basis; Table 3.1). The MSP used in this experiment was analyzed to contain 0.09% Ca and 27.10% P. The ingredient composition of the experimental diets without microbial phytase is presented in Table 3.2 (as-fed basis). The concentration of Ca in basal diets without and with microbial phytase was 0.33 and 0.31%, respectively, and the concentration of P was 0.42 and 0.40%, respectively (Tables 3.3 and 3.4). The analyzed concentration of Ca in diets containing MCP, DCP, Ca carbonate, *L. calcareum* Ca, and the sugar beet co-product was between 0.70 and 0.86% if phytase was not used and between 0.75 and 0.86% in diets that contained microbial phytase. The concentration of P was between 0.40 and 0.67% if phytase was not used and between 0.42 and 0.69% if phytase was used. The concentration of Ca and P in the Ca-free diet was 0.02% and 0.23%, respectively. The concentration of Ca and P in the diets was close to expected values, with the exception that the concentration of Ca in the Ca carbonate diet without phytase was slightly less than expected, but it was assumed that this small difference did not affect the results. The concentration of phytase in most diets was close to expected values, although the concentration of phytase in MCP and *L. calcareum* Ca diets were somewhat greater than formulated.

Digestibility of Ca in Diets

All pigs remained healthy and consumed their assigned diets without apparent problems. The ADFI and basal endogenous losses (mg/d) of Ca were not affected by Ca source or microbial phytase (Table 3.5). The intake of Ca increased ($P < 0.001$) in pigs fed diets containing DCP or Ca carbonate if microbial phytase was used, but Ca intake was not affected by microbial

phytase in the basal diet or in the diets containing MCP, *L. calcareum* Ca, or the sugar beet co-product (Ca source × phytase interaction, $P < 0.001$). Regardless of phytase inclusion, pigs fed diets containing DCP or the sugar beet co-product had greater ($P < 0.05$) fecal output than pigs fed Ca carbonate or the basal diets, but fecal output was not different from MCP and *L. calcareum* Ca. Regardless of Ca source, pigs fed diets containing microbial phytase had greater ($P < 0.05$) fecal output than pigs fed diets that did not contain microbial phytase. Regardless of phytase inclusion, the concentration of Ca was greater ($P < 0.05$) in feces from pigs fed Ca carbonate, *L. calcareum* Ca, or sugar beet co-product than from pigs fed the basal, MCP, or DCP diets. Inclusion of microbial phytase decreased ($P < 0.05$) the concentration of Ca in feces, regardless of the Ca source. The amount of Ca output was not affected by the inclusion of microbial phytase, but pigs fed the *L. calcareum* Ca or the sugar beet co-product diets had a greater ($P < 0.05$) amount of Ca output than pigs fed the basal, MCP, DCP, or Ca carbonate diets. The amount of Ca absorbed in pigs fed the basal, *L. calcareum* Ca, or the sugar beet co-product diets was not affected by microbial phytase inclusion, but pigs fed MCP, DCP, or Ca carbonate had a greater ($P < 0.05$) amount of Ca absorbed if microbial phytase was included in the diets than if no microbial phytase was used (Ca source × phytase interaction, $P < 0.01$). Regardless of phytase inclusion, pigs fed the MCP diets had the greatest ($P < 0.05$) ATTD of Ca, but pigs fed the basal or DCP diets had a greater ($P < 0.05$) ATTD of Ca than pigs fed the Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets. Regardless of phytase inclusion, pigs fed MCP diets had greater ($P < 0.05$) STTD of Ca than pigs fed the DCP, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, but STTD of Ca was not different between the MCP and basal diets. Regardless of Ca source, the ATTD and STTD of Ca increased ($P < 0.05$) if microbial phytase was added to the diets.

Digestibility of Ca in Ingredients

Inclusion of microbial phytase did not affect the ATTD or STTD of Ca in the Ca sources (Table 3.6). Regardless of inclusion of microbial phytase, pigs fed MCP had the greatest ($P < 0.05$) ATTD and STTD values of Ca, and pigs fed DCP had greater ($P < 0.05$) ATTD and STTD of Ca than pigs fed Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product, but no differences were observed among ATTD and STTD of Ca in Ca carbonate, *L. calcareum* Ca, and the sugar beet co-product.

Digestibility of P in Diets

Regardless of Ca source, inclusion of microbial phytase increased ($P < 0.05$) the amount of P intake, percentage of P absorbed, and ATTD of P (Table 3.7). The percentage of P in the feces and amount of P output decreased ($P < 0.05$) if microbial phytase was included in the diets. Regardless of phytase inclusion, pigs fed MCP or DCP had greater ($P < 0.05$) P intake than pigs fed the basal, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets. Pigs fed the basal diets had less ($P < 0.05$) concentration of P in feces than pigs fed MCP, DCP, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, regardless of phytase inclusion. The amounts of P output from pigs fed MCP, DCP, *L. calcareum* Ca, or the sugar beet co-product were greater ($P < 0.05$) than from pigs fed Ca carbonate or the basal diets, regardless of phytase inclusion, but pigs fed Ca carbonate diets had greater ($P < 0.05$) P output than pigs fed the basal diets. The percentage of P absorbed was greater ($P < 0.05$) in pigs fed MCP or DCP diets than pigs fed basal, Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets, but pigs fed basal diets had greater ($P < 0.05$) percentage of P absorbed than pigs fed Ca carbonate, *L. calcareum* Ca, or the sugar beet co-product diets. The ATTD of P was greater ($P < 0.05$) in pigs fed basal, MCP, or DCP diets than pigs fed Ca carbonate, *L. calcareum* Ca, or the sugar beet

co-product diets, regardless of phytase inclusion, but pigs fed Ca carbonate had greater ($P < 0.05$) ATTD of P than pigs fed *L. calcareum* Ca or the sugar beet co-product diets.

DISCUSSION

The concentration of Ca and P in MCP and the concentration of P in DCP used in this experiment are in agreement with reported values (Sauvant et al., 2004; Rostagno et al., 2011; NRC, 2012), but the concentration of Ca in DCP was less than published values (Sauvant et al., 2004; Rostagno et al., 2011; NRC, 2012). The reason for variations in the concentration of Ca in MCP and DCP is that what is commercially known as MCP and DCP, in fact are mixtures of a number of P-containing compounds (Baker, 1989; Petersen and Stein, 2006) as a result of the way feed grade MCP and DCP are produced. Production of MCP and DCP starts with the addition of phosphoric acid to Ca carbonate and the reaction between these 2 components is stopped according to the amount of total P desired in the final product. The reaction will be stopped at 18.5% P if DCP is produced and at 21.0% P if MCP is produced. Therefore, the final product is a mixture of unreacted calcium carbonate, MCP, hydrated DCP, anhydrous DCP, and other reactive P compounds. Phosphorus compounds such as ferrous phosphate, aluminum phosphate, magnesium phosphate, sodium phosphate, and unreacted phosphoric acid represents between 15 and 17% of the total P in the final products (Baker, 1989). Calcium is also present as Ca fluoride and Ca sulfate, which represents 36.2 to 42.3% of the total Ca in the final products (Baker, 1989). In general, what is commercially known as MCP contains 50 to 70% MCP and 10 to 15% DCP, and DCP may contain approximately 29% MCP and 57% DCP (Petersen and Stein, 2006).

The concentrations of Ca and P in Ca carbonate used in this experiment were greater than reported values (Sauvant et al., 2004; NRC 2012), but the concentrations of Ca and P in MSP were within the range of reported values (Sauvant et al., 2004; González-Vega et al., 2013, 2014; NRC, 2012). *Lithothamnium calcareum* may be used as a Ca supplement for poultry (Walk et al., 2012) and pigs (Melo and Moura, 2009; González-Vega et al., 2014). The concentrations of Ca and P in *L. calcareum* Ca used in this experiment were slightly greater than reported values (Melo and Moura, 2009; Walk et al., 2012; González-Vega et al., 2014).

The sugar beet co-product is mostly used in agriculture for soil conditioning and for correction of soil acidity (Associated British Foods Annual Report and Accounts, 2008). The concentrations of Ca and P in the sugar beet co-product used in this experiment were close to expected values.

The concentration of Ca and P in corn concur with reported values (Sauvant et al., 2004; Rostagno et al., 2011; Almeida and Stein, 2012; NRC, 2012), but the concentration of phytate-bound P was slightly less than reported values (Sauvant et al., 2004; Almeida and Stein, 2012). The concentration of Ca, P, and phytate in potato protein isolate were close to previous data (González-Vega et al., 2013). The concentrations of Ca and P in the diets were close to the expected values, but varied among the diets due to the way the diets were formulated. Because of the different concentration of Ca and P in the test ingredients used in this experiment, it was not possible to maintain a constant concentration of Ca and P among all diets. However, the ATTD of Ca is not influenced by Ca concentration in the diets if the Ca concentration is between 0.33 and 0.80% (Stein et al., 2011) and if the P concentration is between 0.44 and 0.66% (Stein et al., 2008), and all diets, except the Ca-free diet, were formulated to stay within these limits.

Phytate and Ca Interactions

Phytate in plant feed ingredients, may bind up to 6 atoms of Ca and promote the formation of Ca-phytate complexes and reduce the digestibility of Ca in the intestinal tract (Selle et al., 2009). There are 2 possible mechanisms that may explain how phytate decreases the digestibility of Ca. One possible mechanism is that phytate binds the intrinsic Ca in the feed ingredient and reduces digestibility. For example, when canola meal was used as the sole source of dietary Ca, the ATTD and true total tract digestibility of Ca were less than that of pigs fed canola meal with microbial phytase (González-Vega et al., 2013). This indicates that intrinsic Ca in canola meal was indeed bound to phytate, and the addition of microbial phytase hydrolyzed the phytate ester bonds, and therefore, phytate had less ability to bind Ca if phytase was added to the diet. The other possible mechanism is that not only intrinsic Ca, but also supplemented dietary Ca, may form complexes with phytate. The ATTD of Ca in diets in which the majority of the Ca was inorganic Ca increased if phytase was added to the diets (Guggenbuhl et al., 2007; Poulsen et al., 2010; Rodríguez et al., 2013). The increase in ATTD of Ca in most of these experiments was greater than what can be explained by release of intrinsic Ca from phytate, which indicates that some of the added dietary Ca may also complex with phytate if phytase is not used in the diet. The fact that the ATTD and STTD of Ca in the diets evaluated in this experiment increased as microbial phytase was added to the diet indicates that some of the Ca in those feed ingredients was bound to phytate. The observation that microbial phytase did not affect the ATTD and STTD of Ca in the Ca supplements, but only in the diets, further indicates that dietary phytate binds to Ca from the inorganic dietary Ca sources.

Differences in Digestibility of Ca among Sources of Ca

The greater ATTD and STTD of Ca in MCP and DCP than in Ca carbonate indicates that Ca in Ca carbonate is more easily bound to phytate or is less soluble. This is likely because Ca in MCP and DCP is already bound to P, which is a result of the reaction between Ca carbonate and phosphoric acid in the production of these ingredients.

We are not aware of other experiments with pigs in which Ca digestibility is compared among MCP, DCP, and calcium carbonate, but in a recent experiment with broilers it was observed that the digestibility of Ca in MCP (67.9%) was greater than in limestone (34.1%; Angel, 2013). Thus, results obtained both with broilers and with pigs indicate that the digestibility of Ca in Ca carbonate or limestone is less than the digestibility of Ca in MCP and DCP.

Concentrations of Ca in *L. calcareum* Ca and the sugar beet co-product were relatively less than in Ca carbonate, however, *L. calcareum* Ca is a very soluble source of Ca (Walk et al., 2012; González-Vega et al., 2014). The ATTD of Ca in Ca carbonate obtained in this experiment was in agreement with reported values (Stein et al., 2011), but the ATTD and STTD of Ca in Ca carbonate and *L. calcareum* Ca were greater than the values reported by González-Vega et al. (2014). The total and available P levels in the diets were similar among experiments, but the main difference among the experiments is that corn-based diets were used by Stein et al. (2011) and in the present experiment, whereas semi-synthetic diets were used by González-Vega et al. (2014). We are not aware of the form in which Ca is present in *L. calcareum* Ca or in the sugar beet co-product, but the observation that ATTD and STTD of Ca in *L. calcareum* Ca and the sugar beet co-product were not different from values for Ca carbonate without or with microbial phytase indicates that Ca-P complexes may have been formed in pigs fed *L. calcareum* Ca (González-Vega et al., 2014) and the sugar beet co-product.

Previous data reported a difference in relative bioavailability among Ca supplements (Ross et al., 1984), which could indicate that STTD of Ca may differ among Ca supplements. Results of the present study demonstrated that there are differences in Ca digestibility among Ca supplements. Therefore, generated values for STTD of Ca in Ca supplements without or with phytase may lead to a more accurate formulation of diets fed to pigs.

Digestibility of P

Phosphorus from corn and potato protein isolate represents 30 to 53% of the total P in the diets used in this experiment, which results in 24 to 43% of the total P in the diets being bound to phytate. Inclusion of microbial phytase in the diets decreases the amount of P excreted in feces (Harper et al., 1997), which is a result of the hydrolysis of phytate by phytase. Therefore, the increase in ATTD of P that was observed as phytase was added to the diets can be explained by the release of P bound to phytate (Poulsen et al., 2010).

Monosodium phosphate was the source of P used in the basal, Ca carbonate, *L. calcareum* Ca, and sugar beet co-product diets and MCP and DCP were the main sources of P in the MCP and DCP diets. The ATTD of P in MSP is greater than in MCP and DCP (Petersen and Stein, 2006), but in the current experiment, the greater ATTD of P observed in diets containing MCP or DCP compared with diets using MSP may be a result of the reduced contribution of P from corn in diets containing MCP or DCP compared with diets containing MSP. In diets containing MCP or DCP, between 24 and 27% of the total P is bound to phytate, whereas in the diets containing MSP, between 38 and 43% of the total P is bound to phytate.

Conclusions

The ATTD and STTD of Ca were greater in MCP and DCP than in Ca carbonate, L. calcareum Ca, and the sugar beet co-product. Inclusion of microbial phytase increased the ATTD

and STTD of Ca in diets, but not in the Ca supplements, confirming that dietary phytate interferes with Ca digestibility. The ATTD of P was greater in the basal, MCP, and DCP diets than in the other diets, and regardless of the Ca source, addition of microbial phytase increased the ATTD of P in all diets.

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TABLES

Table 3.1. Analyzed composition of ingredients, as-fed basis

	Ingredient[1]							
Item	Corn	Potato protein isolate	MCP	DCP	Calcium carbonate	*L. calcareum* Ca	Sugar beet co-product	MSP
GE, kcal/kg	3,925	5,252	-	-	-	-	-	-
DM, %	89.06	91.95	93.50	95.09	99.94	99.27	92.01	98.99
Ash, %	1.22	0.51	81.36	84.66	94.53	92.97	84.21	90.61
CP, %	8.12	81.97	-	-	-	-	-	-
AEE,[2] %	3.73	0.16	-	-	-	-	-	-
ADF, %	2.74	4.59	-	-	-	-	-	-
NDF, %	11.54	1.32	-	-	-	-	-	-
Ca, %	0.01	0.04	18.54	21.61	41.82	34.57	31.70	0.09

Table 3.1. (Cont.)

P, %	0.26	0.15	21.60	19.21	0.16	0.22	0.81	27.10
Phytate, %	0.75	0.34	-	-	-	-	-	-
Phytate-bound P,[3] %	0.16	0.10	-	-	-	-	-	-
Non-phytate P,[4] %	0.10	0.05	-	-	-	-	-	-

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate; MSP = monosodium phosphate. *L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[2]AEE= acid-hydrolyzed ether extract.

[3]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 3.2. Ingredient composition of experimental diets without microbial phytase, as-fed basis

	Diet[1, 2]						
Ingredient, %	Basal	MCP	DCP	Calcium carbonate	*L. calcareum* Ca	Sugar beet co-product	Ca-free
Corn	77.00	77.00	77.00	77.00	77.00	77.00	-
Cornstarch	9.60	8.35	8.20	8.40	8.15	8.00	58.57
Potato protein isolate	8.00	8.00	8.00	8.00	8.00	8.00	10.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	4.00
Calcium carbonate	0.80	0.80	0.80	2.00	0.80	0.80	-
Monocalcium phosphate	-	2.00	-	-	-	-	-
Dicalcium phosphate	-	-	2.15	-	-	-	-
L. calcareum Ca[3]	-	-	-	-	1.45	-	-
Sugar beet co-product[4]	-	-	-	-	-	1.60	-

Table 3.2. (Cont.)

Monosodium phosphate	0.75	-	-	0.75	0.75	0.75	0.95
L-Lys HCl	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	-	-	-	-	-	-	0.11
L-Trp	-	-	-	-	-	-	0.02
Potassium carbonate	-	-	-	-	-	-	0.40
Magnesium oxide	-	-	-	-	-	-	0.10
Solka floc[5]	-	-	-	-	-	-	5.00
Sucrose	-	-	-	-	-	-	20.00
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[6]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 3.2. (Cont.)

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate.

[2]Six additional diets that were similar to the above diets, with the exception that 500 units per kilogram of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK) was included in the diets at the expense of cornstarch were also formulated.

[3]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[4]The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[5]Fiber Sales and Development Corp., Urbana, OH.

[6] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

Table 3.3. Chemical composition of experimental diets without microbial phytase, as-fed basis

	Diet[1]						
Item	Basal	MCP	DCP	Calcium carbonate	*L. calcareum* Ca	Sugar beet co-product	Ca-free
DM, %	90.23	90.24	90.04	90.22	89.90	89.32	92.79
Ash, %	3.43	4.58	5.02	5.28	4.82	5.09	2.34
GE, kcal/kg	4,107	4,101	4,040	4,089	4,080	4,022	4,064
CP, %	13.27	13.12	12.22	12.62	12.76	12.90	8.41
AEE,[2] %	5.75	5.71	5.70	5.87	5.12	4.69	5.78
ADF, %	2.64	2.55	2.56	2.51	2.64	2.41	4.56
NDF, %	9.99	9.34	8.91	8.76	9.28	9.15	8.61
Ca, %	0.33	0.70	0.79	0.75	0.85	0.86	0.02
P, %	0.42	0.64	0.67	0.40	0.42	0.42	0.23
Phytase, FTU/kg[3]	<50	<50	<50	<50	<50	<50	<50

Table 3.3. (Cont.)

Phytate,[4] %	0.60	0.60	0.60	0.60	0.60	0.60	0.03
Phytate-bound P,[5] %	0.17	0.17	0.17	0.17	0.17	0.17	0.01
Non-phytate P,[6]%	0.25	0.47	0.50	0.23	0.25	0.25	0.22

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate. *L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[2]AEE= acid-hydrolyzed ether extract.

[3]FTU= phytase units.

[4]Phytate values were calculated using the values of phytate in the ingredients.

[5]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 3.4. Chemical composition of experimental diets with microbial phytase, as-fed basis

	Diet with 500 FTU/kg[1]					
Item	Basal	MCP	DCP	Calcium carbonate	*L. calcareum* Ca	Sugar beet co-product
DM, %	89.67	90.23	90.22	90.33	90.14	89.89
Ash, %	3.55	3.10	5.40	3.91	4.93	4.39
GE, kcal/kg	4,145	4,071	4,082	4,087	4,020	4,064
CP, %	12.30	12.93	13.01	13.38	12.49	12.74
AEE,[2] %	5.89	5.58	5.76	5.62	5.05	4.69
ADF, %	2.46	2.36	2.19	2.36	2.47	2.42
NDF, %	8.61	9.19	9.08	9.06	9.20	9.45
Ca, %	0.31	0.75	0.86	0.82	0.83	0.83
P, %	0.40	0.69	0.67	0.44	0.43	0.42
Phytase, FTU/kg[3]	470	443	431	679	475	544

Table 3.4. (Cont.)

Phytate,[4] %	0.60	0.60	0.60	0.60	0.60	0.60
Phytate-bound P,[5] %	0.17	0.17	0.17	0.17	0.17	0.17
Non-phytate P,[6] %	0.23	0.52	0.50	0.27	0.26	0.25

[1]MCP = monocalcium phosphate; DCP = dicalcium phosphate. *L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

[2]AEE= acid-hydrolyzed ether extract.

[3]FTU= phytase units.

[4]Phytate values were calculated using the values of phytate in the ingredients.

[5]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 3.5. Apparent and standardized total tract digestibility of Ca in diets containing calcium supplements without or with microbial phytase[1]

Item	ADFI, g/d	Ca intake, g/d	Fecal output, g/d	Ca in feces, %	Ca output, g/d	Ca absorbed, g/d	ATTD of Ca, %	Basal endogenous losses of Ca, mg/d	STTD[2] of Ca, %
No phytase									
Basal[3]	757	2.54^{e}	73.27	1.20	0.91	1.63^{e}	65.51	84.07	68.88
MCP[4]	790	5.53^{cd}	81.73	1.71	1.40	4.13^{c}	75.09	87.72	76.68
DCP[5]	758	5.97^{bc}	85.80	2.05	1.74	4.23^{bc}	71.28	83.94	72.68
Calcium carbonate	687	5.14^{d}	69.43	2.79	1.93	3.21^{d}	62.56	76.22	64.04
L. calcareum Ca[6]	749	6.35^{ab}	80.65	2.84	2.28	4.07^{c}	64.06	82.90	65.36
Sugar beet co-product[7]	743	6.37^{ab}	87.19	2.56	2.17	4.20^{bc}	66.12	81.69	67.40
With phytase									

Table 3.5. (Cont.)

Basal[3]	770	2.43[e]	76.57	0.74	0.59	1.83[e]	76.46	84.93	80.02
MCP[4]	792	5.93[bc]	85.14	1.52	1.28	4.65[ab]	78.56	87.96	80.04
DCP[5]	783	6.72[a]	90.08	2.02	1.81	4.90[a]	73.51	86.99	74.80
Calcium carbonate	763	6.24[ab]	85.33	2.26	1.89	4.35[bc]	69.87	84.84	71.23
L. calcareum Ca[6]	760	6.29[ab]	84.27	2.48	2.07	4.22[bc]	67.22	84.28	68.56
Sugar beet co-product[7]	748	6.20[b]	88.53	2.46	2.20	4.00[c]	65.09	82.79	66.42
SEM	41	0.30	6.22	0.17	0.17	0.23	2.33	4.58	2.33
P-value									
Ca source	0.086	0.003	0.007	<0.001	<0.001	<0.001	<0.001	0.074	<0.001
Phytase	0.092	<0.001	0.026	<0.001	0.160	<0.001	<0.001	0.082	<0.001
Ca source × phytase	0.572	0.001	0.509	0.399	0.564	0.008	0.113	0.565	0.106

[a-e] Values within a column without a common superscript are different ($P < 0.05$).

Table 3.5. (Cont.)

[1]Data are means of 8 observations per treatment, except for the basal diet with phytase that had only 7 observations.

[2]Values for standardized total tract digestibility were calculated by correcting apparent total tract digestibility values for basal endogenous losses. Basal endogenous losses were determined from pigs fed the Ca-free diet as 0.123 ± 0.054 g/kg of DMI.

[3]Basal diet contained corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate, vitamins, and minerals was formulated to contain 0.33% Ca.

[4]MCP = monocalcium phosphate.

[5]DCP = dicalcium phosphate.

[6]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[7]The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

Table 3.6. Apparent and standardized total tract digestibility of Ca in calcium supplements without or with microbial phytase[1]

Item	ATTD of Ca, %	STTD[2] of Ca, %
No phytase		
MCP[3]	82.76	85.86
DCP[4]	75.29	77.80
Calcium carbonate	57.98	60.43
L. calcareum Ca[5]	62.54	64.98
Sugar beet co-product[6]	66.18	68.41
With phytase		
MCP[3]	83.24	86.34
DCP[4]	76.39	78.90
Calcium carbonate	70.62	73.07
L. calcareum Ca[5]	66.24	68.67
Sugar beet co-product[6]	63.18	65.41
SEM	3.80	3.80
P-value		
Ca source	<0.001	<0.001
Phytase	0.173	0.173
Ca source × phytase	0.212	0.212

[1]Data are means of 8 observations per treatment.

[2]Values for standardized total tract digestibility were calculated by correcting apparent total tract digestibility values for basal endogenous losses. Basal endogenous losses were

Table 3.6. (Cont.)

determined from pigs fed the Ca-free diet as 0.123 ± 0.054 g/kg of DMI.

[3]MCP = monocalcium phosphate.

[4]DCP = dicalcium phosphate.

[5]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[6]The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

Table 3.7. Apparent total tract digestibility of P in diets containing different calcium supplements without or with microbial phytase[1]

Item	P intake, g/d	P in feces, %	P output, g/d	P absorbed, g/d	ATTD of P, %
No phytase					
Basal[2]	3.19	1.47	1.09	2.09	66.14
MCP[3]	5.04	1.93	1.56	3.48	69.03
DCP[4]	5.05	1.78	1.48	3.57	70.62
Calcium carbonate	2.76	1.76	1.23	1.53	55.27
L. calcareum Ca[5]	3.16	1.94	1.56	1.60	50.56
Sugar beet co-product[6]	3.13	1.80	1.51	1.62	51.47
With phytase					
Basal[2]	3.09	1.14	0.87	2.22	72.17
MCP[3]	5.44	1.57	1.32	4.12	75.75
DCP[4]	5.23	1.66	1.50	3.73	71.68
Calcium carbonate	3.36	1.51	1.27	2.10	62.29
L. calcareum Ca[5]	3.27	1.59	1.33	1.95	59.45
Sugar beet co-product[6]	3.17	1.60	1.35	1.81	57.28

Table 3.7. (Cont.)

SEM	0.22	0.11	0.11	0.15	2.01
P-value					
Ca source	<0.001	<0.001	<0.001	<0.001	<0.001
Phytase	0.004	<0.001	0.002	<0.001	<0.001
Ca source × phytase	0.065	0.566	0.142	0.103	0.358

[1]Data are means of 8 observations per treatment, except for the basal diet with phytase and the sugar beet co-product diet with phytase, which had only 7 observations.

[2]Basal diet contained corn, cornstarch, potato protein isolate, soybean oil, calcium carbonate, monosodium phosphate, vitamins, and minerals was formulated to contain 0.33% Ca and 0.26% standardized total tract digestible P.

[3]MCP = monocalcium phosphate.

[4]DCP = dicalcium phosphate.

[5]*L. calcareum* Ca is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland).

[6]The sugar beet co-product is a source of Ca in a form of calcium dihydroxide precipitated with carbon dioxide during sugar juice purification (British Sugar PLC, Peterborough, UK).

CHAPTER 4

EFFECT OF PHYTATE, MICROBIAL PHYTASE, FIBER, AND SOYBEAN OIL ON CALCULATED VALUES FOR APPARENT AND STANDARDIZED TOTAL TRACT DIGESTIBILITY OF CALCIUM IN FISH MEAL FED TO GROWING PIGS

ABSTRACT: Two experiments were conducted to determine the effects of phytate, phytase, fiber, and soybean oil on the apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca and on ATTD of P in fish meal fed to growing pigs. In Exp. 1, 40 growing pigs (initial average BW: 19.16 ± 2.04 kg) were randomly allotted to 1 of 5 diets with 8 pigs per treatment and placed in metabolism crates. Four diets were used in a 2 × 2 factorial design with 2 levels of phytate (0 or 0.7%), and 2 levels of microbial phytase (0 or 500 phytase units/kg). The diet containing no phytate was based on sucrose, cornstarch, fish meal, casein, and soybean oil, and the diet containing 0.7% phytate was based on corn, corn germ, fish meal, casein, and soybean oil. A Ca-free diet was used to determine basal endogenous losses of Ca. Feces were collected from d 6 to d 13 after a 5-d adaptation period. Results indicated that the ATTD and STTD of Ca in fish meal and the ATTD of P increased ($P < 0.001$) if phytase was used, and were greater ($P < 0.05$) in the diets based on corn and corn germ. Experiment 2 was conducted to determine effects of fiber and soybean oil on the ATTD and STTD of Ca and the ATTD of P in fish meal. Fifty growing pigs (initial average BW: 19.36 ± 0.99 kg) were randomly allotted to 1 of 5 diets with 10 pigs per treatment. Two diets contained sucrose, cornstarch, fish meal, casein, and either 0 or 8% of a synthetic source of fiber. Two additional diets contained fish meal, casein, corn and either 1 or 7% soybean oil. A Ca-free diet was also used. Pigs were housed individually in metabolism crates and fecal samples were collected.

Results indicated that fiber increased ($P < 0.001$) the ATTD and STTD of Ca and the ATTD of P, but the ATTD and STTD of Ca or the ATTD of P were not affected by soybean oil. In agreement with results of Exp. 1, the ATTD and STTD of Ca and the ATTD of P in the corn-based diet were greater ($P < 0.05$) than in the cornstarch-based diet. In conclusion, phytase and fiber increased the ATTD and STTD of Ca and the ATTD of P in fish meal, but inclusion of soybean oil did not affect digestibility of Ca or P. The observation that values for the ATTD and STTD of Ca and ATTD of P are greater in corn-based diets than in cornstarch-based diets indicates that values for the digestibility of Ca and P obtained in cornstarch-based diets may not always be representative for the digestibility in practical corn-based diets.

Key words: calcium digestibility, fiber, fish meal, phytase, phytate, soybean oil

INTRODUCTION

Phytate may bind Ca from dietary calcium carbonate (González-Vega et al., 2015) and intrinsic Ca in plant ingredients (Selle et al., 2009; González-Vega et al., 2013), but the effect of phytate on the digestibility of Ca in feed ingredients of animal origin has not been reported. Results of experiments in which a synthetic form of phytate was used have been inconsistent (Onyango et al., 2008; González-Vega et al., 2014). However, if natural phytate from an intact feed ingredient is added to a mixed diet that also contains other sources of Ca, phytate-Ca complexes may be formed. When intact feed ingredients are used, not only phytate, but also fiber, fat, and other nutrients are often added, and it is not known to which degree these nutrients influence the standardized total tract digestibility of Ca and the formation of Ca-phytate bonds.

Digestibility of Ca can be determined by the direct procedure that includes the test ingredient as the only source of the nutrient of interest (Adeola, 2001). By formulating diets

based on ingredients that contain virtually no Ca such as cornstarch, corn, or corn germ, it is possible to obtain digestibility values for Ca from the ingredient of interest. However, some fiber and fat is added to the diet if corn or corn germ are used. Fiber may reduce the digestibility of AA and energy (Ma et al., 2008; Zhang et al., 2013), but effects of fiber on the digestibility of minerals is variable (Grieshop et al., 2001). Fat may reduce the absorption of Ca in humans (Agnew and Holdsworth, 1971) and the digestibility of Ca in rats (Frommelt et al., 2014), but to our knowledge, there are no data on the effect of fat on the digestibility of Ca by pigs. The objectives of these experiments were to test the hypothesis that microbial phytase, dietary soybean oil, phytate from corn, and dietary fiber may influence the digestibility of Ca in fish meal.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois (Urbana-Champaign, IL) reviewed and approved the protocol for both experiments. Pigs used in the experiments were the offspring of G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN).

Experiment 1: Effect of Microbial Phytase on Ca Digestibility in the Absence or the Presence of Phytate

Diets, Animals, and Experimental Design. Experiment 1 was designed to determine the effect of phytase on the apparent total tract digestibility (**ATTD**) and the STTD of Ca in fish meal in a diet without phytate as well as a diet with phytate. Forty growing pigs (initial average BW: 19.16 ± 2.04 kg) were randomly allotted to 1 of 5 diets with 8 replicate pigs per diet and housed individually in metabolism crates. The crates were equipped with a slatted floor, a feeder,

a nipple drinker, and a screen floor that allowed for total fecal collection. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

All dietary ingredients were analyzed for nutrient composition prior to diet formulation (Table 4.1). Five diets were formulated. Four diets were used in a 2 × 2 factorial design with 2 levels of phytate (0 and 0.7%) and 2 levels of microbial phytase (0 and 500 phytase units (**FTU**)/kg; Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK). Fish meal was the source of Ca in all diets and corn and corn germ were used to add natural phytate to the diets (Table 4.2). A Ca-free diet that contained cornstarch, potato protein isolate, sucrose, soybean oil, Solka floc, crystalline AA, monosodium phosphate, vitamins, and minerals was also formulated, and this diet was used to determine basal endogenous losses of Ca. All diets except the Ca-free diet were formulated to meet Ca and P requirements of 11 to 25 kg pigs (NRC, 2012; Table 4.3).

Feeding and Sample Collection. Each pig was fed one of the experimental diets for 13 d and an amount of diet equivalent to 2.7 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012) was provided each day. Pigs had free access to water throughout the experiment. The daily allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h. Pigs had a 5-d adaptation period to the diets followed by 5 d of quantitative collection of fecal samples according to the marker-to-marker approach (Adeola, 2001). An indigestible marker (Indigo carmine) was added to the morning meal on d 6 to mark the beginning of fecal collection and ferric oxide was added to the morning meal on d 11 to mark the conclusion of fecal collection. The ferric oxide marker passed on d 12 or d 13, and fecal collections ceased at the time this marker appeared in the feces. Fecal samples were stored at -

20°C immediately after collection. Orts that were collected during the collection period were dried in a forced-air oven at 65ºC and the weight was subtracted from the total feed intake.

Sample Analysis. Fecal samples were dried in a forced-air oven at 65°C, ground in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen, and sub-samples were collected for analysis after all the ground materials had been mixed. Fish meal, casein, corn germ, corn, potato protein isolate, diets, and fecal samples were analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007), and for Ca and P by Inductively Coupled Plasma-Optical Emission Spectroscopy (**ICP-OES**; Method 985.01 A, B, and D; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Fish meal, casein, corn germ, corn, potato protein isolate, and diet samples were analyzed for GE using an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) and for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. These samples were also analyzed for ash (Method 942.05; AOAC Int., 2007), and for acid-hydrolyzed ether extract using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 Automated Analyzer (FOSS North America, Eden Prairie, MN). Corn germ and corn were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Diets were analyzed for ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Corn germ, corn, and potato protein isolate were also analyzed for phytate (Ellis et al., 1977). Diets were analyzed for phytase activity (Engelen et al., 2001). Corn and corn germ were analyzed for insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) using the ANKOM Dietary Fiber Analyzer (Method

991.43; AOAC Int., 2007). Corn germ was de-fatted before analyzed for IDF and SDF (Method 985.29E; AOAC Int., 2007).

Calculations and Statistical Analysis. The concentration of phytate-bound P in corn germ, corn, potato protein isolate, and diets was calculated as 28.2% of phytate (Tran and Sauvant, 2004) and the concentration of non-phytate P was calculated by subtracting phytate-bound P from total P.

The basal endogenous losses of Ca were determined from pigs fed the Ca-free diet and ATTD and STTD values of Ca in each diet were calculated as outlined for calculation of ATTD and STTD of P (Almeida and Stein, 2010; NRC, 2012).

The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to determine normality of residuals and also to identify outliers. One pig fed the fish meal-cornstarch-based diet with phytase was identified as an outlier and removed from the data set. Data were analyzed as a 2 × 2 factorial using the Proc MIXED of SAS. The model included the fixed effects of type of diet, phytase, and the interaction between type of diet and phytase. The LSMEANS procedure was used to calculate the mean values for the treatments and means were separated using the PDIFF option if significant differences were observed. Pig was the experimental unit and an alpha level of 0.05 was used to assess significance among treatments.

Experiment 2: Effects of Type of Diet, Fiber, and Soybean Oil on Ca Digestibility

Diets, Animals, and Experimental Design. Experiment 2 was designed to evaluate effects of fiber and soybean oil on digestibility of Ca in fish meal. Fifty growing pigs (initial average BW: 19.36 ± 0.99 kg) were randomly allotted to 1 of 5 diets. There were 2 blocks of 25 pigs with 5 replicate pigs for each diet in each block. Therefore, a total of 10 pigs were used per treatment. Pigs were housed individually in metabolism crates as described for Exp. 1.

A cornstarch-based diet containing fish meal as the sole source of Ca was formulated and a similar diet was formulated with the exception that 8% Solka floc was included at the expense of cornstarch to evaluate the effects of fiber on ATTD and STTD of Ca (Table 4.4). Two corn-based diets in which fish meal was the sole source of Ca were also formulated with 2 levels of soybean oil (1 or 7%) to evaluate the effect of soybean oil on ATTD and STTD of Ca. All diets were formulated to meet Ca and P requirements for 11 to 25 kg pigs (NRC, 2012; Table 4.5). A Ca-free diet that contained corn, potato protein isolate, soybean oil, monosodium phosphate, crystalline AA, vitamins, and minerals was also formulated to determine basal endogenous losses of Ca. Each pig was fed one of the experimental diets for 13 d with a 5-d adaptation period and a 5-d marker based collection period as explained for Exp. 1. An amount of diet equivalent to 3 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012) was provided each day. Pigs were fed experimental diets for 13 d and fecal samples were collected as explained for Exp. 1.

Fish meal, casein, corn, potato protein isolate, diets, and fecal samples were analyzed for DM, Ca, and P. Diets and ingredients were analyzed for GE, CP, acid-hydrolyzed ether extract, and ash. Corn and diets were analyzed for ADF, NDF, and phytate. Corn was analyzed for SDF and IDF as explained for Exp. 1. Values for ATTD and STTD of Ca in each diet were calculated as explained for Exp. 1.

Statistical Analysis. The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to determine normality of residuals and also to identify outliers. Three extreme outliers were identified and removed from the data (2 pigs fed the fish meal diet and 1 pig fed the fish meal with fiber diet). Data were analyzed using the Proc MIXED of SAS with diet as fixed effect and period as random effect and CONTRAST statements were used to determine differences

between cornstarch and corn-based diets, the effect of fiber, and the effect of soybean oil on response variables. The LSMEANS procedure was used to calculate means as explained for Exp. 1.

RESULTS

Experiment 1: Effect of Microbial Phytase on Ca Digestibility in the Absence or the Presence of Phytate

All pigs remained healthy and consumed their diets during the experiment. Feed intake was greater ($P < 0.01$) for pigs fed corn-corn germ-based diets compared with pigs fed cornstarch-based diets, but ADFI was not affected by the inclusion of phytase (Table 4.6). Daily Ca intake was not affected by the type of diet used or by inclusion of phytase in the diets. Daily fecal output increased ($P < 0.001$) if corn-corn germ-based diets were used, but decreased ($P < 0.05$) if phytase was added to the diets. The concentration of Ca in the feces and the output of Ca decreased ($P < 0.001$) if corn-corn germ-based diets rather than cornstarch-based diets were used, and the output of Ca in feces was less ($P < 0.05$) if phytase was added to the diets than if no phytase was used. The amount of Ca absorbed, ATTD of Ca, and STTD of Ca increased ($P < 0.001$) if corn-corn germ-based diets were used compared with cornstarch-based diets, and were greater ($P < 0.05$) if phytase was added to the diets than if no phytase was used. Daily basal endogenous losses of Ca increased ($P < 0.05$) in cornstarch-based diets if phytase was used, but decreased ($P < 0.05$) in corn-corn germ-based diets if phytase was included (diet × phytase interaction, $P < 0.05$).

Phosphorus intake was greater ($P < 0.001$) if corn-corn germ-based diets were used than if pigs were fed cornstarch-based diets. However, the concentration of P in feces was less ($P <$

0.05) for pigs fed corn-corn germ-based diets than for pigs fed cornstarch-based diets, and not affected by the inclusion of phytase. Daily P output decreased ($P < 0.01$) if phytase was added to the diets to a greater extent if corn-corn germ-based diets were used than if cornstarch-based diets were used (diet × phytase interaction, $P < 0.05$). Therefore, inclusion of phytase increased ($P < 0.01$) the amount of P absorbed in corn-corn germ-based diets, but not in cornstarch-based diets (diet × phytase interaction, $P < 0.05$). The ATTD of P was greater ($P < 0.001$) in corn-corn germ-based diets than in cornstarch-based diets and also greater ($P < 0.05$) if phytase was included in the diets than if no phytase was used.

Experiment 2: Effects of Type of Diet, Fiber, and Soybean Oil on Ca Digestibility

Pigs readily consumed their diets throughout the experiment, but one pig fed the fish meal with fiber diet died on the 4th d of the adaptation period due to meningitis of bacterial origin. All other pigs remained healthy throughout the experiment.

Type of Diet: Cornstarch-Based Diet vs. Corn-Based Diet. Feed intake, Ca intake, fecal output, Ca absorbed, basal endogenous losses of Ca, the ATTD of Ca, and the STTD of Ca were greater ($P < 0.05$) for pigs fed the corn-based diets than for pigs fed the cornstarch-based diets (Table 4.7). However, Ca in feces and the amount of Ca output was greater ($P < 0.05$) from pigs fed the cornstarch-based diets than from pigs fed the corn-based diets. Pigs fed the corn-based diets had greater ($P < 0.05$) P intake, P absorbed, and greater ATTD of P than pigs fed cornstarch-based diets, but P-output in feces was less ($P < 0.05$) for pigs fed corn based diets compared with pigs fed cornstarch-based diets.

Effect of Fiber. Feed intake, daily Ca intake, fecal output, Ca absorbed, basal endogenous losses of Ca, ATTD of Ca, and STTD of Ca increased ($P < 0.001$) if fiber was included in the cornstarch-based diet. Fiber also reduced ($P < 0.001$) the amount and percentage

of Ca in feces. Inclusion of fiber increased ($P < 0.001$) P intake, P absorbed, and the ATTD of P, but decreased ($P < 0.001$) the amount and percentage of P in feces.

Effect of Soybean Oil. Inclusion of soybean oil in the corn-based diet reduced ($P < 0.001$) feed intake, Ca intake, fecal output, amount of Ca absorbed, and basal endogenous losses of Ca, but did not affect the amount and percentage of Ca in feces or the ATTD or STTD of Ca. Phosphorus intake, P output, and amount of P absorbed decreased ($P < 0.001$) if soybean oil was included in the diet, but percentage of P in feces and the ATTD of P were not affected by inclusion of soybean oil in the diet.

DISCUSSION

Chemical Characteristics of Ingredients

The concentration of Ca and P in the fish meal used in these 2 experiments were greater than reported values (Rostagno et al., 2011; NRC, 2012; Rojas and Stein, 2013), but were in agreement with values reported by Sauvant et al. (2004). Increased Ca and P concentration in fish meal is likely a result of inclusion of more bone from the fish filet industry in fish meal, which increases the concentration of ash in fish meal. The concentration of Ca in casein and the concentration of Ca, P, and phytate in potato protein isolate concurred with reported values (Cervantes-Pahm and Stein, 2010; Rostagno et al., 2011; NRC, 2012; González-Vega et al., 2013). Because of the negligible Ca in casein and potato protein isolate, these ingredients can be used as AA sources in diets that are formulated to determine Ca digestibility. The concentration of Ca, P, and phytate in corn were within the range of published values (Rostagno et al., 2011; NRC, 2012; Rojas et al., 2013), and confirm that corn and corn-coproducts contain virtually no Ca and that most P is bound to phytate.

Endogenous Losses of Ca

Basal endogenous loss of Ca obtained from pigs fed the Ca-free diet based on cornstarch, potato protein isolate, and sucrose was 0.22 g/kg DMI (Exp. 1), but the endogenous loss from pigs fed the Ca-free diet based on corn and potato protein isolate was 0.396 g/kg DMI (Exp. 2). The reason for this difference may be that different ingredients used in a nutrient-free diet may result in different endogenous losses of nutrients (Boisen and Moughan, 1996).

Experiment 1: Effect of Microbial Phytase on Ca Digestibility in the Absence or the Presence of Phytate

The reason for the greater feed intake in pigs fed corn-corn germ based diets compared with pigs fed cornstarch-based diets is that these diets contained less ME than the cornstarch-based diets and because pigs were fed to the same daily ME intake, more of the corn-corn germ based diets was provided. The observation that inclusion of microbial phytase increased Ca and P digestibility in corn-corn germ based diets was expected because phytase hydrolyzes phytate, which increases the availability of P and Ca to be absorbed (Poulsen et al., 2010; Almeida et al., 2013; Rodríguez et al., 2013; Almaguer et al., 2014). Because all Ca in the diets was from fish meal, the increased ATTD of Ca that was observed as phytase was added to the diets indicates that Ca from fish meal was bound to phytate, which to our knowledge has not been previously reported. It is, however, not clear why the ATTD and STTD of Ca in the cornstarch diet was increased by phytase because this diet did not contain phytate. The values for ATTD of Ca in fish meal that were calculated were close to reported values for ATTD of Ca in diets containing fish bones fed to pigs (Malde et al., 2010). However, to our knowledge, this is the first time values for the STTD of Ca in fish meal have been reported.

Phytate reduces the digestibility not only of P, but also of Ca (Selle et al., 2009). Pigs fed corn-soybean meal diets had less ATTD of Ca and P than pigs fed corn grits diets, because of the greater concentration of phytate in the corn-soybean meal diets than in corn grits diets (Liu et al., 2014). The ATTD of Ca and P in the corn-corn germ based diets was expected to be less than in the cornstarch-based diets. However, in contrast with our hypothesis, an increase in ATTD and STTD of Ca and in the ATTD of P in the corn-corn germ based diet was observed compared with the cornstarch-based diet. The reason for this observation may be that the fiber in the corn-corn germ based diet increased the motility in the intestinal tract and thereby enhanced absorption and reduced precipitation of minerals, but further research is needed to verify this hypothesis.

Digestibility of Ca and P was possibly increased because of the greater concentration of fat in the corn-corn germ based diets compared with the cornstarch based diets because dietary fat may decrease rate of passage, which may enhance the digestibility of nutrients (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). The Ca:STTD P ratio was similar among all diets, but the Ca:total P ratio was 1.5:1 for the cornstarch-based diets and 1:1 for the corn-corn germ-based diets. Decreasing the Ca:total P ratio from 1.5:1 to 1:1 may increase the ATTD of Ca and P in corn-based diets containing microbial phytase (Liu et al., 1998).

Values for the ATTD of P in the corn-corn germ-based diet were a combination of the ATTD of P in fish meal and the ATTD of P in corn and corn germ. Reported values for the ATTD of P in fish meal range from 62.7 to 90.0% (Rodehutscord et al., 1997; NRC, 2012; Sulabo et al., 2013; Kim et al., 2014). In corn, the range in ATTD of P is from 26.0 to 33.5% (Almeida and Stein, 2012; NRC, 2012; Rojas et al., 2013), and in corn germ, values for ATTD of P range from 33.0 to 37.3% (Almeida and Stein, 2012; NRC, 2012). Therefore, corn-corn germ

based diets were expected to have less ATTD of P than the cornstarch-based diets, because most of the P in the cornstarch-based diets originated from fish meal. The reason we were not able to verify this hypothesis is possibly that the lack of fiber in the cornstarch based diets may have resulted in precipitation of the P from fish meal, which resulted in reduced digestibility in this diet.

Experiment 2: Effects of Type of Diet, Fiber, and Soybean Oil on Ca Digestibility

Experiment 2 was conducted to elucidate possible reasons for the unexpected results from Exp. 1, which indicated that the ATTD and STTD of Ca and the ATTD of P are greater in corn-corn germ based diets than in cornstarch-based diets. Results of Exp. 2 confirmed that values for ATTD and STTD of Ca and the ATTD of P were greater in the corn-based diets than in the cornstarch-based diets. The values for ATTD and STTD of Ca (40.42 and 45.64%, respectively) in fish meal in the cornstarch-based diet obtained in this experiment were close to the values for ATTD and STTD of Ca (51.22 and 53.87%, respectively) in fish meal observed in Exp. 1. In contrast the ATTD and STTD of Ca in the corn-based diet was 84.24 and 88.99%, respectively.

Because all Ca in all diets was from fish meal, the difference in ATTD and STTD of Ca between the 2 types of diets may have been caused by fiber, fat, or other unknown factors. The fact that fiber (Solka floc) and corn increased the ATTD and STTD of Ca in fish meal by 16.6 and 42.5 percentage units, respectively, indicates that fiber possibly prevented some precipitation that may have occurred in the cornstarch-based diet. However, Solka floc is 100% cellulose, which is insoluble fiber, whereas the total dietary fiber in corn is 28% soluble and 72% insoluble (Jaworski et al., 2015). Fermentation of soluble dietary fiber results in production of short chain fatty acids, which may contribute to increased mineral absorption due to a reduction in intestinal pH (Wong et al., 2006; Rose et al., 2007). A low pH may enhance Ca solubility and increase

absorption of Ca in rats (Ohta et al., 1995), humans (Coudray et al., 1997), and pigs (Bird et al., 2000). Synthesis of butyrate may also enhance the growth of epithelial cells in the small and large intestine, which may further enhance absorption of nutrients (Montagne et al., 2003). Therefore, the greater digestibility of Ca observed in the corn-based diet compared with the cornstarch-based diet may be a result of the fermentation of fiber in the corn-based diet.

The high values for STTD of Ca in the corn-based diet indicate that Ca from fish meal has high digestibility in commercial diets. The calculated value for ATTD of Ca in fish meal in the cornstarch-fiber diet was in agreement with reported values, but the value in the corn-based diet was greater than previously reported values of diets containing fish bones (Malde et al., 2010).

A positive effect of fat on digestibility of AA in pigs has been observed, which is presumed to be a result of a reduction in the rate of passage (Cervantes-Pahm and Stein, 2008; Kil and Stein, 2011). However, the effect of fat on ATTD of Ca and P in rats and humans is variable, because depending on the type of fatty acids, different effects on pH and formation of Ca soaps have been reported (Boyd et al., 1932; Agnew and Holdsworth, 1971; Wargovich et al., 1984). In humans, high levels of Ca increased fat excretion (Bendsen et al., 2008), which may lead to a lower body fat content (Soares et al., 2012). In rats, high dietary fat content decreases the digestibility of Ca due to formation of Ca soaps (Frommelt et al., 2014). Saturated fat decreases the absorption of Ca (Gacs and Barltrop, 1977). However, soybean oil is mostly unsaturated fat, which may results in less Ca soaps being formed. This may be the reason for the observation in the present experiment indicating that soybean oil did not influence the ATTD and STTD of Ca or the ATTD of P in pigs.

Conclusions

The digestibility of Ca and P in fish meal is less in cornstarch-based diets than in corn-based diets, but inclusion of microbial phytase in the diet will increase the digestibility of Ca and P regardless of the type of diet used. Inclusion of synthetic fiber in the cornstarch-based diet resulted in an increased digestibility of Ca and P, and it is possible that lack of fiber in cornstarch-based diets results in precipitation of Ca and P in the intestinal tract. Soybean oil did not affect the ATTD and STTD of Ca or the ATTD of P in fish meal. Due to the negative effect of cornstarch-based diets on the ATTD and STTD of Ca, it is recommended that Ca digestibility of feed ingredients should be determined in corn-based diets.

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TABLES

Table 4.1. Analyzed composition of ingredients, as-fed basis

	Ingredient								
	Fish meal		Casein		Corn		Potato protein isolate		Corn germ
Item	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	
GE, kcal/kg	4,274	4,131	5,298	5,337	3,840	3,803	5,241	5,244	6,270
DM, %	92.89	91.47	91.32	92.67	87.73	87.97	91.50	94.43	94.83
CP, %	61.80	62.21	85.37	86.76	7.17	8.62	81.76	81.46	15.03
Ash, %	22.02	22.07	1.69	2.29	1.01	1.43	0.42	3.11	2.42
AEE,[1] %	8.31	8.61	0.91	1.32	2.63	2.88	0.10	0.17	32.88
ADF, %	-	-	-	-	3.66	3.63	-	-	20.23
NDF, %	-	-	-	-	11.40	11.51	-	-	40.15
IDF,[2] %	-	-	-	-	13.00	13.07	-	-	39.56

Table 4.1. (Cont.)

SDF,[3] %	-	-	-	-	1.66	1.94	-	-	2.79
Ca, %	5.69	5.66	0.03	0.03	ND[4]	0.01	0.04	0.03	0.08
P, %	3.26	3.19	0.59	0.57	0.19	0.23	0.13	0.14	0.58
Phytate, %	-	-	-	-	0.52	0.63	0.33	0.36	1.32
Phytate-bound P,[5] %	-	-	-	-	0.15	0.18	0.09	0.10	0.37
Non-phytate P,[6] %	3.26	3.19	0.59	0.57	0.04	0.05	0.04	0.04	0.21

[1]AEE = acid-hydrolyzed ether extract.

[2]IDF = insoluble dietary fiber.

[3]SDF = soluble dietary fiber.

[4]ND = not detected.

[5]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 4.2. Ingredient composition of experimental diets, as-fed basis, Exp. 1

	Diet				
	Fish meal + cornstarch-based diets		Fish meal + corn-corn germ-based diets		
Ingredient, %	0 FTU[1]	500 FTU	0 FTU	500 FTU	Ca-free
Fish meal	12.00	12.00	12.00	12.00	-
Corn	-	-	45.55	45.53	-
Corn germ	-	-	25.00	25.00	-
Cornstarch	50.27	50.25	-	-	47.74
Potato protein isolate	-	-	-	-	20.50
Casein	12.75	12.75	12.75	12.75	-
Sucrose	20.00	20.00	-	-	20.00
Soybean oil	4.00	4.00	4.00	4.00	4.00
Phytase premix[2]	-	0.02	-	0.02	-
Solka floc[3]	-	-	-	-	5.00
L-Lys HCL	-	-	-	-	0.15
DL-Met	0.16	0.16	-	-	0.14
L-Thr	0.12	0.12	-	-	-

Table 4.2. (Cont.)

L-His	-	-	-	-	0.12
Monosodium phosphate	-	-	-	-	1.15
Potassium carbonate	-	-	-	-	0.40
Magnesium oxide	-	-	-	-	0.10
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[4]	0.30	0.30	0.30	0.30	0.30

[1]FTU = phytase units.

[2]The phytase premix contained 5,000 phytase units per g (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[3]Fiber Sales and Development Corp., Urbana, OH.

[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

Table 4.3. Analyzed composition of experimental diets, as-fed basis, Exp. 1

	Diet				
	Fish meal + cornstarch-based diets		Fish meal + corn-corn germ-based diets		
Item	0 FTU	500 FTU[1]	0 FTU	500 FTU	Ca-free
GE, kcal/kg	4,233	4,219	4,861	4,973	4,201
DM, %	92.91	93.13	91.25	91.20	93.19
CP, %	18.24	19.14	25.87	26.97	17.65
Ash, %	3.36	3.33	4.71	4.67	2.05
AEE,[2] %	1.76	1.43	15.68	14.28	1.82
ADF, %	0.39	0.34	4.04	5.96	5.83
NDF, %	3.11	2.61	19.35	21.08	5.35
Ca, %	0.77	0.73	0.64	0.70	0.02
P, %	0.51	0.49	0.66	0.70	0.36
Phytase, FTU/kg	ND[3]	667	ND	712	<50
Phytate,[4] %	ND	ND	0.57	0.57	0.07
Phytate-bound P,[5] %	0.04	0.04	0.17	0.20	0.12
Non-phytate P,[6] %	0.47	0.45	0.49	0.50	0.24

Table 4.3. (Cont.)

Calculated values					
ME, kcal/kg	3,940	3,939	3,659	3,659	3,761
SID Lys, %	1.32	1.32	1.54	1.54	1.24

[1]FTU = phytase units.

[2]AEE= acid-hydrolyzed ether extract.

[3]ND = not detected.

[4]Phytate values were calculated using the values of phytate in the ingredients.

[5]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 4.4. Ingredient composition of experimental diets, as-fed basis, Exp. 2

Ingredient, %	Diet				
	Fish meal-cornstarch	Fish meal-cornstarch + fiber	Fish meal-corn	Fish meal-corn + soybean oil	Ca-free
Fish meal	12.00	12.00	12.00	12.00	-
Casein	12.75	12.75	12.75	12.75	-
Potato protein isolate	-	-	-	-	17.00
Cornstarch	53.37	45.37	-	-	-
Corn	-	-	73.65	67.65	80.24
Sucrose	20.00	20.00	-	-	-
Soybean oil	1.00	1.00	1.00	7.00	1.00
Solka floc[1]	-	8.00	-	-	-
Monosodium phosphate	-	-	-	-	0.97
L-Lys HCL	-	-	-	-	0.16
DL-Met	0.16	0.16	-	-	0.01
L-Thr	0.12	0.12	-	-	-

Table 4.4. (Cont.)

L-His	-	-	-	-	0.02
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20

[1]Fiber Sales and Development Corp., Urbana, OH.

[2] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

Table 4.5. Analyzed composition of experimental diets, as-fed basis, Exp. 2

	Diet				
Item	Fish meal-cornstarch	Fish meal-cornstarch + fiber	Fish meal-corn	Fish meal-corn + soybean oil	Ca-free
DM, %	93.54	93.88	88.77	89.89	89.27
Ash, %	3.44	3.28	4.14	4.06	2.64
GE, kcal/kg	4,044	4,034	4,027	4,407	4,277
CP, %	19.01	18.70	23.68	24.28	22.19
NDF, %	1.15	6.32	7.08	8.10	7.47
ADF, %	0.09	3.74	2.40	2.39	3.34
AEE,[1] %	2.30	2.06	4.20	9.22	3.74
Ca, %	0.71	0.72	0.74	0.68	0.03
P, %	0.48	0.49	0.64	0.60	0.48
Phytate,[3] %	ND[2]	ND	0.46	0.43	0.57
Phytate-bound P,[4] %	0.04	0.04	0.14	0.14	0.28
Non-phytate P,[5] %	0.44	0.45	0.50	0.46	0.20

Table 4.5. (Cont.)

Calculated values					
ME, kcal/kg	3,811	3,492	3,476	3,775	3,488
SID Lys, %	1.32	1.32	1.47	1.46	1.22

[1]AEE = acid-hydrolyzed ether extract.

[2]ND = not detected.

[3]Phytate values were calculated using the values of phytate in the ingredients.

[4]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 4.6. Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of Ca in diets containing fish meal-cornstarch or fish meal-corn-corn germ without or with microbial phytase, Exp. 1

Item	Diet		Phytase			*P*- value	
	Fish meal-cornstarch	Fish meal-corn-corn germ	0 FTU[1]	500 FTU	SEM	Diet	Phytase
ADFI, g/d	762	824	824	825	14	0.004	0.361
Ca intake, g/d	5.71	5.52	5.50	5.73	0.10	0.186	0.094
Fecal output, g/d	25.67	148.13	91.11	82.69	2.86	<0.001	0.049
Ca in feces, %	10.74	0.85	6.09	5.50	0.29	<0.001	0.161
Fecal Ca output, g/d	2.60	1.17	2.09	1.68	0.11	<0.001	0.016
Ca absorbed, g/d	3.11	4.35	3.41	4.05	0.15	<0.001	0.004
ATTD of Ca, %	54.24	78.54	62.15	70.64	2.12	<0.001	0.009
Basal ECaL,[2, 3] mg/d	156	168	161	163	2.06	<0.001	0.697
STTD of Ca, %	56.97	81.54	65.04	73.48	2.12	<0.001	0.009
P intake, g/d	3.81	5.70	4.71	4.80	0.06	<0.001	0.311
P in feces, %	5.47	0.88	3.35	3.00	0.13	<0.001	0.063

Table 4.6. (Cont.)

Fecal P output,[3] g/d	1.30	1.23	1.43	1.11	0.07	0.484	0.003
P absorbed,[3] g/d	2.50	4.37	3.19	3.69	0.09	<0.001	<0.001
ATTD of P, %	65.62	77.88	68.21	75.29	1.57	<0.001	0.004

[1]FTU = phytase units.

[2]ECaL = endogenous losses of Ca. Values for standardized total tract digestibility were calculated by correcting apparent total tract digestibility values for basal endogenous losses. Basal endogenous losses were determined from pigs fed Ca-free diets as 0.22 ± 0.074 g/kg of DMI.

[3]Diet by phytase interaction was significant ($P < 0.05$).

Table 4.7. Apparent total tract digestibility (ATTD) of Ca and P and standardized total tract digestibility (STTD) of Ca, Exp. 2

Item	Diet				SEM	Contrast *P*-value		
	Fish meal-cornstarch (1)	Fish meal-cornstarch-fiber (2)	Fish meal-corn (3)	Fish meal-corn-soybean oil (4)		Cornstarch vs. corn (1 vs. 3)	Fiber (1 vs. 2)	Soybean oil (3 vs. 4)
ADFI, g/d	867	907	982	905	14.57	<0.001	0.049	<0.001
Ca intake, g/d	6.16	6.53	7.26	6.15	0.10	<0.001	0.011	<0.001
Fecal output, g/d	28.97	72.63	87.43	72.46	2.29	<0.001	<0.001	<0.001
Ca in feces, %	12.48	3.87	1.32	1.45	0.16	<0.001	<0.001	0.443
Fecal Ca output, g/d	3.66	2.82	1.15	1.05	0.19	<0.001	<0.001	0.616
Ca absorbed, g/d	2.48	3.71	6.12	5.10	0.14	<0.001	<0.001	<0.001
ATTD of Ca, %	40.42	57.08	84.24	82.91	2.51	<0.001	<0.001	0.623

Table 4.7. (Cont.)

Basal ECaL,[1] mg/d	321	337	345	322	5.30	0.001	0.031	<0.001
STTD of Ca, %	45.64	62.23	88.99	88.14	2.51	<0.001	<0.001	0.754
P intake, g/d	4.16	4.44	6.28	5.43	0.08	<0.001	0.011	<0.001
P in feces, %	6.52	2.14	1.71	1.74	0.12	<0.001	<0.001	0.765
Fecal P output, g/d	1.88	1.55	1.49	1.27	0.10	0.001	0.009	0.043
P absorbed, g/d	2.27	2.89	4.79	4.16	0.08	<0.001	<0.001	<0.001
ATTD of P, %	54.66	65.18	76.29	76.70	1.84	<0.001	<0.001	0.845

[1]ECaL = endogenous losses of Ca. Values for standardized total tract digestibility were calculated by correcting apparent total tract digestibility values for basal endogenous losses. Basal endogenous losses were determined from pigs fed Ca-free diets as 0.396 ± 0.099 g/kg of DMI.

CHAPTER 5

REQUIREMENT FOR DIGESTIBLE CALCIUM BY 11 TO 25 KG PIGS AS DETERMINED BY GROWTH PERFORMANCE, BONE ASH CONCENTRATIONS, CALCIUM AND PHOSPHORUS BALANCES, AND EXPRESSION OF GENES INVOLVED IN TRANSPORT OF CALCIUM IN INTESTINAL AND KIDNEY CELLS

ABSTRACT: Two experiments were conducted to determine the standardized total tract digestible (STTD) Ca requirement by 11 to 25 kg pigs based on growth performance, bone ash, or Ca retention, and to determine the effect of dietary Ca on expression of genes related to Ca transport in the jejunum and kidneys. Six diets were formulated to contain 0.36% STTD P. These diets were formulated to contain 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca, by including increasing quantities of calcium carbonate at the expense of cornstarch. Two additional diets contained 0.72% STTD Ca and 0.33% or 0.40% STTD P to determine if 0.36% STTD P had negative effects on the Ca requirement. The same batch of diets was used in both experiments. In experiment 1, 256 pigs (initial BW: 11.39 ± 1.21 kg) were randomly allotted to the 8 diets with 4 pigs per pen and 8 replicate pens per diet in a randomized complete block design. On the last day of the experiment, 1 barrow from each pen was euthanized and the right femur and intestine and kidney samples were collected. Results indicated that ADG and G:F started to decline (linear and quadratic, $P < 0.05$) at 0.54 and 0.50% STTD Ca, respectively. In contrast, bone ash increased as dietary Ca increased and reached a plateau indicating the requirement for STTD Ca to maximize bone ash was 0.48%. Growth performance was not affected by the level of P in the diets, but bone ash increased (linear, $P < 0.01$) as STTD P increased in the diets. The mRNA expression of genes related to transcellular Ca transport decreased (linear, $P < 0.01$) in jejunum and (linear and

quadratic, $P < 0.01$) in kidneys as dietary Ca increased. In experiment 2, 80 pigs (initial BW: 13.12 ± 1.79 kg) were placed in metabolism crates and randomly allotted to the 8 diets with 10 replicate pigs per diet in a randomized complete block design. Fecal and urine samples were collected using the marker-to-marker approach. Results indicated that Ca retained (g/d) (linear and quadratic, $P < 0.05$) and P retained (g/d) (linear and quadratic, $P < 0.15$) increased as the dietary STTD Ca increased. The requirement for STTD Ca to maximize Ca and P retention (g/d) was 0.60 and 0.49%, respectively. In conclusion, the STTD Ca requirement by 11 to 25 kg to maximize bone ash under these experimental conditions was 0.48%, however, ADG and G:F declined at 0.54 and 0.50% STTD Ca, respectively. Increasing dietary Ca decreased the mRNA expression of several genes related to transcellular Ca transport in the jejunum and kidneys.

Key words: bone ash, calcium balance, calcium requirement, calcium retention, digestible calcium, pigs

INTRODUCTION

A maximum utilization of Ca and P by pigs is expected if diets are formulated to meet requirements for standardized total tract digestible (**STTD)** Ca and STTD P. Requirements for total Ca in diets fed to growing pigs may be obtained by multiplying the requirements for STTD P by 2.15 (NRC, 2012), but no STTD Ca requirements have been reported because of a lack of data for STTD of Ca (NRC, 2012). However, recent experiments have generated values for STTD of Ca in most commonly used Ca sources (González-Vega et al., 2014, 2015a; b). Thus, requirements for digestible Ca may be established. Concentration of STTD Ca needed to maximize growth performance is expected to be less than the concentration needed to maximize bone ash and Ca retention (NRC, 2012). However, because 96 to 99% of Ca is believed to be

deposited in skeletal tissue (Crenshaw, 2001), no differences between concentrations of STTD Ca needed to maximized bone ash and Ca retention are expected.

Vitamin D may influence the expression of genes involved in transcellular transport of Ca, which is mainly active if dietary Ca is relatively low (Kutuzova and DeLuca, 2004). Extensive research has been conducted in broiler chickens, rats, and mice to evaluate the effect of dietary Ca concentrations on expression of genes involved in transcellular transport of Ca in the small intestine and kidneys (Armbrecht et al., 1980, 2003; Rosenberg et al., 1986; Hurwitz et al., 1995; Van de Graaf et al., 2004; Healy et al., 2005; Ko et al., 2009), but limited data have been reported for pigs. Therefore, the objectives of the present experiments were to determine the requirement for STTD Ca by 11 to 25 kg pigs to maximize growth performance, bone ash, and Ca retention, and to determine the effect of dietary Ca on the expression of genes involved in transcellular transport of Ca in the jejunum and kidneys in pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for 2 experiments. Pigs used in both experiments were the offspring of G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN).

Experiment 1: Growth Performance, Bone Ash, and Gene Expression

Animals, Diets, and Feeding. Two hundred and fifty six pigs (initial average BW: 11.39 ± 1.21 kg) were randomly allotted to 8 diets with 8 replicate pens per diet in a randomized complete block design. Pen was the experimental unit and each pen had 2 barrows and 2 gilts. The experiment was conducted in 2 blocks, 1 block with 3 replicate pens per diet and 1 block with 5 replicate pens per diet. The Experimental Animal Allotment Program (Kim and Lindemann,

2007) was used to allot pigs to experimental diets. Pigs were housed in pens with fully slatted floors, and room temperature was controlled (maximum temperature: 27.5 ± 2.1ºC; minimum temperature: 24.1 ± 2.2ºC).

Diets were based on corn, soybean meal, and lactose (Table 5.1). A diet containing 0.36% STTD P and 0.32% STTD Ca was formulated (Table 5.2). Five additional diets were formulated to contain 0.36% STTD P and 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca, by including increasing quantities of calcium carbonate in the diets at the expense of cornstarch. The 3 diets with the least concentrations of Ca contained total less Ca than the requirement according to NRC (2012) and the 3 diets with the greatest concentrations of Ca met or exceeded the Ca requirement, but the concentration of STTD P in all diets was 10% above the requirement (NRC, 2012; Table 5.3). Two additional diets were formulated to contain 0.72% STTD Ca and 0.33% or 0.40% STTD P.

Growth Performance and Bone Measurements. Pigs were allowed ad libitum access to feed and water throughout the experiment, and pigs were weighed at the beginning of the experiment, at d 10, and at the conclusion of the experiment (d 22). The amount of feed offered was recorded every day, and the amount of feed left in the feeder at the conclusion of the experiment was subtracted from the total feed offered. On the last day of the experiment, 1 barrow in each pen that had a BW closest to the average BW of the pen was euthanized via captive bolt stunning. The right front foot and the right hind leg were removed and stored at -20ºC. The right front foot and the right hind leg of each pig were autoclaved separately at 125ºC for 55 min. The 3rd and 4th metacarpals were removed from the feet and femurs were removed from the hind legs. The marrow of the broken metacarpals and femurs was removed and the bones were dried and soaked in petroleum ether under a chemical hood for 72 h to remove the

remaining marrow and fat. Bones were dried overnight at 130ºC and ashed at 600ºC for 16 h to calculate the concentration of bone ash.

Sample Analysis. Corn, soybean meal, lactose, calcium carbonate, monocalcium phosphate, diets, and bones were analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007), for ash (Method 942.05; AOAC Int., 2007), and for Ca and P by inductively coupled plasma-optical emission spectrometry (**ICP-OES**; Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC Int., 2007). Corn, soybean meal, and lactose were analyzed for GE using an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL). Benzoic acid was used as the standard for calibration. Corn, soybean meal, lactose, and diets were analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. Corn, soybean meal, and diets were also analyzed for ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Corn and soybean meal were analyzed for phytic acid (Megazyme method; AB Vista, Ystrad Mynach, UK), and diets were analyzed for phytate-P using a Foss near-infrared spectrometer with the phytate-P levels predicted using AUNIR calibration standards (AB Vista, Memphis, TN).

Sample Collection for Gene Expression. From 48 euthanized pigs that were fed diets containing 0.36% STTD P and 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca, 5-cm jejunum tissue samples were collected from the middle region of the small intestine, cut longitudinally, washed with PBS, and scraped with microscope slides to recover the mucosa layer. Kidney samples were collected from these pigs between the renal cortex and the renal medulla. Scraped mucosal layer

and kidney samples were snap-frozen in liquid N immediately after collection and stored at -80°C.

RNA Extraction and Quantitative Reverse Transcription-PCR. The RNA was extracted from 100 mg of tissue using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. The RNA quantity and quality were assessed using the ND-1000 Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), respectively (average RNA integrity number = 9.63 ± 0.36). The RNA was subjected to reverse transcription by using the Superscript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA) to synthesize the double-stranded cDNA. Double-stranded cDNA was diluted and used for quantitative reverse transcription (**qRT-PCR**). Each 10 µL reaction consisted of 5 µL SYBR Green (Applied Biosystems, Foster City, CA), 4 µL diluted cDNA sample, 0.4 µL of 10 µM forward and reverse primer, and 0.2 µL DNase/RNase free water. The reactions were performed in an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. An additional dissociation stage was added to verify the presence of a single PCR product. All reactions were run in triplicate. Data were analyzed using the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, Foster City, CA).

Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) and hydroxymethylbilane synthase (***HMBS***) were used to normalize the expression of tested genes (Vigors et al., 2014). The selected tested genes are involved into the active transcellular transport of Ca and these tested genes included S100 calcium binding protein G (***S100G***), transient receptor potential cation channel, subfamily V, member 6 (***TRPV6***), ATPase, Ca^{++} transporting,

plasma membrane 1 (***ATP2B1*** or ***PMCA1***), and vitamin D (1, 25-dihydroxyvitamin D_3) receptor (***VDR***) in jejunum samples; and S100G, calbindin 1, 28kDa (***CALB1***), TRPV6, transient receptor potential cation channel, subfamily V, member 5 (***TRPV5***), ATP2B1, and VDR in kidney samples. Primers are listed in Table 5.4 and primers for TRPV5 and CALB1 were designed using the Primer3 program (Ye et al., 2012). All primers were commercially synthesized by Applied Biosystems (Foster, CA). Primers for TRPV5 and CALB1 were verified by gel electrophoresis and sequencing.

Calculations and Statistical Analysis. The percentage of phytate-bound P in corn and soybean meal was calculated as 28.2% of phytate (Tran and Sauvant, 2004). The percentage of phytate in diets was calculated by dividing the analyzed phytate-bound P by 0.282, and to calculate the percentage of non-phytate P, the amount of phytate-bound P was subtracted from the amount of total P. Dietary cation-anion difference (**DCAD**) was calculated using the following equation:

DCAD, mEq/kg = [(Na*10000)/23] + [(K*10000)/39] – [(Cl*10000)/35.5], [1]

where Na, K, and Cl are expressed in percentage of inclusion in diet.

The ADG, ADFI, and G:F were calculated for each pen and treatment group. Bone ash percentage was calculated by dividing the quantity of bone ash by the quantity of fat-free dried bone and multiplied by 100. To obtain the relative gene expression data, the average of quantity of triplicate samples were calculated and divided by the geometric mean of the 2 internal control genes. The gene expression data were expressed as a fold change relative to the average of the diet containing the least concentration of Ca (0.32% STTD Ca).

Normality of residuals and identification of outliers were determined by the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Gene expression data were log-10

transformed to align measures to a normal distribution. Body weight, ADG, ADFI, G:F, bone variables, and log-scale gene expression data were analyzed using the Proc MIXED of SAS (SAS Inst. Inc., Cary, NC). The model included diet as the fixed effect and block as the random effect. The LSMEANS procedure was used to calculate mean values for treatments. Gene expression data presented in figures were back-transformed using antilog. Linear and quadratic effects of increasing levels of STTD Ca were determined using CONTRAST statements. The PROC NLIN of SAS was used for broken line analysis if the linear effect was significant, and for quadratic analyses if the quadratic effect was significant. If both linear and quadratic effects were significant, the intersection of the broken line and the quadratic line was determined (Baker et al., 2002). Pen was the experimental unit and results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.15$.

Experiment 2. Calcium and P Balance

Animals, Diets, and Feeding. Eighty pigs (initial average BW: 13.12 ± 1.79 kg) were randomly allotted to 8 diets with 10 replicate pigs per diet in a randomized complete block design. The 8 diets used in Exp. 1 were also used in Exp. 2, and the amount of each diet that was needed for both experiments was mixed in 1 batch. This experiment was conducted in 2 blocks with 40 pigs and 5 replicate pigs per diet in each block. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. Pigs were housed individually in metabolism crates that were equipped with a slatted floor, a feeder, and a nipple drinker. A screen floor and a urine pan were placed under each crate, and a bucket was placed under each urine pan, which allowed for total collection of feces and urine. Pigs had free access to water throughout the experiment. The room temperature was controlled (maximum temperature: 27.5 ± 1.6ºC; minimum temperature: 23.8 ± 1.1ºC). Pigs were fed 3 times the daily

maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The daily allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h. Pigs were fed each diet for 13 d and the initial 5 d were an adaptation period to the diets and fecal samples were collected quantitatively from the feed provided from d 6 to 11 using the marker-to-marker approach (Adeola, 2001). The beginning of fecal collections was marked by adding an indigestible 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 11. Urine samples were collected every morning from d 6 to 11 and 50 mL of 6*N* HCl was added to each bucket after they were emptied. Fecal samples and 20% of the collected urine were stored at -20°C immediately after collection. Orts collected during the collection period were dried in a forced-air oven at 65ºC and feed intake was calculated by subtracting the orts from feed allowance.

Sample Analysis and Statistical Analysis. Before analysis, urine samples were thawed at room temperature, thoroughly mixed, and a subsample of 10 mL was collected. Urine samples were analyzed for Ca and P as explained for Exp. 1. Fecal samples were dried in a forced-air oven at 65°C and then ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen. Fecal samples were analyzed for DM as explained for Exp. 1 and for Ca and P by ICP-OES (Method 965.17; 985.01 mod. AOAC Int., 2007). The apparent total tract digestibility (**ATTD**) of Ca and P was calculated according to standard procedures (NRC, 2012). Values for absorption and retention of Ca and P were calculated as explained by González-Vega et al. (2013). Data were analyzed using PROC MIXED of SAS as explained for Exp. 1. Pig was the experimental unit and results were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.15$.

RESULTS

Experiment 1: Growth Performance, Bone Ash, and Gene Expression

All pigs remained healthy and consumed their diets without apparent problems, but 1 gilt died due to meningitis and ADG, ADFI, and G:F of the pen for this pig were adjusted (Lindemann and Kim, 2007). There were no effects of increasing concentration of STTD Ca on final BW or ADFI (Table 5.5). However, overall ADG decreased (quadratic, $P < 0.05$) as the concentration of STTD Ca increased (Fig. 5.1). The concentration of STTD Ca at which ADG started to decline was 0.54% as determined by the intersection between the quadratic and the broken line analysis. Likewise, overall G:F decreased (quadratic, $P < 0.01$) as the concentration of STTD Ca increased (Fig. 5.2). The G:F intersection between the quadratic and the broken line analysis was at 0.50% STTD Ca. In contrast, bone ash and bone Ca in grams in femurs increased linearly ($P < 0.05$) and quadratically ($P < 0.05$) as the concentration of STTD Ca increased (Fig. 5.3 and 5.4, respectively). Likewise, bone P in grams in femurs and bone ash in grams in metacarpals increased quadratically ($P < 0.05$) as the concentration of STTD Ca increased (Fig. 5.5 and 5.6, respectively). The concentrations of STTD Ca to maximize bone ash, bone Ca, and bone P in femurs, and bone ash in metacarpals were 0.48, 0.50, 0.56, and 0.54%, respectively.

There was no effect of increasing concentration of STTD P on BW, ADG from d 1 to 10 or from d 1 to 22, ADFI, G:F from d 1 to 10, or bone P percentage in femurs (Table 5.6). However, ADG from d 10 to 22, G:F from d 10 to 22 and from d 1 to 22, bone ash, bone Ca, and bone P in grams in femurs and bone ash in grams in metacarpals increased linearly ($P < 0.05$) as the concentration of STTD P increased. Bone Ca percentage in femurs tended to increase ($P = 0.144$) as concentration of STTD P increased. Bone ash in percent of dried bone weight in

metacarpals increased linearly ($P < 0.01$) and quadratically ($P < 0.01$) as the concentration of STTD P increased.

Gene expression. The effect of increasing concentration of STTD Ca was evaluated for the internal control genes in jejunum and kidney, and as it was expected, the mRNA expression of internal control genes was not affected by the concentration of STTD Ca in diets. In jejunum, the mRNA expression of *TRPV6* linearly decreased ($P < 0.01$) as the concentration of STTD Ca increased (Fig. 5.7). There was a tendency ($P < 0.10$) for a linear and quadratic decrease in the mRNA expression of *S100G* as the concentration of STTD Ca increased. However, the mRNA expression of *ATP2B1* quadratically increased ($P < 0.001$) as dietary STTD Ca increased, reaching the maximum expression for the diet containing 0.64% STTD Ca and 0.36% STTD P. Likewise, the mRNA expression of *VDR* quadratically increased ($P < 0.01$) as the concentration of STTD Ca increased, reaching the maximum expression for the diet containing 0.64% STTD Ca and 0.36% STTD P. However in kidney, the mRNA expression of *TRPV6* (linear, $P < 0.001$), *TRPV5* (linear, $P < 0.001$), *S100G* (linear, $P < 0.001$, and quadratic $P = 0.056$), and *CALB1* (linear and quadratic, $P < 0.01$) decreased as the concentration of STTD Ca increased (Fig. 5.8). Increasing concentration of STTD Ca quadratically increased ($P < 0.01$) the mRNA expression of *VDR* in the kidney, reaching the maximum expression for the diet containing 0.56% STTD Ca and 0.36% STTD P. However, increasing concentration of STTD Ca had no effect on the mRNA expression of *ATP2B1* in the kidney.

Experiment 2. Calcium and P Balance

Balance of Ca and ATTD of Ca. Pigs consumed the diets and remained healthy throughout the experiment. There was no effect of increasing concentration of STTD Ca on feed intake (Table 5.7). However, Ca intake, Ca absorption, and Ca excretion in percentage of intake

increased linearly ($P < 0.001$) as dietary STTD Ca increased. Likewise, fecal and urine Ca output and Ca excreted in g/d increased linearly ($P < 0.001$) and quadratically ($P < 0.05$) as the concentration of dietary STTD Ca increased. However, Ca retention in percentage of intake and ATTD of Ca decreased linearly ($P < 0.001$) as dietary STTD Ca increased. The quantity of Ca retained in g/d increased linearly ($P < 0.001$) and quadratically ($P < 0.05$) as dietary concentration of STTD Ca increased. The concentration of STTD Ca needed to maximize Ca retention in g per d was 0.60% (Fig. 5.9). Increasing dietary STTD P did not affect feed intake, Ca intake, absorbed Ca, or ATTD of Ca. However, increasing concentration of STTD P decreased (linear and quadratic, $P < 0.05$) fecal Ca output, (linear and quadratic, $P < 0.05$) Ca excreted in g/d, (linear, $P < 0.001$) urine Ca output, and (linear, $P < 0.001$) Ca excretion in percentage of intake. However, Ca retention in percentage of intake linearly increased ($P < 0.001$) as the concentration of STTD P increased.

Balance of P and ATTD of P. Intake, absorption, and excretion of P in g/d were not affected by increasing the concentration of STTD Ca in the diet (Table 5.8). However, urine P output and P excretion in percent of intake decreased linearly ($P < 0.05$) and quadratically ($P < 0.01$) as the concentration of STTD Ca increased. There was a tendency ($P < 0.15$) for an increase in the quantity of P retained in g/d as the concentration of STTD Ca increased. The concentration of STTD Ca needed to maximize P retention in g/d was 0.49% (Fig. 5.10). The ATTD of P decreased linearly ($P < 0.001$) as dietary STTD Ca increased. However, fecal P output increased linearly ($P < 0.01$) and P retention (% of intake) increased linearly ($P < 0.05$) and quadratically ($P < 0.01$) as the concentration of STTD Ca increased. There were no effects of increasing concentration of STTD P in the diet on fecal or urine output of P or P excretion in g/d. However, the quantity of absorbed and retained P in g/d and ATTD of P increased linearly ($P <$

0.05) as dietary STTD P increased. There was a tendency ($P < 0.15$) for an increase in P intake and P retention in percent of intake as concentration of STTD P increased, however, P excretion in percent of intake tended to decrease ($P = 0.07$) as concentration of STTD P increased.

DISCUSSION

The concentration of Ca and P in corn and monocalcium phosphate were in agreement with reported values (de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012), but the concentration of Ca and P in soybean meal and calcium carbonate were greater than reported values (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012). However, all ingredients were analyzed before diet mixing so these differences did not influence concentrations of Ca and P in the diets, and the Ca and P in all diets were close to calculated values.

The ratio of Ca and P in the diets is important to consider because an excess or deficiency of one mineral may affect the utilization of the other (Crenshaw, 2001). In the present experiment, 0.36% STTD P was used, which is 10% above the requirement (NRC, 2012) to make sure pigs were not deficient in P. Results obtained for the diet containing 0.33% STTD P indicated that there were no differences in growth performance between pigs fed 0.36% STTD P and pigs fed 0.33% STTD P. The reduced quantity of bone ash in pigs fed the diet containing 0.33% STTD P was expected because P requirements are established to maximize growth performance, but not bone ash (NRC, 2012). The observation that pigs fed 0.40% STTD P had greater G:F and Ca retention than pigs fed 0.36% STTD P indicates that pigs fed 0.36% STTD P may have been marginally deficient in STTD P despite receiving 10% more P than the

requirement. However, this may at least partly be a result of the reduced ATTD of P that was observed as STTD Ca in the diets increased.

Results illustrate that the quantity of STTD Ca that is needed to maximize retention of Ca and maximize bone ash is different from the quantity needed to maximize growth performance. Increasing concentrations of Ca may increase formation of Ca-P complexes in the gastrointestinal tract, which reduces digestibility of P (Clark, 1969; Brink et al., 1992; Stein et al., 2011; González-Vega et al., 2014). This negative effect on digestibility of P may be one of the reasons for the negative effect of increasing concentrations of dietary STTD Ca on ADG and G:F. This response was also observed in 25 kg pigs that were fed increasing concentrations of true total tract digestible Ca and a constant concentration of true total tract digestible P (Fan and Archbold, 2012). However, the increase in bone ash that was observed as the STTD Ca increased indicates that although ATTD of P was reduced at the greatest concentrations of dietary STTD Ca, pigs were able to maximize bone ash if there was enough Ca in the diet. The observation that ADG and G:F were not negatively affected by the least concentration of STTD Ca indicates that pigs were able to mobilize Ca from the bones to compensate for the deficiency of dietary Ca. It is possible that 22 d was too short a period to deplete bone Ca stores and therefore observe negative effects on growth performance. Similar results were observed in pigs fed low levels of Ca for 32 d (Eklou-Kalonji et al., 1999) and in rats fed high levels of Ca and low levels of P for 28 d (Shapiro and Heaney, 2003).

In this experiment, the STTD Ca:STTD P ratios ranged from 0.88:1 to 2.00:1. Dietary Ca above 0.50 or 0.54% STTD Ca negatively affected G:F and ADG, which corresponds to digestible Ca:digestible P ratios between 1.39:1 and 1.50:1. To maximize bone ash, bone Ca, and bone P, dietary STTD Ca above 0.48% were needed, and to maximize retention of Ca and P, a

minimum of 0.60 and 0.49% STTD Ca, respectively, were needed, which correspond to digestible Ca:digestible P ratios between 1.33:1 and 1.67:1. These values are within the range of values for the Ca:P ratio in the whole body (Rymarz et al., 1982; Hendriks and Moughan, 1993; Mahan and Shields, 1998; Wiseman et al., 2009; Pettey et al., 2015).

In studies aimed at determining Ca or P requirements in pigs, the dietary concentration of one mineral may be constant while responses to graded levels of the other mineral is observed (Fan and Archbold, 2012). Alternatively, a constant Ca:P ratio may be used (Zhai and Adeola, 2013). In this experiment, a constant concentration of dietary STTD P was used in diets with varying dietary Ca. In studies that used a constant concentration of dietary Ca, increasing concentrations of STTD P increased growth performance (Ekpe et al., 2002; Saraiva et al., 2009; Viana et al., 2013; Zhai and Adeola, 2013). However, in this experiment, increasing concentrations of STTD Ca decreased growth performance, which is in agreement with results observed by Fan and Archbold (2012). This observation indicates that to maximize growth performance in pigs the ratio of STTD Ca to STTD P likely is more important than the absolute concentration of both minerals.

Calcium may be absorbed by paracellular or transcellular routes. If luminal Ca concentration is high, most Ca is absorbed by passive absorption using the paracellular route, (Hurwitz, 1996; Fleet and Schoch, 2010). In contrast, if luminal Ca concentration is low, most Ca is absorbed by active transport using the transcellular route, which requires Ca channels, transporters, and energy (Hurwitz, 1996; Bouillon et al., 2003; Fleet and Schoch, 2010). Calcium crosses the brush border membrane using Ca channels (*TRPV6* and/or *TRPV5*), and in the cytoplasm, Ca is bound to Ca binding proteins (calbindin-D9k and/or calbindin-D28k), which transport Ca to the basolateral membrane (Schröder et al., 1996; Bouillon et al., 2003; Schwaller,

2010), where it is released from the cell via *PMCA1* or by a Na^+/Ca^{2+} exchanger (Bouillon et al., 2003; Prozkowiec-Weglarz and Angel, 2013). Therefore, dietary Ca concentration may affect the expression of genes related to Ca transport. As indicated by the results from the current experiment, the mRNA expression of Ca binding proteins and Ca channel proteins were regulated by dietary Ca concentrations in both the jejunum and the kidneys. These results are in close agreement with data obtained in mice, rats, and broiler chickens (Armbrecht et al., 1980, 2003; Rosenber et al., 1986; Hurwitz et al., 1995; Van de Graaf et al., 2004; Ko et al., 2009).

Calcium homeostasis is mainly regulated by 2 hormones, parathyroid hormone (**PTH**) and calcitonin (Crenshaw, 2001). Secretion of PTH increases at low dietary Ca concentrations due to low plasma Ca concentrations (Eklou-Kalonji et al., 1999). Parathyroid hormone increases the production of 1-hydroxylase in the kidneys, which forms 1,25-dihydroxycholecalciferol (**1,25-$(OH)_2D_3$**), the active form of vitamin D (Hurwitz, 1996; Crenshaw et al., 2011). If 1,25-$(OH)_2D_3$ is bound to *VDR*, up-regulation of several genes involved in transcellular Ca transport may occur (Healy et al., 2005), which may increase Ca absorption in the small intestine and Ca reabsorption in the kidneys (Bouillon et al., 2003). In broiler chickens, rats, and mice, the expression of *TRPV6*, *TRPV5*, *S100G*, and/or *PMCA1* was increased in the small intestine and/or in the kidneys at low dietary Ca concentrations (Armbrecht et al., 1980, 2003; Rosenber et al., 1986; Hurwitz et al., 1995; Van de Graaf et al., 2004; Ko et al., 2009). Results from this experiment support this observation because the mRNA expression of *TRPV6* and *S100G* in the jejunum, and the mRNA expression of *TRPV5*, *TRPV6*, *S100G*, and *CALB1* in the kidneys were down-regulated at high concentrations of dietary Ca. In mice, high concentrations of dietary Ca increased mRNA expression of *VDR* in the kidney, but not in the duodenum (Healy et al., 2005). Our results for the kidney support this observation. Thus, results of this experiment indicate that

although, at high dietary concentrations of Ca, mRNA expression of Ca transporters was down-regulated in jejunum, most Ca may have been absorbed using the paracellular route. As a consequence, Ca that was not needed for bone tissue synthesis was subsequently excreted in the urine, indicating that Ca homeostasis was regulated mainly at the renal level. In contrast, at low concentrations of dietary Ca, most Ca is likely absorbed using the transcellular route. As a consequence, vitamin D-dependent transport may play a role in increasing Ca absorption from the small intestine and in Ca reabsorption in the kidneys at low concentrations of dietary Ca.

Conclusions

Growth performance of pigs was reduced if dietary Ca exceeded approximately 0.50% STTD Ca in diets containing 0.36% STTD P. Bone ash, bone Ca, and bone P were maximized by dietary Ca above 0.48% STTD Ca, and retention of Ca and P was maximized if dietary Ca was above 0.60 or 0.49% STTD Ca, respectively. Based on these results, it is likely that the requirement for STTD Ca for 11 to 25 kg pigs is approximately 1.35 times the required STTD P, but further experiments need to be conducted to verify this value. Increasing concentrations of dietary Ca decreased expression of genes related to Ca transport in the jejunum and kidneys of pigs, and although Ca absorption is negatively affected by increasing concentrations of dietary Ca, the main site for regulation of Ca homeostasis appears to be in the kidneys.

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FIGURES

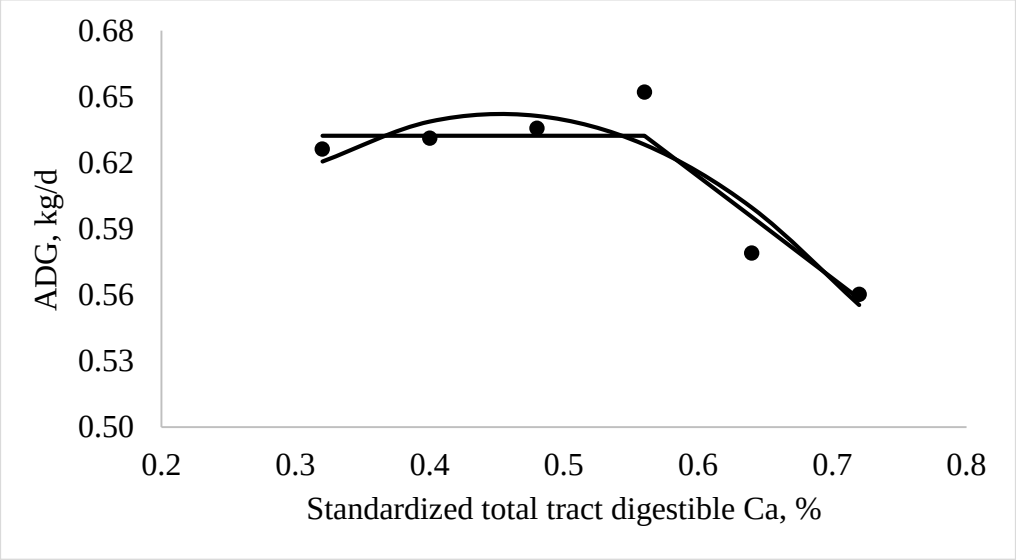

Figure 5.1. Fitted broken line of ADG (kg/d, d 1 to 22) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 1). The mean of each diet (●) represents the mean of 8 replicated pens per diet. The breakpoint was 0.56 ± 0.10% STTD Ca if the broken line analysis was used (plateau = 0.63kg/d ADG). The maximum ADG determined from the quadratic analysis was at 0.45 ± 0.04% STTD Ca. The intersections between the broken line and quadratic analysis were 0.36 and 0.54% STTD Ca.

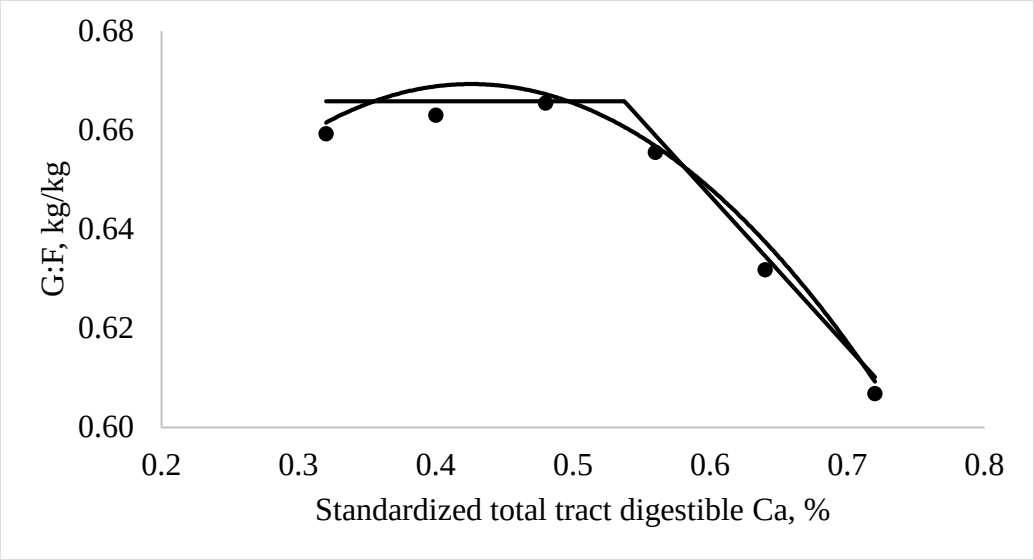

Figure 5.2. Fitted broken line of G:F (kg/kg, d 1 to 22) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 1). The mean of each diet (●) represents the mean of 8 replicated pens per diet. The break point for G:F was at 0.54 ± 0.04% STTD Ca (plateau = 0.67 kg/kg G:F). The maximum G:F determined from the quadratic analysis was at 0.43 ± 0.04% STTD Ca. The intersections between the broken line and quadratic analysis were 0.35 and 0.50% STTD Ca.

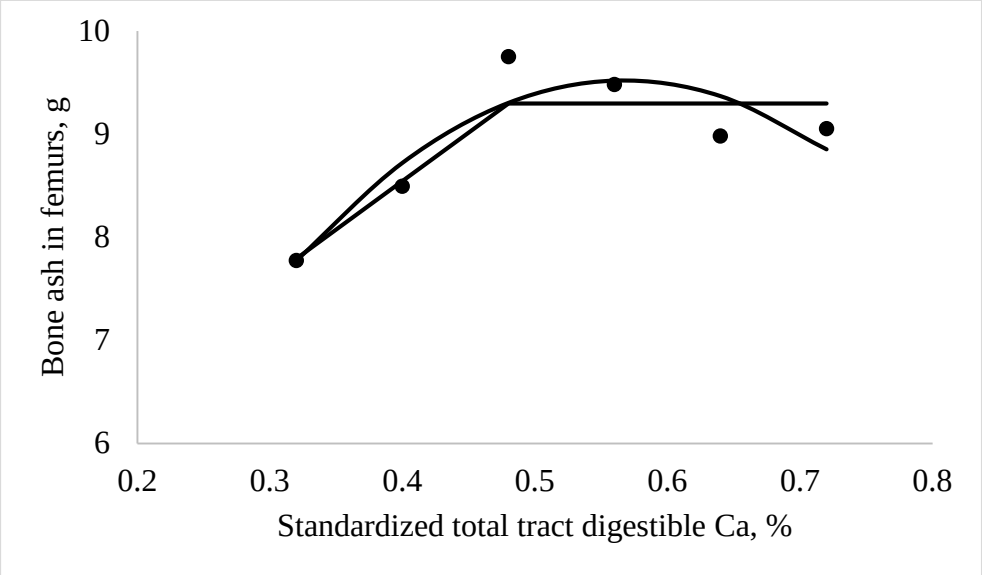

Figure 5.3. Fitted broken line of bone ash in femurs (g) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 1). The mean of each diet (●) represents the mean of 8 replicated pens per diet. The break point for g bone ash was at 0.48 ± 0.11% STTD Ca if the broken line analysis was used (plateau = 9.30g bone ash). The maximum concentration of bone ash determined from the quadratic analysis was 0.57 ± 0.03% STTD Ca. The intersections between the broken line and quadratic analysis were 0.48 and 0.66% STTD Ca.

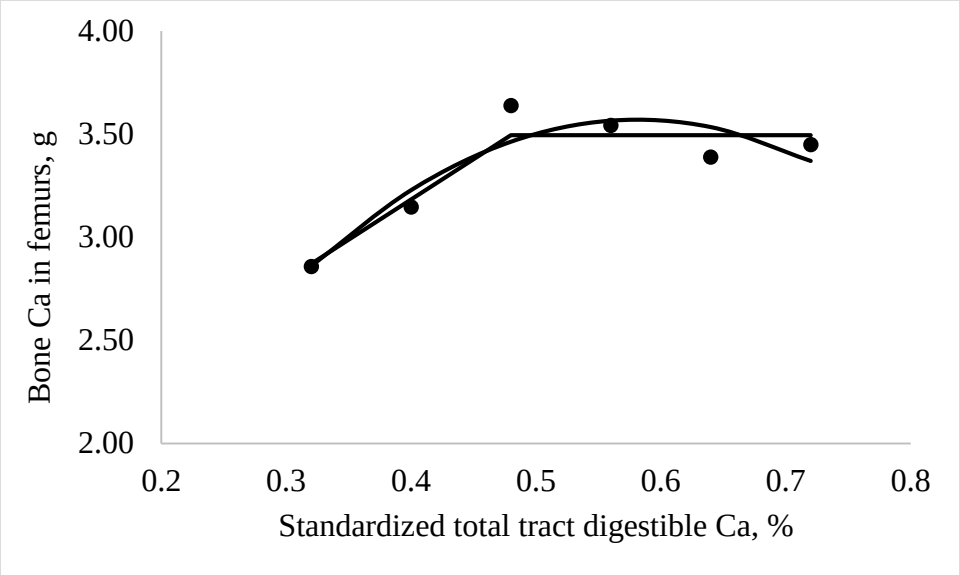

Figure 5.4. Fitted broken line of bone Ca in femurs (g) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 1). The mean of each diet (●) represents the mean of 8 replicated pens per diet. The break point for bone Ca was at 0.48 ± 0.11% STTD Ca if the broken line analysis was used (plateau = 3.49g bone Ca). The maximum quantity of bone Ca determined from the quadratic analysis was at 0.58 ± 0.04% STTD Ca. The intersections between the broken line and quadratic analysis were 0.50 and 0.67% STTD Ca.

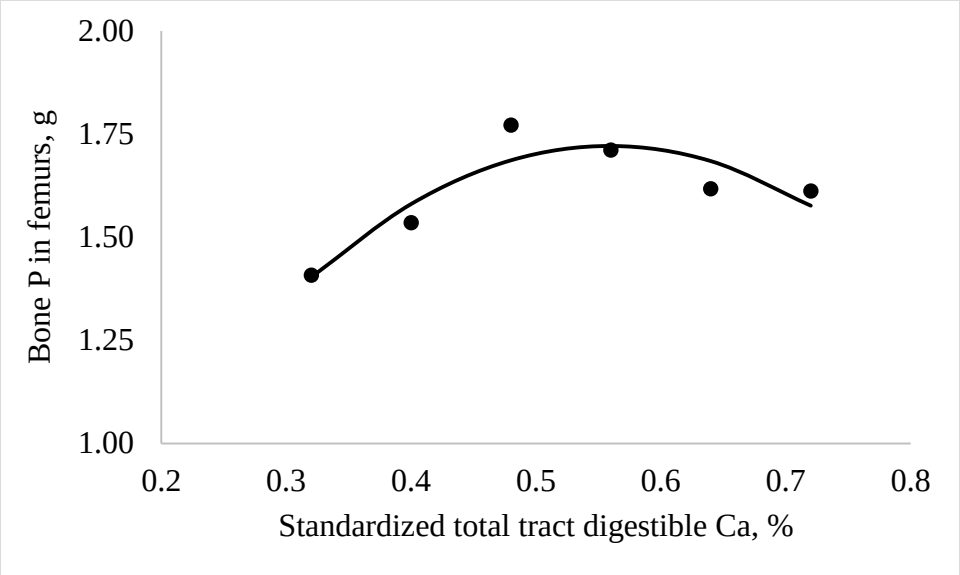

Figure 5.5. Fitted quadratic line of bone P in femurs (g) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 1). The mean of each diet (●) represents the mean of 8 replicated pens per diet. The maximum quantity of bone P was at 0.56 ± 0.03% STTD Ca.

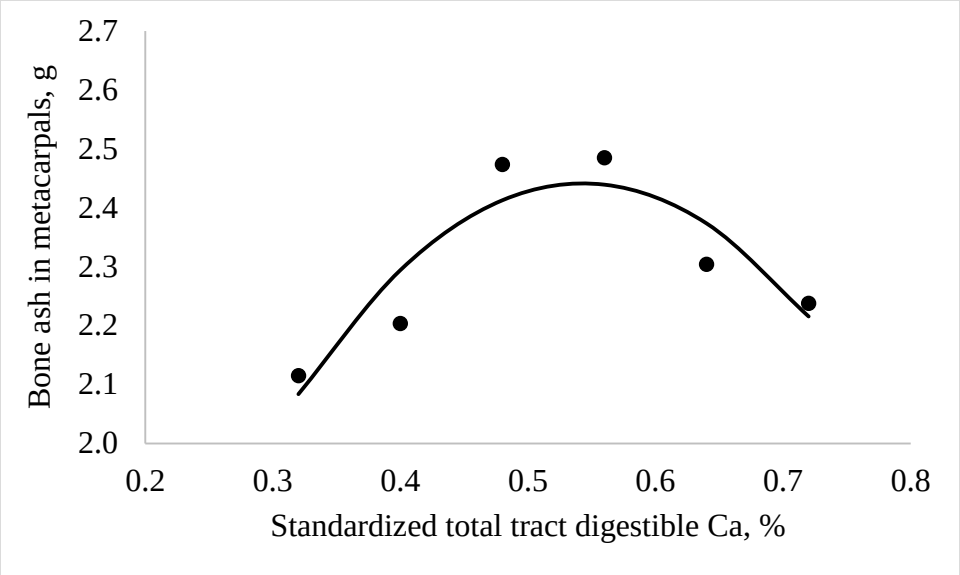

Figure 5.6. Fitted quadratic line of bone ash in metacarpals (g) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 1). The mean of each diet (●) represents the mean of 8 replicated pens per diet. The maximum concentration of bone ash in metacarpals was at 0.54 ± 0.02% STTD Ca.

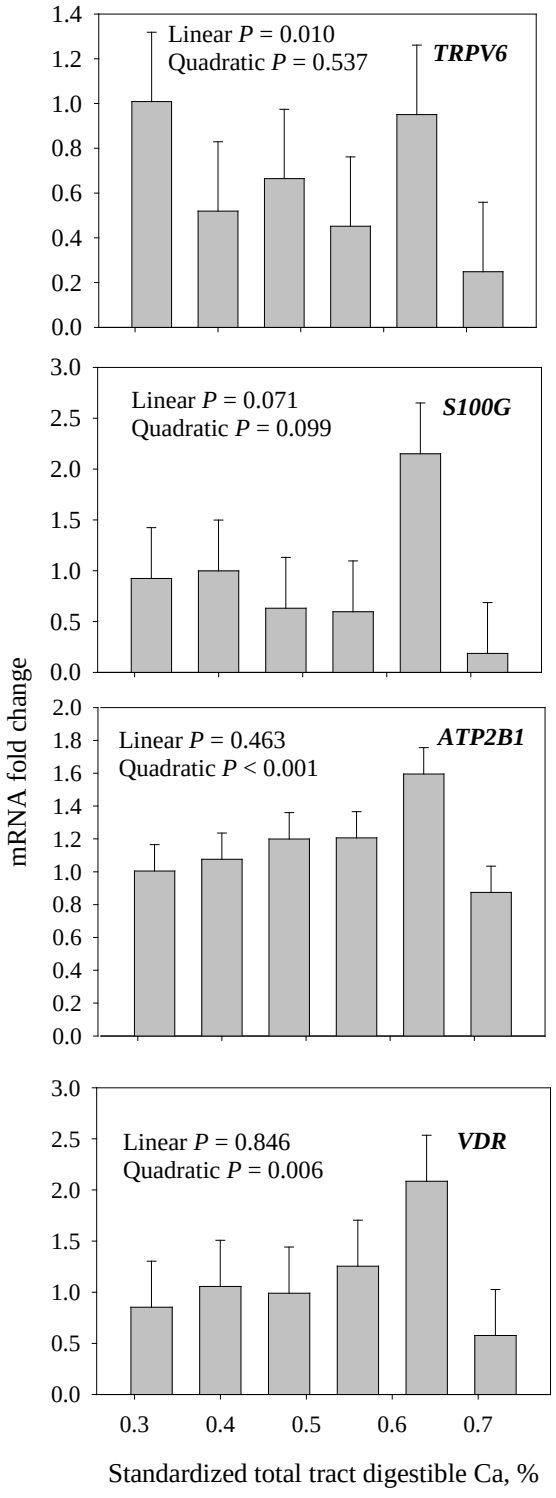

Figure 5.7. Expression of genes related to transcellular transportation of Ca in jejunum of pigs fed 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca and 0.36% STTD P. The *P*-values for linear

Figure 5.7. (Cont.)

and quadratic effect of increasing concentration of STTD Ca, and means for each diet and standard errors (vertical bars) are indicated. TRPV6 = transient receptor potential cation channel, subfamily V, member 6; S100G = S100 calcium binding protein G; ATP2B1 = ATPase, Ca^{++} transporting, plasma membrane 1; VDR = vitamin D (1, 25-dihydroxyvitamin D_3) receptor.

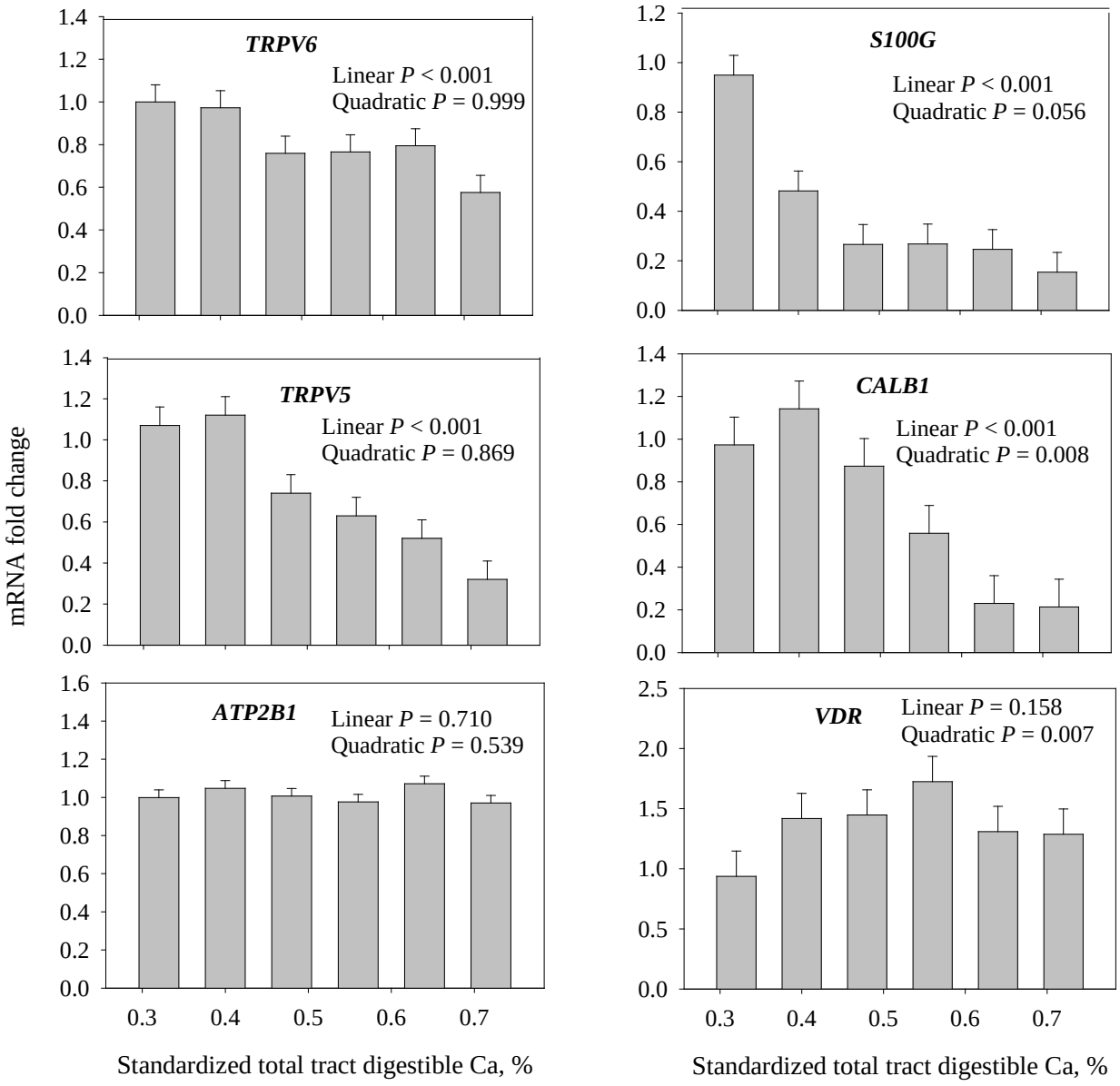

Figure 5.8. Expression of genes related to transcellular transportation of Ca in kidney of pigs fed 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca and 0.36% STTD P. The *P*-values for linear and quadratic effect of increasing concentration of STTD Ca and means for each diet and

Figure 5.8. (Cont.)

standard errors (vertical bars) are indicated. TRPV6 = transient receptor potential cation channel, subfamily V, member 6; TRPV5 = transient receptor potential cation channel, subfamily V, member 5; ATP2B1 = ATPase, Ca^{++} transporting, plasma membrane 1; S100G = S100 calcium binding protein G; CALB1 = calbindin 1, 28kDa; VDR = vitamin D (1, 25-dihydroxyvitamin D_3) receptor.

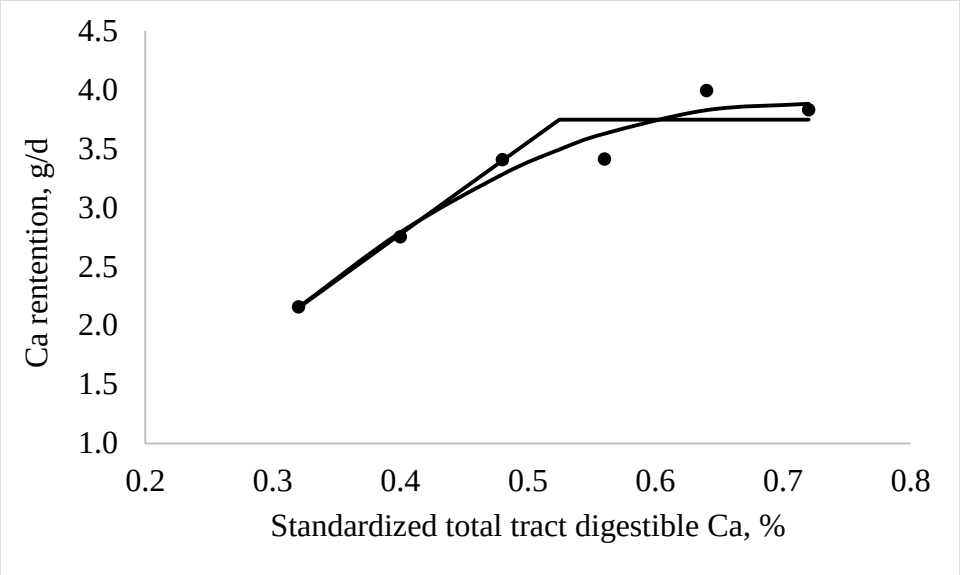

Figure 5.9. Fitted broken line of Ca retention (g/d) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 2). The mean of each diet (●) represents the mean of 10 replicated pigs per diet. The break point for Ca retention was at 0.52 ± 0.03% STTD Ca (plateau = 3.75g/d of Ca retention). The maximum Ca retention determined from the quadratic analysis was at 0.71 ± 0.08% STTD Ca. The intersection between the broken line and quadratic analysis was at 0.60% STTD Ca.

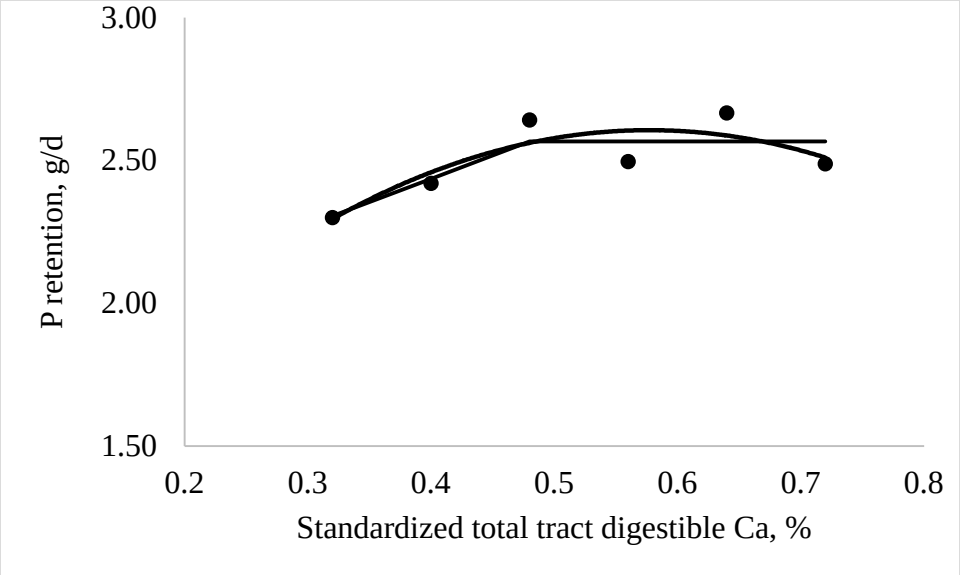

Figure 5.10. Fitted broken line of P retention (g/d) as a function of standardized total tract digestible Ca (STTD Ca; Exp. 2). The mean of each diet (●) represents the mean of 10 replicated pigs per diet. The break point for P retention was at 0.48 ± 0.16% STTD Ca (plateau = 2.57g/d of P retention). The maximum P retention determined from the quadratic analysis was at 0.58 ± 0.05% STTD Ca. The intersection between the broken line and quadratic analysis was at 0.49% STTD Ca.

TABLES

Table 5.1. Composition of ingredients, as-fed basis, Exp. 1 and 2

	Ingredient				
Item	Corn	Soybean meal	Lactose	Calcium carbonate	Monocalcium phosphate
GE, kcal/kg	3,913	4,160	3,685	-	-
DM, %	90.70	90.45	95.13	99.93	94.62
CP, %	8.96	47.69	0.13	-	-
NDF, %	8.49	7.42	-	-	-
ADF, %	3.42	5.86	-	-	-
Ash, %	1.40	8.11	0.46	93.30	80.93
Ca, %	0.01	0.52	0.04	41.16	19.03
P, %	0.25	0.58	0.01	1.13	22.48
Phytate, %	0.60	1.38	-	-	-
Phytate-bound P,[1] %	0.17	0.39	-	-	-
Nonphytate P,[2] %	0.08	0.19	-	-	-

[1]Phytate-bound P was calculated as 28.2 % of phytate (Tran and Sauvant, 2004).

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

Table 5.2. Ingredient composition of experimental diets, as-fed basis, Exp. 1 and 2

	0.36% STTD[1] P						0.33% STTD P	0.40% STTD P
Ingredient, %	0.32% STTD Ca	0.40% STTD Ca	0.48% STTD Ca	0.56% STTD Ca	0.64% STTD Ca	0.72% STTD Ca	0.72% STTD Ca	0.72% STTD Ca
Ground corn	53.00	53.00	53.00	53.00	53.00	53.00	53.00	53.00
Soybean meal, 47% CP	29.50	29.50	29.50	29.50	29.50	29.50	29.50	29.50
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cornstarch	3.79	3.13	2.49	1.89	1.25	0.61	0.76	0.48
Choice white grease	0.90	1.21	1.52	1.80	2.10	2.41	2.33	2.47
Calcium carbonate	0.05	0.40	0.73	1.05	1.39	1.72	1.80	1.59
Monocalcium phosphate	1.15	1.15	1.15	1.15	1.15	1.15	1.00	1.35
L-Lys HCL	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

Table 5.2. (Cont.)

DL-Met	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Thr	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Val	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Sodium chloride	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]STTD = standardized total tract digestible.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

Table 5.3. Composition of experimental diets, as-fed basis, Exp. 1 and 2

Item	0.36% STTD[1] P						0.33% STTD P	0.40% STTD P
	0.32% STTD Ca	0.40% STTD Ca	0.48% STTD Ca	0.56% STTD Ca	0.64% STTD Ca	0.72% STTD Ca	0.72% STTD Ca	0.72% STTD Ca
Analyzed composition								
DM, %	90.07	90.08	90.13	90.12	90.10	90.08	90.51	90.23
Ash, %	4.13	4.62	4.93	4.74	5.79	5.36	5.87	5.59
CP, %	18.64	18.98	18.61	18.77	19.72	18.33	18.89	19.60
NDF, %	7.76	8.31	8.01	7.98	7.91	7.55	9.07	8.71
ADF, %	4.79	4.95	4.36	4.47	4.78	4.02	3.96	4.62
Ca, %	0.38	0.50	0.72	0.77	0.86	1.03	1.02	1.06
P, %	0.56	0.58	0.58	0.56	0.58	0.56	0.54	0.63
Phytate,[2] %	0.50	0.60	0.60	0.60	0.60	0.67	0.67	0.71
Phytate-bound P, %	0.14	0.17	0.17	0.17	0.17	0.19	0.19	0.20
Nonphytate P,[3] %	0.42	0.41	0.41	0.39	0.41	0.37	0.35	0.43
Calculated composition								

Table 5.3. (Cont.)

Na, %	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
K, %	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Cl, %	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
DCAD,[4] mEq/kg	182.4	182.4	182.4	182.4	182.4	182.4	182.4	182.4

[1]STTD = standardized total tract digestible.

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

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

[4]DCAD = dietary cation-anion difference. The DCAD was calculated as Na + K – Cl.

Table 5.4. Gene-specific primer sets, Exp. 1

Gene[1]	Forward	Reverse	Source
TRPV5	5'- AGGGTCGGTTTCTCTCGCTA-3'	5'- GGCATAGGTGATGGTGATGACA-3'	This study
TRPV6	5'- TCCAGACAGAGGACCCTAACAAG-3'	5'- GTGAGAAACAGCTCAAAGGTGCTA-3'	Vigors et al., 2014
S100G	5'- CGCAACAGTCCCATTTAAGGA-3'	5'- TCAGCAGAGACATGGGTGGTT-3'	Vigors et al., 2014
CALB1	5'- ACGCTGACGGAAGTGGTTAC-3'	5'- ATCCAGCCTTCTTTCGTGCC-3'	This study
ATP2B1	5'- GGGCGGGCAGGTCATT-3'	5'- CCGCCGGGAGAAGATCA-3'	Vigors et al., 2014
VDR	5'- AGGCTTCTTCAGACGGAGCATGAA-3'	5'- ACTCCTTCATGCCGATGTCCA-3'	Gupta et al., 2012
Internal			
control gene			
GAPDH	5'- CAGCAATGCCTCCTGTACCA-3'	5'- ACGATGCCGAAGTTGTCATG-3'	Vigors et al., 2014
HMBS	5'- CTGAACAAAGGTGCCAAGAACA-3'	5'- GCCCCGCAGACCAGTTAGT-3'	Vigors et al., 2014

[1]TRPV5 = transient receptor potential cation channel, subfamily V, member 5; TRPV6 = transient receptor potential cation channel, subfamily V, member 6; S100G = S100 calcium binding protein G; CALB1 = calbindin 1, 28kDa; ATP2B1 = ATPase, Ca^{++} transporting, plasma membrane 1; VDR = vitamin D (1, 25-dihydroxyvitamin D_3) receptor; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HMBS = hydroxymethylbilane synthase.

Table 5.5. Body weight and ADFI of pigs fed diets containing 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD P, Exp. 1

	Diets							*P*-Value	
STTD Ca, %	0.32	0.40	0.48	0.56	0.64	0.72	SEM	Linear	Quadratic
BW, kg									
d 1	11.39	11.35	11.44	11.37	11.45	11.33	0.42	0.982	0.909
d 10	16.44	16.90	16.81	16.47	16.28	15.65	0.65	0.195	0.256
d 22	25.21	25.29	25.47	25.76	24.23	23.74	0.83	0.117	0.172
ADFI, g/d									
d 1-10	790	830	778	778	776	762	56	0.233	0.738
d 10-22	1,097	1,070	1,117	1,189	1,048	1,075	53	0.785	0.223
d 1-22	957	961	963	1,003	927	934	52	0.506	0.316

Table 5.6. Growth performance and bone mineralization of pigs fed diets containing 0.72% standardized total tract digestible (STTD) Ca and 0.33, 0.36, or 0.40% STTD P, Exp. 1

	Diets				*P*-Value	
STTD P, %	0.33	0.36	0.40	SEM	Linear	Quadratic
BW, kg						
d 1	11.42	11.33	11.42	0.43	0.987	0.868
d 10	16.07	15.65	15.96	0.85	0.937	0.659
d 22	24.32	23.74	25.05	1.00	0.517	0.412
ADG, g/d						
d 1-10	466	435	453	54	0.833	0.535
d 10-22	689	674	759	22	0.027	0.113
d 1-22	587	565	622	31	0.230	0.183
ADFI, g/d						
d 1-10	779	762	748	80	0.618	0.939
d 10-22	1,127	1,075	1,129	57	0.909	0.363
d 1-22	970	934	957	64	0.867	0.536
G:F						
d 1-10	0.60	0.56	0.60	0.02	0.928	0.281
d 10-22	0.61	0.63	0.68	0.03	0.008	0.751
d 1-22	0.61	0.61	0.65	0.01	0.003	0.127
Femurs						
Bone ash, %	55.46	57.21	57.59	0.60	<0.001	0.076
Bone ash, g	8.11	9.05	10.23	0.42	0.002	0.941

Table 5.6. (Cont.)

Bone Ca, %	37.70	38.07	37.29	0.37	0.307	0.144
Bone Ca, g	3.05	3.45	3.82	0.17	0.006	0.747
Bone P, %	17.93	17.84	17.93	0.28	1.000	0.535
Bone P, g	1.45	1.62	1.84	0.09	0.003	0.989
Metacarpals						
Bone ash, %	51.29	53.43	53.08	0.36	0.004	0.006
Bone ash, g	2.19	2.25	2.55	0.11	0.022	0.492

[a,b]Means of diets containing 0.72% STTD Ca within a row without common superscript differ ($P < 0.05$).

Table 5.7. Calcium balance and apparent total tract digestibility (ATTD) of Ca for pigs fed diets containing between 0.32 and 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD P, and diets containing 0.72% STTD Ca and 0.33 or 0.40% STTD P, Exp. 2[1]

Item	Diets								Diets with 0.36% STTD P			Diets with 0.72% STTD Ca		
STTD Ca, %	0.32	0.40	0.48	0.56	0.64	0.72	0.72	0.72		*P*-Value			*P*-Value	
STTD P, %	0.36	0.36	0.36	0.36	0.36	0.36	0.33	0.40	SEM	L^2	Q^2	SEM	L	Q
Feed intake, g/d	771	759	781	746	782	761	748	717	29	0.964	0.953	30	0.412	0.465
Ca intake, g/d	3.10	4.14	5.33	6.07	7.45	8.30	8.19	7.70	0.26	<0.001	0.985	0.26	0.158	0.326
Fecal Ca output, g/d	0.82	1.19	1.54	1.93	2.34	3.03	2.89	2.56	0.10	<0.001	0.039	0.11	0.021	0.035
Urine Ca output, mg/d	122	204	383	731	1124	1534	1677	1096	65	<0.001	<0.001	73	<0.001	0.243
Absorbed Ca, g/d	2.28	2.96	3.79	4.14	4.95	5.25	5.09	5.03	0.19	<0.001	0.283	0.20	0.774	0.450

Table 5.7. (Cont.)

Ca retention, % of intake	69.74	66.34	64.03	56.17	53.11	47.83	44.63	54.51	1.62	<0.001	0.385	1.65	<0.001	0.611
Ca excretion, g/d	0.94	1.39	1.92	2.66	3.46	4.55	4.69	3.47	0.14	<0.001	<0.001	0.14	<0.001	0.031
Ca excretion, % of intake	30.26	33.66	35.97	43.83	46.89	52.17	55.37	45.49	1.62	<0.001	0.385	1.65	<0.001	0.611
ATTD of Ca, %	73.68	71.35	71.13	68.15	68.25	65.24	65.88	67.95	1.52	<0.001	0.889	1.57	0.322	0.431

[1]Each least squares means represents 10 observations.

[2]L = linear response; Q = quadratic response

Table 5.8. Phosphorus balance and apparent total tract digestibility (ATTD) of P for pigs fed diets containing between 0.32 and 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD P, Exp. 2[1]

Item	Diets								Diets with 0.36% STTD P			Diets with 0.72% STTD Ca		
STTD Ca, %	0.32	0.40	0.48	0.56	0.64	0.72	0.72	0.72		*P*-Value			*P*-Value	
STTD P, %	0.36	0.36	0.36	0.36	0.36	0.36	0.33	0.40	SEM	L^2	Q^2	SEM	L	Q
P intake, g/d	4.34	4.31	4.46	4.29	4.53	4.35	4.11	4.56	0.17	0.699	0.842	0.17	0.066	0.809
Fecal P output, g/d	1.52	1.60	1.69	1.71	1.76	1.78	1.72	1.75	0.07	0.006	0.489	0.07	0.801	0.668
Urine P output, mg/d	548	287	131	82	84	88	93	84	15	<0.001	<0.001	12	0.567	0.951
Absorbed P, g/d	2.82	2.70	2.77	2.58	2.77	2.58	2.39	2.80	0.13	0.246	0.892	0.13	0.023	0.951
P retention, g/d	2.30	2.42	2.64	2.50	2.67	2.49	2.29	2.72	0.12	0.144	0.148	0.12	0.017	0.941

Table 5.8. (Cont.)

P retention, % of intake	53.09	56.37	59.12	58.16	58.55	57.18	55.77	59.21	1.33	0.021	0.009	1.33	0.070	0.972
P excretion, g/d	1.95	1.89	1.82	1.80	1.86	1.87	1.82	1.84	0.08	0.415	0.217	0.08	0.867	0.687
P excretion, % of intake	46.91	43.63	40.88	41.84	41.45	42.82	44.24	40.79	1.33	0.021	0.009	1.33	0.070	0.972
ATTD of P, %	65.89	62.80	62.08	60.08	60.83	59.14	58.07	62.08	1.13	<0.001	0.206	1.14	0.014	0.639

[1]Each least squares means represents 10 observations.

[2]L = linear response; Q = quadratic response

CHAPTER 6

REQUIREMENT FOR DIGESTIBLE CALCIUM BY 25 TO 50 KG PIGS AT DIFFERENT DIETARY CONCENTRATIONS OF PHOSPHORUS BY GROWTH PERFORMANCE, BONE ASH CONCENTRATION, AND CALCIUM AND PHOSPHORUS BALANCES

ABSTRACT: Two experiments were conducted to determine the requirement for standardized total tract digestible (STTD) Ca by 25 to 50 kg pigs at different concentrations of STTD P. Twenty corn-soybean meal based diets were formulated with diets containing 4 concentrations of STTD P (0.15, 0.31, 0.39, or 0.47%) and 5 concentrations of STTD Ca (0.13, 0.27, 0.42, 0.57, or 0.72%). Diets were mixed in 1 batch and were used in both experiments. In Exp. 1, 240 pigs (initial average BW: 24.70 ± 1.27 kg) were randomly allotted to 20 diets in 6 blocks with 1 pen per diet in each block using a 4 × 5 factorial design. There was 1 gilt and 1 barrow in each pen. At the conclusion of the 28 d experiment, all barrows were euthanized and the right femur was collected. Results indicated that there were interactions ($P < 0.001$) between concentration of STTD Ca and concentration of STTD P in diets for ADG, G:F, and bone ash. The predicted maximum ADG at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 0.76, 0.87, 0.90, and 0.92 kg at STTD Ca concentrations of 0.12, 0.36, 0.47, and 0.59%, respectively, which correspond to STTD Ca:STTD P ratios of 0.80:1, 1.16:1, 1.21:1, and 1.26:1. The predicted maximum G:F ratio at the 4 STTD P concentrations were 0.43, 0.46, 0.48, and 0.50 kg/kg at STTD Ca concentrations of 0.09, 0.38, 0.52, and 0.67%, respectively, and these values correspond to STTD Ca:STTD P ratios of 0.60:1, 1.23:1, 1.33:1, and 1.43:1. The predicted maximum bone ash at the 4 STTD P concentrations were 14.5, 21.0, 23.1, and 24.5 g at STTD

Ca concentrations of 0.41, 0.56, 0.64, and 0.72%, respectively, which correspond to STTD Ca:STTD P ratios of 2.73:1, 1.81:1, 1.64:1, and 1.53:1. In Exp. 2, 120 pigs (initial average BW: 29.45 ± 2.15 kg) were placed in metabolism crates and randomly allotted to the 20 diets in 6 blocks with 1 pig per diet in each block. Fecal and urine samples were collected. Results indicated that the predicted maximum retention of Ca in the body at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 4.7, 7.1, 8.6, and 10.2 g/d at STTD Ca concentrations of 0.77, 0.96, 1.06, and 1.15%, respectively, which correspond to STTD Ca:STTD P ratios of 5.13:1, 3.10:1, 2.72:1, and 2.45:1. These observations indicate that if STTD P meets or exceed the requirement, the STTD Ca:STTD P ratio needed to maximize ADG and G:F by 25 to 50 kg pigs appears to be between 1.16:1 and 1.43:1. However, a greater ratio may be needed to maximize bone ash.

Key words: bone ash, calcium, digestible calcium, growth performance, pigs, requirements

INTRODUCTION

The disadvantage of formulating diets to meet total Ca requirement is that excess of dietary Ca may increase P excretion due to formation of Ca-P complexes (Clark, 1969; Brink et al., 1992; Stein et al., 2011). Therefore, if diets are formulated to meet requirements for standardized total tract digestible (**STTD**) Ca and STTD P requirements, a maximum utilization of both minerals is expected. Requirements for STTD P by different categories of pigs have been reported (NRC, 2012), but requirements for STTD Ca have not been reported because of a lack of information about Ca digestibility in feed ingredients. However, values for STTD of Ca in some Ca sources have been recently reported (González-Vega et al., 2014, 2015a; b; c;

Merriman and Stein, 2015), and as a consequence, requirements for STTD Ca can be determined. An attempt to determine the requirement for STTD Ca by 11 to 25 kg pigs was made (González-Vega et al., 2015c), and results indicated that the STTD Ca to STTD P ratio was important in the diet. A negative effect of increasing concentration of dietary STTD Ca was observed for ADG and G:F, and ADG and G:F were maximized at a STTD Ca:STTD P ratio below 1.39:1 and 1.50:1, respectively. However, bone ash and the quantity of Ca and P retained in the body were maximized if the STTD Ca: STTD P ratio was between 1.33:1 and 1.67:1. These ratios are in agreement with the ratio between Ca and P in the body of pigs, which has been reported to be in the range between 1.20:1 and 1.60:1 (Rymarz et al., 1982; Hendriks and Moughan, 1993; Mahan and Shields, 1998; Wiseman et al., 2009; Pettey et al., 2015). There are, however, no estimates for the STTD Ca requirement by 25 to 50 kg pigs. Thus, the present experiments were conducted to test the hypothesis that a wide range of STTD Ca:STTD P negatively affects growth performance, therefore, the requirement for STTD Ca by 25 to 50 kg pigs needed to maximize ADG, G:F, bone ash, and Ca retention will be determined at different concentrations of STTD P.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for 2 experiments. Pigs used in both experiments were the offspring of PIC 359 boars and F-46 sows (Pig Improvement Company, Hendersonville, TN).

Experiment 1: Requirements for Digestible Ca to Maximize Growth Performance and Bone Ash

Animals, Diets, and Feeding. Two hundred and forty pigs (initial average BW: 24.70 ± 1.27 kg) were randomly allotted to 20 diets in 6 blocks with 1 pen per diet in each block using a 4 × 5

factorial design. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. Pen was the experimental unit and there was 1 gilt and 1 barrow in each pen. Room temperature was controlled and each pen had a feeder, a nipple drinker, and a fully slatted concrete floor. Feed and water were available at all times.

Twenty corn-soybean meal based diets were formulated to contain different Ca and P concentrations, but to keep the concentration of phytate constant, all diets contained the same amount of corn and soybean meal (Table 6.1). Diets were formulated to contain 0.15, 0.31, 0.39, or 0.47% STTD P and 0.18, 0.42, 0.66, 0.90, or 1.14% total Ca (0.13, 0.27, 0.42, 0.57, or 0.72% STTD Ca), respectively; Tables 6.2 and 6.3. Concentrations of P ranged from 48 to 152% of the STTD P requirement (NRC, 2012) and concentrations of Ca ranged from 27 to 173% of the total Ca requirement (NRC, 2012). The concentration of P was increased in the diets by adding increased concentrations of monosodium phosphate (**MSP**), but to maintain the same Na concentration among diets, inclusion of sodium bicarbonate was reduced as MSP increased in the diets.

Growth Performance and Bone Measurements. Pig weights were recorded on d 1 and 28. Pigs were allowed ad libitum access to feed and the amount of feed offered each day was recorded. To calculate feed intake, the amount of feed in the feeders on d 28 was subtracted from the total amount of feed offered. On d 28, all barrows were euthanized via captive bolt stunning. Stomach contents were immediately collected to measure pH. The right femurs were removed, cleaned, and stored at -20ºC. The frozen bones were later thawed, broken, and soaked in petroleum ether under a chemical hood for 72 h to remove bone marrow and fat. Bones were dried overnight at 130ºC and then ashed at 600ºC for 24 h.

Sample Analysis. Corn, soybean meal, calcium carbonate, MSP, monocalcium phosphate (**MCP**), NaCl, and diets were analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007), for ash (Method 942.05; AOAC Int., 2007), for Na by flame emission photometry (Method 956.01; AOAC Int., 2007), and for Cl by manual titration (Method 9.15.01, 943.01; AOAC, 2006). Ingredients, diets, and bone ash were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (**ICP-OES**; Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Corn, soybean meal, and diets were analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. Corn and soybean meal were analyzed for GE using an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) and for phytic acid (Megazyme method; AB Vista, Ystrad Mynach, UK). Diets were analyzed for phytate-P using a Foss near-infrared spectrometer with the phytate-P levels predicted using AUNIR calibration standards (AB Vista, Memphis, TN). The pH of the stomach content was measured using a pH meter (Oakton pH 11 Standard Portable Meter, Fisher Scientific, Pittsburgh, PA, USA).

Calculations and Statistical Analyses. The percentage of phytate-bound P in corn and soybean meal was calculated as 28.2% of phytate (Tran and Sauvant, 2004). The percentage of phytate in diets was calculated by dividing the analyzed phytate-bound P by 0.282, and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. Dietary cation-anion difference (**DCAD**) was calculated using the following equation:

$$\text{DCAD, mEq/kg} = [(\text{Na}*10000)/23] + [(\text{K}*10000)/39] - [(\text{Cl}*10000)/35.5], \quad [1]$$

where Na, K, and Cl are expressed in percentage of inclusion in diet. The ADG, ADFI, and G:F

were calculated. Bone ash percentage was calculated by dividing the quantity of bone ash by the quantity of fat-free dried bone and multiplying by 100.

Normality of residuals and identification of outliers were determined by the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data for BW, ADG, ADFI, G:F, bone ash, bone Ca, bone P, and stomach pH were analyzed using the Proc MIXED of SAS (SAS Inst. Inc., Cary, NC). The fixed effects of the model were dietary concentration of STTD Ca, dietary concentration of STTD P, and the interaction between STTD Ca and STTD P; the random effect was block. Effect of dietary STTD Ca, STTD P, or the interaction between STTD Ca and STTD P were considered significant at $P \leq 0.05$. If the interaction or the main effects were significant, the program NLREG was used to determine parameter estimates for the surface response model to increasing concentrations of STTD Ca and STTD P. The parameter estimates of the model that were not significant ($P > 0.05$) and were not included in a significant interaction were removed from the model and the estimates were recalculated. The surface response full model was:

$$Y = a + b \times \text{STTD Ca} + c \times \text{STTD Ca}^2 + d \times \text{STTD P} + e \times \text{STTD P}^2 + f \times \text{STTD Ca} \times \text{STTD P}, \quad [2]$$

where Y was the dependent variable, a was the intercept, b, c, d, e, and f were the coefficients, STTD Ca and STTD P were the percentage concentrations of dietary STTD Ca and STTD P. If parameter estimate c was significant ($P \leq 0.05$), the concentrations of STTD Ca at the maximum response values were calculated using the following equation:

$$\text{STTD Ca max} = [(- f \times \text{STTD P}) - b] / (2 \times c), \quad [3]$$

where STTD Ca max is the concentration of STTD Ca at the maximum response and STTD P is the percentage concentration of STTD P in the diet. The maximum response values were,

therefore, calculated using the respective model equations with the concentrations of STTD Ca at the maximum response in each concentration of STTD P. The LSMEANS procedure was used to calculate mean values for treatments and the pen was the experimental unit for all analyses.

Experiment 2. Calcium and P Balance

Animals, Diets, and Feeding. One hundred and twenty pigs (initial average BW: 29.45 ± 2.15 kg) were randomly allotted to 20 diets in 6 blocks with 1 pig per diet in each block using a 4 × 5 factorial design. The 20 diets used in Exp. 1 were also used in Exp. 2, and the amount of each diet that was needed for both experiments was mixed in 1 batch. Pigs were housed individually in metabolism crates that were equipped with a slatted floor, a feeder, and a nipple drinker. Pigs had free access to water throughout the experiment. Each pen had a screen floor for fecal collection and a urine pan with a urine bucket for total urine collection.

Pigs were fed 3 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The daily allotments of feed were divided into 2 equal meals and provided at 0800 and 1700 h. Pigs were fed each diet for 13 d, the initial 5 d were an adaptation period to the diets and fecal samples were collected quantitatively from the feed provided from d 6 to 11 using the marker-to-marker approach (Adeola, 2001). 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 11. Urine samples were collected every morning from d 6 to d 11 and 50 mL of 6*N* HCl was added to each bucket after they were emptied. Fecal samples and 20% of the collected urine were stored at -20°C immediately after collection. Orts collected during the collection period were dried in a forced-air oven at 65ºC and feed intake was calculated by subtracting the orts from feed allowance. Blood samples were collected on d 1 and d 13 by jugular venipuncture. Samples

were immediately centrifuged and plasma samples were collected and stored at -20°C for Ca and P analysis.

Sample Analysis and Statistical Analyses. Fecal samples were dried in a forced-air oven at 65°C and ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen. Fecal samples were analyzed for DM as explained for Exp. 1. Fecal and urine samples were analyzed for Ca and P as explained for Exp. 1. Plasma samples were analyzed for Ca and P (Method 968.08 D(b); AOAC Int., 2007). The apparent total tract digestibility (**ATTD**) of Ca and P were calculated according to standard procedures (NRC, 2012). Values for retention of Ca and P were calculated as explained by González-Vega et al. (2013). Data were analyzed using PROC MIXED of SAS and parameter estimates for the surface response model to increasing levels of STTD Ca and STTD P were determined as explained for Exp. 1. For the urine Ca output and urine P output data, the surface response full model was:

$$Y = a + b \times \text{STTD Ca} + c \times \text{STTD Ca}^2 + d \times \text{STTD P} + e \times \text{STTD P}^2 + f \times \text{STTD Ca} \times \text{STTD P} + g \times \text{STTD Ca}^2 \times \text{STTD P} + h \times \text{STTD Ca} \times \text{STTD P}^2 + i \times \text{STTD Ca}^2 \times \text{STTD P}^2, \quad [4]$$

where Y was the dependent variable, a was the intercept, b, c, d, e, f, g, h, and i were the coefficients, STTD Ca and STTD P are the percentage concentrations of dietary STTD Ca and STTD P. The concentrations of STTD Ca at the maximum or minimum response values were calculated using the following equation:

$$\text{STTD Ca max/min} = [- (h \times \text{STTD P}^2 + f \times \text{STTD P} + b)] / [2 \times (i \times \text{STTD P}^2 + g \times \text{STTD P} + c)], \quad [5]$$

where STTD Ca max/min is the percentage concentration of STTD Ca at the maximum or minimum response and STTD P is the percentage concentration of STTD P in the diet. The maximum or minimum response values were, therefore, calculated using the respective model

equations with the concentrations of STTD Ca at the maximum or minimum response in each concentration of STTD P. Pig was the experimental unit for all analyses.

RESULTS

Experiment 1: Requirements for Digestible Ca to Maximize Growth Performance and Bone Ash

All pigs consumed their diets without apparent problems, but 1 gilt and 1 barrow fed the diet containing 0.13% STTD Ca and 0.47% STTD P died from blocks 3 and 4, respectively. The ADG, ADFI, and G:F of the pen for these pigs were adjusted (Lindemann and Kim, 2007). However, values of bone ash, bone Ca, and bone P from this barrow were missing.

The predicted maximum BW at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% was 46.0, 49.0, 49.9, and 50.5 kg at STTD Ca concentrations of 0.14, 0.35, 0.45, and 0.56%, respectively (Table 6.4). These values correspond to STTD Ca:STTD P ratios of 0.93:1, 1.13:1, 1.15:1, and 1.19:1 (Fig. 6.1). The predicted maximum ADG at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% was 0.76, 0.87, 0.90, and 0.92 kg at STTD Ca concentrations of 0.12, 0.36, 0.47, and 0.59%, respectively, which correspond to STTD Ca:STTD P ratios of 0.80:1, 1.16:1, 1.21:1, and 1.26:1 (Fig. 6.2). The predicted maximum ADFI at the 4 STTD P concentrations was 1.80, 1.90, 1.89, and 1.85 kg at STTD Ca concentrations of 0.14, 0.33, 0.43, and 0.52%, respectively, and these values correspond to STTD Ca:STTD P ratios of 0.93:1, 1.06:1, 1.10:1, and 1.11:1 (Fig. 6.3). The predicted maximum G:F at the 4 STTD P concentrations was 0.43, 0.46, 0.48, and 0.50 kg/kg at STTD Ca concentrations of 0.09, 0.38, 0.52, and 0.67%, respectively, which correspond to STTD Ca:STTD P ratios of 0.60:1, 1.23:1, 1.33:1, and 1.43:1 (Fig. 6.4).

The predicted maximum bone ash at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% was 14.5, 21.0, 23.1, and 24.5 g at STTD Ca concentrations of 0.41, 0.56, 0.64, and 0.72%, respectively (Table 6.5). These values correspond to STTD Ca:STTD P ratios of 2.73:1, 1.81:1, 1.64:1, and 1.53:1 (Fig. 6.5). The predicted maximum bone Ca and bone P at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 5.7, 8.2, 9.1, and 9.6 g Ca at STTD Ca concentrations of 0.42, 0.57, 0.65, and 0.73%, and 2.7, 3.9, 4.3, and 4.6 g P at STTD Ca concentrations of 0.40, 0.55, 0.63, and 0.71%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.80:1, 1.84:1, 1.67:1, and 1.55:1 for bone Ca (Fig. 6.6) and 2.67:1, 1.77:1, 1.62:1, and 1.51:1 for bone P (Fig. 6.7). The stomach pH was less ($P = 0.016$) in pigs fed the diet containing 0.13% STTD Ca than in pigs fed diets containing 0.27, 0.57, or 0.72% STTD Ca, but not different from the pH of the diet containing 0.42% STTD Ca.

Experiment 2. Calcium and P Balance

Pigs consumed the diets without problems throughout the experiment, but 1 barrow fed the diet containing 0.57% STTD Ca and 0.31% STTD P was euthanized on d 9 of the experiment due to an injured leg. Therefore, this diet had 1 missing replication.

The ATTD of Ca in diets linearly increased ($P = 0.009$) as concentration of STTD Ca increased, but was not affected by the concentration of STTD P (Table 6.6). However, the ATTD of P in diets linearly decreased ($P < 0.001$) as the concentration of STTD Ca increased, but linearly increased ($P < 0.001$) as the concentration of STTD P increased. Due to linear responses for ATTD of Ca and ATTD of P, maximum values were not estimated. The predicted maximum urine Ca output at 0.15% STTD P was 8.8 g/d at STTD Ca concentration of 0.64%, which correspond to STTD Ca:STTD P ratio of 4.27:1 (Fig. 6.8). However, the predicted minimum urine Ca output at STTD P concentrations of 0.31, 0.39, and 0.47% were 0.4, -0.3, and 0.2 g/d at

STTD Ca concentrations of 0.24, 0.29, and 0.29%, respectively, which correspond to STTD Ca:STTD P ratios of 0.77:1, 0.74:1, and 0.62:1. The predicted minimum urine P output at STTD P concentrations of 0.15, 0.31, and 0.39% were 0.0, 0.2, and 0.7 g/d at STTD Ca concentrations of 0.49, 0.62, and 0.78%, respectively, which correspond to STTD Ca:STTD P ratios of 3.27:1, 2.00:1, and 2.00:1, respectively (Fig. 6.9). The values for minimum urine P output at 0.47% STTD P was not estimated due to a nearly linear response. The predicted maximum retention of Ca in the body at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 4.7, 7.1, 8.6, and 10.2 g/d at STTD Ca concentrations of 0.77, 0.96, 1.06, and 1.15%, respectively, and these values correspond to STTD Ca:STTD P ratios of 5.13:1, 3.10:1, 2.72:1, and 2.45:1 (Fig. 6.10). These STTD Ca concentrations needed to maximize retention of Ca in the body were extrapolations of the model. The predicted maximum retention of P in the body at the 4 STTD P concentrations were 1.8, 2.6, 3.2, and 4.0 g/d at STTD Ca concentrations of 0.15, 0.45, 0.61, and 0.76%, respectively, which correspond to STTD Ca:STTD P ratios of 1.00:1, 1.46:1, 1.55:1, and 1.61:1 (Fig. 6.11).

Calcium in plasma increased ($P < 0.001$) in pigs fed diets containing 0.15% STTD P as the concentration of dietary Ca increased, but for pigs fed diets containing 0.31, 0.39, or 0.47% STTD P no change in plasma concentration of Ca was observed as dietary Ca increased (interaction, $P < 0.001$; Fig. 6.12). However, P in plasma linearly decreased ($P = 0.003$) as the concentration of STTD Ca increased, and linearly increased ($P = 0.003$) as the concentration of STTD P increased (Fig. 6.13). Due to the linear response for Ca and P in plasma, maximum values were not estimated.

DISCUSSION

The concentration of Ca, P, Na, and Cl in corn, calcium carbonate, MSP, MCP, and NaCl were in agreement with reported values (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012). The concentration of P, Na, and Cl in soybean meal were close to reported values (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012), but the concentration of Ca was greater than expected (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012). However, diets were formulated based on analyzed values for Ca and P in all ingredients and the concentration of Ca, P, Na, and Cl in all diets were, therefore, close to calculated values.

Utilization of Ca and P in the body is dependent on the excess or deficiency of one mineral or the other (Crenshaw, 2001), therefore, the ratio between Ca and P needs to be considered in diet formulations. Studies that are design to determine Ca or P requirements usually are designed to contain a constant dietary concentration of one mineral with graded levels of the other mineral, or alternatively, a constant Ca:P ratio may be used. Results obtained from a previous study that aimed at determining STTD Ca requirements by 11 to 25 kg pigs, indicated that the ratio between STTD Ca and STTD P was important for maximizing ADG, G:F, the quantity of deposited bone ash, Ca retained, and P retained (González-Vega et al., 2015c). Due to the negative effect of increasing the concentration of STTD Ca in diets with a constant concentration of STTD P on ADG and G:F (González-Vega et al., 2015c), the current experiments were designed to include 4 concentrations of STTD P and 5 concentrations of STTD Ca, which allowed us to determine requirements of STTD Ca at different concentrations of STTD P.

The fact that increasing concentrations of STTD Ca had a negative effect on growth performance of pigs fed diets containing concentrations of STTD P between 48.4 and 151.6% of the requirement (NRC, 2012), especially at low P concentration, is in agreement with results obtained in our previous study with 11 to 25 kg pigs (González-Vega et al., 2015c). The negative effect of increasing dietary Ca on growth performance was also observed in fast-growing Cobb broiler chickens (Hurwitz et al., 1995). This may be caused by formation of Ca-P complexes in the gastrointestinal tract due to excess Ca in diets, which reduces digestibility of P (Clark, 1969; Brink et al., 1992; Stein et al., 2011; González-Vega et al., 2014). However, the negative effect of increasing concentration of STTD Ca on growth performance of pigs was ameliorated as concentration of STTD P increased, which further indicates that the negative effects of high dietary concentrations of STTD Ca may have been caused by reduced P digestibility.

Regardless of the concentration of STTD P in the diet, final BW, ADG, and G:F were less affected by Ca concentrations below the requirement than if Ca was provided above the requirement, which indicates that at low concentrations of dietary Ca, pigs were able to mobilize Ca from Ca stores to compensate for the deficiency of dietary Ca. These results are in agreement with previous reports (Eklou-Kalonji et al., 1999; González-Vega et al., 2015c). The implications of the results obtained for growth performance is that the STTD Ca:STTD P ratio is important in diet formulation because excess Ca needs to be avoided.

Results from our study with 11 to 25 kg pigs indicated that the quantity of STTD Ca that was needed to maximize bone ash and retention of Ca was greater than the quantity needed to maximize growth performance (González-Vega et al., 2015c). Results obtained from the current experiments supported this observation, and these results are also in agreement with data from studies that aimed at determining P requirements in pigs (Ketaren et al., 1993; Partanen et al.,

2010; Adeola et al., 2015). It is possible that these responses are a result of the fact that deposition of Ca and P in bones and in soft tissue are independent (Crenshaw, 2001). However, it is also possible that the results for bone ash and Ca retention simply reflect that the pig is able to deposit significantly more Ca and P in bones than what is needed to maximize ADG and G:F. Thus, bones seem to be the default storage site for Ca and P if availability exceeds the requirement to maximize growth performance.

If diets met or exceeded STTD P requirement (NRC, 2012), the STTD Ca values to maximize ADG and G:F ranged from 0.36 to 0.67%, which corresponded to a STTD Ca:STTD P ratios between 1.16:1 and 1.43:1. However, the STTD Ca values to maximize bone ash, bone Ca, and bone P ranged from 0.55 to 0.73%, which corresponded to a STTD Ca:STTD P ratio between 1.55:1 and 1.77:1. These values are close to the Ca:P ratio in the body, which ranges between 1.25:1 and 1.70:1 for a 50 kg pig (Rymarz et al., 1982; Hendriks and Moughan, 1993; Mahan and Shields, 1998; Wiseman et al., 2009; Pettey et al., 2015).

Requirements for STTD Ca to maximize bone ash were expected to be close to requirements to maximize Ca retention, because 96 to 99% of Ca is believed to be stored in skeletal tissue (Crenshaw, 2001). However, results from Exp. 2 indicated that the STTD Ca values to maximize Ca retention ranged from 0.96 to 1.15%, which corresponded to a STTD Ca:STTD P ratio between 2.45:1 and 3.10:1. There is not a clear explanation for the greater quantities needed to maximize Ca retention than the quantities needed to maximize bone ash, however, similar results were observed previously in 11 to 25 kg pigs (González-Vega et al., 2015c). Thus, results from our 2 experiments indicate that there may be Ca stored in tissues other than bones, that the Ca:P ratio in bone ash is not constant, or that retention of ash in the femur is not representative of deposition of ash in all skeletal tissue. Results from the present experiment

demonstrate that plasma Ca is influenced by dietary Ca if P is deficient, but plasma Ca concentration was not increased as dietary Ca increased for pigs fed a diet that was not deficient in P, which indicates that it is unlikely that significant quantities of Ca are stored in plasma if dietary Ca is increased. Deposition of minerals in bones is a result of the exchange of minerals among 3 compartments: hydration shell, crystal surface, and crystal interior (Szymendera, 1970). Because bone ash accounts only for the crystalline mineral, minerals present in the hydration shell are not included in values for bone ash. This may explain the difference between the concentration of Ca needed to maximize bone ca concentration and the Ca needed to maximize Ca retention, however, it is not clear, why this difference was not also observed for P retention. Accumulation of minerals in the cecum has been observed in rats fed diets varying in the concentration of inulin (Levrat et al., 1991). However, if minerals are deposited in the cecum it is expected that not only Ca, but also P, are deposited. In the present experiment, the quantity of P required to maximize P in bone ash was in very good agreement with the quantity needed to maximize P retention. It is, therefore, unlikely, that deposition of minerals in the cecum contributed to the discrepancy between Ca needed to maximize Ca retention and Ca needed to maximize Ca in bone ash.

Although, deposition of Ca in soft tissue is negligible (Crenshaw, 2001; Pettey et al., 2015), calcification of soft tissue has been reported. A tenfold increase of Ca concentration in kidney was observed in dogs fed phosphates (Laflamme and Jowsey, 1972), and calcification of soft tissues has been observed in rats and rabbits (Spaulding and Walser, 1970; Jowsey and Balasubramaniam, 1972). The Ca:P ratios in the bones in 11 to 25 kg pigs were between 2.03:1 and 2.14:1 (González-Vega et al., 2015c) and the Ca:P ratios in the bones from pigs in the current study ranged between 2.03:1 and 2.20:1, which are close to the ratio of 2.15:1 that is

needed to form hydroxyapatite [$Ca_5(PO_4)_3(OH)$; Crenshaw, 2001]. Bones differ in size and mineral content (Han et al., 2015), which may result in challenges to quantify deposition of ash in all skeletal tissue, however, data from Crenshaw et al. (1981, 2009) indicated that femur is a good indicator of whole body bone mineral content. Therefore, the greater quantities of STTD Ca needed to maximize Ca retention than the quantities needed to maximize bone ash, is likely a result of accumulation of Ca in soft tissues, however, further research needs to be conducted to elucidate this difference.

The fact that increased concentration of dietary Ca negatively affects the ATTD of P is in agreement with previous reports (Stein et al., 2011; González-Vega et al., 2015c), which is likely a consequence of formation of Ca-P complexes in the intestine (Clark, 1969; Brink et al., 1992; Stein et al., 2011; González-Vega et al., 2014, 2015c). However, the effect of dietary Ca on ATTD of Ca in diets varies. At low concentrations of dietary Ca, ATTD of Ca increases as a result of reduced contribution of endogenous Ca to total Ca output as dietary Ca increases (González-Vega et al., 2013), which explains the increase in ATTD of Ca from diets containing 0.13 to 0.27% STTD Ca. No effect of dietary Ca on ATTD of Ca has been reported in diets containing from 55 to 173% of the requirement of Ca (Stein et al., 2011), however, negative effects of increasing dietary Ca was observed in diets containing from 54 to 147% of the requirement of Ca (González-Vega et al., 2015c). In the current experiment, it was observed that for diets containing 0.15% STTD P and from 0.27 to 0.72% STTD Ca (from 64 to 173% of the requirement of Ca), ATTD of Ca was negatively affected by dietary Ca concentration. This is possibly because excess Ca formed Ca-P complexes (Clark, 1969; Brink et al., 1992; Stein et al., 2011; González-Vega et al., 2014, 2015c). The fact that ATTD of Ca in diets was not affected by the concentration of dietary P is in agreement with previous reports (Stein et al., 2008; González-

Vega et al., 2015c). Likewise, the increase in ATTD of P with increased dietary P was previously reported (Stein et al., 2008; González-Vega et al., 2015c), which is likely a consequence of a reduced contribution of endogenous P.

The fact that in diets deficient in either Ca or P there was a minimum excretion of Ca and P in urine indicates that there are obligatory losses of Ca and P in urine. A minimum urinary loss of P of 7 mg/kg BW per day was reported by NRC (2012), therefore, a minimum of 0.21 g per day was expected in Exp. 2. This is in agreement with the predicted value (0.20 g/d) for the diet containing 0.31% STTD P. There is also an obligatory loss of Ca in humans (Nordin and Morris, 1989), but, to our knowledge, the amount of obligatory losses of Ca in pigs has not been estimated.

The observation that the concentration of Ca in plasma from pigs fed diets containing the least concentration of STTD P increased as concentration of STTD Ca increased, may be a result of deficiency of P, which limited Ca deposition in bones. Therefore, absorbed Ca that could not be deposited in bones was circulating in blood to be later excreted in the urine. Increased concentration of Ca in plasma as the concentration of dietary Ca increased has also been observed in chickens (Hurwitz et al., 1995).

Conclusions

Because STTD Ca negatively influenced the digestibility of P, but dietary STTD P did not affect the digestibility of Ca, interactions between STTD Ca and STTD P need to be considered to formulate diets. Data from these experiments indicate that utilization of both minerals is influenced by STTD Ca and STTD P ratio. Therefore, if STTD P is below recommended requirement (NRC, 2012), the STTD Ca:STTD P ratio needed to maximize ADG and G:F is between 0.60:1 and 0.80:1, whereas, the STTD Ca:STTD P ratio needed to maximize

bone ash, bone Ca, and bone P is between 2.67:1 and 2.80:1. However, these ratios will negatively affect growth performance of pigs. In contrast, if STTD P meets or exceeds the requirement (NRC, 2012), a STTD Ca:STTD P ratio between 1.16:1 and 1.43:1 is needed to maximize ADG and G:F. A greater ratio will reduce growth performance, although a ratio between 1.55:1 and 1.77:1 is needed to maximize bone ash, bone Ca, and bone P. The implication of this observation is that the ratios needed to maximize bone ash, bone Ca, and bone P may reduce growth performance, therefore, it is recommended that, for commercial growing-finishing pigs, diets should be formulated using a ratio of STTD Ca: STTD P between 1.16:1 and 1.43:1.

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FIGURES

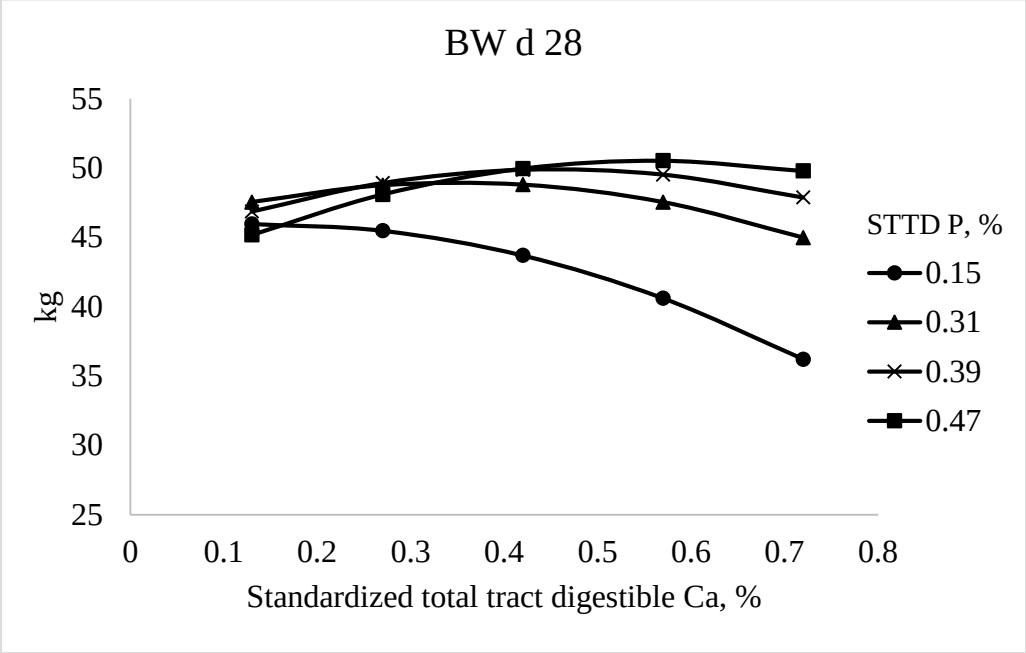

Figure 6.1. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for BW at d 28 in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum BW at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 46.0, 49.0, 49.9, and 50.5 kg at STTD Ca concentrations of 0.14, 0.35, 0.45, and 0.56%, respectively. These values correspond to STTD Ca:STTD P ratios of 0.93:1, 1.13:1, 1.15:1, and 1.19:1.

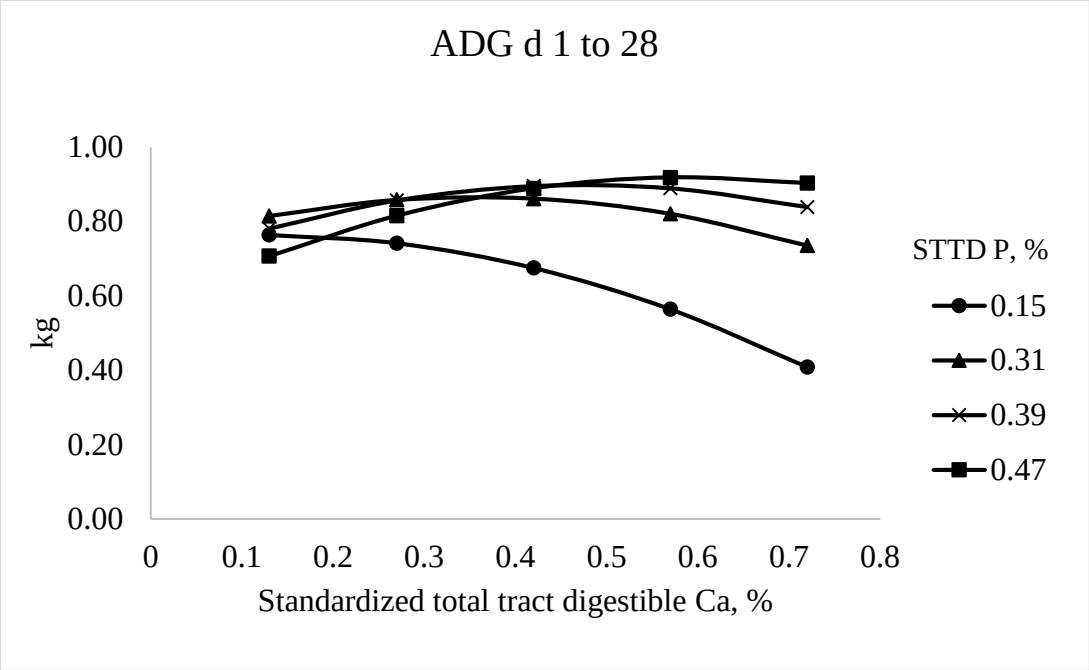

Figure 6.2. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for average daily gain (ADG) from d 1 to 28 in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum ADG at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 0.76, 0.87, 0.90, and 0.92 kg at STTD Ca concentrations of 0.12, 0.36, 0.47, and 0.59%, respectively. These values correspond to STTD Ca:STTD P ratios of 0.80:1, 1.16:1, 1.21:1, and 1.26:1.

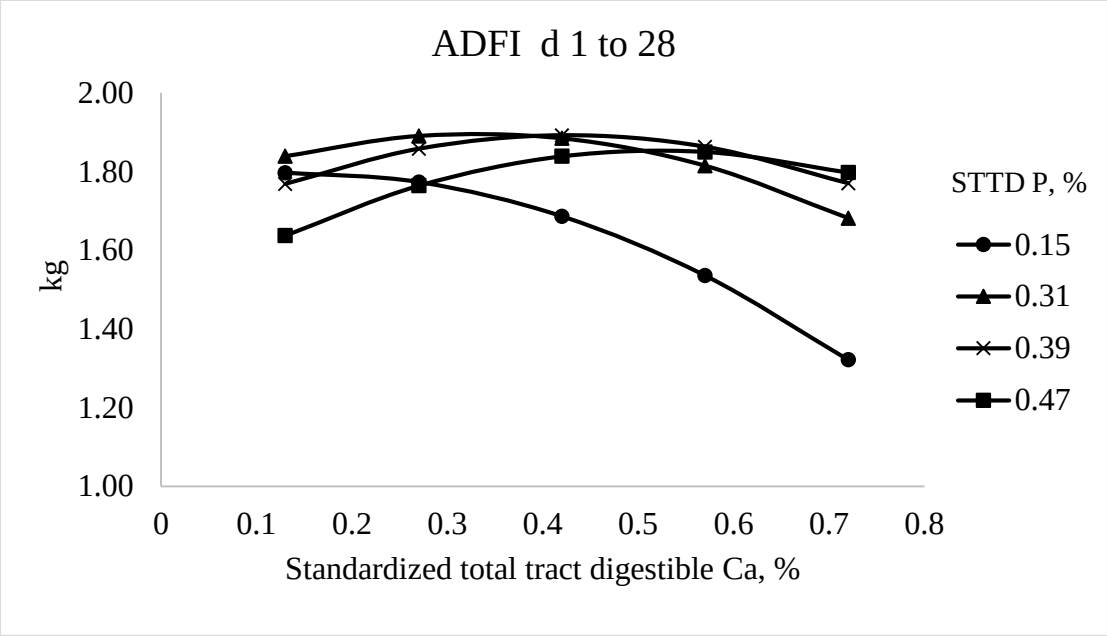

Figure 6.3. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for average daily feed intake (ADFI) from d 1 to 28 in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum ADFI at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 1.80, 1.90, 1.89, and 1.85 kg at STTD Ca concentrations of 0.14, 0.33, 0.43, and 0.52%, respectively. These values correspond to STTD Ca:STTD P ratios of 0.93:1, 1.06:1, 1.10:1, and 1.11:1.

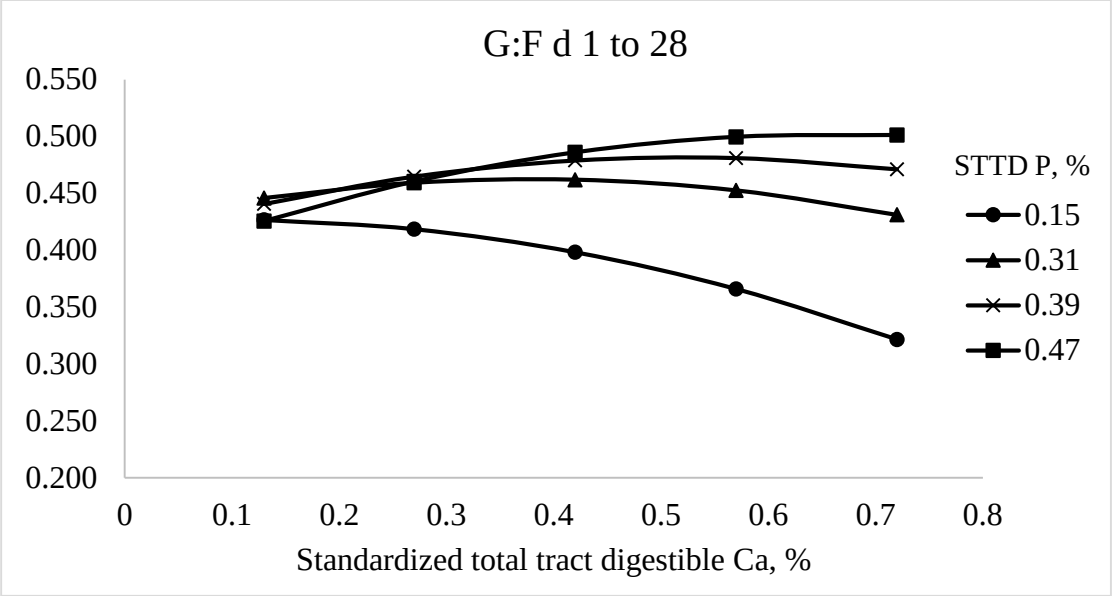

Figure 6.4. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for gain to feed ratio (G:F) from d 1 to 28 in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum G:F ratio at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 0.43, 0.46, 0.48, and 0.50 kg/kg at STTD Ca concentrations of 0.09, 0.38, 0.52, and 0.67%, respectively. These values correspond to STTD Ca:STTD P ratios of 0.60:1, 1.23:1, 1.33:1, and 1.43:1.

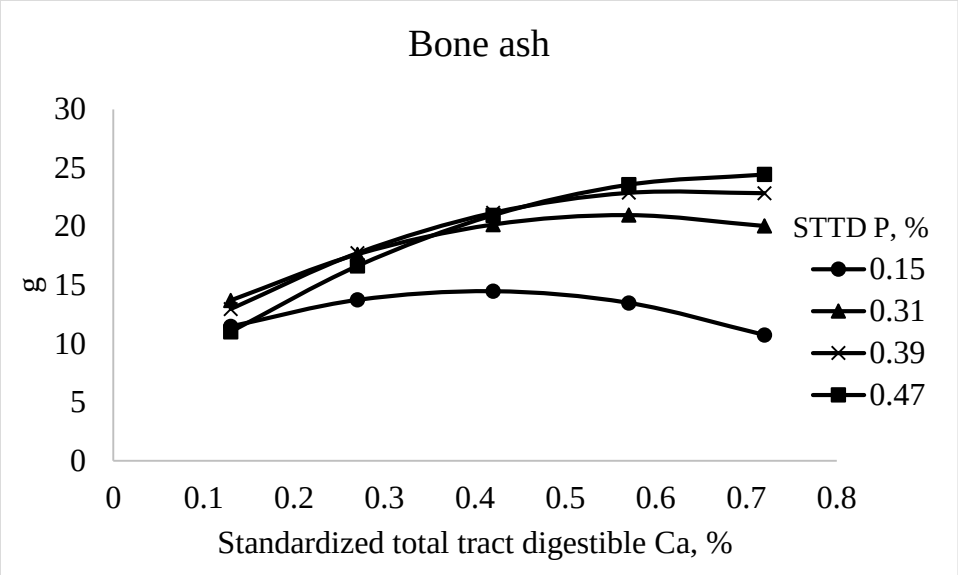

Figure 6.5. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for bone ash (g) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum bone ash at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 14.5, 21.0, 23.1, and 24.5 g at STTD Ca concentrations of 0.41, 0.56, 0.64, and 0.72%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.73:1, 1.81:1, 1.64:1, and 1.53:1.

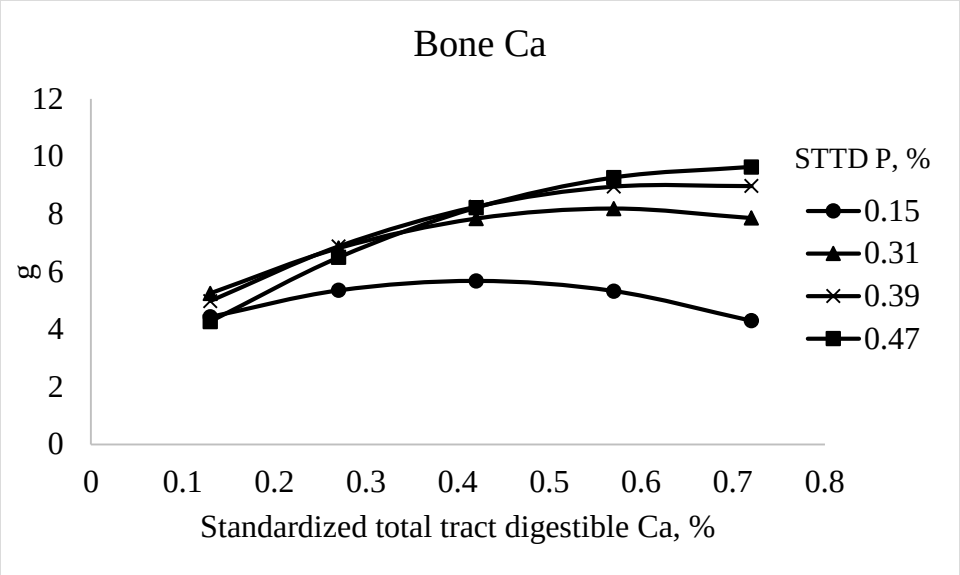

Figure 6.6. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for bone Ca (g) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum bone Ca at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 5.7, 8.2, 9.1, and 9.6 g at STTD Ca concentrations of 0.42, 0.57, 0.65, and 0.73%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.80:1, 1.84:1, 1.67:1, and 1.55:1.

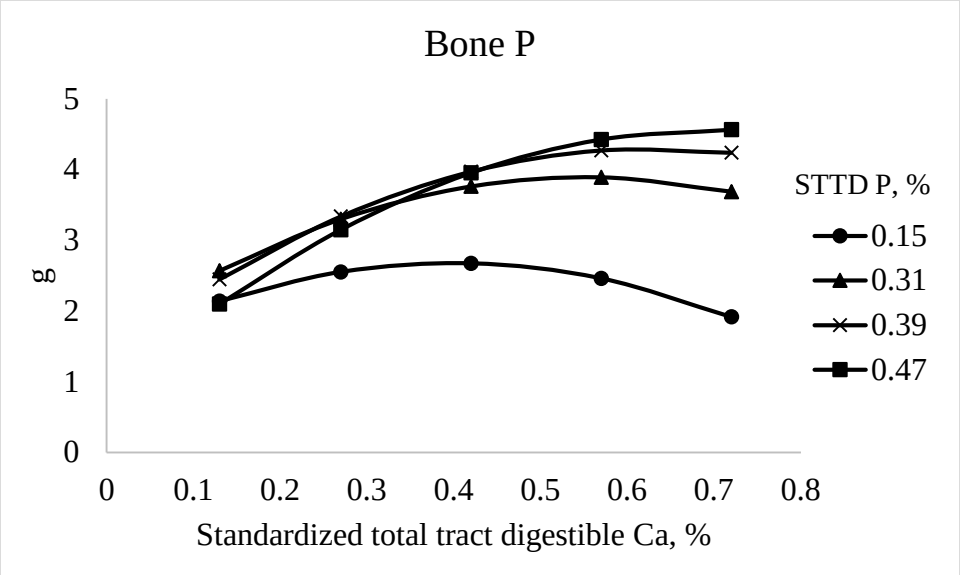

Figure 6.7. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for bone P (g) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum bone P at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 2.7, 3.9, 4.3, and 4.6 g at STTD Ca concentrations of 0.40, 0.55, 0.63, and 0.71%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.67:1, 1.77:1, 1.62:1, and 1.51:1.

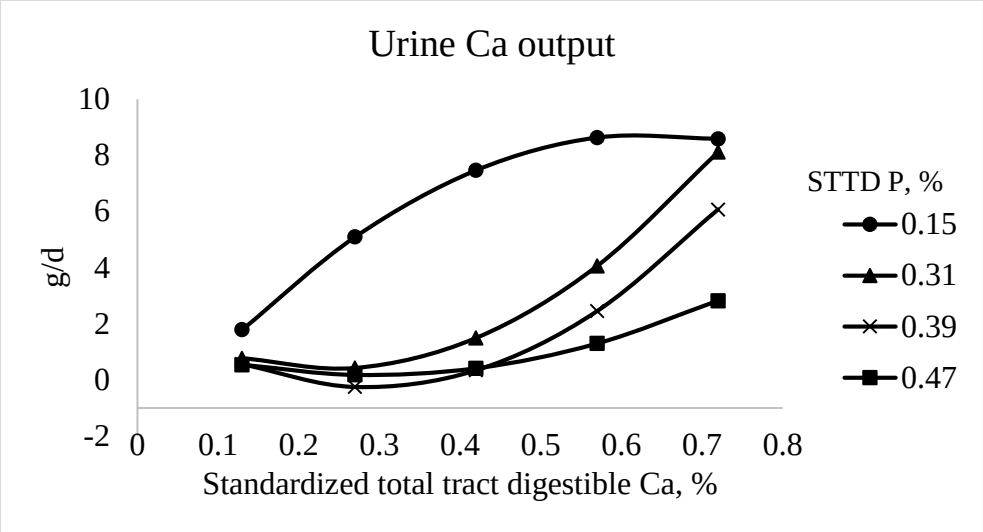

Figure 6.8. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for urine Ca output (g/d) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum urine Ca output at STTD P concentrations of 0.15% was 8.8 g/d at STTD Ca concentrations of 0.64%, which correspond to STTD Ca:STTD P ratios of 4.27:1. However, the predicted minimum urine Ca output at STTD P concentrations of 0.31, 0.39, and 0.47% were 0.4, -0.3, and 0.2 g/d at STTD Ca concentrations of 0.24, 0.29, and 0.29%, respectively. These values correspond to STTD Ca:STTD P ratios of 0.77:1, 0.74:1, and 0.62:1.

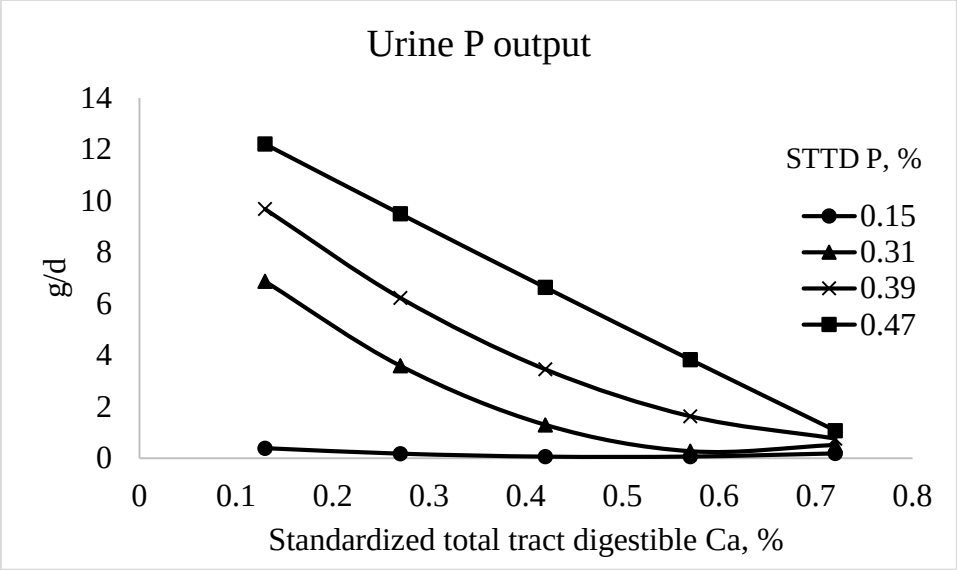

Figure 6.9. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for urine P output (g/d) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted minimum urine P output at STTD P concentrations of 0.15, 0.31, and 0.39% were 0.0, 0.2, and 0.7 g/d at STTD Ca concentrations of 0.49, 0.62, and 0.78%, respectively. These values correspond to STTD Ca:STTD P ratios of 3.27:1, 2.00:1, and 2.00:1. The values for minimum urine P output at 0.47% STTD P was not estimated due to a nearly linear response.

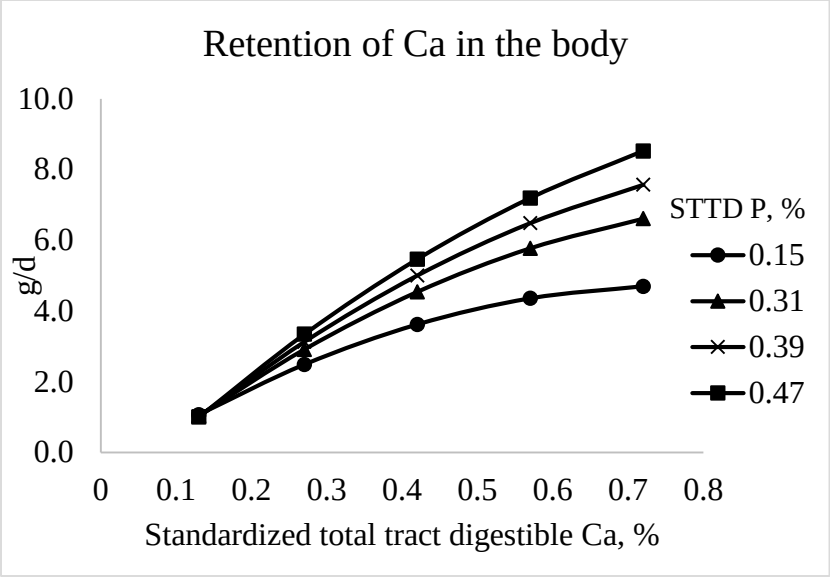

Figure 6.10. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for retention of Ca in the body (g/d) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum retention of Ca in the body at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 4.7, 7.1, 8.6, and 10.2 g/d at STTD Ca concentrations of 0.77, 0.96, 1.06, and 1.15%, respectively. These values correspond to STTD Ca:STTD P ratios of 5.13:1, 3.10:1, 2.72:1, and 2.45:1. These STTD Ca concentrations needed to maximize retention of Ca in the body were extrapolations of the model.

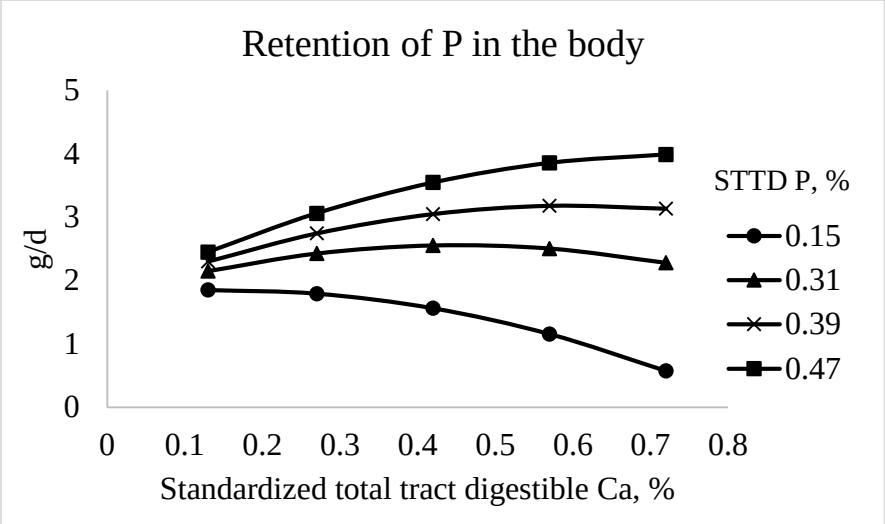

Figure 6.11. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for retention of P in the body (g/d) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. The predicted maximum retention of P in the body at STTD P concentrations of 0.15, 0.31, 0.39, and 0.47% were 1.8, 2.6, 3.2, and 4.0 g/d at STTD Ca concentrations of 0.15, 0.45, 0.61, and 0.76%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.00:1, 1.46:1, 1.55:1, and 1.61:1.

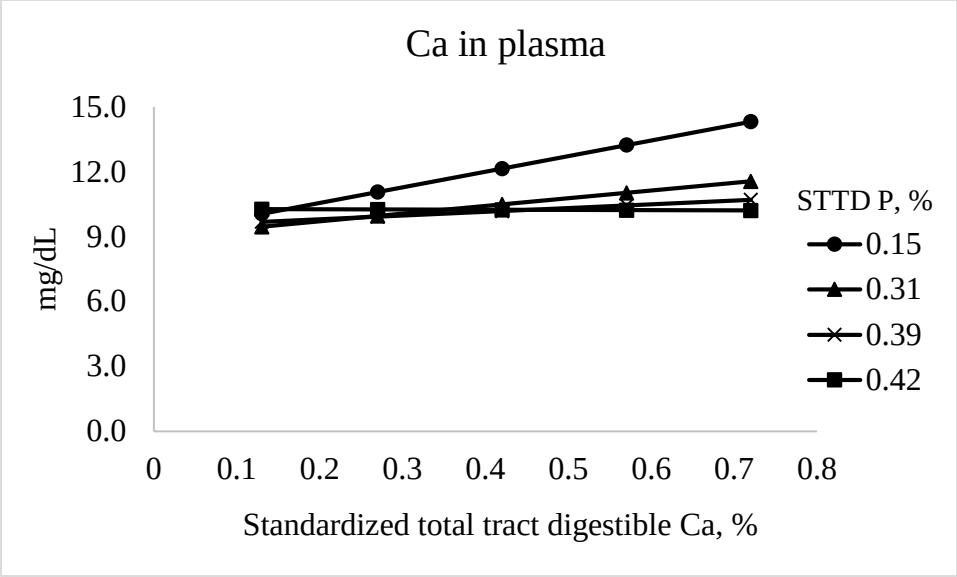

Figure 6.12. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for Ca in plasma (ppm) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. All responses were linear, therefore, no maximum values were estimated.

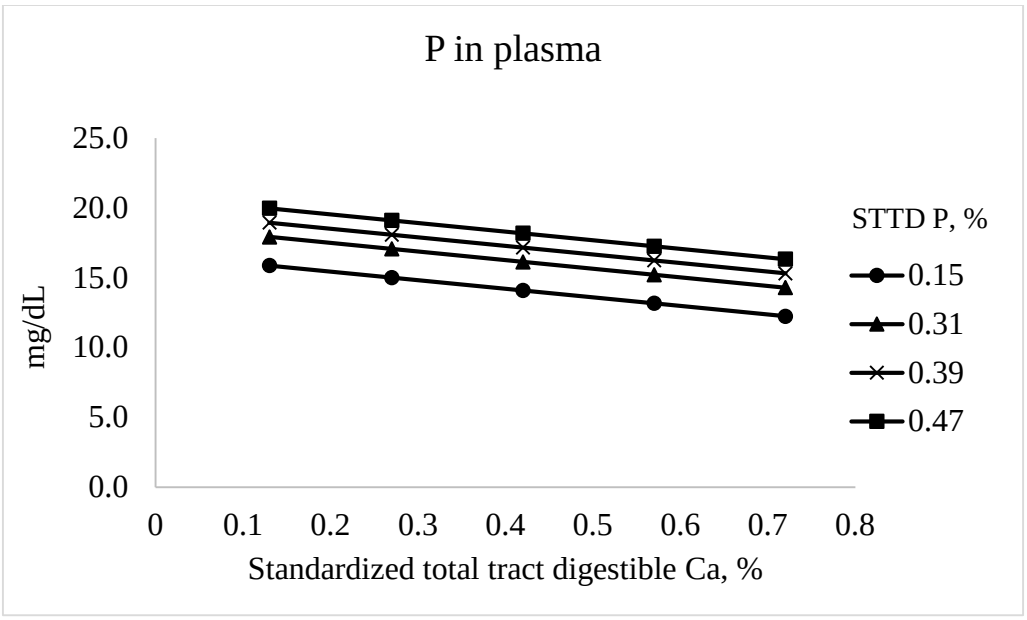

Figure 6.13. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for P in plasma (ppm) in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P. All responses were linear, therefore, no maximum values were estimated.

TABLES

Table 6.1. Analyzed composition of ingredients, as-fed basis, Exp. 1 and 2

Item	Ingredient					
	Corn	Soybean meal	Calcium carbonate	Monosodium phosphate	Monocalcium phosphate	Sodium chloride
GE, kcal/kg	3,810	4,199	-	-	-	-
DM, %	85.58	88.33	99.88	99.13	91.59	99.86
Ash, %	1.43	7.55	93.12	91.30	80.34	100.00
CP, %	7.91	48.49	-	-	-	-
Ca, %	0.02	0.52	38.20	0.17	17.10	ND
P, %	0.23	0.63	0.06	24.60	20.60	ND
Na, %	ND	0.02	0.17	20.30	0.25	38.80
Cl, %	ND	0.01	ND	ND	ND	58.52
Phytate, %	0.69	1.60	-	-	-	-
Phytate-bound P,[1] %	0.19	0.45	-	-	-	-
Nonphytate P,[2] %	0.04	0.18	-	-	-	-

[1]Phytate-bound P was calculated as 28.2 % of phytate (Tran and Sauvant, 2004).
[2]Nonphytate P was calculated as the difference between total P and phytate-bound P.
[3]ND = not detectable.

Table 6.2. Ingredient composition and analyzed composition of experimental diets containing 0.15 or 0.31% standardized total tract digestible (STTD) P, as-fed basis, Exp. 1 and 2

Ingredient, %	0.15% STTD P					0.31% STTD P				
Total Ca, %:	0.18	0.42	0.66	0.90	1.14	0.18	0.42	0.66	0.90	1.14
STTD Ca, %:	0.13	0.27	0.42	0.57	0.72	0.13	0.27	0.42	0.57	0.72
STTD Ca:STTD P:	0.87:1	1.80:1	2.80:1	3.80:1	4.80:1	0.42:1	0.87:1	1.35:1	1.84:1	2.32:1
Ground corn	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00
Soybean meal	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Cornstarch	8.95	7.13	5.30	3.47	1.63	8.35	6.58	4.78	2.93	1.10
Soybean oil	-	1.20	2.40	3.60	4.80	0.40	1.55	2.73	3.95	5.15
Calcium carbonate	0.03	0.65	1.28	1.91	2.55	0.03	0.65	1.28	1.91	2.55
Monocalcium phosphate	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Monosodium phosphate	-	-	-	-	-	0.70	0.70	0.70	0.70	0.70
Sodium bicarbonate	1.05	1.05	1.05	1.05	1.05	0.55	0.55	0.54	0.54	0.53
L-Lys HCl	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
DL-Met	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04

Table 6.2. (Cont.)

L-Thr	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Sodium chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition										
NE, kcal/kg	2552	2552	2552	2552	2552	2552	2552	2552	2552	2552
CP, %	16.81	16.81	16.81	16.81	16.81	16.81	16.81	16.81	16.81	16.81
Indispensable SID[2] AA, %										
Arg	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
His	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Ile	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Leu	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
Lys	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Met	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Phe	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72

Table 6.2. (Cont.)

Thr	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Trp	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Val	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
P, %	0.34	0.34	0.34	0.34	0.34	0.51	0.51	0.51	0.51	0.51
Na, %	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Cl, %	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
K, %	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Analyzed composition										
DM, %	85.83	86.50	86.61	86.66	86.80	86.64	86.83	86.83	85.21	86.21
CP, %	15.65	15.30	15.46	15.65	16.29	15.88	15.53	16.22	15.38	15.95
Ash, %	3.63	4.02	4.62	5.01	6.45	3.85	5.02	5.28	5.85	6.65
Ca, %	0.18	0.38	0.61	0.90	1.10	0.16	0.44	0.68	0.94	1.16
P, %	0.38	0.36	0.37	0.39	0.38	0.52	0.53	0.52	0.53	0.53
Phytate,[3] %	0.71	0.71	0.71	0.71	0.71	0.67	0.67	0.57	0.57	0.78
Phytate-bound P, %	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.16	0.16	0.22

Table 6.2. (Cont.)

Non-phytate P,[4] %	0.18	0.16	0.17	0.19	0.18	0.33	0.34	0.36	0.37	0.31
Na, %	0.37	0.35	0.37	0.36	0.38	0.40	0.36	0.35	0.39	0.33
Cl, %	0.20	0.20	0.19	0.20	0.18	0.20	0.20	0.16	0.18	0.17
DCAD,[5] mEq/kg	297	288	300	292	307	310	292	299	311	288

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

[2]SID = standardized ileal digestible.

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

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

[5]DCAD = dietary cation-anion difference. The DCAD was calculated as Na + K – Cl.

Table 6.3. Ingredient composition and analyzed composition of experimental diets containing 0.39 or 0.47% standardized total tract digestible (STTD) P, as-fed basis, Exp. 1 and 2

Ingredient, %	0.39% STTD P					0.47% STTD P				
Total Ca, %:	0.18	0.42	0.66	0.90	1.14	0.18	0.42	0.66	0.90	1.14
STTD Ca, %:	0.13	0.27	0.42	0.57	0.72	0.13	0.28	0.42	0.57	0.72
STTD Ca:STTD P:	0.33:1	0.69:1	1.08:1	1.46:1	1.85:1	0.28:1	0.60:1	0.89:1	1.21:1	1.53:1
Ground corn	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00
Soybean meal	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Cornstarch	8.13	6.36	4.53	2.70	0.86	7.90	6.13	4.30	2.53	0.63
Soybean oil	0.55	1.70	2.90	4.10	5.30	0.70	1.85	3.05	4.20	5.45
Calcium carbonate	0.03	0.65	1.28	1.91	2.55	0.03	0.65	1.28	1.90	2.55
Monocalcium phosphate	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Monosodium phosphate	1.05	1.05	1.05	1.05	1.05	1.40	1.40	1.40	1.40	1.40
Sodium bicarbonate	0.27	0.27	0.27	0.27	0.27	-	-	-	-	-
L-Lys HCl	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
DL-Met	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04

Table 6.3. (Cont.)

L-Thr	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Sodium chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition										
NE, kcal/kg	2552	2552	2552	2552	2552	2552	2552	2552	2552	2552
CP, %	16.81	16.81	16.81	16.81	16.81	16.81	16.81	16.81	16.81	16.81
Indispensable SID[2] AA, %										
Arg	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
His	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Ile	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Leu	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31	1.31
Lys	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Met	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Phe	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72

Table 6.3. (Cont.)

Thr	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Trp	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Val	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
P, %	0.59	0.59	0.59	0.60	0.59	0.68	0.68	0.68	0.68	0.68
Na, %	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Cl, %	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
K, %	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Analyzed composition										
DM, %	85.68	85.38	86.51	86.44	85.99	86.16	86.32	86.44	86.90	87.12
CP, %	14.82	15.85	15.76	15.88	15.55	16.59	15.27	15.92	15.69	16.27
Ash, %	3.99	4.84	5.80	5.96	6.54	4.21	4.96	5.68	6.72	6.92
Ca, %	0.20	0.42	0.64	0.87	1.25	0.18	0.45	0.62	1.03	1.27
P, %	0.59	0.65	0.64	0.65	0.59	0.67	0.70	0.72	0.73	0.79
Phytate,[3] %	0.78	0.78	0.78	0.85	0.78	0.71	0.71	0.78	0.78	0.78
Phytate-bound P, %	0.22	0.22	0.22	0.24	0.22	0.20	0.20	0.22	0.22	0.22

Table 6.3. (Cont.)

Non-phytate P,[4] %	0.37	0.43	0.42	0.41	0.37	0.47	0.50	0.50	0.51	0.57
Na, %	0.36	0.40	0.38	0.37	0.36	0.36	0.41	0.34	0.35	0.37
Cl, %	0.17	0.20	0.20	0.20	0.20	0.20	0.20	0.16	0.16	0.17
DCAD,[5] mEq/kg	301	310	301	297	292	292	314	295	299	305

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

[2]SID = standardized ileal digestible.

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

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

[5]DCAD = dietary cation-anion difference. The DCAD was calculated as Na + K – Cl.

Table 6.4. Least squares means for BW at d 28, and ADG, ADFI, and G:F from d 1 to 28 in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P, Exp. 1

	STTD Ca, %				
Item	0.13	0.27	0.42	0.57	0.72
BW, kg[1, 2]					
0.15% STTD P	46.96	44.98	42.69	41.15	35.89
0.31% STTD P	47.03	48.68	50.17	49.28	44.47
0.39% STTD P	46.13	48.53	48.43	48.89	48.53
0.47% STTD P	45.42	49.89	49.05	50.84	49.36
ADG, kg[3, 4]					
0.15% STTD P	0.80	0.72	0.64	0.58	0.41
0.31% STTD P	0.80	0.86	0.90	0.88	0.71
0.39% STTD P	0.78	0.85	0.85	0.86	0.86
0.47% STTD P	0.69	0.89	0.86	0.93	0.89
ADFI, kg[5, 6]					
0.15% STTD P	1.82	1.74	1.66	1.54	1.35
0.31% STTD P	1.81	1.88	1.98	1.90	1.61
0.39% STTD P	1.79	1.88	1.82	1.79	1.78
0.47% STTD P	1.59	1.85	1.79	1.85	1.85
G:F, kg:kg[7, 8]					
0.15% STTD P	0.44	0.41	0.39	0.38	0.30
0.31% STTD P	0.44	0.46	0.46	0.46	0.44

Table 6.4. (Cont.)

0.39% STTD P	0.43	0.45	0.47	0.48	0.49
0.47% STTD P	0.43	0.48	0.48	0.50	0.48

[1]Results indicated that BW at different combinations of STTD Ca and STTD P can be described by the following model: 41.8265 - 3.30995 × Ca - 28.9672 × Ca^2 + 35.4607 × P-77.1011 × P^2 + 76.0425 × Ca × P ($P < 0.001$).

[2]Standard error of the within treatments least squares means = 1.37.

[3]Results indicated that ADG at different combinations of STTD Ca and STTD P can be described by the following model: 0.61476 - 0.20212 × Ca - 0.98679 × Ca^2 + 1.36823 × P-3.10677 × P^2 + 2.92181 × Ca × P ($P < 0.001$).

[4]Standard error of the within treatments least squares means = 0.04.

[5]Results indicated that ADFI at different combinations of STTD Ca and STTD P can be described by the following model: 1.5745 - 0.1117 × Ca - 1.41036 × Ca^2 + 2.01723 × P-4.76539 × P^2 + 3.36731 × Ca × P ($P < 0.001$).

[6]Standard error of the within treatments least squares means = 0.08.

[7]Results indicated that G:F at different combinations of STTD Ca and STTD P can be described by the following model: 0.3896 - 0.09482 × Ca - 0.26695 × Ca^2 + 0.35043 × P - 0.77095 × P^2 + 0.95727 × Ca × P ($P < 0.001$).

[8]Standard error of the within treatments least squares means = 0.02.

Table 6.5. Least squares means for bone ash, bone Ca, bone P, and stomach pH in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P, Exp. 1

	STTD Ca, %				
Item	0.13	0.27	0.42	0.57	0.72
Bone ash, g[1, 2]					
0.15% STTD P	12.49	13.48	12.95	13.11	11.63
0.31% STTD P	13.59	17.44	21.76	21.71	19.49
0.39% STTD P	12.98	15.71	21.14	23.86	22.02
0.47% STTD P	12.44	18.56	20.29	23.67	24.37
Bone Ca, g[3, 4]					
0.15% STTD P	4.80	5.30	5.13	5.14	4.63
0.31% STTD P	5.16	6.76	8.49	8.47	7.65
0.39% STTD P	5.02	6.12	8.20	9.24	8.74
0.47% STTD P	4.82	7.26	7.95	9.32	9.58
Bone P, g[5, 6]					
0.15% STTD P	2.32	2.51	2.41	2.37	2.10
0.31% STTD P	2.53	3.29	4.09	4.03	3.57
0.39% STTD P	2.47	2.95	3.93	4.42	4.11
0.47% STTD P	2.35	3.53	3.82	4.46	4.55
Ca:P in bone					
0.15% STTD P	2.07	2.11	2.13	2.17	2.20
0.31% STTD P	2.04	2.05	2.08	2.10	2.14

Table 6.5. (Cont.)

0.39% STTD P	2.03	2.07	2.09	2.09	2.13
0.47% STTD P	2.05	2.06	2.08	2.09	2.11
Stomach pH[7, 8]	2.27[b]	2.86[a]	2.73[ab]	3.19[a]	3.07[a]

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Results indicated that bone ash at different combinations of STTD Ca and STTD P can be described by the following model: $2.9550 + 20.3139 \times Ca - 38.5764 \times Ca^2 + 48.1313 \times P - 95.6806 \times P^2 + 75.0046 \times Ca \times P$ ($P < 0.001$).

[2]Standard error of the within treatments least squares means = 1.15.

[3]Results indicated that bone Ca at different combinations of STTD Ca and STTD P can be described by the following model: $1.2282 + 8.2355 \times Ca - 15.0966 \times Ca^2 + 17.3926 \times P - 34.9669 \times P^2 + 29.1245 \times Ca \times P$ ($P < 0.001$).

[4]Standard error of the within treatments least squares means = 0.46.

[5]Results indicated that bone Ca at different combinations of STTD Ca and STTD P can be described by the following model: $0.5601 + 3.7735 \times Ca - 7.3960 \times Ca^2 + 8.8805 \times P - 17.5142 \times P^2 + 14.2338 \times Ca \times P$ ($P < 0.001$).

[6]Standard error of the within treatments least squares means = 0.22.

[7]Concentration of STTD Ca in diet affect ($P < 0.016$) pH in stomach.

[8]Standard error of means = 0.24.

Table 6.6. Least squares means for apparent total tract digestibility (ATTD) of Ca, ATTD of P, and retention of Ca and P in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P, Exp. 2

	STTD Ca, %				
Item	0.13	0.27	0.42	0.57	0.72
ATTD of Ca, %[1, 2]					
0.15% STTD P	53.59	68.00	66.69	60.17	63.09
0.31% STTD P	56.26	64.90	66.31	64.03	59.34
0.39% STTD P	47.00	59.50	64.67	69.45	63.99
0.47% STTD P	52.92	59.04	63.54	63.76	63.42
ATTD of P, %[3, 4]					
0.15% STTD P	45.39	36.76	33.90	33.10	27.45
0.31% STTD P	57.08	51.82	51.61	45.05	40.03
0.39% STTD P	59.18	52.44	43.52	54.11	48.99
0.47% STTD P	63.34	57.91	54.09	53.22	48.49
Urine Ca output, g/d[5, 6]					
0.15% STTD P	1.30	6.12	7.73	8.78	9.13
0.31% STTD P	0.22	0.31	1.93	4.16	8.73
0.39% STTD P	0.89	0.39	0.61	1.64	5.90
0.47% STTD P	0.28	0.30	0.46	1.07	3.13
Urine P output, g/d[7, 8]					
0.15% STTD P	0.28	0.13	0.16	0.20	0.15
0.31% STTD P	7.79	3.29	0.50	0.47	0.42

Table 6.6. (Cont.)

0.39% STTD P	8.45	6.85	3.76	2.33	0.71
0.47% STTD P	14.83	10.03	5.95	3.57	1.17
Retention of Ca in the body, g/d[9, 10]					
0.15% STTD P	0.94	2.39	3.77	4.17	5.13
0.31% STTD P	1.26	3.23	5.00	6.44	6.67
0.39% STTD P	0.90	3.22	5.45	6.99	7.63
0.47% STTD P	1.11	3.00	5.28	6.92	8.38
Retention of P in the body, g/d[11, 12]					
0.15% STTD P	1.86	1.55	1.36	1.21	0.87
0.31% STTD P	2.21	2.53	3.15	2.95	2.45
0.39% STTD P	2.76	2.71	3.37	3.33	3.35
0.47% STTD P	2.37	2.88	3.53	3.79	3.87

[1]Results indicated that ATTD of Ca of diets at different combinations of STTD Ca and STTD P can be described by the following model: 55.6492 + 12.6427 × Ca (P = 0.009).

[2]Standard error of the within treatments least squares means = 3.55.

[3]Results indicated that ATTD of P of diets at different combinations of STTD Ca and STTD P can be described by the following model: 36.42009 - 23.6654 × Ca + 65.43595 × P (P < 0.001).

[4]Standard error of the within treatments least squares means = 2.95.

[5]Results indicated that urine Ca output at different combinations of STTD Ca and STTD P can be described by the following model: - 11.2788 + 133.541 × Ca - 154.747 × Ca^2 + 76.1041

Table 6.6. (Cont.)

× P – 104.612 × P^2 – 830.115 × Ca × P + 1084.04 × Ca^2 × P + 1124.03 × Ca × P^2 – 1541.08 × Ca^2 × P^2 ($P < 0.001$).

[6]Standard error of the within treatments least squares means = 0.72.

[7]Results indicated that urine P output at different combinations of STTD Ca and STTD P can be described by the following model: - 14.6788 + 70.134 × Ca – 68.942 × Ca^2 + 121.048 × P – 124.258 × P^2 – 622.705 × Ca × P + 631.354 × Ca^2 × P + 917.568 × Ca × P^2 – 1026.05 × Ca^2 × P^2 ($P < 0.001$).

[8]Standard error of the within treatments least squares means = 0.63.

[9]Results indicated that retention of Ca in the body at different combinations of STTD Ca and STTD P can be described by the following model: - 0.1145 + 10.5371 × Ca – 8.8014 × Ca^2 – 2.8922 × P + 20.6228 × Ca × P ($P < 0.001$).

[10]Standard error of the within treatments least squares means = 0.35.

[11]Results indicated that retention of P in the body at different combinations of STTD Ca and STTD P can be described by the following model: 1.7735 – 1.0789 × Ca – 3.9067 × Ca^2 – 0.0664 × P + 14.9090 × Ca × P ($P < 0.001$).

[12]Standard error of the within treatments least squares means = 0.23.

Table 6.7. Least squares means for Ca and P in plasma (mg/dL) at d13 in pigs fed diets containing from 0.13 to 0.72% standardized total tract digestible (STTD) Ca and from 0.15 to 0.47% STTD P, Exp. 2

	STTD Ca, %				
Item	0.13	0.27	0.42	0.57	0.72
Plasma Ca, mg/dL[1, 2]					
0.15% STTD P	10.1	11.0	12.6	12.7	14.7
0.31% STTD P	9.4	10.3	10.2	10.9	12.5
0.39% STTD P	10.1	10.3	10.3	10.2	11.3
0.47% STTD P	10.1	10.1	10.2	10.1	10.2
Plasma P, mg/dL[3, 4]					
0.15% STTD P	15.8	17.1	14.9	12.7	9.7
0.31% STTD P	16.7	19.2	17.6	15.4	14.5
0.39% STTD P	18.8	18.6	18.5	16.4	16.0
0.47% STTD P	19.0	16.9	17.0	18.5	18.0

[1]Results indicated that Ca in plasma at different combinations of STTD Ca and STTD P can be described by the following model: $10.5277 + 10.6516 \times Ca - 13.5555 \times P + 27.7143 \times P^2 - 22.8713 \times Ca \times P$ ($P < 0.001$).

[2]Standard error of the within treatments least squares means = 0.32.

[3]Results indicated that P in plasma at different combinations of STTD Ca and STTD P can be described by the following model: $14.7573 - 6.16262 \times Ca + 12.7857 \times P$ ($P = 0.003$).

[4]Standard error of the within treatments least squares means = 2.59.

GENERAL CONCLUSIONS

Most diets fed to pigs are currently formulated to meet requirements for total Ca and requirements for digestible P. Formulating using values for total Ca has 2 disadvantages: one is that the amount of Ca that will be absorbed is unknown, and the other disadvantage is that excess dietary Ca decreases the digestibility of P, which increases P excretion. Excretion of Ca and P in feces may be reduced if diets are formulated to meet requirements for standardized total tract digestible (STTD) Ca and STTD P. Therefore, determination of STTD values for Ca in feed ingredients and determination of requirements for STTD Ca by pigs may result in a reduction in excretion of P from pigs.

Due to the relatively low concentration of Ca in plant ingredients, Ca supplements or animal ingredients need to be included in diets. Results of the present research indicated that the STTD of Ca vary among Ca supplements, and STTD of Ca was greater in monocalcium phosphate and dicalcium phosphate than in Ca carbonate. It appears that phytate is able to bind more Ca from Ca carbonate than Ca from monocalcium phosphate and dicalcium phosphate. This hypothesis was supported by the observation that STTD of Ca in diets containing calcium carbonate increased if microbial phytase was used, but that was not the case for Ca from monocalcium phosphate or dicalcium phosphate. Likewise, the STTD of Ca in fish meal increased if microbial phytase was included in the diet, which indicates that Ca from fish meal may also be bound to phytate and subsequently released if phytase is added to the diet. Thus, the effect of microbial phytase is dependent on the source of Ca in the diet. Most commercial diets contain fiber and oil, and results from this research indicates that fiber may increase STTD of Ca and ATTD of P, whereas soybean oil did not affect the STTD of Ca or the ATTD of P in fish meal. It was also observed that cornstarch-based diets had a negative effect on the STTD of Ca

compared with corn-based diets, and it is, therefore, recommended that Ca digestibility in feed ingredients is determined in corn-based diets because these values are more representative of commercial diets.

For 11 to 25 kg pigs, it was observed that growth performance was negatively affected if diets contained more than 0.50% STTD Ca and 0.36% STTD P. However, bone ash, bone Ca, bone P, retention of Ca, and retention of P were maximized by dietary Ca above 0.48% STTD Ca. Based on these results, it is concluded that it is likely that the requirement for STTD Ca for 11 to 25 kg pigs is approximately 1.35 times the required STTD P, but further experiments need to be conducted to verify this value. It was also observed that although expression of genes related to Ca transport in the jejunum and kidneys of pigs were decreased as dietary Ca increased, data indicated that kidneys appear to be the main site for regulation of Ca homeostasis.

For 25 to 50 kg pigs, a negative effect on ADG and G:F of increasing concentration of STTD Ca in diets was observed, especially for diets containing 0.15% STTD P. However, this negative effect was ameliorated as concentration of STTD P in diets increased. Based on these results, it was concluded that a STTD Ca:STTD P ratio between 1.16:1 and 1.43:1 was needed to maximize ADG and G:F, but a ratio between 1.53:1 and 1.81:1 was needed to maximize bone ash. One of the main reasons for the negative effect of excess Ca on growth performance is the negative effect of excess Ca on the digestibility of P. Therefore, it is recommended to formulate diets using the ratio between STTD Ca:STTD P. One surprising observation from this work was that it appears that Ca can be retained in the body in excess of what is retained in bones, indicating that at high levels of Ca intake some Ca maybe stored in soft tissue.

In conclusion, results from these experiments indicate that values for STTD of Ca vary among different sources of Ca. As a consequence, values for STTD of Ca should be used to formulate diets fed to pigs. Inclusion of microbial phytase may increase STTD of Ca of some Ca sources. To maximize the utilization of Ca and P and to maximize growth performance of pigs, diets should be formulated to meet the requirements for STTD Ca and STTD P, because this will result in more efficient utilization of both P and Ca. It is likely that in diets where STTD P meets or exceeds the requirement, growth performance will be maximized if the STTD Ca: STTD P ratio in the diet is between 1.15:1 and 1.45:1.