REQUIREMENTS FOR DIGESTIBLE CALCIUM BY GROWING PIGS

BY

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THESIS

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ABSTRACT

Two experiments were conducted to determine the digestible Ca requirements by pigs from 11 to 25 kg and from 50 to 85 kg as indicated by growth performance and bone ash. In Exp. 1, the hypothesis was that the requirement to maximize growth performance of 50- to 85-kg pigs expressed as the STTD Ca:STTD P ratio, is less than 1.35:1. Fifteen corn-soybean meal based diets were formulated to contain 0.14, 0.27, or 0.41% STTD P and 0.13, 0.25, 0.38, 0.50, or 0.63% STTD Ca. Ninety barrows (50.2 ± 2.1 kg) were individually housed and randomly allotted to the 15 diets. On d 30, the amount of feed left in the feeders and the weight of the pigs were recorded. Pigs were euthanized on d 31, the right femur was removed, and ash, Ca, and P were determined in dried defatted femurs. Data were analyzed using the response surface model in NLREG. Results indicated that there were interactions ($P < 0.10$) between Ca and P for final BW, ADG, G:F, and bone ash. The predicted maximum final BW, ADG and bone ash at 0.27% STTD P was obtained at STTD Ca:STTD P ratios of 1.20:1, 1.25:1, and 2.03:1, respectively, and the STTD Ca to STTD P ratio needed to assure adequate bone mineralization without affecting growth performance was about 1.23:1 if the concentration of P was at the requirement. In Exp. 2 the hypothesis was that the requirement for dietary Ca to maximize growth performance of 11- to 25-kg pigs expressed as the STTD Ca:STTD P ratio, is less than 1.40:1. A second objective was to test the hypothesis that increasing dietary Ca increases plasma Ca concentration, and downregulates expression of genes related to intestinal Ca absorption (i.e., *TRPV6* and *S100G*) and tight junction proteins (i.e., *OCLN* and *ZO1*) in the duodenum. Twenty corn-soybean meal based diets were formulated to contain 0.16, 0.33, 0.42, or 0.50% STTD P and 0.14, 0.29, 0.44, 0.59, or 0.74% STTD Ca. A total of 640 pigs (11.1 ± 1.4 kg) were allotted to 20 diets in a randomized complete block design. On day 21, weights of pigs and feed left in feeders were

recorded and blood, duodenal tissue, and the right femur were collected from one pig per pen. Gene expression was determined in duodenal tissue via quantitative RT-PCR. Data were analyzed using a response surface model in NLREG. Results indicated that there were interactions ($P < 0.01$) between Ca and P for ADG, ADFI, G:F, and bone ash. The predicted maximum ADG, G:F, and bone ash at 0.33% STTD P was obtained at STTD Ca:STTD P ratios of 1.39:1, 1.25:1 and 1.66:1, respectively. Plasma Ca concentration was positively affected by increasing dietary Ca (quadratic, $P < 0.01$) and negatively affected by increasing dietary P (linear, $P < 0.01$). There was a linear negative effect ($P < 0.05$) of dietary Ca on the expression of *S100G*, *TRPV6*, *OCLN*, and *Z01*. In conclusion, the STTD Ca:STTD P ratio needed to maximize growth performance of 11- to 25-kg pigs is less than 1.40:1, if P is at the requirement. Increasing dietary Ca decreases expression of genes related to transcellular Ca absorption but stimulates paracellular absorption of Ca by decreasing the expression of tight junction proteins in the duodenum.

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

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CHAPTER 1: INTRODUCTION

Calcium is the most abundant mineral in the body. Around 98% of the total-body Ca is present in the skeleton where it has a structural function. The remaining Ca is located in the blood, within the cell, and in the extracellular fluid, where it performs several physiological and regulatory functions (Crenshaw, 2001). Calcium needs to be supplied in diets for pigs due to the relatively low concentration of Ca in commonly used plant ingredients. Dietary Ca is, therefore, mainly supplied using low-cost inorganic sources, which is likely the reason for the limited research that has been conducted on Ca inclusion in pig diets. However, interest in Ca requirements has increased due to the close relationship between Ca and P and the fact that excess Ca reduces the digestibility of P and increases P excretion in manure (Stein et al., 2011). The NRC (2012) recommends formulating diets based on standardized total tract digestible (**STTD**) P values. However, although it is believed that digestible Ca values will increase the efficiency of Ca and P utilization, a lack of data for the digestibility of Ca in feed ingredients has prevented the use of STTD Ca values in diet formulation (NRC, 2012).

Recent research, however, has determined the STTD of Ca in most calcium-containing feed ingredients (González-Vega et al., 2015a, b; Merriman and Stein, 2016; Merriman et al., 2016), allowing for formulation of diets based on STTD Ca values. It is, therefore, also possible to estimate requirements for STTD Ca by growing pigs. González-Vega et al. (2016a) used a constant concentration of STTD P and 6 concentrations of STTD Ca to determine the STTD Ca requirements by 11- to 25-kg pigs. Results indicated a decrease in growth performance as STTD Ca concentration increased, but a minimum requirement for STTD Ca could not be estimated. Therefore, 2 experiments were conducted using 5 concentrations of STTD Ca and different concentrations of STTD P to estimate the STTD Ca requirements by 25- to 50-kg and 100- to

130-kg pigs (González-Vega et al., 2016b; Merriman et al., 2017). Results of these studies demonstrated that there is a negative effect of excess Ca on growth performance, but that this effect could be ameliorated by including dietary P in excess of the requirement, indicating that the ratio between STTD Ca and STTD P is important to optimize growth performance and Ca and P utilization. The estimated digestible Ca requirement expressed as STTD Ca:STTD P ratio in pigs from 25 to 50 kg and from 100 to 130 kg is less than 1.35:1.

Based on the existing data, 2 gaps regarding the digestible Ca requirements by growing pigs were identified. Therefore, the objective of the work included in this thesis was to determine requirements for digestible Ca in pigs from 11 to 25 kg and from 50 to 85 kg, as indicated by growth performance and bone mineralization.

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CHAPTER 2: DIGESTIBILITY OF CALCIUM AND REQUIREMENTS FOR CALCIUM BY GROWING PIGS: REVIEW OF LITERATURE

INTRODUCTION

Calcium is an essential macro mineral that plays important physiological roles in the synthesis and maintenance of skeletal tissue, and in many biochemical processes (Georgievskii, 1981; Kiarie and Nyachoti, 2010). Macro minerals are inorganic elements that are required by animals in amounts greater than 100 ppm to support normal growth and reproductive performance (Ewing and Charlton, 2007). The concentration of minerals in the body is 3 to 4% and Ca and P are the most abundant minerals in the body (Hendriks and Moughan, 1993; Perry et al., 2003). The skeletal tissue stores 96 to 99% of Ca and 60 to 80% of P (Maynard et al., 1979; Crenshaw, 2001). Bone ash contains 36 to 39% Ca and 17 to 19% P and the Ca to P ratio is relatively constant at approximately 2.1:1 (Crenshaw, 2001). The Ca that is not stored in skeletal tissue is distributed in blood, secretions, and body fluids (Gillespie, 1987) and along with P, it is involved in physiological functions such as enzymatic activation, muscle contraction, membrane structure, blood clotting, hormone secretion, and nerve impulses (Ewing and Charlton, 2007; Suttle, 2010). The extracellular concentration of Ca is maintained at a stable concentration via hormonal control, but the intracellular concentration of Ca is usually 4 times lower than the extracellular concentration (Crenshaw, 2001; Perry et al., 2003).

Calcium deficiency is caused by low dietary Ca intake, reduced absorption of Ca, increased losses of Ca, or a combination of the above (Gropper and Smith, 2013). Symptoms of Ca deficiency include lameness, rickets, and fractures (Crenshaw, 2001); however, excess dietary Ca also may be detrimental to growth performance, calcification of soft body tissues,

utilization of P and Zn, and may also increase the risk of formation of kidney stones (Maynard et al., 1979; Georgievskii, 1981; Crenshaw, 2001; NRC, 2012). Whereas P utilization has been widely studied because of the economic, environmental, and animal welfare impact of P in pork production (Poulsen, 2000), much less research has addressed the need for Ca by pigs. However, to achieve optimal growth and development of growing pigs, it is necessary that inclusion of not only P, but also Ca, is at the levels required by the animal (NRC, 2012).

Because bone mineralization occurs only if both Ca and P are present in adequate concentrations in diets, deficiency of one mineral will reduce deposition of both Ca and P in bone (Cromwell, 1998; Crenshaw, 2001). Likewise, excess of one mineral may reduce the utilization and metabolism of the other (Veum, 2010). As a consequence, deposition of Ca and P depends on the dietary concentrations of both minerals, and the ratio between Ca and P may be more important than the absolute dietary concentrations. A wide Ca to P ratio in the diet results in a decrease in P utilization, and the effect is more evident if the dietary concentration of P is low than if P is included at greater concentrations (Crenshaw, 2001; NRC, 2012; González-Vega et al., 2016c).

Another factor that affects Ca requirement is the concentration of vitamin D in the diet due to the regulatory function of vitamin D in Ca and P metabolism and dietary Ca absorption (Bondi, 1987; Veum, 2010). The age of the animal as well as the physiological condition also affect the requirement for Ca, because if the animal is older, the requirement for Ca as a percentage of the diet is less than in younger animals. Likewise, the requirements for gestating and lactating sows are greater than for growing pigs due to the need for Ca for fetal growth and milk production and the fact that the requirement for Ca to maximize bone ash is greater than to maximize growth performance (NRC, 2012; González-Vega et al., 2016a, c; Merriman et al.,

2017). Inclusion of microbial phytase in diets may also affect the requirement for Ca because phytase can degrade Ca-phytate complexes and thereby increase the digestibility of dietary Ca (Traylor et al., 2001; Guggenbuhl et al., 2007; González-Vega et al., 2015b). Other factors such as the genetic background of the animal, energy concentration in the diet, management strategies, and the use of antibiotics and other feed additives in diets may also affect the requirement for Ca (Suttle, 2010).

Thus, Ca is an indispensable nutrient that plays many roles in the body. The amount of Ca supplied in the diet to growing pigs must meet requirements without providing an excess to maximize P utilization, avoid health problems, and maximize feed intake and BW gain. Because there are many factors affecting the requirement for Ca, it is important to take all factors into account as requirements are established. This review is focused on Ca digestibility and requirements for growing pigs, but due a lack of data, digestibility of Ca by sows or requirements for Ca by sows are not addressed in this review.

SOURCES OF CALCIUM

Calcium in swine diets may be supplied by inorganic sources, plant feed ingredients, and animal feed ingredients. The inorganic sources provide most dietary Ca because of the high Ca concentration compared with plant feed ingredients. As an example, a corn-soybean meal based diet contains less than 0.1% Ca from corn and soybean meal (Soares, 1995). The content of Ca in inorganic sources ranges from 16.9 to 38.5% (Table 2.1), whereas the concentration of Ca in commonly used plant feed ingredients ranges from 0.01 to 0.69% (Table 2.2).

The most common Ca supplements used in swine diets are calcium carbonate, limestone, and calcium phosphates. The slope ratio method and the mean ratio comparison assay using

calcium carbonate as the standard have been used to calculate the relative bioavailability (**RBV**) of Ca in inorganic sources of Ca fed to swine (Ross et al., 1984; Soares, 1995; Kiarie and Nyachoti, 2010). Most inorganic sources of Ca have an RBV that is close to that of calcium carbonate, but dolomitic limestone has the lowest RBV (51 to 78%), which is likely due to the presence of Mg that reduces the absorption of Ca (Ross et al., 1984).

Potential animal feed ingredients include bone meal, meat meal, milk products, fish meal, poultry byproduct meal, and blood products. Some of these have greater concentration of Ca than plant ingredients but contain less Ca than inorganic sources of Ca, with concentrations of Ca in the range from 0.05 to 10.94% (Table 2.3).

DIGESTIBILITY OF CALCIUM IN FEED INGREDIENTS

Bioavailability

Bioavailability of nutrients is an indirect measurement of the estimated percentage of the ingested nutrient that is absorbed and used for body metabolism (Stein et al., 2007b), and it is usually expressed as RBV. For estimating RBV of nutrients, a standard substance is used, and assays such as slope ratio, three point design, standard curve, or mean ratio comparison are conducted (Littell et al., 1995). Growth performance, bone strength, and concentrations and quantities of bone ash are parameters usually used for determining Ca availability in diets and calcium carbonate is usually the standard (Soares, 1995). However, RBV is not a measurement of the quantity of the nutrient that is available for metabolism. Instead, values for RBV are relative values, and experiments are somewhat complex and expensive to conduct. In addition, values for RBV are not additive in mixed diets (NRC, 2012). As a consequence, digestibility

values are often used instead of RBV values because these values are easier and less expensive to generate.

Digestibility

Digestibility is defined as nutrient disappearance through the gastrointestinal tract and is measured as the amount of a nutrient that is excreted by the animal subtracted from the amount of nutrient that was ingested (Stein et al., 2007b). Digestibility values represent the amount of the nutrient that is available for metabolism (Stein et al., 2007b). Nutrient digestibility can be expressed as duodenal, ileal, or total tract digestibility. The digestibility of AA, for example, is expressed as ileal digestibility because fecal excretion of AA is influenced by fermentation in the large intestine (Stein et al., 2007b). By contrast, Ca and P digestibility can be expressed as ileal or total tract digestibility because there is no net absorption or secretion of either Ca or P in the hindgut (Bohlke et al., 2005; González-Vega et al., 2014). Values for total tract digestibility are most often determined because they are believed to be more accurate and less expensive to determine than values for ileal digestibility.

There are endogenous losses of most nutrients from the gastrointestinal tract as a result of the DMI by the animal and sometimes also due to specific diet features. These are known as basal endogenous losses and diet specific endogenous losses, respectively, and total endogenous losses represent the sum of basal and diet-specific losses (Stein et al., 2007a). A Ca-free diet is usually used to determine basal endogenous losses of Ca (González-Vega et al., 2014) and the regression procedure may be used to estimate total endogenous losses of P and Ca (Fan et al., 2001; González-Vega et al., 2013).

The apparent total tract digestibility (**ATTD**) of Ca is calculated by subtracting Ca output in feces from the amount of Ca ingested by the pig (NRC, 2012) using equation [1]:

$$\text{ATTD of Ca} = [(\text{Ca ingested} - \text{Ca in feces})/\text{Ca ingested}] \times 100 \quad [1]$$

Feces are collected for a specific period of time using an indigestible marker to indicate the start and the conclusion of collection (Adeola, 2001). In the calculation of values for ATTD, the endogenous losses are not taken into account. By contrast, values for the standardized total tract digestibility (**STTD**) of Ca are calculated by subtracting the basal endogenous losses from Ca output in feces (NRC, 2012) and equation [2] is used for this calculation:

$$\text{STTD of Ca} = [(\text{Ca ingested} - (\text{Ca in feces} - \text{basal endogenous losses of Ca}))/\text{Ca ingested}] \times 100 \quad [2]$$

The true total tract digestibility (**TTTD**) is calculated if the total endogenous losses are subtracted from the nutrient output in feces (NRC, 2012) using equation [3]:

$$\text{TTTD of Ca} = [(\text{Ca ingested} - (\text{Ca in feces} - \text{total endogenous losses of Ca}))/\text{Ca ingested}] \times 100 \quad [3]$$

When mixed diets are prepared, the ATTD in single ingredients are not always additive (Stein et al., 2005; NRC, 2012). As a consequence, values for STTD of nutrients are preferred when formulating diets for growing pigs because values for STTD of nutrients are additive in mixed diets. Data for ATTD and STTD of Ca in inorganic sources, as well as plant and animal feed ingredients are presented in Table 2.4.

FACTORS AFFECTING THE DIGESTIBILITY OF CALCIUM

Phytate

Phytate, a salt of phytic acid (*myo*-inositol-hexakis dihydrogen phosphate; **IP6**), is the main storage form of P in plants and is considered an anti-nutrient for pigs (Wilcock and Walk, 2016). Diets for pigs usually contain 0.2 to 0.3% phytate-bound P (Rodehutscord and

Rosenfelder, 2016) and the concentration of P in phytate is 282 g per kg (Selle et al., 2009). Phosphorus that is bound to phytate is largely unavailable to pigs (Kerr et al., 2010) and is excreted in the manure. The reduced availability of P bound to phytate is a consequence of the lack of phytase activity in the small intestine of pigs (Pointillart et al., 1987). Phytase is the enzyme that hydrolyzes phytate, producing available P and inositol phosphate (**IP**) isomers, such as IP5, IP4, and IP3 (Sandberg and Andlid, 2002; Rodehutscord and Rosenfelder, 2016). These molecules need to be further hydrolyzed to reduce their potential anti-nutritional effects (Bedford and Walk, 2016) and to obtain the possible benefits from the end product, *myo*-inositol (Huber, 2016).

Phytic acid may carry up to 12 negative charges and chelates 6 Ca atoms and although phytate has more affinity for Zn and Cu, the fact that the concentration of Ca in swine diets is high, make the linkage possible (Selle et al., 2009). As pH increases, IP6 becomes more negatively charged and the affinity of IP6 for cations increases (Bedford and Rousseau, 2017). It was demonstrated that Ca forms insoluble complexes with phytate, which makes Ca less available to broiler chickens (Nelson and Kirby, 1987).

Bohlke et al. (2005) obtained greater ATTD of Ca in diets with reduced level of phytate compared with diets with greater concentrations of phytate when fed to growing pigs, concluding that less inorganic Ca should be included in diets with low level of phytate. However, low phytate does not appear to always increase ATTD and STTD of Ca (Kemme et al., 1999; González-Vega et al., 2014), but the reason for this apparent contradiction has not been elucidated.

Phytase

Phytase, the enzyme that hydrolyzes phytate, improves the digestibility of P (Jongbloed

et al., 1992) by making phytate-bound P available for absorption. This process involves a stepwise hydrolysis of ester bonds that bind the phosphates to the inositol ring (Rodehutscord and Rosenfelder, 2016). Phytase has been commonly included in diets for pigs with the main objective of increasing available P and reducing P excretion in the manure. Microbial phytase is usually included in diets because it is more stable under intestinal conditions (Sandberg and Andlid, 2002) and 40% more efficient than phytase from cereals grains (Zimmermann et al., 2002). Cations such as Ca that are bound to phytate may also be released if phytase is included in the diet, which results in increased digestibility of Ca (Jongbloed et al., 1992). The positive effect of including phytase in diets for pigs on the ATTD of Ca has been previously demonstrated (Kemme et al., 1999; Almeida et al., 2013). Phytase also improved the ATTD and STTD of Ca in diets where the main source of Ca was inorganic Ca, indicating that some inorganic Ca may be bound to phytate in the stomach of pigs if no phytase is used (González-Vega et al., 2015b). Likewise, phytase increased the ATTD, STTD, and TTTD of Ca in plant feed ingredients (González-Vega et al., 2013; Rodríguez et al., 2013) and fish meal (González-Vega et al., 2015a). However, an improved digestibility of Ca in animal proteins has not always been observed (Merriman et al., 2016b).

Phytase inclusion in diets for pigs is usually 500 to 1,000 phytase units (**FTU**). One FTU is defined as the phytase activity that releases 1µmol per minute of P from sodium phytate at pH 5.5 and 37°C (Jones et al., 2010). Inclusion of more than 2,500 FTU is known as super-dosing and may have benefits in the digestibility of nutrients other than minerals (Adeola and Cowieson, 2011), as well as in the degradation of lower IP esters such as IP4 and IP3 (Rodehutscord and Rosenfelder, 2016). Increasing phytase from 500 to 1,500 FTU linearly increased the STTD of Ca in soybean meal (Traylor et al., 2001), but increasing phytase from 1,500 to 15,000 FTU only

improved the ATTD of Ca by 4 percentage units (Kies et al., 2006), indicating that super-dosing of phytase may not be necessary to maximize the digestibility of Ca. Likewise, in diets containing from 0 to 4,000 FTU of phytase, the maximum digestibility of Ca was observed in diets containing approximately 1,000 FTU (Almeida et al., 2013).

High concentration of Ca and a wide Ca to P ratio have a negative effect on phytase activity (Lei et al., 1994; Qian et al., 1996; Selle et al., 2009). This may be a consequence of the increase in pH in the intestinal tract caused by excess Ca due to its high acid buffering capacity (Bosi et al., 1999), the formation of insoluble Ca-phytate complexes that are unapproachable to phytase (Qian et al., 1996), or competition between Ca and phytase for the binding site (González-Vega and Stein, 2014). Therefore, interactions between Ca and phytase need to be evaluated because inclusion of excess Ca is common in swine diets as a result of the low cost of Ca supplements (Walk, 2016).

Dietary Ca Level

It has been suggested that the digestibility of Ca is influenced by the dietary level of Ca because the active mechanism of absorption of Ca is modified depending on the concentration of Ca in the diet, which is a consequence of the hormonal regulation of Ca in plasma by the active form of vitamin D (1,25-dihydroxycholecalciferol; calcitriol) and calcitonin (Crenshaw, 2001). Activation of vitamin D in the kidney is stimulated by the parathyroid hormone (**PTH**) which is released by the parathyroid gland as a result of low concentration of Ca in plasma (Crenshaw, 2001). The activated vitamin D increases the transcellular intestinal absorption of Ca and the reabsorption of Ca from the renal tubules and bones (Veum, 2010). Transcellular absorption is increased by increasing the expression of apical membrane channels (transient receptor potential vanilloid; **TRPV**), which allow Ca to enter the cell (Christakos, 2012), Ca binding proteins

(**CaBP**; calbindin), which transport Ca through the cell (Veum, 2010), and basolateral membrane channels (Ca-ATPase), which allow Ca to exit the cell (Kutuzova and DeLuca, 2004). Calcitonin, by contrast, is released by the C cells of the thyroid gland in response to high concentrations of plasma Ca and reduces the plasma concentration of Ca by increasing the excretion of Ca in urine and preventing the resorption of Ca from bones (Crenshaw, 2001). Paracellular absorption, the passive mechanism of absorption of Ca, is increased by high concentration of Ca in the lumen of the intestine because it creates a gradient of Ca across the epithelium (Gropper and Smith, 2013).

Increased dietary Ca increases the ATTD of Ca, but not the TTTD of Ca in diets containing Ca below the requirement. This is a result of the reduction of the proportion of endogenous Ca in the feces that is observed as dietary Ca increases (González-Vega et al., 2013). However, the ATTD of Ca in diets where Ca was mostly supplied by calcium carbonate was not affected by increasing dietary Ca from 55 to 173% of the requirement (Stein et al., 2011). Likewise, the ATTD and STTD of Ca in calcium carbonate was not affected by the level of dietary Ca (González-Vega et al., 2014). The reason the digestibility of Ca is not affected by the concentration of Ca in diets containing Ca above the requirement is likely that the paracellular absorption of Ca is not under hormonal regulation (Crenshaw, 2001). As a consequence, Ca digestibility is mostly constant regardless of the concentration of Ca in the diet, although extreme excess of dietary Ca may result in a slight changes in the digestibility of Ca (González-Vega et al., 2016b).

Particle Size

Reduction of particle size of feed ingredients is sometimes beneficial for growth performance and nutrient digestibility in growing pigs due to an increase in the surface area

(Giesemann et al., 1990; Rojas and Stein, 2015). However, according to Ross et al. (1984), the availability of Ca is not influenced by the particle size of Ca supplements if the diameter is between 0.10 and 0.54 mm. A similar result was reported by Merriman and Stein (2016), who concluded that calcium carbonate can be offered in diets at particle sizes from 200 to 1,125 µm without having any effect on Ca digestibility or pig growth performance. In dairy cows, it was also observed that the particle size of calcium carbonate had no effect on growth performance, ruminal fermentation, or nutrient digestibility (Clark et al., 1989). These results are likely a consequence of calcium carbonate being very soluble in the liquid environment of the stomach.

Fat and Fiber

The presence of dietary fat in the gastrointestinal tract of pigs reduces the passage rate and improves the digestibility of some nutrients such as AA (Azain, 2001; Cervantes-Pahm and Stein, 2008). However, experiments in humans have demonstrated a negative effect of fat on Ca absorption (Agnew and Holdsworth, 1971) and bioavailability (Bandali et al., 2015). This is likely a result of formation of insoluble Ca soaps in the gastrointestinal tract (Soares et al., 2012). A similar response has been observed in rats with a reduction in absorption and digestibility of Ca if high levels of fat are supplied, and a greater negative effect from saturated fatty acids than from unsaturated fatty acids (Gacs and Barltrop, 1977; Frommelt et al., 2014). This interaction between fat and Ca also reduces the digestibility of fat in humans if the intake of Ca is high (Bendsen et al., 2008). Postprandial lipidemia decreases as a result of the reduced absorption of fat if the intake of Ca from dairy products is high, but the effect is not observed if calcium carbonate is used as the source of Ca (Lorenzen et al., 2007).

However, addition of a blend of soybean, sunflower, and rapeseed oil in high levels did not affect the AID or ATTD of Ca in growing pigs (Steiner et al., 2006) and no effect of addition

of pure soybean oil on the ATTD or STTD of Ca was observed in growing pigs (González-Vega et al., 2015a). The fact that most of the fat in vegetable oils is unsaturated may explain the lack of an effect of fat on the digestibility of Ca in these experiments. However, inclusion of tallow, palm oil, corn oil, or soybean oil increased the ATTD of Ca in calcium carbonate, and did not result in excretion of Ca-fat complexes (Merriman et al., 2016a). Thus, there is no evidence of interactions between Ca and dietary fat in diets fed to pigs.

Dietary fiber may increase passage rate in the gastrointestinal tract of pigs (Azain, 2001) and different effects of fiber on the bioavailability of Ca in humans have been reported (Kies, 1985). Likewise, effects of fiber on mineral metabolism in pigs are not consistent (Grieshop et al., 2001). Based on studies with rats, it is believed that high dietary concentrations of fermentable dietary fiber may shift the absorption of Ca and P from the small intestine to the large intestine where the pH in digesta is lower, improving the solubility of the minerals and increasing the paracellular absorption in the large intestine (Metzler and Mosenthin, 2008). Synthesis of short chain fatty acids in the large intestine contributes to a reduced pH and the presence of butyrate may increase absorption of nutrients by stimulating growth of epithelial cells (Montagne et al., 2003). Indeed, González-Vega et al. (2015a) demonstrated an improvement in the ATTD and STTD of Ca by the inclusion of synthetic fiber to a fish meal-cornstarch diet fed to growing pigs, which supports the hypothesis that dietary fiber has a positive impact on absorption of Ca. However, unlike rats, there is no evidence that there is absorption of Ca from the hindgut of pigs (González-Vega et al., 2014).

Phosphorus and Zinc

Minerals tend to interact more than other nutrients because of their propensity for forming bonds. The interactions may be synergistic or antagonistic and may take place in the

gastrointestinal tract (Georgievskii, 1981). Changes in the mechanism of absorption of a mineral is one result of interactions among minerals. Calcium interacts with P in a synergistic way and with Zn in an antagonistic way (Georgievskii, 1981).

Despite the negative effect of high levels of Ca on P digestibility, P does not affect the digestibility of Ca. The ATTD of Ca by pigs fed diets with an adequate concentration of P was not different from that of pigs fed a P-free diet (Stein et al., 2006). Likewise, no differences in the ATTD of Ca were observed if dietary P increased from 0.26 to 0.64% in diets fed to growing pigs (Stein et al., 2008).

Inclusion of zinc oxide at pharmacological levels has a negative effect on the absorption of Ca (Meyer et al., 2002) as well as on the ATTD and STTD of Ca in weanling pigs (Blavi et al., 2017). This may be a consequence of the competition between Ca and Zn for a shared channel mechanism in the brush border membrane of the intestinal cells whose affinity is greater for Zn than for Ca (Bertolo et al., 2001). Phytase improves the digestibility of Ca if pharmacological levels of zinc oxide are included in the diet (Blavi et al., 2017), which may be explained by formation of Zn-Ca-phytate complexes in the intestinal tract which are more stable than simple Zn-phytate or Ca-phytate complexes (Maenz et al., 1999). However, in diets where phytase was included the ATTD Ca was reduced by increasing pharmacological levels of zinc oxide, an effect that was not observed in the absence of phytase (Walk et al., 2015). This may indicate a reduction of the positive effect of phytase on the digestibility of Ca by the presence of Zn. Thus, there is an interaction between Ca and Zn, and there are other interactions among Ca, Zn, phytate, and phytase that should be taken into account during the formulation of swine diets.

REQUIREMENTS FOR TOTAL CALCIUM BY GROWING PIGS

Requirements for Ca by pigs have been expressed on the basis of total Ca because of a lack of data about the digestibility of Ca in feed ingredients (NRC, 2012). Empirical measurements have been used to determine the requirements for total Ca and total P using response criteria such as growth performance, skeletal development, carcass characteristics, and blood composition in response to different concentrations of dietary Ca and P (Aubel et al., 1941; Chapman et al., 1962; Cromwell et al., 1970; Stockland and Blaylock, 1973). Similar experiments have also been conducted to evaluate suggested recommendations (NRC, 1973, 1979) for total Ca and total P, either using a constant concentration of one mineral and different concentrations of the other mineral (Thomas and Kornegay, 1981) or maintaining a constant Ca:P ratio (Maxson and Mahan, 1983). However, the requirement values may be influenced by different factors such as sex (Thomas and Kornegay, 1981), age (NRC, 2012), and the ratio between total Ca and total P (Qian et al., 1996; Liu et al., 2000). Results from these studies have also demonstrated that requirements for total Ca and total P to maximize bone development are greater than requirements to maximize growth performance.

Phosphorus requirements are most correctly expressed as requirements for STTD P (NRC, 2012) and commercial diets for pigs are usually formulated based on STTD P because of the interest in reducing P excretion in the manure. In addition, STTD P values are believed to more accurately meet the P requirement of growing pigs (NRC, 2012). The Ca:P ratio has also been widely studied because of the close relationship between Ca and P in metabolism (Veum, 2010). The current recommendation for total Ca:total P is 1.1:1 to 1.25:1 and the recommended ratio between total Ca and STTD P is 2.15:1 (NRC, 2012). Because of the lack of data for digestibility of Ca in feed ingredients, at the time when the NRC (2012) was developed no

recommendation for the STTD Ca:STTD P ratio was provided, but it was pointed out that a more accurate way of formulating diets for pigs would be to use a ratio between STTD Ca and STTD P (NRC, 2012).

The NRC (2012) utilized a factorial approach to calculate the requirement for total Ca and digestible P by growing pigs. The STTD P requirement was based on maximizing P retention, reducing P excretion in the manure, and maximizing growth performance (NRC, 2012), and eq. [4] was used for this calculation:

$$\text{STTD P requirements} = 0.85 \times [(\text{maximum whole-body P retention}) / 0.77 + 0.19 \times \text{feed dry matter intake} + 0.007 \times \text{BW}], \quad [4]$$

where it was assumed that 1) 85% of P requirement for maximum P retention will maximize growth performance; 2) P body mass is directly related to body protein mass; 3) 77% of the STTD P intake is used for P retention; 4) 190 mg/kg DMI are estimated as endogenous losses of P; and 5) 7 mg/kg BW are considered the minimum P urine losses. The body P mass (g) is obtained by using eq. [5]:

$$\text{Body P mass} = 1.1613 + 26.012 \times \text{body protein mass} + 0.2299 \times (\text{body protein mass})^2. \quad [5]$$

The requirement for total Ca was calculated by multiplying the STTD P requirement by 2.15. The basis for this value, however, has not been elucidated and more research is required to accurately establish the requirement for Ca. Data for requirements for total Ca, total P, and STTD P in pigs with different BW are presented in Table 2.5.

REQUIREMENTS FOR DIGESTIBLE CALCIUM BY GROWING PIGS

The importance of the Ca:P ratio and the recognized needs for determining requirements for Ca and P based on STTD values was the background for a number of studies conducted

during recent years to estimate the STTD of Ca in different feed ingredients (González-Vega et al., 2015a, b; Merriman and Stein, 2016; Merriman et al., 2016b). Having determined the values for STTD Ca in most Ca-containing feed ingredients allowed for determining the requirements for STTD Ca and a number of studies have been conducted to estimate this requirement by growing pigs (González-Vega et al., 2016a, c; Merriman et al., 2017).

In a 22-d study, a constant concentration of STTD P, and 6 concentrations of STTD Ca were used to determine the amount of STTD Ca required to maximize growth performance, bone ash, and Ca retention in 11- to 25-kg pigs (González-Vega et al., 2016a). Results indicated a reduction in ADG and G:F if the concentration of STTD Ca was above 0.50%, but a minimum requirement for STTD Ca could not be estimated. However, bone ash and Ca retention were maximized at STTD Ca concentrations of minimum 0.48% and 0.60%, respectively. An increase in growth performance was also observed in high STTD Ca diets as the concentration of STTD P increased, indicating the importance of the Ca:P ratio in diet formulation. Therefore, a second experiment was conducted to estimate the requirement for STTD Ca by 25- to 50-kg pigs by using 5 dietary concentrations of STTD Ca and 4 concentrations of STTD P for a total of 20 different STTD Ca:STTD P ratios (González-Vega et al., 2016c). This experiment confirmed the negative effect of increasing dietary concentrations of STTD Ca on growth performance and demonstrated that the impact is more evident if STTD P is at or below the requirement than if P is supplied above the requirement, again demonstrating the importance of formulating diets based on the correct Ca:P ratio. Growth performance, bone ash, and Ca retention were maximized at STTD Ca:STTD P ratios of 1.16:1 to 1.43:1, 1.55:1 to 1.77:1, and 2.45:1 to 2.72:1, respectively (González-Vega et al., 2016c). As has been previously reported, these data confirmed that the Ca requirement to maximize bone ash is greater than the requirement to

maximize growth performance. The greater estimated requirements for Ca retention than the requirement to maximize bone ash may indicate that Ca is not only stored in bones (González-Vega et al., 2016c). A similar experiment was performed in 100 to 130 kg pigs by using 5 dietary concentrations of STTD Ca and 3 dietary concentrations of STTD P to estimate STTD Ca requirements needed to maximize growth performance and bone ash (Merriman et al., 2017). Increasing dietary concentrations of STTD Ca linearly decreased ADFI of pigs regardless of the concentration of STTD P in the diet whereas the reduction of ADG of pigs was more evident if the concentration of STTD P was below or at the requirement. Bone ash, by contrast, was linearly increased as the dietary concentration of Ca increased. These observations are in agreement with the results obtained for 25- to 50-kg pigs by González-Vega et al. (2016c) and indicates that even if growth performance of pigs is reduced due to increased dietary concentrations of Ca, bone ash is being synthetized in increasing quantities. This observation also indicates that the regulation of bone ash is different from the regulation of growth performance.

CONCLUSIONS

Calcium is an important macro mineral required for bone and teeth formation and several physiological functions of the body. The digestibility of Ca in pigs may be influenced by different dietary factors that should be taken into account during diet formulation. The presence of phytate and Zn potentially affect the digestibility of Ca whereas if phytase and fiber are included in diets, the digestibility of Ca in pigs may be enhanced. Factors such as dietary Ca level, particle size of Ca supplements, and fat concentration in diet appear to have no influence on the digestibility of Ca by pigs. Requirements for total Ca have been used in formulation of

swine diets because data for the digestibility of Ca in feed ingredients were not available. Values for the STTD Ca in most calcium-containing feed ingredients have been determined in recent years and it is, therefore, possible to formulate diets based on values for STTD of Ca. It has been clearly demonstrated that excess dietary Ca is detrimental to growth performance of pigs. However, the concentration of bone ash may be increased in pigs fed diets that reduce ADFI, ADG, and G:F. The detrimental effects of excess dietary Ca are most pronounced in pigs fed diets that are limited or marginal in dietary P and those negative effects may be ameliorated by providing dietary P above the requirement. It is, therefore, clear that the ratio between STTD Ca and STTD P is important to optimize growth performance of pigs. Based on recent data, it appears that a ratio between STTD Ca and STTD P that is less than 1.35:1 is needed to maximize growth performance if dietary P is at the requirement. However, more research is needed to determine the optimum ratio for different age group of pigs. Specifically, there is a need to determine the STTD Ca:STTD P ratio required by pigs in the weight range of 11 to 25 kg and 50 to 100 kg.

TABLES

Table 2.1. Calcium concentration and bioavailability in inorganic sources of calcium

Source	Calcium (%)	Relative bioavailability (%)
Calcium carbonate	38.5[1]	-
Calcium chloride, anhydrate	36.0[2]	86.0[4]
Calcium chloride, dihydrate	27.0[2]	94.0[4]
Calcium sulfate, dihydrate	21.9[1]	83.0[4]
Dolomite limestone	22.0[2]	51.0-78.0[3]
Limestone	35.8[1]	99.0[3]
Monocalcium phosphate	16.9[1]	-
Dicalcium phosphate	24.8[1]	-
Tricalcium phosphate	17.7[1]	-
Oyster shell, ground	34-36[2]	98[3]

[1]Values from NRC (2012).

[2]Values from Sauvant et al. (2004).

[3]Values from Ross et al. (1984).

[4]Values from Kuznetsov et al. (1987).

Table 2.2. Concentrations of calcium, phosphorus and phosphorus bound to phytate in plant feed ingredients[1]

Source	Calcium (%)	Phosphorus (%)	Phytate P (%)
Bakery meal	0.13	0.25	0.06[3]
Barley	0.06	0.35	0.22
Canola meal	0.69	1.08	0.65
Corn	0.02	0.26	0.21
Corn germ	0.02	1.27	1.07
Corn gluten meal	0.03	0.49	-
DDGS[2]	0.12	0.73	0.26
Oats	0.03	0.35	0.19
Field peas	0.09	0.42	0.17
Rice	0.09	0.34	0.18
Rice bran	0.22	2.16	1.74
Rye	0.08	0.30	0.20
Sorghum	0.02	0.27	0.18
Soybean meal	0.33	0.71	0.38
Sunflower meal	0.38	0.95	0.84
Triticale	0.04	0.33	0.21
Wheat	0.06	0.39	0.22

[1]Values from NRC (2012).

[2]DDGS = Distillers dried grains with solubles.

[3]Calculated as 28.2% of phytate (Tran and Sauvant, 2004); phytate value from Stein et al. (2016).

Table 2.3. Concentrations of calcium and phosphorus in animal feed ingredients[1]

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

[1]Values from NRC (2012).

Table 2.4. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of calcium in inorganic sources of Ca and feed ingredients from plant or animal origin, without (W/o) or with phytase (W)

	ATTD of Ca (%)		STTD of Ca (%)	
Source	W/o phytase	W phytase	W/o phytase	W phytase
Inorganic sources				
Calcium carbonate[1]	57.98	70.62	60.43	73.07
Calcium carbonate[2]	60.90-70.90	-	-	-
Calcium carbonate[3]	69.98-74.29	-	74.15-78.45	-
Monocalcium phosphate[1]	82.76	83.24	85.86	86.34
Dicalcium phosphate[1]	75.29	76.39	77.80	78.90
Plant feed ingredients				
Canola meal[4]	47.93	64.19	-	-
Corn[5]	49.60	-		
Soybean meal[5]	46.70	-	-	-
Animal feed ingredients				
Fish meal[6]	73.07	84.01	76.21	86.88
Meat and bone meal[7]	74.54	79.66	76.83	81.94
Meat meal[7]	74.61	83.25	76.97	85.75
Poultry meal[7]	80.74	74.31	82.41	76.06
Poultry byproduct[7]	85.34	83.51	87.76	86.66

[1]Values from González-Vega et al. (2015b).

[2]Values from Stein et al. (2011).

[3]Values from Merriman and Stein (2016).

Table 2.4. (Cont.)

[4]Values from González-Vega et al. (2013).

[5]Values from Bohlke et al. (2005).

[6]Values from González-Vega et al. (2015a).

[7]Values from Merriman et al. (2016b).

Table 2.5. Requirements for total Ca, total P, and standardized total tract digestible (STTD) P for growing and finishing pigs[1]

	Body weight range, kg				
Item	11-25	25-50	50-75	75-100	100-135
Total Ca, %	0.70	0.66	0.59	0.52	0.46
Total P, %	0.60	0.56	0.52	0.47	0.43
STTD P, %	0.33	0.31	0.27	0.24	0.21

[1]Values from NRC (2012).

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CHAPTER 3: EFFECTS OF DIETARY DIGESTIBLE CALCIUM ON GROWTH PERFORMANCE AND BONE ASH IN 50- TO 85-KG GROWING BARROWS FED DIETS WITH DIFFERENT CONCENTRATIONS OF PHOSPHORUS

ABSTRACT

An experiment was conducted to determine the requirement for standardized total tract digestible (STTD) Ca by 50- to 85-kg pigs and to test the hypothesis that the requirement to maximize growth performance expressed as the STTD Ca:STTD P ratio, is less than 1.35:1. Fifteen corn-soybean meal based diets were formulated using a 3 × 5 factorial design with diets containing 0.14, 0.27, or 0.41% STTD P and 0.13, 0.25, 0.38, 0.50, or 0.63% STTD Ca. Ninety barrows (BW: 50.2 ± 2.1 kg) were individually housed and randomly allotted to the 15 diets with 6 replicate pigs per diet. Diets were fed for 30 d and the amount of feed offered was recorded daily. At the conclusion of the experiment, pig weights were recorded and ADG, ADFI, and G:F were calculated for each diet. On d 31, pigs were euthanized, the right femur was removed, and concentrations and percentages of ash, Ca, and P were determined in the dried defatted femurs. Data were analyzed using a response surface model in NLREG. Results indicated interactions ($P < 0.10$) between dietary concentrations of STTD Ca and STTD P for final BW, ADG, G:F, and the concentration (g per femur) and percentage of bone ash, Ca, and P. The full model was used to predict final BW and ADG, whereas for G:F, the model only included the linear terms for STTD Ca and STTD P and the interaction between the linear terms. The 3 models contained a negative linear effect of STTD Ca ($P < 0.10$). The predicted maximum BW (87.20 kg) and ADG (1.23 kg) at 0.27% STTD P was obtained at STTD Ca:STTD P ratios of 1.20:1 and 1.25:1, respectively. However, a predicted maximum G:F was not obtained because of the linear nature

of the equation. A decrease in ADFI (main effect of Ca, $P < 0.05$) regardless of the concentration of STTD P in the diets was observed as the concentration of STTD Ca increased. The model to predict bone ash (g) was not reduced and the estimated maximum values at STTD P concentrations of 0.27 and 0.41% were 55.8 and 60.1 g. These values were obtained at STTD Ca:STTD P ratios of 2.03:1 and 1.59:1, respectively. In conclusion, excess Ca is detrimental to growth performance of 50- to 85-kg pigs if the concentration of P is at or below the requirement. The amount of STTD Ca needed to maximize bone ash is greater than the amount needed to maximize growth performance. The STTD Ca to STTD P ratio needed to assure adequate bone mineralization without affecting growth performance is about 1.23:1 if the concentration of P is at the requirement.

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

INTRODUCTION

Calcium is the most abundant mineral in the animal body and plays important roles in many physiological processes (Vitti and Kebreab, 2010). Reduction of growth performance and bone or kidney diseases have been reported as a result of excess or deficiency of Ca (Crenshaw, 2001; González-Vega and Stein, 2014). The concentration of dietary Ca also influences P digestibility (Stein et al., 2011).

The requirement for Ca by pigs is usually expressed on the basis of total Ca and the current requirement for total Ca for 50- to 75-kg pigs is 0.59% (NRC, 2012). However, data for the standardized total tract digestibility (**STTD**) of Ca in a number of feed ingredients have been recently reported (González-Vega et al., 2015a, b; Merriman and Stein, 2016; Merriman et al., 2016). Therefore, it is now possible to formulate diets for pigs based on values for STTD of Ca,

which may make it possible to establish Ca requirements for pigs based on values for STTD of Ca. Values for STTD of Ca may reflect Ca utilization more accurately than values for total Ca, and these values are also believed to be additive in mixed diets (NRC, 2012). Estimates for STTD Ca requirements for pigs from 25 to 50 kg and from 100 to 130 kg have been reported (González-Vega et al., 2016b; Merriman et al., 2017). In both of these experiments, it was demonstrated that increasing dietary Ca reduces ADFI and therefore ADG. It was also concluded that the requirement for STTD Ca should be expressed as a ratio between STTD Ca and STTD P, and that a ratio greater than 1.35:1 may be detrimental to pig growth performance if STTD P is supplied at the requirement. However, the requirement for STTD Ca has not been reported for pigs from 50 to 85 kg. Therefore, the objective of this experiment was to test the hypothesis that the requirement to maximize growth performance of 50- to 85-kg pigs for STTD Ca, expressed as the STTD Ca:STTD P ratio, is less than 1.35:1.

MATERIALS AND METHODS

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

Animals and Housing

Ninety barrows with an initial BW of 50.2 ± 2.1 kg were randomly allotted to 15 diets and 5 blocks in a randomized complete block design. Blocks 1, 2, 4, and 5 had 1 replicate per treatment (15 pigs), and block 3 had 2 replicates per treatment (30 pigs). Therefore, there were a total of 6 replicate pigs per treatment. There was 1 pig per pen (0.9 × 1.8 m) and pens had fully slatted concrete floors, a feeder, and a cup waterer. Feed and water were available at all times.

The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding

Fifteen diets based on corn and soybean meal were formulated to contain different Ca and P concentrations, but to keep the concentration of phytate constant among diets, all diets contained the same quantities of corn and soybean meal (Table 3.1). Diets were formulated using a 3 × 5 factorial design with diets containing 0.14, 0.27, or 0.41% STTD P, and 0.18, 0.38, 0.59, 0.80, or 1.00% total Ca, corresponding to 0.13, 0.25, 0.38, 0.50, and 0.63% STTD Ca, respectively (Table 3.2). Concentrations of STTD P ranged from 50 to 150% of the requirement (NRC, 2012) and Ca concentrations ranged from 30 to 170% of the requirement for total Ca (NRC, 2012). All diets were formulated to have equal concentrations of Na, and inclusion of sodium bicarbonate was, therefore, reduced as inclusion of monosodium phosphate increased to increase the concentration of dietary P (Table 3.3).

Growth Performance and Bone Measurements

Pigs were allowed ad libitum access to feed for 30 d and they had a final BW of 83.2 ± 5.4 kg. Pig weights were recorded at the beginning of the experiment and the amount of feed offered was recorded daily. On d 30 in the afternoon, individual pig weights were recorded and the amount of feed in the feeders was also recorded. Pigs were then transported to the Meat Science Laboratory at the University of Illinois (3 km) and kept in lairage overnight with free access to water.

On d 31 in the morning, all pigs were humanely slaughtered as described by Overholt et al. (2016). The right femur was collected from all pigs and autoclaved at 125ºC for 55 min. Femurs were broken, dried, and soaked for 72 h in petroleum ether under a chemical hood to remove marrow and fat. Femurs were dried for 2 h at 135ºC and then ashed at 600ºC for 16 h.

Sample Analysis

Corn, soybean meal, calcium carbonate, monosodium phosphate, monocalcium phosphate, sodium bicarbonate, NaCl, and diets were analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007), ash (Method 942.05; AOAC Int., 2007), Na and K by flame emission photometry (Method 956.01; AOAC Int., 2007), and Cl by manual titration (Method 9.15.01, 943.01; AOAC Int., 2006). Ingredients, diets, and bone ash were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Corn, soybean meal, and diets were also analyzed for GE using bomb calorimetry (Model 6300, Parr Instruments, Moline, IL), CP (Method 990.03; AOAC Int., 2007), acid hydrolyzed ether extract (Method 2003.06; AOAC Int., 2007), and ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Diets were analyzed for phytate-bound P by wet chemistry using the Megazyme kit (Megazyme Inc., Chicago, IL).

Calculations and Statistical Analyses

The percentage of phytate in diets was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004), and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. Dietary cation-anion difference (**DCAD**) was calculated using the following equation (González-Vega et al., 2016b):

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

where Na, K, and Cl were expressed as percentages of the diet. The ADG, ADFI, and G:F were calculated for each diet and concentrations of bone Ca and bone P in grams per femur were

calculated by multiplying the total quantity of bone ash by the percentage of Ca and P in the bone ash.

Normality of data was tested using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data for growth performance and concentration and percentage of bone ash, bone Ca, and bone P were analyzed using the PROC MIXED of SAS with the experimental unit being the pig. 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. Assumptions of the model were tested using PROC GPLOT and influence options of SAS. Effects of dietary STTD Ca, STTD P, and the interaction between STTD Ca and STTD P were considered significant at $P \leq 0.05$. If the interaction or the main effects was significant, the software NLREG version 6.5 (Sherrod, 2008) was used to determine parameter estimates for the second-order response surface model to increasing concentrations of STTD Ca and STTD P as described by Khuri and Cornell (1996). Parameter estimates of the model that were not significant ($P > 0.10$) and were not included in a significant interaction were removed from the model and the estimates were recalculated. The surface response full model was:

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

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

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

where STTD Ca_{max} is the percentage 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 for each concentration of STTD P.

RESULTS

All pigs completed the experiment and consumed their diets without apparent problems and no health problems were observed during the experiment. The full model was used to predict final BW and ADG (interactions, $P < 0.10$; Table 3.4). The predicted maximum BW and ADG at STTD P concentration of 0.27% were 87.2 and 1.23 kg at STTD Ca concentrations of 0.33 and 0.34%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.20:1 and 1.25:1, respectively (Fig. 3.1 and Fig. 3.2). However, maximum BW and ADG values at STTD P concentrations of 0.14 and 0.41% were not estimated because of the nature of the responses. In the case of ADFI, the model only contained the negative linear term for STTD Ca ($P < 0.05$; Fig. 3.3). For G:F, only the linear STTD Ca and STTD P terms and the interaction ($P < 0.01$) between STTD Ca and STTD P were included in the model (Fig. 3.4). However, a predicted maximum G:F was not obtained because of the linear nature of the equation.

For concentration of bone ash, bone Ca, and bone P, the model was not reduced (interactions, $P < 0.10$; Table 3.5). The predicted maximum bone ash in grams per femur (g) at STTD P concentrations of 0.27, and 0.41% were 55.8 and 60.1 g at STTD Ca concentrations of

0.55 and 0.65%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.03:1 and 1.59:1 (Fig. 3.5). The predicted maximum bone Ca in grams per femur (g) at STTD P concentrations of 0.27 and 0.41% were 20.7 and 22.7 g at STTD Ca concentrations of 0.57 and 0.65%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.11:1 and 1.58:1 (Fig. 3.6). The predicted maximum bone P in grams per femur (g) at STTD P concentrations of 0.27 and 0.41% were 9.6 and 10.5 g at STTD Ca concentrations of 0.53 and 0.64%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.95:1 and 1.55:1 (Fig. 3.7). However, maximum bone ash, bone Ca, and bone P in g per femur at STTD P concentration of 0.14% were not estimated because of the nature of the responses.

The model for percentage of bone ash and bone P only contained the linear STTD Ca and STTD P terms and the interaction ($P < 0.05$) between STTD Ca and STTD P (Table 3.6). A predicted maximum percentage of bone ash and bone P was not obtained because of the linear nature of the equations (Fig. 3.8 and Fig. 3.9). In the case of percentage of bone Ca, the model was not reduced (interactions, $P < 0.01$; Fig. 3.10) and the predicted maximum bone Ca at STTD P concentrations of 0.14 and 0.41% were 37.6 and 37.8% at STTD Ca concentrations of 0.44 and 0.53%, respectively. These values correspond to STTD Ca:STTD P ratios of 3.16:1, and 1.30:1. A maximum percentage of bone Ca value at STTD P concentration of 0.27% was not estimated because of the nature of the response.

DISCUSSION

Values reported by NRC (2012) for Ca and P in ingredients were used in diet formulation, which may be the reason for differences between analyzed and predicted values in diets. However, the intended differences among diets were obtained for both Ca and P. For corn,

soybean meal, calcium carbonate, monosodium phosphate, sodium bicarbonate, and NaCl, the concentration of Ca, P, Na, Cl, and K were within the range of reported values (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; Stein et al., 2016). Likewise, concentrations of Na, Cl, and K in monocalcium phosphate were in agreement with reported values, but concentrations of Ca and P were lower than expected (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011). The DCAD values for all diets ranged from 249 to 289 mEq/kg; these values are within the range of DCAD for optimal growth performance of growing and finishing pigs (i.e., 250 to 400 mEq/kg; Haydon et al., 1990).

Response surface models allow interactions between several independent variables and one or more response variables to be quantified to determine values for independent variables that optimize the response (Khuri and Cornell, 1996). The chosen model is recommended for factorial experiments where the independent variables are values of quantities of treatments such as diet components (Box and Wilson, 1951; Steel et al., 1996), and this model has been used to determine the optimal Ca:P ratio to maximize growth performance by White Pekin ducklings (Xie et al., 2009) and growing pigs (González-Vega et al., 2016b; Merriman et al., 2017).

The observation that increasing dietary STTD Ca linearly reduces ADFI of pigs regardless of the level of STTD P in the diet is in agreement with data for 100- to 130-kg pigs (Merriman et al., 2017). The reason for this observation may be that as Ca increases in the diet more P will be bound in undigestible Ca-P complexes in the intestinal tract and thereby inducing a P deficiency or increasing an already existing P-deficiency. Growing-finishing pigs will respond to P-deficiency by reducing ADFI (Aubel et al., 1936; Sørensen et al., 2018).

As a consequence of the reduction in ADFI, a decline in ADG and final BW was observed as dietary STTD Ca increased, and this observation is also in agreement with previous

data (González-Vega et al., 2016b; Merriman et al., 2017). A reduction in growth performance in broiler chickens and in White Pekin ducklings as dietary Ca levels increased has also been reported (Hurwitz et al., 1995; Xie et al., 2009; Akter et al., 2017; Kim et al., 2017). Likewise, a reduction in ADG for 11- to 25-kg pigs fed diets with increasing levels of STTD Ca and constant STTD P was observed (González-Vega et al., 2016a). However, results of the current experiment demonstrated that the negative effects of increasing dietary Ca on ADG and final BW is greater if the concentration of P is below the requirement than if dietary P is at the requirement, which is in agreement with data reported in White Pekin ducklings (Xie et al., 2009) and pigs (González-Vega et al., 2016b; Merriman et al., 2017). It is possible that the reason for the negative impact of dietary Ca on ADG is that complexes between Ca and P may be formed in the gastrointestinal tract, which prevents P from being properly absorbed (Stein et al., 2011; González-Vega and Stein, 2014). Indeed, as was demonstrated in this study, if the concentration of dietary STTD P is above the requirement, increasing concentration of STTD Ca increased ADG and final BW, which may be an indication that the negative impact of Ca at low dietary concentrations of P is a result of reduced absorption of dietary P as has been previously demonstrated (Stein et al., 2011). In fact, the observation that if the concentration of STTD Ca is low and the concentration of STTD P is above the requirement, the ADG was less than if the concentration of STTD Ca is at the requirement, indicates that binding of Ca by excess P in the intestinal tract may also take place. This demonstrates how Ca and P interact in the intestinal tract of pigs and indicates the need for formulating diets with an appropriate ratio between the 2 minerals. The observation that G:F in response to increased STTD Ca was reduced, not affected, or improved in diets with STTD P below, at, or above the requirement, respectively, supports the hypothesis that the dietary Ca:P ratio is important for ADFI and ADG of pigs.

Diets that include distiller's dried grains with solubles or other co-products may sometimes contain more P than required by growing-finishing pigs and the present data indicate that diets with STTD P above the requirement also need to contain STTD Ca above the requirement to maximize G:F. This observation further demonstrates that the ratio between STTD Ca and STTD P may be more important than the absolute concentration in the diets.

Despite the fact that no maximum response for final BW and ADG was obtained if dietary STTD P was below the requirement (NRC, 2012), data indicated that a reduced concentration of STTD Ca will maximize final BW and ADG. Therefore, if P is provided below the requirement, dietary Ca also needs to be reduced. The observation that if the diet met the requirement for STTD P (NRC, 2012), the STTD Ca value to maximize final BW and ADG ranged from 0.33 to 0.34%, corresponding to STTD Ca:STTD P ratios from 1.20:1 to 1.25:1, respectively, indicates that if the concentration of STTD P is at the requirement, a concentration of STTD Ca that is lower than the current requirement may be beneficial to growth. Data from this experiment also indicated that if STTD P exceeds the requirement (NRC, 2012), STTD Ca:STTD P ratios above 1.5:1 will maximize growth.

The linear nature of the response to increased dietary STTD Ca for G:F obtained in this study prevents prediction of a maximum response; and therefore, it was not possible to estimate an optimal concentration of STTD Ca at each concentration of STTD P for this parameter. However, data indicated that if the STTD P in the diet is below the requirement, a reduced concentration of STTD Ca will increase G:F; whereas if the STTD P in the diet is above the requirement, greater concentrations of STTD Ca are needed to maximize G:F. The Ca to P ratio is crucial if either Ca or P is deficient, but becomes less important if the concentration of the 2

minerals in diets is above the requirement (Crenshaw, 2001; NRC, 2012), and the present data support this hypothesis.

The STTD Ca required to maximize bone ash is greater than the requirement to maximize growth performance (González-Vega et al., 2016a; González-Vega et al., 2016b; Merriman et al., 2017) and results obtained from this experiment confirm this observation. This response implies that after the requirement for Ca for growth is met, pigs are able to utilize Ca and P to synthesize bone tissue. It is, therefore, concluded that to assure adequate bone mineralization without affecting growth performance of pigs, a STTD Ca:STTD P ratio that is about 1.23:1 is needed if the concentration of STTD P is at the requirement. At this ratio, the concentration of bone ash (g per femur) will be 92% of that present if the ratio that maximizes bone ash is used. However, if the dietary STTD P is below or above the requirement, different ratios may need to be used.

Adequate concentrations of both Ca and P in diets are needed for bone mineralization to occur (Cromwell, 1998; Crenshaw, 2001). Results from this experiment are in agreement with this observation as indicated by the large value for the interaction in the bone ash (g per femur) model. Bone ash (g per femur) and bone ash percentage were increased as the dietary concentration of Ca increased, but the increase was greater if the concentration of dietary P also increased. However, increasing dietary concentrations of P considerably increased bone ash (g per femur) only if the concentration of Ca was at or above the requirement and this observation is in agreement with previous data (González-Vega et al., 2016b). This may indicate that low concentrations of dietary Ca are more limiting for bone tissue synthesis than dietary P. Bone Ca and bone P in grams per femur responded to dietary changes in STTD Ca and STTD P in a way that was similar to the responses for bone ash (g per femur).

The mineral portion of the bone is mainly composed of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], a mineral salt that has a Ca:P ratio of 2.1:1 (Crenshaw, 2001; Veum, 2010). In the current experiment, the Ca:P ratio in bone ash ranged from 2.09:1 to 2.20:1. This ratio is close to the 2.03:1 STTD Ca to STTD P ratio that was estimated in this experiment as needed to maximize bone ash (g per femur) if the concentration of STTD P is at the requirement. However, the STTD Ca:STTD P ratio to maximize bone ash (g per femur) decreased as the concentration of dietary P increased. Thus, despite feeding diets with greatly varying Ca:P ratios, the Ca:P ratio remains constant; whereas the STTD Ca:STTD P ratio to maximize bone ash varies depending on the concentration of dietary P.

Only small differences among treatments were observed for bone ash as a percentage of the dried defatted bone, which indicates that bone tissue synthesis primarily is regulated by changing the size of the bone; whereas the composition of ash in the bone remains constant. The interaction between Ca and P in the model to describe percentage of bone ash also indicates the need for both minerals in bone mineralization.

Conclusions

Growth performance is negatively affected by increasing dietary concentrations of STTD Ca if dietary P is at or below the requirement. However, the effect may be ameliorated if dietary STTD P is included above the requirement. This demonstrates the importance of the STTD Ca:STTD P ratio in formulating diets for growing pigs from 50 to 85 kg, which based on the current results, should be around 1.23:1 if the concentration of STTD P is at the requirement. In contrast, maximizing bone ash requires a greater STTD Ca:STTD P ratio, which indicates that pigs are able to utilize Ca and P for synthesis of bone tissue after the requirement for growth performance has been met.

FIGURES

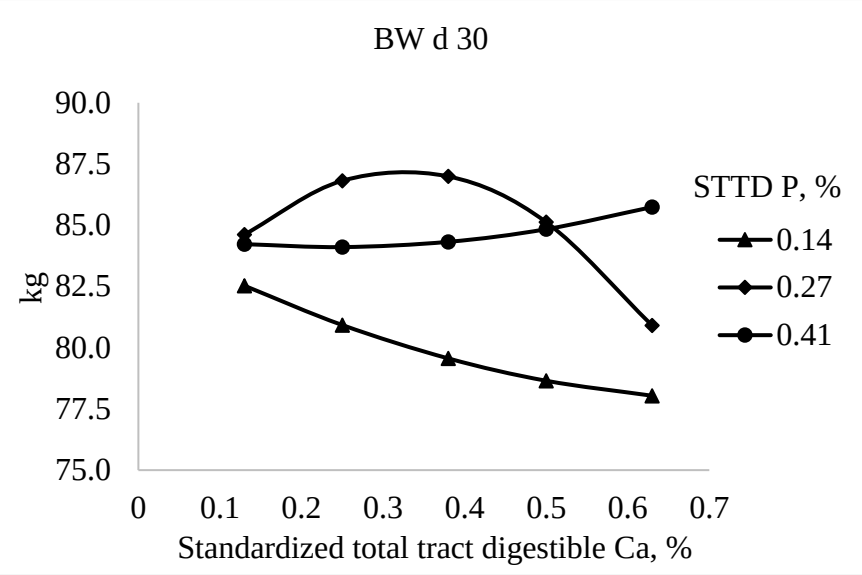

Figure 3.1. Predicted values, based on interactions between Ca and P ($P \leq 0.10$), for body weight (BW) at d 30 in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD of P. The predicted maximum BW a STTD P concentration of 0.27% was 87.2 kg at STTD Ca concentration of 0.33%. This value corresponds to a STTD Ca:STTD P ratio of 1.20:1. A maximum BW value at STTD P concentrations of 0.14 and 0.41% was not estimated because of the nature of the responses.

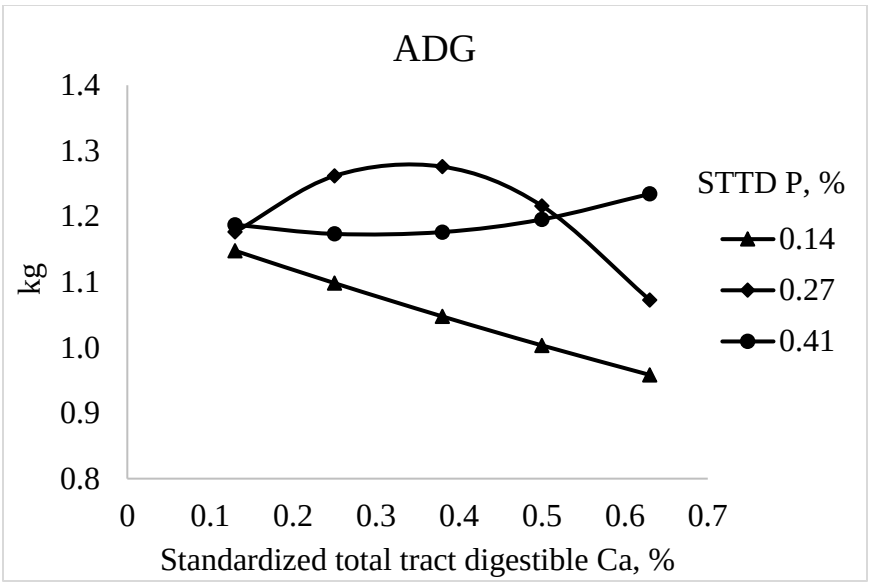

Figure 3.2. Predicted values, based on interactions between Ca and P ($P < 0.10$), for average daily gain (ADG) from d 1 to 30 in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD of P. The predicted maximum ADG at STTD P concentration of 0.27% was 1.23 kg at STTD Ca concentration of 0.34%. This value corresponds to a STTD Ca:STTD P ratio of 1.25:1. A maximum ADG value at STTD P concentrations of 0.14 and 0.41% was not estimated because of the nature of the responses.

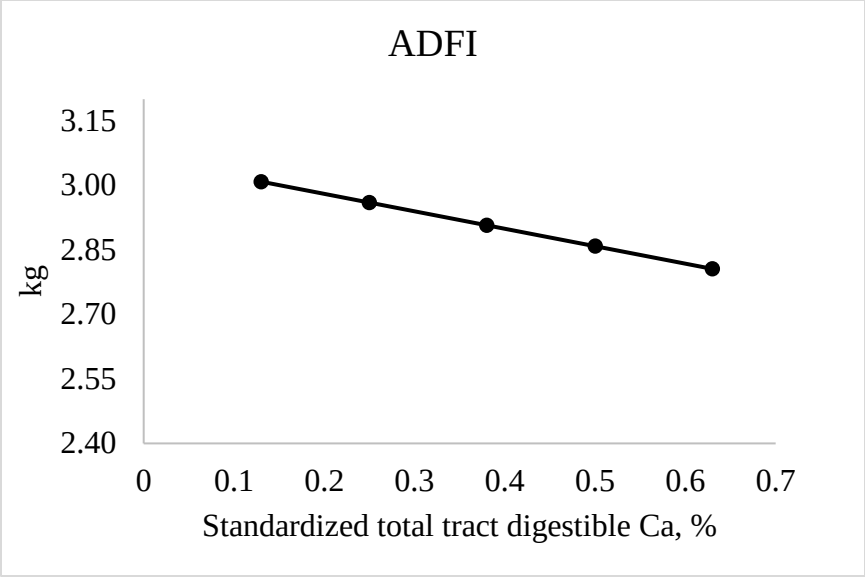

Figure 3.3. Predicted values, based on the linear effect of Ca ($P < 0.05$), for average daily feed intake (ADFI) from d 1 to 30 in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD of P.

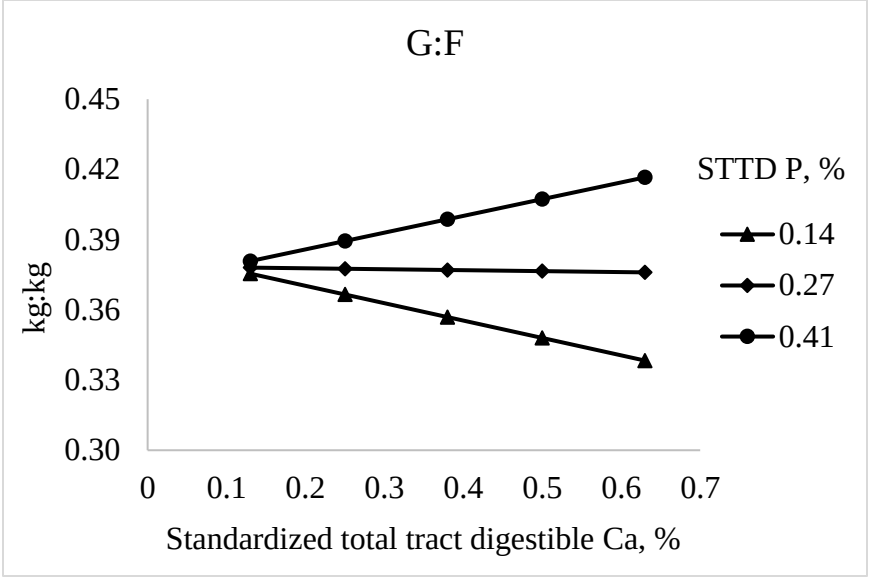

Figure 3.4. Predicted values, based on the interaction between Ca and P ($P < 0.01$), for gain to feed ratio (G:F) from d 1 to 30 in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD of P. Maximum values were not estimated because all responses were linear.

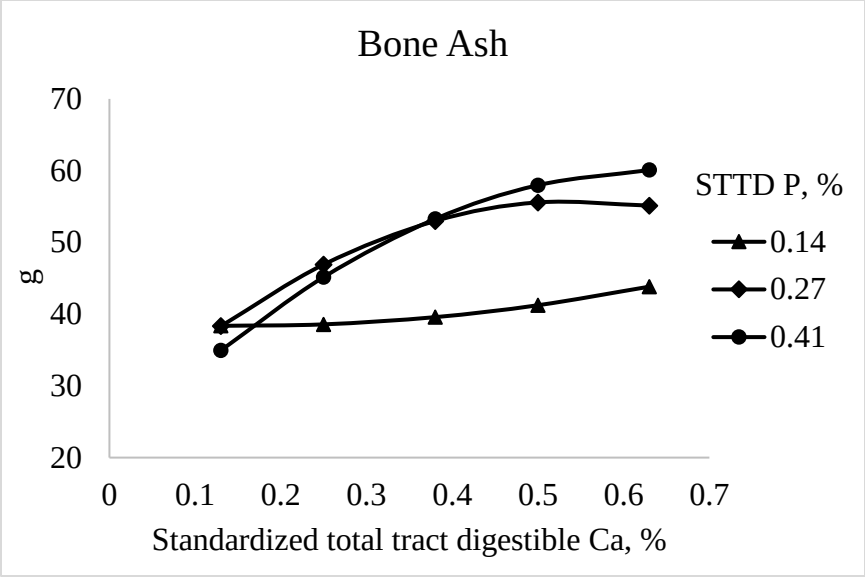

Figure 3.5. Predicted values, based on interactions between Ca and P ($P < 0.10$), for bone ash in grams per femur (g) in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD P. The predicted maximum bone ash at STTD P concentrations of 0.27 and 0.41 were 55.8 and 60.1 g at STTD Ca concentrations of 0.55, and 0.65%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.03:1, and 1.59:1. A maximum bone ash value at STTD P concentration of 0.14% was not estimated because of the nature of the response.

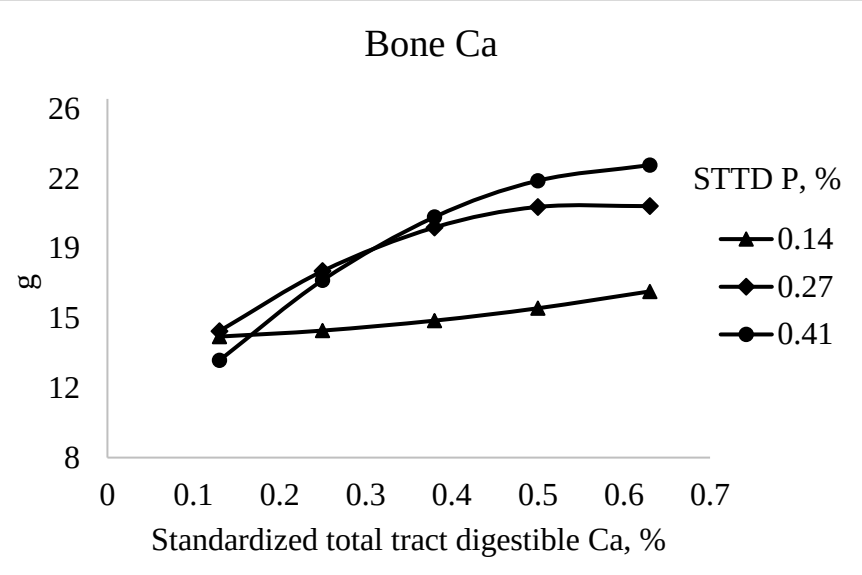

Figure 3.6. Predicted values, based on interactions between Ca and P ($P < 0.10$), for bone Ca in grams per femur (g) in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD P. The predicted maximum bone Ca at STTD P concentrations of 0.27 and 0.41 were 20.7 and 22.7 g at STTD Ca concentrations of 0.57, and 0.65%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.11:1, and 1.58:1. A maximum bone Ca value at STTD P concentration of 0.14% was not estimated because of the nature of the response.

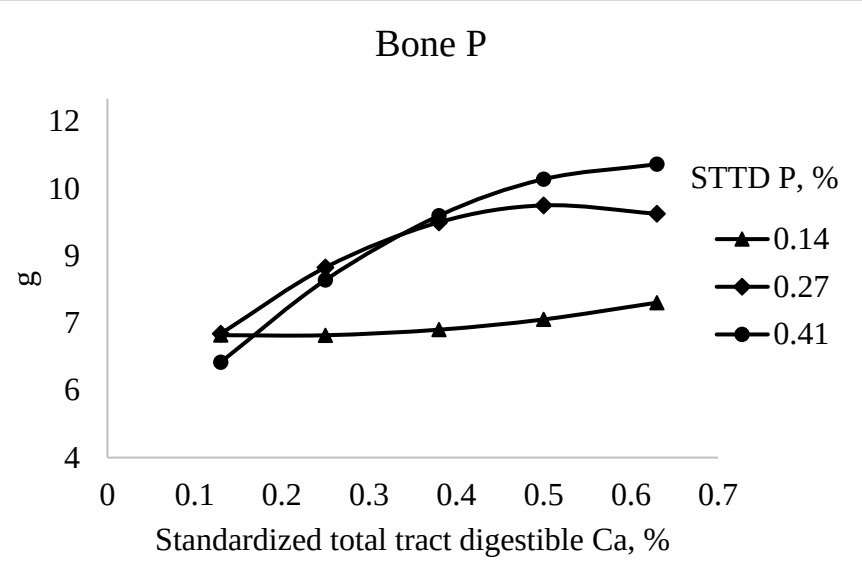

Figure 3.7. Predicted values, based on interactions between Ca and P ($P < 0.10$), for bone P in grams per femur (g) in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD P. The predicted maximum bone P at STTD P concentrations of 0.27 and 0.41 were 9.6 and 10.5 g at STTD Ca concentrations of 0.53, and 0.64%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.95:1, and 1.56:1. A maximum bone P value at STTD P concentration of 0.14% was not estimated because of the nature of the response.

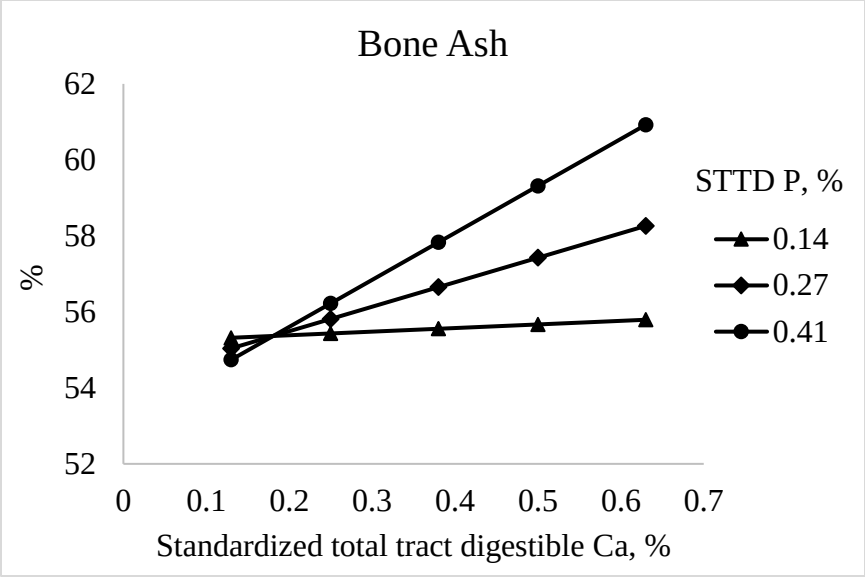

Figure 3.8. Predicted values, based on the interaction between Ca and P ($P < 0.05$), for percentage of bone ash (%) in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD of P. Maximum values were not estimated because all responses were linear.

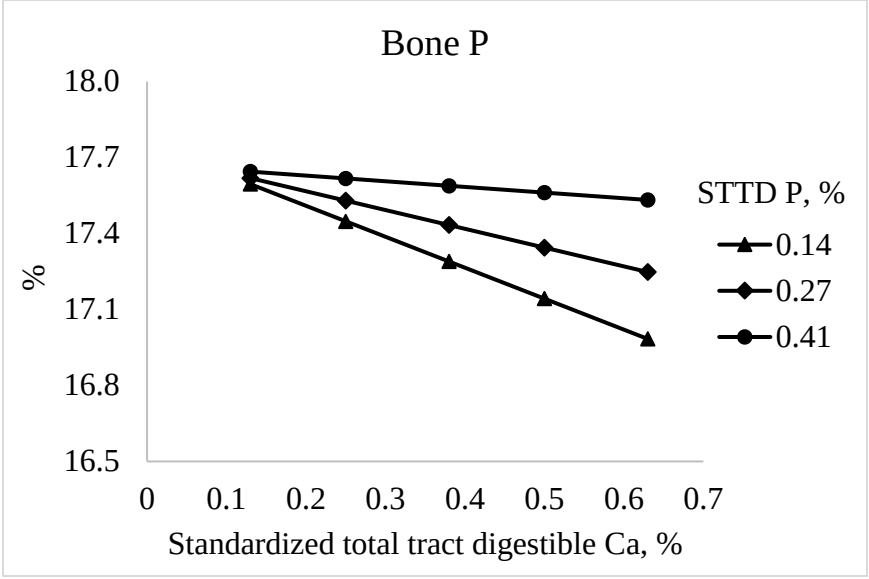

Figure 3.9. Predicted values, based on the interaction between Ca and P ($P < 0.05$), for percentage of bone P (%) in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD of P. Maximum values were not estimated because all responses were linear.

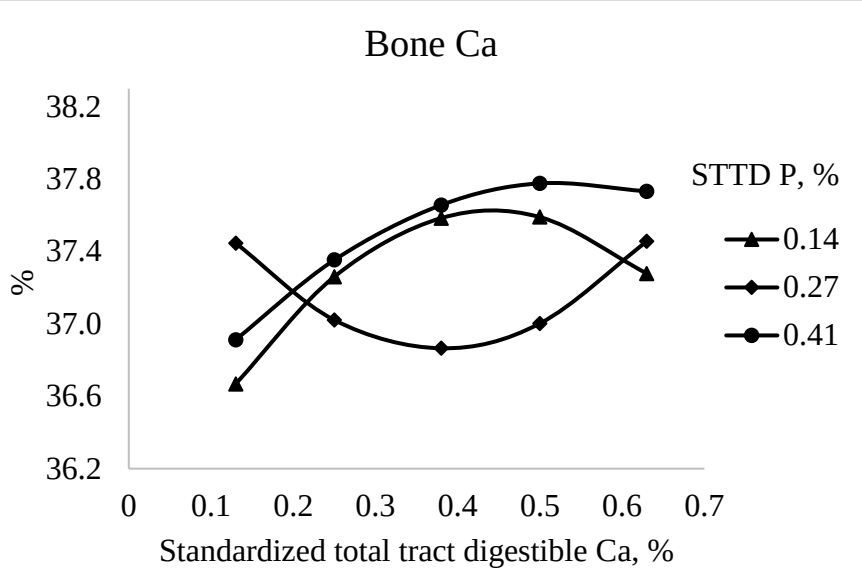

Figure 3.10. Predicted values, based on interactions between Ca and P ($P < 0.01$), for percentage of bone Ca (%) in pigs fed diets containing from 0.13 to 0.63% standardized total tract digestible (STTD) Ca and from 0.14 to 0.41% STTD P. The predicted maximum bone Ca at STTD P concentrations of 0.14 and 0.41 were 37.6 and 37.8% at STTD Ca concentrations of 0.44, and 0.53%, respectively. These values correspond to STTD Ca:STTD P ratios of 3.16:1, and 1.30:1. A maximum percentage of bone Ca value at STTD P concentration of 0.27% was not estimated because of the nature of the response.

TABLES

Table 3.1. Ingredient composition of experimental diets containing 0.14%, 0.27%, or 0.41% standardized total tract digestible (STTD) P, as-fed basis

Ingredient, %	0.14% STTD P					0.27% STTD P					0.41% STTD P				
Total Ca, % :	0.18	0.38	0.59	0.80	1.00	0.18	0.38	0.59	0.80	1.00	0.18	0.38	0.59	0.80	1.00
STTD Ca, % :	0.13	0.25	0.37	0.50	0.63	0.13	0.25	0.37	0.50	0.63	0.13	0.25	0.37	0.50	0.63
Corn	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00	65.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Cornstarch	12.96	11.36	9.84	8.29	6.74	12.48	10.90	9.38	7.85	6.29	12.01	10.43	8.92	7.39	5.82
Choice white grease	-	1.06	2.05	3.05	4.07	0.32	1.36	2.35	3.33	4.37	0.63	1.67	2.65	3.63	4.68
Calcium carbonate	0.08	0.62	1.15	1.70	2.23	0.08	0.62	1.15	1.70	2.22	0.08	0.62	1.15	1.70	2.22
Monocalcium phosphate	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Monosodium phosphate	-	-	-	-	-	0.58	0.58	0.58	0.58	0.58	1.21	1.21	1.21	1.21	1.21
Sodium bicarbonate	1.05	1.05	1.05	1.05	1.05	0.63	0.63	0.63	0.63	0.63	0.16	0.16	0.16	0.16	0.16
L-Lys HCl	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
DL-Met	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L-Thr	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Sodium chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

Table 3.1. (Cont.)

Vitamin mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[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, 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 3.2. Analyzed composition of experimental diets containing 0.14%, 0.27%, or 0.41% standardized total tract digestible (STTD) P, as-fed basis[1]

Item	0.14% STTD P					0.27% STTD P					0.41% STTD P				
Total P, % :	0.31	0.31	0.31	0.31	0.31	0.45	0.45	0.45	0.45	0.45	0.61	0.61	0.61	0.61	0.61
Total Ca, % :	0.18	0.38	0.59	0.80	1.00	0.18	0.38	0.59	0.80	1.00	0.18	0.38	0.59	0.80	1.00
STTD Ca, % :	0.13	0.25	0.37	0.50	0.63	0.13	0.25	0.37	0.50	0.63	0.13	0.25	0.37	0.50	0.63
GE, kcal/kg	3,831	3,888	3,912	3,968	3,979	3,869	3,930	3,969	3,958	4,062	3,904	3,886	3,920	3,969	4,005
DM, %	87.56	88.07	89.01	88.55	88.70	88.22	87.96	88.02	87.99	89.33	88.07	88.14	87.68	87.70	88.16
CP, %	14.47	14.68	15.07	13.90	14.91	14.72	14.38	14.18	14.56	14.48	14.56	14.90	14.94	15.33	14.44
Ash, %	3.12	3.46	3.88	4.76	5.37	3.27	3.57	4.13	4.65	5.20	3.76	4.13	4.55	5.14	5.64
AEE[2], %	2.46	3.03	3.64	4.96	5.97	2.56	3.26	4.45	5.58	6.85	2.82	3.82	5.32	5.97	6.87
ADF, %	2.44	2.27	2.12	2.26	2.38	2.25	2.41	2.48	2.47	2.85	2.91	2.71	3.17	2.93	2.78
NDF, %	6.16	6.24	6.14	6.21	6.52	6.24	6.16	7.04	7.32	6.74	7.23	7.24	7.36	7.10	7.05
Ca, %	0.18	0.35	0.58	0.76	0.98	0.18	0.39	0.53	0.84	1.02	0.14	0.31	0.54	0.74	0.99
P, %	0.35	0.35	0.37	0.36	0.37	0.51	0.47	0.47	0.50	0.49	0.65	0.63	0.63	0.65	0.62
Phytate[3], %	1.00	0.80	0.85	0.66	0.81	0.71	0.81	0.73	0.87	0.92	0.94	0.79	0.77	0.83	0.96
Phytate-bound P, %	0.28	0.23	0.24	0.19	0.23	0.20	0.23	0.21	0.25	0.26	0.27	0.22	0.22	0.23	0.27
Non-phytate P[4], %	0.07	0.13	0.13	0.17	0.14	0.31	0.24	0.26	0.25	0.23	0.39	0.41	0.41	0.41	0.35
Na, %	0.32	0.33	0.34	0.33	0.31	0.33	0.27	0.31	0.33	0.34	0.33	0.32	0.33	0.33	0.33

Table 3.2. (Cont.)

Cl, %	0.17	0.16	0.18	0.16	0.17	0.17	0.18	0.15	0.16	0.16	0.15	0.15	0.16	0.17	0.15
K, %	0.72	0.71	0.72	0.74	0.74	0.76	0.72	0.70	0.70	0.71	0.71	0.69	0.69	0.70	0.67
DCAD[5], mEq/kg	277	281	285	289	278	293	249	273	277	286	283	270	275	277	272
Total Ca: total P	0.58:1	1.23:1	1.90:1	2.58:1	3.23:1	0.40:1	0.84:1	1.31:1	1.78:1	2.22:1	0.30:1	0.62:1	0.97:1	1.31:1	1.64:1
Total Ca: STTD P	1.29:1	2.71:1	4.21:1	5.71:1	7.14:1	0.67:1	1.41:1	2.19:1	2.96:1	3.70:1	0.44:1	0.93:1	1.44:1	1.95:1	2.44:1
STTD Ca: STTD P	0.93:1	1.79:1	2.64:1	3.57:1	4.50:1	0.48:1	0.93:1	1.41:1	1.85:1	2.33:1	0.32:1	0.61:1	0.93:1	1.22:1	1.54:1

[1]All diets were formulated to have the following quantities of NE (kcal/kg), CP (%), AA (expressed as standardized ileal digestible AA; %), and minerals (%): NE, 2,609; CP, 14.90; Arg, 0.86; His, 0.36; Ile, 0.53; Leu, 1.18; Lys, 0.85; Met, 0.24; Phe, 0.64; Thr, 0.52; Trp, 0.15; Val, 0.59; Na, 0.37; Cl, 0.20; and K, 0.68.

[2]AEE = acid ether extract.

[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 3.3. Analyzed composition of ingredients used in diets, as fed basis

Item	Corn	Soybean meal	Calcium carbonate	Monocalcium phosphate	Monosodium phosphate	Sodium bicarbonate	Sodium chloride
GE, kcal /kg	3,721	4,278	-	-	-	-	-
DM, %	83.70	89.75	99.98	91.83	98.50	63.21	99.99
Ash, %	1.18	6.78	93.78	80.56	90.08	63.10	100.00
CP, %	7.05	50.97	-	-	-	-	-
AEE[1], %	3.60	1.50	-	-	-	-	-
ADF, %	2.37	4.51	-	-	-	-	-
NDF, %	7.32	9.33	-	-	-	-	-
Ca, %	0.03	0.33	38.70	16.85	0.14	0.05	0.26
P, %	0.29	0.71	0.06	20.81	24.10	0.04	0.03
Na, %	0.01	ND[2]	0.05	0.05	19.80	26.70	39.10
Cl, %	0.07	0.05	0.05	0.05	0.05	0.05	59.09
K, %	0.47	2.49	0.04	0.12	0.20	0.01	0.02

[1]AEE = acid hydrolyzed ether extract.

[2]ND = not detectable.

Table 3.4. Least squares means for growth performance of pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and STTD P for 30 d[1]

	STTD Ca, %				
Item	0.13	0.25	0.38	0.50	0.63
Initial BW, kg[2]					
0.14% STTD P	49.53	49.72	49.32	50.25	50.75
0.27% STTD P	50.72	50.73	50.07	50.08	50.28
0.41% STTD P	49.87	50.92	50.43	50.13	50.40
ADG, kg[3,4]					
0.14% STTD P	1.07	1.10	0.94	0.95	0.91
0.27% STTD P	1.10	1.24	1.20	1.15	1.02
0.41% STTD P	1.14	1.10	1.10	1.19	1.16
ADFI, kg[5,6]					
0.14% STTD P	2.94	3.00	2.54	2.83	2.76
0.27% STTD P	3.02	3.14	3.10	3.00	2.70
0.41% STTD P	3.00	2.85	2.81	2.93	2.87
G:F, kg:kg[7,8]					
0.14% STTD P	0.36	0.37	0.37	0.34	0.33
0.27% STTD P	0.37	0.40	0.39	0.38	0.38
0.41% STTD P	0.38	0.39	0.39	0.41	0.41
Final BW, kg[9,10]					
0.14% STTD P	81.52	82.65	77.59	78.72	77.90
0.27% STTD P	83.79	87.87	86.05	84.49	80.94

Table 3.4. (Cont.)

0.41% STTD P	84.10	83.82	83.27	85.62	85.10

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

[2]Standard error of the within treatment least squares means = 0.91.

[3]Results indicated that ADG from d 1 to 30 at different combinations of STTD Ca and STTD P could be described by the following model: $1.799 – 6.886 \times Ca + 8.473 \times Ca^2 – 6.200 \times P + 11.383 \times P^2 + 61.560 \times Ca \times P – 80.933 \times Ca^2 \times P – 111.128 \times Ca \times P^2 + 150.289 \times Ca^2 \times P^2$ ($P < 0.001$).

[4]Standard error of the within treatment least squares means = 0.07.

[5]Results indicated that ADFI from d 1 to 30 at different combinations of STTD Ca and STTD P could be described by the following model: $3.061 – 0.406 \times Ca$ ($P < 0.05$).

[6]Standard error of the within treatment least squares means = 0.14.

[7]Results indicated that G:F from d 1 to 30 at different combinations of STTD Ca and STTD P could be described by the following model: $0.392 – 0.150 \times Ca – 0.050 \times P + 0.540 \times Ca \times P$ ($P < 0.001$).

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

[9]Results indicated that final BW at different combinations of STTD Ca and STTD P could be described by the following model: $99.218 – 199.878 \times Ca + 260.540 \times Ca^2 – 139.734 \times P + 254.456 \times P^2 + 1{,}728.893 \times Ca \times P – 2{,}384.581 \times Ca^2 \times P – 3{,}057.698 \times Ca \times P^2 + 4{,}329.226 \times Ca^2 \times P^2$ ($P < 0.001$).

[10]Standard error of the within treatment least squares means = 2.13.

Table 3.5. Least squares means for concentration of bone ash, bone Ca, and bone P in grams per femur from pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and P[1]

	STTD Ca, %				
Item	0.13	0.25	0.38	0.50	0.63
Bone ash, g[2,3]					
0.14% STTD P	37.28	40.62	39.78	39.03	44.87
0.27% STTD P	38.75	46.57	51.40	57.95	54.06
0.41% STTD P	35.07	44.85	53.43	58.05	60.02
Bone Ca, g[4,5]					
0.14% STTD P	13.68	15.10	14.95	14.70	16.71
0.27% STTD P	14.53	17.21	18.92	21.54	20.19
0.41% STTD P	12.96	16.72	20.10	22.03	22.60
Bone P, g[6,7]					
0.14% STTD P	6.56	7.05	6.89	6.73	7.62
0.27% STTD P	6.87	8.12	8.96	10.12	9.21
0.41% STTD P	6.19	7.83	9.43	10.29	10.50
Ca:P in bone[8]					
0.14% STTD P	2.09:1.00	2.14:1.00	2.17:1.00	2.18:1.00	2.20:1.00
0.27% STTD P	2.12:1.00	2.12:1.00	2.11:1.00	2.13:1.00	2.19:1.00
0.41% STTD P	2.09:1.00	2.14:1.00	2.13:1.00	2.14:1.00	2.16:1.00

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

Table 3.5. (Cont.)

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[2]Results indicated that bone ash at different combinations of STTD Ca and STTD P could be described by the following model: $62.457 – 249.502 \times Ca + 300.275 \times Ca^2 – 202.482 \times P + 246.359 \times P^2 + 2{,}156.740 \times Ca \times P – 2{,}497.209 \times Ca^2 \times P – 3{,}060.602 \times Ca \times P^2 + 3{,}756.777 \times Ca^2 \times P^2$ ($P < 0.001$).

[3]Standard error of the within treatment least squares means = 1.76.

[4]Results indicated that bone Ca at different combinations of STTD Ca and STTD P could be described by the following model: $19.645 – 69.705 \times Ca + 84.267 \times Ca^2 – 46.505 \times P + 40.071 \times P^2 + 613.039 \times Ca \times P – 703.160 \times Ca^2 \times P – 797.651 \times Ca \times P^2 + 994.936 \times Ca^2 \times P^2$ ($P < 0.001$).

[5]Standard error of the within treatment least squares means = 0.68.

[6]Results indicated that bone P at different combinations of STTD Ca and STTD P could be described by the following model: $10.819 – 43.785 \times Ca + 53.044 \times Ca^2 – 33.838 \times P + 39.557 \times P^2 + 376.109 \times Ca \times P – 445.098 \times Ca^2 \times P – 527.786 \times Ca \times P^2 + 669.295 \times Ca^2 \times P^2$ ($P < 0.001$).

[7]Standard error of the within treatment least squares means = 0.32.

[8]Standard error of the within treatment least squares means = 0.01.

Table 3.6. Least squares means for percentage of bone ash, bone Ca, and bone P in pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and P[1]

	STTD Ca, %				
Item	0.13	0.25	0.38	0.5	0.6
Bone ash, %[2,3]					
0.14% STTD P	55.20	55.94	53.40	54.80	56.32
0.27% STTD P	54.51	56.02	58.39	58.45	57.48
0.41% STTD P	53.65	56.15	57.77	60.05	59.40
Bone Ca, %[4,5]					
0.14% STTD P	36.70	37.20	37.58	37.65	37.25
0.27% STTD P	37.50	36.93	36.82	37.15	37.38
0.41% STTD P	36.97	37.27	37.60	37.93	37.67
Bone P, %[6,7]					
0.14% STTD P	17.56	17.35	17.30	17.21	16.96
0.27% STTD P	17.71	17.41	17.41	17.43	17.04
0.41% STTD P	17.65	17.43	17.63	17.70	17.46

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

[2]Results indicated that percentage of bone ash at different combinations of STTD Ca and STTD P could be described by the following model: $56.265 – 4.973 \times Ca – 7.645 \times P + 42.296 \times Ca \times P$ ($P < 0.001$).

[3]Standard error of the within treatment least squares means = 1.10.

[4]Results indicated that percentage of bone Ca at different combinations of STTD Ca and STTD P could be described by the following model: $28.368 + 55.397 \times Ca – 65.744 \times Ca^2 +$

Table 3.6. (Cont.)

$69.642 \times P - 122.900 \times P^2 - 444.172 \times Ca \times P + 530.648 \times Ca^2 \times P + 787.728 \times Ca \times P^2 - 934.965 \times Ca^2 \times P^2$ ($P < 0.001$).

[5]Standard error of the within treatment least squares means = 0.25.

[6]Results indicated that percentage of bone P at different combinations of STTD Ca and STTD P could be described by the following model: $17.795 - 1.740 \times Ca - 0.298 \times P + 3.698 \times Ca \times P$ ($P < 0.001$).

[7]Standard error of the within treatment least squares means = 0.11.

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CHARTER 4: INFLUENCE OF DIGESTIBLE CALCIUM AND PHOSPHORUS ON GROWTH PERFORMANCE, BONE MINERALIZATION, PLASMA CALCIUM, AND EXPRESSION OF GENES INVOLVED IN INTESTINAL ABSORPTION OF CALCIUM IN PIGS FROM 11 TO 25 KG

ABSTRACT

A 21-day experiment was conducted to test the hypothesis that the requirement for Ca to maximize growth performance expressed as the standardized total tract digestible (STTD) Ca to STTD P ratio is less than 1.40:1. The second hypothesis was that increasing dietary Ca increases plasma Ca concentration and downregulates expression of genes related to intestinal Ca absorption (i.e., *TRPV6*, *S100G*, and *ATP2B1*) and tight junction proteins (i.e., *OCLN*, *CLDN1*, and *ZO1*) in the duodenum. Twenty corn-soybean meal diets were formulated using a 4 × 5 factorial design with diets containing 0.16, 0.33, 0.42, or 0.50% STTD P, and 0.14, 0.29, 0.44, 0.59, or 0.74% STTD Ca. Six hundred and forty pigs (initial BW: 11.1 ± 1.4 kg) were allotted to 20 diets and 5 blocks in a randomized complete block design. On day 21, weights of pigs and feed left in feeders were recorded and ADG, ADFI, and G:F were calculated for each diet. One pig per pen was euthanized and blood, duodenal tissue, and the right femur were collected. Concentration and percentage of ash, Ca, and P were determined in the dried defatted femurs. Gene expression was determined in duodenal tissue via quantitative RT-PCR. Data were analyzed using a response surface model in NLREG by removing the terms that were not significant ($P > 0.10$). Results indicated interactions ($P < 0.01$) between dietary concentrations of STTD Ca and STTD P for ADG, ADFI, G:F, concentration (g per femur) of bone ash, Ca, and P, and percentage of bone ash. The predicted maximum ADG (614 g), G:F (0.65 g:g), and bone ash

(11.68 g) at 0.33% STTD P was obtained at STTD Ca:STTD P ratios of 1.39:1, 1.25:1, and 1.66:1, respectively. Plasma Ca concentration was positively affected by increasing STTD Ca (9.64 to 13.30 mg/dL; quadratic, $P < 0.01$) and negatively affected by increasing STTD P (13.03 to 11.06 mg/dL; linear, $P < 0.01$). There was a linear negative effect ($P < 0.05$) of STTD Ca on the expression of *S100G*, *TRPV6*, *OCLN*, and *Z01*, indicating that increased dietary Ca reduces transcellular absorption of Ca and increases paracellular absorption of Ca. In conclusion, the STTD Ca:STTD P ratio needed to maximize growth performance of 11- to 25-kg pigs is less than 1.40:1, if P is at the requirement. Increasing dietary Ca decrease expression of genes related to Ca absorption and transport and tight junctions proteins in the duodenum.

Key words: requirement, digestible calcium, growth, bone ash, calcium absorption, pigs

INTRODUCTION

Requirements for P has been expressed as standardized total tract digestible (**STTD**) P, and although it is believed that diets for pigs will be more accurately formulated based on a STTD Ca:STTD P ratio, Ca requirements have been expressed as total Ca because of a lack of data about the digestibility of Ca in feed ingredients (NRC, 2012). However, recent work has generated values for STTD of Ca in most Ca containing feed ingredients (Stein et al., 2016b), which has allowed for formulating diets based on STTD Ca. It has also been demonstrated that a ratio between STTD Ca and STTD P that is less than 1.35:1, 1.25:1, and 1.10:1 maximizes growth performance of pigs from 25 to 50 kg (González-Vega et al., 2016b), 50 to 85 kg (Lagos et al., 2018), and 100 to 130 kg (Merriman et al., 2017), respectively, if STTD P is provided at the requirement (NRC, 2012). It was also demonstrated that the STTD Ca:STTD P ratio needed to maximize bone ash is greater than the ratio needed to maximize growth performance

(González-Vega et al., 2016b; Merriman et al., 2017; Lagos et al., 2018). An attempt to estimate the requirement for STTD Ca by pigs from 11 to 25 kg was also made, but due to a reduction in ADG and G:F as dietary Ca increased, an optimal STTD Ca:STTD P ratio could not be estimated (González-Vega et al., 2016a).

Calcium is absorbed by transcellular or paracellular transport (Bronner, 2003). Transcellular transport is the primary route if dietary Ca is low and this absorption is stimulated by vitamin D (Bouillon et al., 2003). In contrast, if dietary Ca is adequate or high, increased quantities of Ca are absorbed using paracellular absorption via the tight junctions (Pérez et al., 2008). Data for effects of dietary Ca on expression of transcellular transporters for Ca in the jejunum and kidneys of pigs were reported (González-Vega et al., 2016a), but there are limited data demonstrating effects of dietary Ca on the expression of genes related to paracellular transport of Ca in pigs. Therefore, the objectives of this experiment were to test the hypotheses that a STTD Ca:STTD P ratio less than 1.40:1 maximizes growth performance of pigs from 11 to 25 kg and that increasing dietary Ca increases plasma concentration of Ca and downregulates expression of genes related to transcellular absorption and transport of Ca and tight junction proteins in the duodenum.

MATERIAL AND METHODS

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

Animals and Housing

Six hundred and forty pigs (initial average BW: 11.1 ± 1.4 kg) were randomly allotted to 20 diets and 5 blocks in a randomized complete bock design. Blocks 1, 4, and 5 had 2 replicate pens per diet (40 pens) and blocks 2 and 3 had 1 replicate pen per diet (20 pens). Therefore, there were 8 replicate pens per diet in total. There were 2 barrows and 2 gilts in each pen and pens had fully slatted floors, a feeder, and a nipple drinker. Feed and water were available at all times. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding

Twenty corn-soybean meal based diets were formulated to have different concentrations of Ca and P, but the amount of corn and soybean meal was constant among diets to keep the concentration of phytate constant (Table 4.1). Diets were formulated using a 4 × 5 factorial design with diets containing 0.16, 0.33, 0.42, or 0.50% STTD P, and 0.21, 0.45, 0.70, 0.94, or 1.19% total Ca, which correspond to 0.14, 0.29, 0.44, 0.59, and 0.74% STTD Ca, respectively (Table 4.2 and Table 4.3). Concentrations of dietary P ranged from 50 to 150% of the STTD P requirement (NRC, 2012), and dietary Ca ranged from 30 to 170% of the requirement (NRC, 2012). The concentration of P was increased in the diets by increasing the monosodium phosphate inclusion, but to maintain a constant concentration of Na among diets, inclusion of sodium bicarbonate was reduced as the concentration of monosodium phosphate increased.

Growth Performance, Sample collection, and Bone Measurements

Pigs were allotted to the experimental diets at d 20 post-weaning and they were allowed ad libitum access to feed for 21 d. The amount of feed offered was recorded daily and at the conclusion of the experiment, the amount of feed in the feeders was recorded. Pig weights were recorded on d 1 and 21 when pigs had an average BW of 22.4 ± 3.3 kg.

On the last day of the experiment, 1 barrow in each pen with a BW closest to the average BW of the pen was euthanized via captive bolt stunning. Blood samples were collected and immediately centrifuged and plasma samples were collected and stored at -20ºC for analysis of Ca and P. The gastrointestinal tract was removed and duodenal tissue samples were collected, immediately snap-frozen in liquid N, and stored at -80ºC for gene expression analysis.

The right femur was collected and autoclaved at 125ºC for 55 min. Femurs were broken, dried, and soaked for 72 h in petroleum ether under a chemical hood to remove marrow and fat. Femurs were dried for 2 h at 135ºC and then ashed at 600ºC for 16 h.

Sample Analysis

Corn, soybean meal, lactose, calcium carbonate, monocalcium phosphate, monosodium phosphate, sodium bicarbonate, NaCl, and diets were analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007). Ingredients (except lactose) and diets were analyzed for Na and K by flame emission photometry (Method 956.01; AOAC Int., 2007) and Cl by manual titration (Method 9.15.01, 943.01; AOAC Int., 2006). Ingredients, diets, bone ash, and plasma samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and D; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Corn, soybean meal, lactose, and diets were analyzed for GE using bomb calorimetry (Model 6400, Parr Instruments, Moline, IL). Corn, soybean meal, and diets were also analyzed for CP using the Kjeldahl method by quantifying N and using a conversion factor of 6.25 to calculate CP (Method 984.13; AOAC Int., 2007); a Kjeltec^{TM} 8400 (FOSS, Eden Prairie, MN) was used. Acid hydrolyzed ether extract (Method 2003.06; AOAC Int., 2007) was analyzed on an Ankom^{XT15} (Ankom Technology, Macedon, NY), and ADF and NDF was analyzed using Ankom

Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Phytate-bound P was predicted in corn, soybean meal, and diets by near infra-red reflectance spectroscopy (ESC Standard Analytical Method, SAM120; AB Vista, Memphis, TN).

RNA Extraction and Quantitative Reverse-Transcription PCR.

The RNA was extracted from 30 ± 0.2 mg of frozen duodenal tissue using β-mercaptoethanol (Alfa Aesar, Tewksbury, MA) according to the RNeasy Mini Kit (QIAGEN, Germantown, MD) manufacturer's instructions. Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The RNA quality was determined using a Fragment Analyzer™ Automated CE System (Method DNF-471-33 - SS Total RNA 15nt; Advanced Analytical, Ankeny, IA) and RNA samples with an RNA quality number greater than 7 were used for cDNA synthesis.

A portion of the RNA was diluted to 100 ng/μL with DNase/RNase-free water for cDNA synthesis as described by Vailati-Riboni et al. (2015) using 4 μl of diluted RNA from duodenal tissue. The cDNA was then diluted 1:4 with DNase/RNase-free water, prior to qPCR analysis.

Quantitative PCR was performed using 4 μL of diluted cDNA combined with 6 μL of a mixture composed of 5 μL of SYBR Green master mix (PerfeCTa SYBR Green FastMix, ROX™; Quanta BioSciences, Beverly, MA), 0.4 μL each of 10 μM forward and reverse primers, and 0.2 μL DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA). All samples were run in duplicate using a 7-point standard curve that was developed with samples run in triplicate. Reactions were performed in a QuantStudio™ 7 Flex Real-Time PCR System (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. The presence of a single PCR product was verified by the dissociation protocol using

incremental temperatures to 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. Data were analyzed using the QuantStudio™ Real-Time PCR Software (version 1.3; Applied Biosystems, Foster City, CA).

Three internal control genes, β-actin (**ACTB**; Metzler-Zebeli et al., 2015), glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**), and hydroxymethylbilane synthase (**HMBS**; Vigors et al., 2014) were used to normalize the expression of tested genes. Tested genes included S100 calcium binding protein G (**S100G**), transient receptor potential cation channel, subfamily V, member 6 (**TRPV6**), ATPase, Ca2+ transporting, plasma membrane-1 (**ATP2B1**), Occludin (**OCLN**), Zonula occludens-1 (**ZO1**), and Claudin-1 (**CLDN1**). All these genes are important for transcellular or paracellular transport of Ca. Primers are listed in Table 4.4 and were commercially synthesized by Integrated DNA Technologies (Skokie, IL).

Calculations and Statistical Analyses

The percentage of phytate in corn, soybean meal, and diets was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004), and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. Dietary cation-anion difference (**DCAD**) was calculated using the following equation (González-Vega et al., 2016b):

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

where Na, K, and Cl were expressed as percentages of the diet. The ADG, ADFI, and G:F were calculated for each diet and concentrations of bone Ca and bone P in grams per femur were calculated by multiplying the total quantity of bone ash by the percentage of Ca and P in the bone ash. The final data from the expression of tested genes were normalized using the

geometric mean of the 3 internal control genes and the real-time quantitative PCR data were log2 transformed before statistical analysis to obtain a normal distribution.

Normality of data was tested using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data for growth performance, concentration and percentage of bone ash, bone Ca, and bone P, concentration of Ca and P in plasma, and gene expression were analyzed using the PROC MIXED of SAS with the experimental unit being the pen. 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. Assumptions of the model were tested using PROC GPLOT and influence options of SAS. Effects of dietary STTD Ca, STTD P, and the interaction between STTD Ca and STTD P were considered significant at $P \leq 0.10$. If the interaction or the main effects was significant, the software NLREG version 6.5 (Sherrod, 2008) was used to determine parameter estimates for the second-order response surface model to increasing concentrations of STTD Ca and STTD P as described by Khuri and Cornell (1996). Parameter estimates of the model that were not significant ($P > 0.10$) and were not included in a significant interaction were removed from the model and the estimates were recalculated. The surface response full model was:

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

where Y is the dependent variable, a is the intercept, b, c, d, e, f, g, h, and i are the coefficients, and STTD Ca and STTD P are the percentage concentrations of dietary STTD Ca and STTD P.

The percentage concentrations of STTD Ca at the maximum response values were calculated using the following equation:

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

where STTD Ca_{max} is the percentage 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 in the variables of interest using the respective model equations with the concentrations of STTD Ca at the maximum response for each concentration of STTD P. The LSMEANS procedure was used to calculate mean values for treatments.

RESULTS

There was 1.9% mortality observed in 6 different diets through the 5 blocks of the experiment. The ADG, ADFI, and G:F of the pen for these pigs were adjusted (Lindemann and Kim, 2007). The remaining animals consumed their diets without apparent problems and remained healthy throughout the experiment. The model to predict final BW, ADG, ADFI, and G:F was reduced because only an interaction ($P < 0.01$) between STTD Ca and STTD P was observed (Table 4.5). The predicted maximum BW at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 21.18, 23.92, 24.40, and 24.27 kg at STTD Ca concentrations of 0.31, 0.46, 0.54, and 0.61%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.93:1, 1.39:1, 1.28:1, and 1.21:1 (Fig. 4.1). The predicted maximum ADG at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 481, 614, 635, and 624 g at STTD Ca concentrations of 0.31, 0.46, 0.54, and 0.61%, respectively, corresponding to STTD Ca:STTD P ratios of 1.93:1, 1.39:1,

1.28:1, and 1.22:1 (Fig. 4.2). The predicted maximum ADFI at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 867, 946, 953, and 940 g at STTD Ca concentrations of 0.36, 0.50, 0.57, and 0.64%, respectively, which correspond to STTD Ca:STTD P ratios of 2.24:1, 1.51:1, 1.36:1, and 1.28:1 (Fig. 4.3). The predicted maximum G:F at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 0.556, 0.652, 0.671, and 0.668 g:g at STTD Ca concentrations of 0.24, 0.41, 0.50, and 0.59%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.51:1, 1.25:1, 1.20:1, and 1.17:1 (Fig. 4.4).

The full model was used to predict the concentration of bone ash, bone Ca, and bone P in g per femur (interactions, $P < 0.01$; Table 4.6). The predicted maximum bone ash at STTD P concentrations of 0.33, 0.42, and 0.50% were 11.68, 13.03, and 15.68 g at STTD Ca concentrations of 0.55, 0.63, and 1.15%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.66:1, 1.50:1, and 2.30:1 (Fig. 4.5). The predicted maximum bone Ca at STTD P concentrations of 0.33, 0.42, and 0.50% were 4.51, 5.03, and 6.41 g at STTD Ca concentrations of 0.55, 0.64, and 1.31%, respectively, corresponding to STTD Ca:STTD P ratios of 1.67:1, 1.52:1, and 2.62:1 (Fig. 4.6). The predicted maximum bone P at STTD P concentrations of 0.33, 0.42, and 0.50% were 2.14, 2.38, and 2.89 g at STTD Ca concentrations of 0.53, 0.61, and 1.19%, respectively, which correspond to STTD Ca:STTD P ratios of 1.61:1, 1.45:1, and 2.38:1 (Fig. 4.7). However, maximum bone ash, bone Ca, and bone P in g per femur at STTD P concentration of 0.16% were not estimated because of the nature of the response.

The model to predict percentage of bone ash was not reduced (interactions, $P < 0.01$; Fig. 4.8) and the predicted maximum bone ash at STTD P concentrations of 0.33, 0.42, and 0.50% were 49.84, 51.79, and 54.27% at STTD Ca concentrations of 0.56, 0.62, and 0.98%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.70:1, 1.47:1, and 1.97:1.

However, a maximum percentage of bone ash at STTD P concentration of 0.16% was not estimated because of the nature of the response. The reduced model to predict percentage of bone Ca only contained the linear ($P < 0.10$) STTD Ca and STTD P terms, whereas the reduced model to predict percentage of bone P contained the linear ($P < 0.05$) STTD Ca and STTD P terms and the quadratic ($P < 0.05$) STTD P term (Fig. 4.9 and Fig. 4.10).

In the model to predict the concentration of plasma Ca in mg/dL, there were no interactions between STTD Ca and STTD P (Table 4.7). The reduced model only contained the linear and quadratic ($P < 0.01$) STTD Ca terms and the linear ($P < 0.01$) STTD P term (Fig. 4.11). The model to predict the concentration of plasma P in mg/dL was reduced because the interactions between linear STTD Ca and STTD P terms and between linear STTD Ca and quadratic STTD P terms were not significant; however, interactions ($P < 0.01$) between quadratic STTD Ca and linear STTD P terms and between quadratic STTD Ca and STTD P terms were observed (Fig. 4.12).

Neither Ca nor P could be used to predict the expression of *ATP2B1* or *CLDN1* in the duodenum (Table 4.8). The model to predict the expression of *TRPV6*, *S100G*, *OCLN*, and *ZO1* only contained the linear ($P < 0.05$) STTD Ca term (Fig. 4.13 and Fig. 4.14).

DISCUSSION

The analyzed concentration of Ca, P, Na, Cl, and K in corn, soybean meal, calcium carbonate, monosodium phosphate, sodium bicarbonate, and NaCl was within the range of previously published values (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; Stein et al., 2016a). Diets were formulated using NRC (2012) values for Ca and P in ingredients. This may explain the differences observed between calculated and analyzed values in some diets.

However, the objective of obtaining differences in both Ca and P among diets was met. The calculated values for DCAD, which ranged from 327 to 369 mEq/kg, were within the range of DCAD values that improve growth performance in young pigs (200 to 500 mEq/kg; Dersjant-Li et al., 2001).

In an experiment that aimed at determining STTD Ca requirements by 11- to 25-kg pigs, a fixed concentration of 0.36% STTD P and 6 increasing concentrations of STTD Ca, from 0.32 to 0.72% were used (González-Vega et al., 2016a). Results indicated a negative effect of increasing dietary Ca on ADG and G:F which prevented the estimation of a STTD Ca concentration that maximized growth performance. Therefore, in this study, different concentrations of STTD P (below, at, or above the requirement; NRC, 2012) were used to estimate STTD Ca requirements at different concentrations of STTD P. The reason for including 4 concentrations of STTD P was that in a previous experiment (Lagos et al., 2018), where only 3 STTD P concentrations were used, the response to increasing dietary Ca on growth performance was different if STTD P was at or below the requirement than if STTD P was above the requirement. Therefore, in the current experiment, 2 concentrations of STTD P that were above the requirement were used.

The surface response model was chosen to determine the optimal STTD Ca:STTD P ratio following recommendations about the use of this model in factorial experiments with independent variables such as quantities of dietary components (Box and Wilson, 1951; Steel et al., 1996). By using this model, interactions between the independent and response variables can be quantified to estimate the values for the independent variables that maximize the response (Khuri and Cornell, 1996).

Results obtained in the current experiment indicated a detrimental effect on growth performance as the concentrations of Ca increased in diets that were deficient or marginal in P. This observation concurs with data reported for broiler chickens (Hurwitz et al., 1995) and pigs (González-Vega et al., 2016a). However, the negative effect of Ca was ameliorated by including P above the requirement, and this was also observed in research conducted in poultry (Xie et al., 2009; Akter et al., 2017) and pigs (González-Vega et al., 2016b; Merriman et al., 2017; Wu et al., 2017; Lagos et al., 2018). The reason for the negative response in growth performance by increasing concentrations of dietary Ca is likely the formation of Ca-P complexes in the intestinal tract due to excess Ca, which causes a reduction in P digestibility (Brink et al., 1992; Stein et al., 2011; Walk et al., 2012; González-Vega et al., 2014). As a consequence increased dietary Ca induced a P-deficiency even if dietary P was at the requirement. The fact that inclusion of P above the requirement reduces the negative effect of increasing concentrations of dietary Ca on growth performance, further indicates that the detrimental effect of excess Ca may be a result of P being improperly absorbed. The negative effect of excess Ca on the digestibility of P has been previously observed in pigs (Stein et al., 2011) and broiler chickens (Mutucumarana et al., 2014).

The observation that the concentration of STTD Ca that maximized BW, ADG, and G:F ranged from 0.24 to 0.31% corresponding to STTD Ca:STTD P ratios from 1.51:1 to 1.93:1 if dietary P was below the requirement, indicates that if STTD P is deficient in the diet, the concentration of STTD Ca also needs to be supplied below the requirement to avoid a reduction in growth performance. In contrast, if STTD P was above the requirement, Ca deficiency became the limiting factor for growth performance, as demonstrated by the gradual improvement in growth performance as dietary Ca increased from 30 to 140% of the requirement. The

observation that if STTD P concentrations were above the requirement, a concentration of STTD Ca to maximize BW, ADG, and G:F that ranged from 0.50 to 0.61%, with corresponding STTD Ca:STTD P ratios from 1.17:1 to 1.28:1 was needed, indicates that if the concentration of STTD P is above the requirement, STTD Ca also needs to be supplied above the requirement. This observation is relevant in finishing pigs fed diets with high concentration of P-rich coproducts such as distiller's dried grains with solubles, because such diets often contain P in concentrations that exceed the requirement and the current data indicate that in this situation, dietary Ca also needs to be increased to maximize growth performance.

The fact that if STTD P was included at the requirement, BW, ADG, and G:F was maximized at 0.41 to 0.46% STTD Ca, indicates that the current NRC (2012) requirement for total Ca (0.70%) likely is accurate because this level of total Ca corresponds to 0.44% STTD Ca. However, this level of dietary Ca will maximize growth performance only if dietary STTD P is at the requirement (i.e., 0.33%). The corresponding STTD Ca:STTD P ratios that maximize growth performance ranged from 1.25:1 to 1.39:1, which supports our hypothesis that a STTD Ca:STTD P ratio less than 1.40:1 maximizes growth performance for 11- to 25-kg pigs. These data also support the notion that a STTD Ca:STTD P ratio greater than 1.50:1 and 1.39:1 is detrimental to ADG and G:F, respectively (González-Vega et al., 2016a). These results further indicate that the requirement for Ca and P expressed as STTD Ca:STTD P decreases as the pig gets older.

The femur was used to estimate bone mineralization because it is believed to be an accurate indicator of the body mineral content of pigs (Crenshaw et al., 2009). The observation that bone ash, bone Ca, and bone P in g per femur were maximized at STTD Ca:STTD P ratios between 1.61:1 to 1.67:1 if STTD P was at the requirement is in agreement with data

demonstrating that a greater STTD Ca:STTD P ratio is required to maximize bone ash than to maximize growth performance (González-Vega et al., 2016b; Merriman et al., 2017; Lagos et al., 2018). The implication of this observation is that once the Ca requirement for growth has been met, Ca along with P are used to synthesize more skeletal tissue. However, if a ratio STTD Ca:STTD P of 1.35:1 is used, the concentration of bone ash (g per femur) will be 97% of that observed if a ratio of 1.66:1 is used. This further indicates that formulating diets based on STTD Ca:STTD P ratios that maximize growth performance does not dramatically affect bone mineralization.

Because the concentration (g per femur) of bone Ca and bone P was calculated by multiplying the concentration of bone ash in g per femur by the percentage of Ca and P in bone ash, the responses for concentration of bone Ca and bone P were similar to results observed for bone ash. The fact that bone ash (g per femur) was not affected by dietary Ca if STTD P was below the requirement, indicates that P deficiency was limiting bone deposition and addition of extra Ca did not ameliorate this situation. In contrast, if the concentration of STTD P was at or above the requirement (NRC, 2012), Ca deficiency limited bone deposition; thus, increasing STTD Ca increased bone ash (g per femur) up to the point were P became the limiting factor. This point was reached at around 0.50 and 0.60% STTD Ca if STTD P was at the requirement or at 130% of the requirement, respectively. For STTD P at 150% of the requirement, it appeared that Ca was the limiting nutrient for bone tissue synthesis. These responses in the concentration of bone ash (g per femur) at changing dietary Ca and P concentrations demonstrate the interaction between these 2 minerals in the skeletal tissue and the need for both Ca and P for bone deposition, which has also been previously demonstrated (Crenshaw, 2001).

The increased percentage of bone ash in pigs fed diets deficient in P as more Ca was included in the diet indicates that the lack of response in bone ash (g per femur) to increasing concentrations of Ca was a result of the size of the bones, which was smaller in pigs fed high Ca diets compared with pigs fed low Ca diets. In contrast, in pigs fed diets with adequate or excess P, the response in percentage of bone ash was similar to that observed for concentration of bone ash (g per femur). Calcium was limiting bone mineralization in low Ca diets, but P became limiting in high Ca diets, except at the highest concentration of P.

The Ca to P ratio in bone ash ranged from 2.01:1 to 2.21:1, these values are in agreement with reported data (González-Vega et al., 2016b; Merriman et al., 2017; Lagos et al., 2018) and are close to the ratio of 2.1:1 that is needed to form hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] (Crenshaw, 2001; Suttle, 2010). Although the concentration of Ca and P in bone ash is relatively constant, data from this study indicate that percentages of Ca and P in bone ash are responsive to the level of Ca in the diet. Regardless of the level of dietary P, the percentage of Ca in bone ash was increased by increasing concentrations of dietary Ca, whereas, increased dietary Ca decreased the percentage of P in bone ash, indicating a negative effect of Ca on P deposition. However, the changes in the percentage of Ca and P in bone ash were relatively small.

As mentioned, if STTD P is at the requirement, the STTD Ca:STTD P ratio that maximizes growth performance reduces as pig BW increases. However, it was also observed that the STTD Ca:STTD P ratio needed to maximize bone ash (g per femur) increases as the animal becomes heavier. Bone ash (g) was maximized at STTD Ca:STTD P ratios of 1.66:1, 1.81:1, 2.03:1, and 2.33:1 in pigs from 11 to 25 kg, 25 to 50 kg (González-Vega et al., 2016b), 50 to 85 kg (Lagos et al., 2018), and 100 to 130 kg (Merriman et al., 2017), respectively, if STTD P was provided at the requirement. A possible explanation for this observation is that young pigs were

supplied sufficient Ca through the milk while they were nursing and as a consequence when this experiment started, bone ash was already maximized and a dietary Ca:P ratio below that in bones was sufficient to maintain bone composition. In contrast, growing and finishing pigs are supplied diets that are deficient in Ca (in relation to what is needed to maximize bone ash), which limits bone tissue synthesis. Therefore, older pigs have greater capacity to increase skeletal tissue than younger pigs and a greater dietary Ca:P ratio is, therefore, needed to maximize bone ash. However, further research is needed to confirm this hypothesis.

Results from plasma analysis indicated that plasma Ca concentration is responsive to dietary Ca. The observation that as STTD P increased, plasma Ca was reduced indicates that if P is available, more bone tissue synthesis can occur, and less Ca is present in plasma. Similar effects of varying concentrations of dietary Ca and P on plasma Ca concentration were observed in 25 kg pigs (Nicodemo et al., 1998). In a similar experiment, 4 levels of dietary P and 5 levels of dietary Ca were fed to pigs from 25 to 50 kg, but dietary Ca only influenced the concentration of Ca in plasma when dietary P was deficient (González-Vega et al., 2016b). In the current experiment, the greatest variability in plasma Ca concentration was observed in the P deficient diets, whereas in diets with adequate dietary P, a narrow range of values was observed. The physiological range of serum Ca concentration is from 8 to 12 mg/dL (Amundson et al., 2017). Plasma Ca concentration is regulated by parathyroid hormone, vitamin D, and calcitonin (Veum, 2010). Phosphorus concentration in plasma, however, is less tightly regulated than Ca concentrations (Veum, 2010), and data from this experiment confirm this hypothesis.

Although it appears that Ca balance in pigs is mainly regulated in the kidneys (González-Vega et al., 2016a), the process of Ca absorption in the intestine is important for maintaining Ca homeostasis (Schröder and Breves, 2007). This process is carried out by 2 routes: transcellular

and paracellular absorption of Ca and the relative absorption during each route is dependent on the concentration of dietary Ca (Hurwitz, 1996; Taylor and Bushinsky, 2009). Transcellular absorption is the primary route under low dietary Ca conditions whereas paracellular transport is preferred if dietary Ca is at adequate or high levels (Bronner, 2003; Pérez et al., 2008).

Transcellular absorption of Ca is a 3-step saturable process that occurs mainly in the duodenum and upper jejunum and requires energy, Ca channels, and Ca-binding proteins (Gropper and Smith, 2013). First, Ca is absorbed in the enterocyte through Ca channels such as TRPV6 located in the brush border membrane (Bouillon et al., 2003; van de Graaf et al., 2004). Once in the cytosol, Ca is bound to Ca-binding proteins (calbindin-D9k), which move Ca towards the basolateral membrane (Kaune, 1996; Schwaller, 2010) to finally release it via plasma membrane Ca-ATPase activity, although a Na^{+}/Ca^{2+} exchanger has also been identified (Schröder and Breves, 2007). This process is regulated by the active form of vitamin D (1, 25-dihydroxyvitamin D3), also known as calcitriol, whose activation is stimulated by the parathyroid hormone released as a result of a low plasma Ca concentration (Eklou-Kalonji et al., 1999; Bouillon et al., 2003; Fleet and Schoch, 2010). Thus, calcitriol increases the absorption of Ca by upregulating genes related to Ca channels and transporters including TRPV6, Ca-binding proteins, and plasma membrane Ca-ATPase (Cai et al., 1993; Armbrecht et al., 2003; Kutuzova and DeLuca, 2004; Ko et al., 2009). Results from this experiment support this observation as demonstrated by the decrease in the expression of *S100G* and *TRPV6* in duodenal tissue as more Ca was included in the diet, indicating reduced transcellular absorption of Ca in the duodenum of pigs fed high Ca diets. This can also be linked to the quadratic increase in plasma Ca concentration by increasing dietary Ca, which indicates that transcellular absorption is increased in low Ca diets to elevate the concentration of Ca in plasma and is inhibited as the upper limit for

plasma Ca is approached. However, dietary Ca appeared not to influence *ATP2B1* expression. These observations are in agreement with data for Ca absorption in the jejunum of 50 kg pigs fed diets with increasing levels of Ca (González-Vega et al., 2016a). The lack of a response in *ATP2B1* expression is not surprising because the influence of calcitriol on plasma membrane Ca-ATPase activity has not been consistently observed (Schröder and Breves, 2007).

Paracellular transport of Ca is a non-saturable passive process that mainly occurs in the jejunum and ileum sections (Pérez et al., 2008). The paracellular pathway is the intracellular space that allows the movement of small molecules and ions, therefore, it needs to be regulated to maintain selective permeability (Hoenderop et al., 2005). The tight junctions are located in the apical region of the intracellular space and function as a semipermeable barrier to the passage of ions and molecules (Chiba et al., 2008). Claudins and occludin are integral membrane proteins that conform the tight junction structure and zonula occludens-1 is a peripheral membrane that binds the integral membrane proteins (Chiba et al., 2008). Paracellular absorption of Ca is, therefore, a result of high concentration of Ca in the lumen that generates an electrochemical gradient across the epithelium and influences the flux of Ca through the tight junctions (Gropper and Smith, 2013). This observation concurs with results obtained in this experiment that demonstrated that expression of *OCLN* and *ZO1* in the duodenum was reduced as more Ca was included in the diet. Even though Ca absorption via the paracellular pathway is mainly performed in the jejunum and ileum, the present data indicate that passive absorption of Ca may start in the duodenum. A negative effect of high dietary Ca on the expression of tight junction proteins was previously observed in the jejunum of young pigs (Metzler-Zebeli et al., 2015). This response indicates increased paracellular absorption of Ca by increasing dietary Ca and may imply a risk to the intestinal integrity if Ca is supplied above the requirement.

Conclusions

If the concentration of P is deficient or adequate in diets for 11- to 25-kg pigs, increasing concentrations of Ca are detrimental to growth performance. However, if P is above the requirement, the negative effect of increasing concentrations of dietary Ca is ameliorated, and increased dietary Ca may increase G:F of pigs. The STTD Ca to STTD P ratio that maximizes bone ash is greater than the ratio that maximizes growth performance, indicating that pigs can utilize more Ca and P for bone tissue synthesis than for growth performance. The STTD Ca:STTD P ratio needed to maximize growth performance of 11- to 25-kg pigs is less than 1.40:1 and about 1.35:1. Increasing dietary Ca increases plasma Ca concentration and decreases expression of genes related to transcellular absorption and transport of Ca, but increases paracellular absorption of Ca by decreasing expression of tight junction proteins in the duodenum.

FIGURES

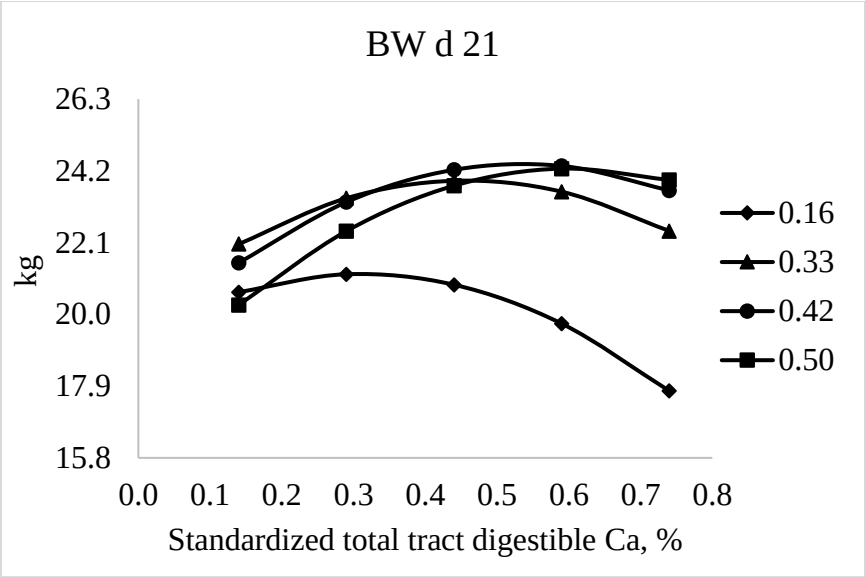

Figure 4.1. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for body weight (BW) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum BW at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 21.18, 23.92, 24.40, and 24.27 kg at STTD Ca concentrations of 0.31, 0.46, 0.54, and 0.61%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.93:1, 1.39:1, 1.28:1, and 1.21:1.

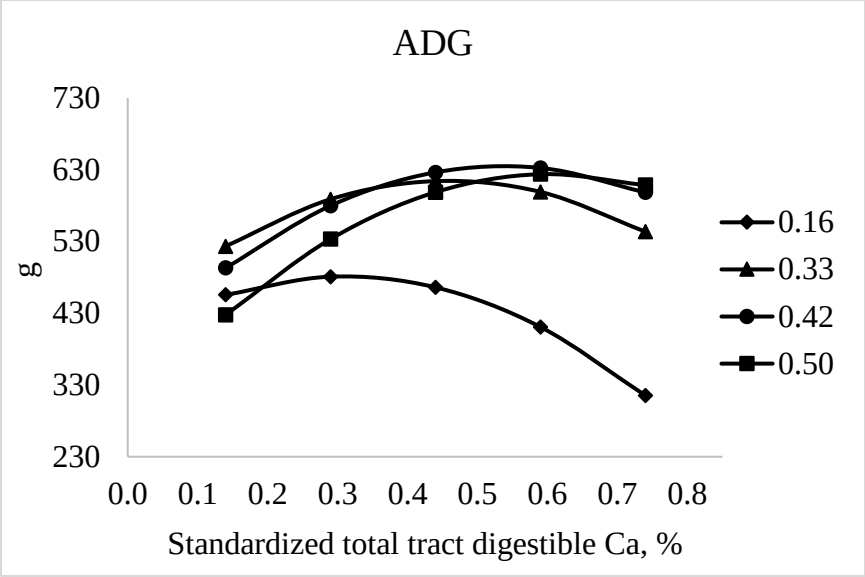

Figure 4.2. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for average daily gain (ADG) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum ADG at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 481, 614, 635, and 624 g at STTD Ca concentrations of 0.31, 0.46, 0.54, and 0.61%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.93:1, 1.39:1, 1.28:1, and 1.22:1.

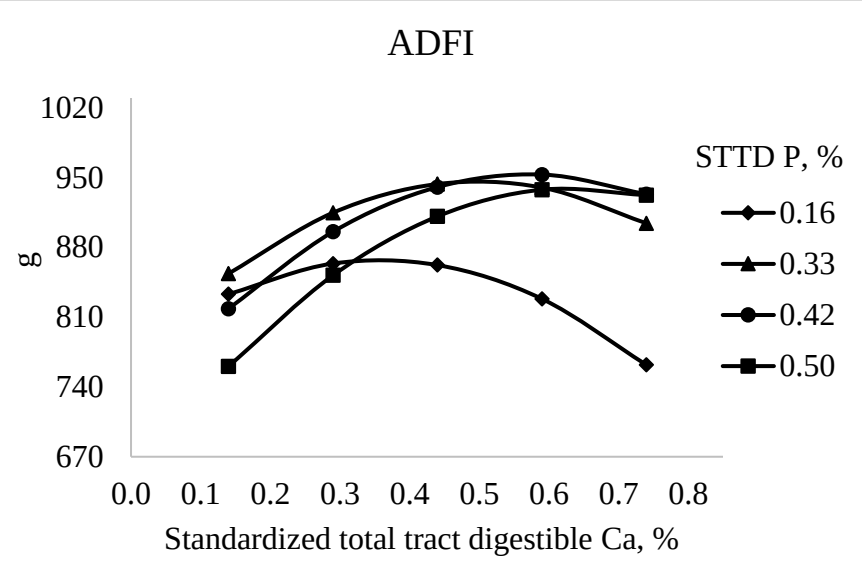

Figure 4.3. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for average daily feed intake (ADFI) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum ADFI at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 867, 946, 953, and 940 g at STTD Ca concentrations of 0.36, 0.50, 0.57, and 0.64%, respectively. These values correspond to STTD Ca:STTD P ratios of 2.24:1, 1.51:1, 1.36:1, and 1.28:1.

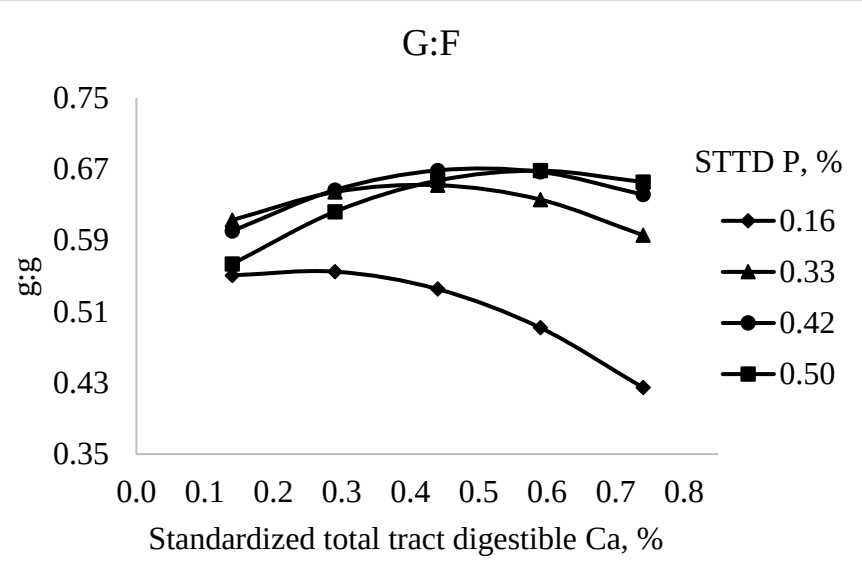

Figure 4.4. Predicted values, based on the interaction between Ca and P ($P < 0.001$), for gain to feed ratio (G:F) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum G:F at STTD P concentrations of 0.16, 0.33, 0.42, and 0.50% were 0.556, 0.652, 0.671, and 0.668 g:g at STTD Ca concentrations of 0.24, 0.41, 0.50, and 0.59%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.51:1, 1.25:1, 1.20:1, and 1.17:1.

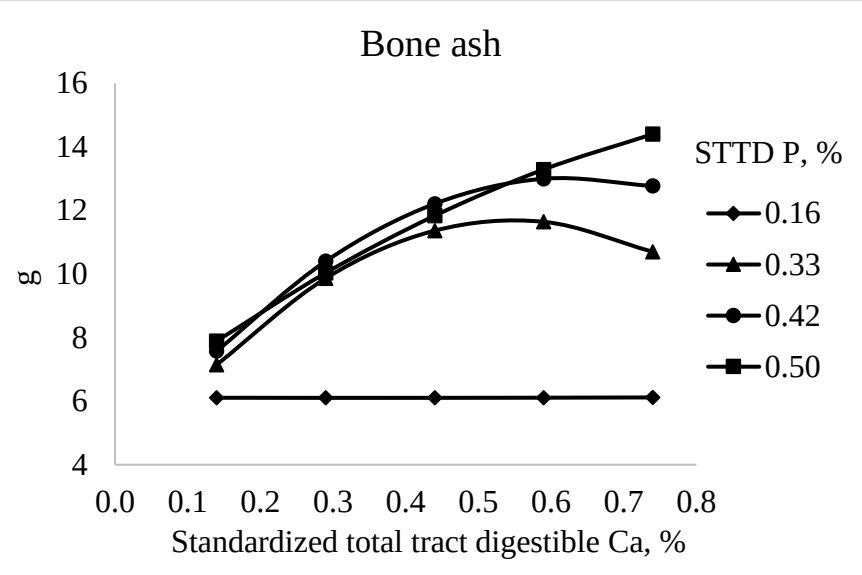

Figure 4.5. Predicted values, based on interactions between Ca and P ($P < 0.001$), for bone ash in grams per femur (g) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum bone ash at STTD P concentrations of 0.33, 0.42, and 0.50% were 11.68, 13.03, and 15.68 g at STTD Ca concentrations of 0.55, 0.63, and 1.15%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.66:1, 1.50:1, and 2.30:1. A maximum bone ash value at STTD P concentration of 0.16% was not estimated because of the nature of the response.

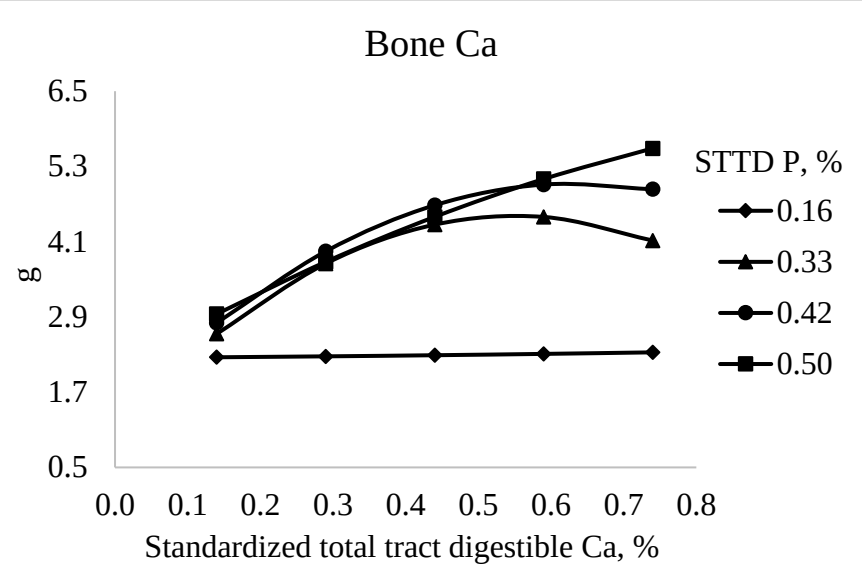

Figure 4.6. Predicted values, based on interactions between Ca and P ($P < 0.001$), for bone Ca in grams per femur (g) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum bone Ca at STTD P concentrations of 0.33, 0.42, and 0.50% were 4.51, 5.03, and 6.41 g at STTD Ca concentrations of 0.55, 0.64, and 1.31%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.67:1, 1.52:1, and 2.62:1. A maximum bone Ca value at STTD P concentration of 0.16% was not estimated because of the nature of the response.

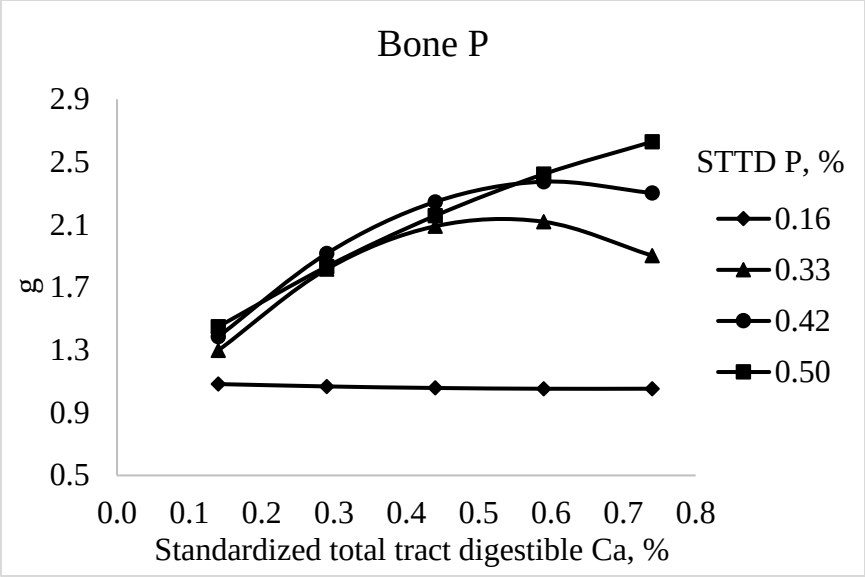

Figure 4.7. Predicted values, based on interactions between Ca and P ($P < 0.001$), for bone P in grams per femur (g) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum bone P at STTD P concentrations of 0.33, 0.42, and 0.50% were 2.14, 2.38, and 2.89 g at STTD Ca concentrations of 0.53, 0.61, and 1.19%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.61:1, 1.45:1, and 2.38:1. A maximum bone P value at STTD P concentration of 0.16% was not estimated because of the nature of the response.

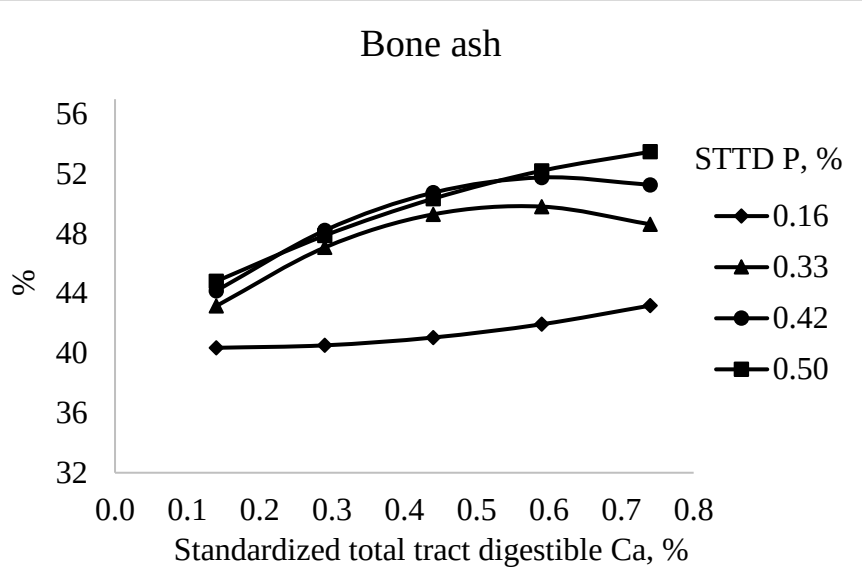

Figure 4.8. Predicted values, based on interactions between Ca and P ($P < 0.01$), for percentage of bone ash (%) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. The predicted maximum bone ash at STTD P concentrations of 0.33, 0.42, and 0.50% were 49.84, 51.79, and 54.27% at STTD Ca concentrations of 0.56, 0.62, and 0.98%, respectively. These values correspond to STTD Ca:STTD P ratios of 1.70:1, 1.47:1, and 1.97:1. A maximum bone ash value at STTD P concentration of 0.16% was not estimated because of the nature of the response.

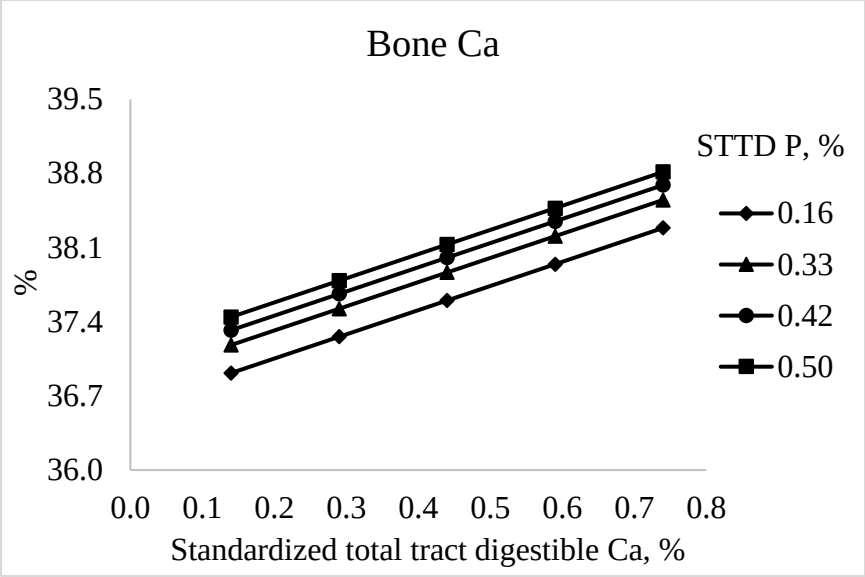

Figure 4.9. Predicted values, based on the linear effect of Ca ($P < 0.001$) and P ($P < 0.10$), for percentage of bone Ca (%) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. Maximum values were not estimated because all responses were linear.

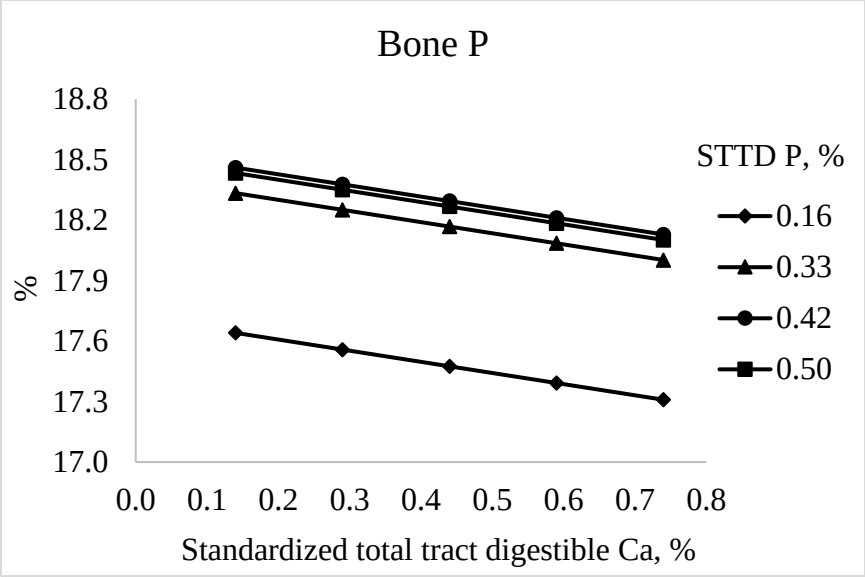

Figure 4.10. Predicted values, based on the linear effect of Ca and P ($P < 0.05$) and the quadratic effect of P ($P < 0.05$), for percentage of bone P (%) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P. Maximum values were not estimated because all responses were linear.

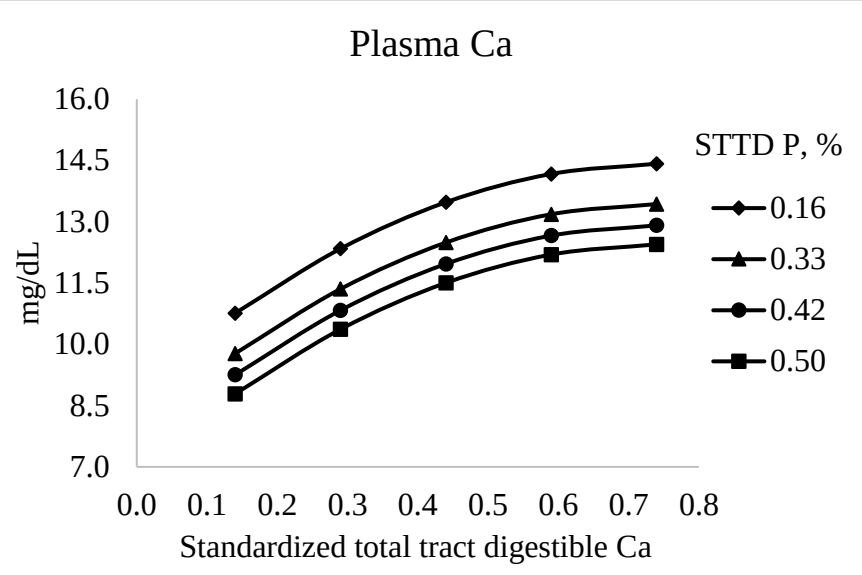

Figure 4.11. Predicted values, based on the linear and quadratic effect of Ca ($P < 0.001$) and the linear effect of P ($P < 0.001$), for plasma Ca (mg/dL) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P.

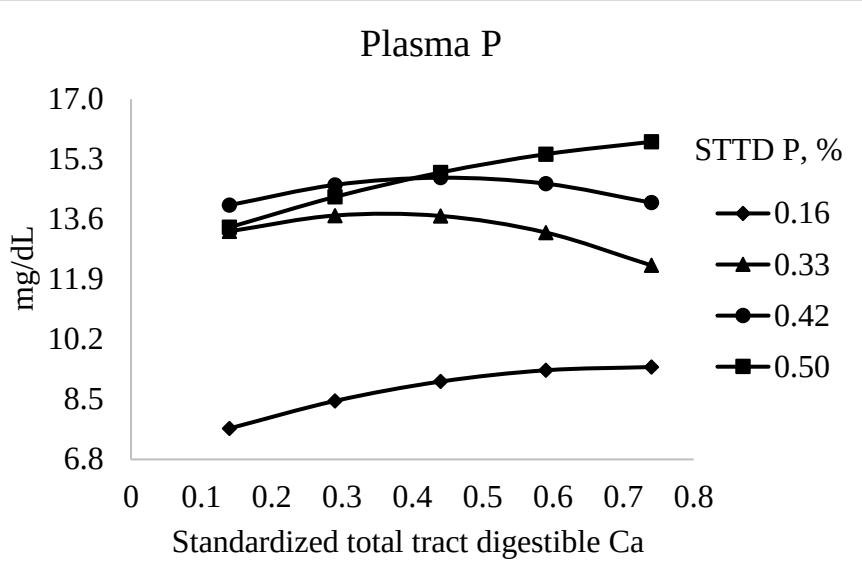

Figure 4.12. Predicted values, based on interactions between Ca and P ($P < 0.001$) for plasma P (mg/dL) at d 21 in pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca and from 0.16 to 0.50% STTD of P.

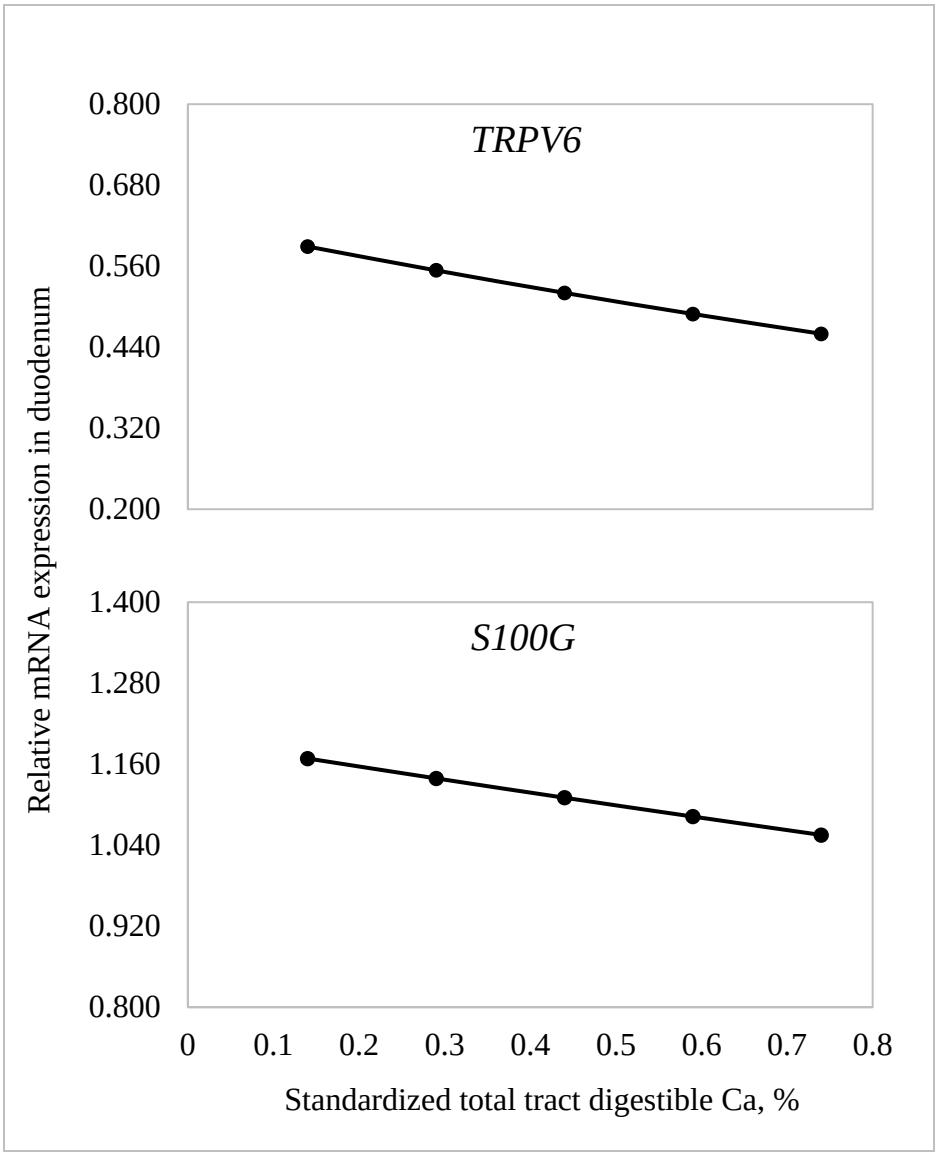

Figure 4.13. Predicted values, based on the linear effect of Ca ($P < 0.05$), for expression of genes related to transcellular transport and absorption of Ca in the duodenum of pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca. *TRPV6* = transient receptor potential cation channel, subfamily V, member 6; *S100G* = S100 calcium binding protein G.

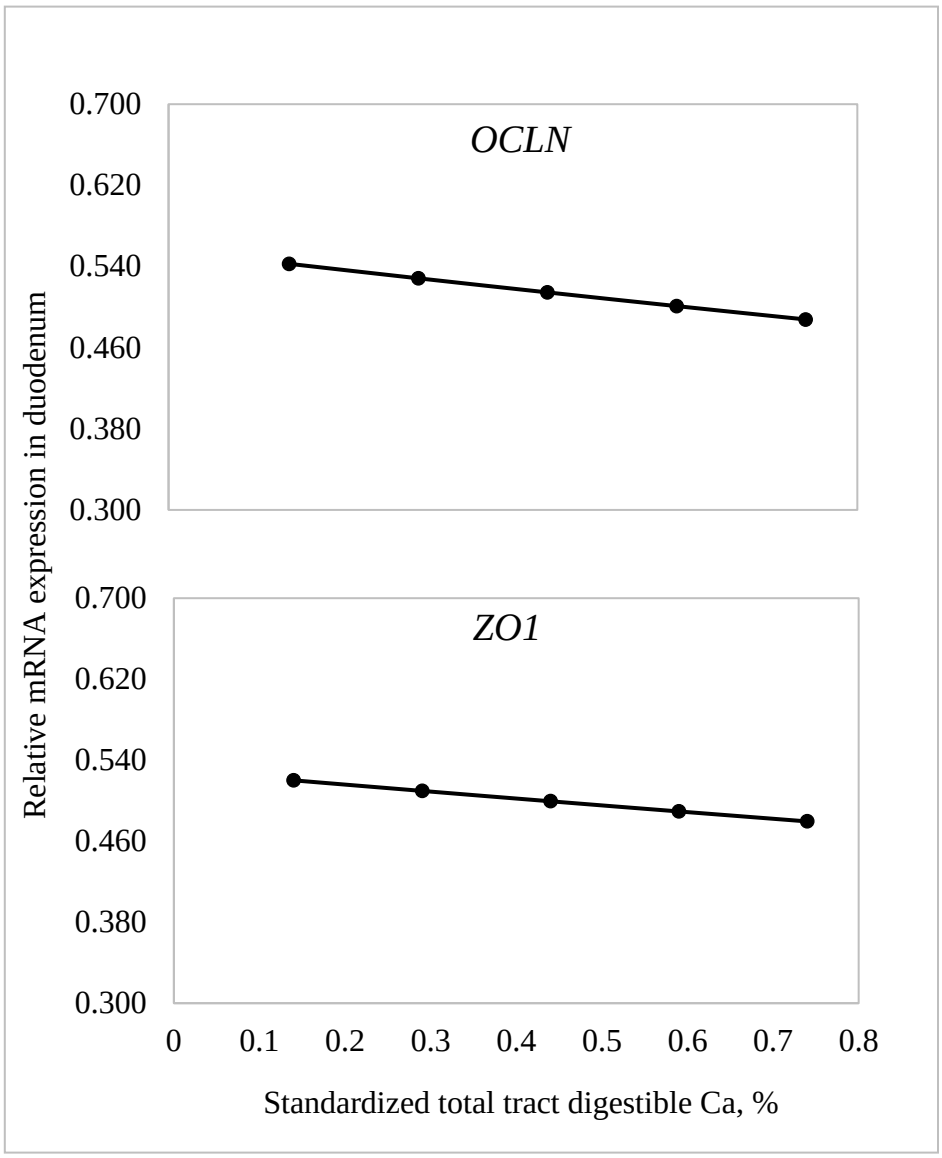

Figure 4.14. Predicted values, based on the linear effect of Ca ($P < 0.05$), for expression of genes related to tight junction proteins in the duodenum of pigs fed diets containing from 0.14 to 0.74% standardized total tract digestible (STTD) Ca. *OCLN* = Occludin; *ZO1* = Zonula occludens-1.

TABLES

Table 4.1. Analyzed composition of ingredients used in diets, as fed basis

Item	Corn	Soybean meal	Lactose	Calcium carbonate	Monocalcium phosphate	Monosodium phosphate	Sodium bicarbonate	Sodium chloride
GE, kcal /kg	3,769	4,173	3,694	-	-	-	-	-
DM, %	85.29	86.97	94.91	99.94	92.47	99.83	63.33	99.96
Ash, %	1.49	7.11	0.19	91.53	81.89	91.85	63.30	100.18
CP, %	6.43	46.60	-	-	-	-	-	-
AEE[1], %	4.31	1.45	-	-	-	-	-	-
ADF, %	3.02	6.60	-	-	-	-	-	-
NDF, %	9.16	7.08	-	-	-	-	-	-
Ca, %	0.02	0.30	0.02	39.23	18.59	0.04	0.01	0.19
P, %	0.29	0.67	0.01	0.02	21.59	25.44	0.03	ND[2]
Phytate[3], %	0.74	1.49	-	-	-	-	-	-
Phytate-bound P, %	0.21	0.42	-	-	-	-	-	-
Non-phytate P[4], %	0.08	0.25	-	-	-	-	-	-

Table 4.1. (Cont.)

Na, %	0.02	0.02	-	0.05	0.09	19.04	27.10	39.45
Cl, %	0.10	0.10	-	ND	0.01	ND	ND	58.77
K, %	0.44	2.49	-	0.10	0.16	0.22	ND	ND

[1]AEE = acid hydrolyzed ether extract.

[2]ND = not detectable.

[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.

Table 4.2. Ingredient composition and analyzed composition of experimental diets containing 0.16% and 0.33% standardized total tract digestible (STTD) P, as-fed basis[1]

Ingredient, %	0.16% STTD P					0.33% STTD P				
Total P, % :	0.35	0.35	0.35	0.35	0.35	0.53	0.53	0.53	0.53	0.53
Total Ca, % :	0.21	0.45	0.70	0.95	1.19	0.21	0.45	0.70	0.95	1.19
STTD Ca, % :	0.14	0.29	0.44	0.59	0.74	0.14	0.29	0.44	0.59	0.74
Corn	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00
Soybean meal	32.50	32.50	32.50	32.50	32.50	32.50	32.50	32.50	32.50	32.50
Cornstarch	8.85	7.05	5.11	3.26	1.43	8.39	6.59	4.71	2.81	0.97
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Choice white grease	-	1.17	2.45	3.65	4.85	0.30	1.47	2.70	3.95	5.15
Calcium carbonate	0.16	0.79	1.45	2.10	2.73	0.16	0.79	1.44	2.10	2.73
Monocalcium phosphate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Monosodium phosphate	-	-	-	-	-	0.75	0.75	0.75	0.74	0.75
Sodium bicarbonate	1.15	1.15	1.15	1.15	1.15	0.56	0.56	0.56	0.56	0.56
L-Lys HCl	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
DL-Met	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14

Table 4.2. (Cont.)

Thr	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Val	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Analyzed composition										
GE, kcal/kg	3,910	3,846	3,842	3,966	3,984	3,846	3,869	3,926	3,954	3,991
DM, %	87.04	87.48	87.70	87.88	88.04	87.92	88.08	88.06	87.24	87.63
CP, %	18.80	19.00	19.23	18.93	19.50	19.42	19.15	18.47	19.17	18.86
Ash, %	3.95	5.03	5.22	6.84	6.73	4.20	4.64	5.55	6.57	6.75
AEE[3], %	2.67	3.33	5.18	5.94	7.46	3.03	3.60	5.27	6.31	7.56
ADF, %	3.67	4.22	2.84	4.87	3.43	3.25	3.40	3.21	3.23	3.59
NDF, %	6.80	7.26	6.52	7.73	7.00	6.12	6.20	6.41	6.59	6.40
Ca, %	0.26	0.46	0.74	0.93	1.27	0.21	0.46	0.72	1.02	1.24
P, %	0.36	0.36	0.36	0.37	0.37	0.54	0.60	0.58	0.56	0.59
Phytate[4], %	0.43	0.46	0.50	0.53	0.57	0.39	0.43	0.53	0.57	0.57

Table 4.2. (Cont.)

Phytate-bound P, %	0.12	0.13	0.14	0.15	0.16	0.11	0.12	0.15	0.16	0.16
Non-phytate P[5], %	0.24	0.23	0.22	0.22	0.21	0.43	0.48	0.43	0.40	0.43
Na, %	0.46	0.47	0.47	0.49	0.47	0.42	0.50	0.47	0.46	0.47
Cl, %	0.28	0.33	0.32	0.32	0.34	0.33	0.31	0.32	0.33	0.33
K, %	0.92	0.91	0.89	0.92	0.95	0.93	0.93	0.92	0.90	0.90
DCAD[6], mEq/kg	357	348	342	357	349	327	367	349	339	344
Total Ca: total P	0.60:1	1.29:1	2.00:1	2.71:1	3.40:1	0.40:1	0.85:1	1.32:1	1.79:1	2.25:1
Total Ca: STTD P	1.31:1	2.81:1	4.38:1	5.94:1	7.44:1	0.64:1	1.36:1	2.12:1	2.88:1	3.61:1
STTD Ca: STTD P	0.88:1	1.81:1	2.75:1	3.69:1	4.63:1	0.42:1	0.88:1	1.33:1	1.79:1	2.24:1

[1]All diets were formulated to have the following quantities of NE (kcal/kg), CP (%), AA (expressed as standardized ileal digestible AA; %), and minerals (%): NE, 2,520; CP, 19.30; Arg, 1.20; His, 0.47; Ile, 0.72; Leu, 1.42; Lys, 1.23; Met, 0.40; Phe, 0.84; Thr, 0.73; Trp, 0.22; Val, 0.78; Na, 0.47; Cl, 0.33; and K, 0.90.

[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, 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.

[3]AEE = acid ether extract.

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

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

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

Table 4.3. Ingredient composition and analyzed composition of experimental diets containing 0.42% and 0.50% standardized total tract digestible (STTD) P, as-fed basis[1]

Ingredient, %	0.42% STTD P					0.50% STTD P				
Total P, % :	0.63	0.63	0.63	0.63	0.63	0.71	0.71	0.71	0.71	0.71
Total Ca, % :	0.21	0.45	0.70	0.95	1.19	0.21	0.45	0.70	0.95	1.19
STTD Ca, % :	0.14	0.29	0.44	0.59	0.74	0.14	0.29	0.44	0.59	0.74
Corn	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00
Soybean meal	32.50	32.50	32.50	32.50	32.50	32.50	32.50	32.50	32.50	32.50
Cornstarch	8.08	6.25	4.35	2.44	0.67	7.85	6.02	4.12	2.21	0.44
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Choice white grease	0.50	1.70	2.95	4.20	5.35	0.65	1.85	3.10	4.35	5.50
Calcium carbonate	0.16	0.79	1.44	2.10	2.72	0.16	0.79	1.44	2.10	2.72
Monocalcium phosphate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Monosodium phosphate	1.15	1.15	1.15	1.15	1.15	1.50	1.50	1.50	1.50	1.50
Sodium bicarbonate	0.27	0.27	0.27	0.27	0.27	-	-	-	-	-
L-Lys HCl	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
DL-Met	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14

Table 4.3. (Cont.)

Thr	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Val	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Analyzed composition										
GE, kcal/kg	3,818	3,869	3,922	4,014	4,018	3,845	3,896	3,928	4,015	4,008
DM, %	87.47	87.72	87.73	87.87	88.17	87.44	87.44	87.92	87.72	87.86
CP, %	19.33	19.01	17.91	18.78	18.43	19.59	18.86	18.89	18.41	18.81
Ash, %	4.82	5.44	5.54	6.71	7.82	5.80	5.51	6.45	7.16	8.06
AEE[3], %	3.02	3.97	5.56	7.38	8.53	3.63	5.29	5.80	7.24	7.89
ADF, %	3.69	3.31	3.23	4.55	3.52	3.81	4.10	3.89	4.34	3.55
NDF, %	6.88	6.38	5.70	7.46	6.14	5.57	6.24	5.29	7.75	5.66
Ca, %	0.20	0.47	0.66	0.91	1.21	0.20	0.47	0.63	0.97	1.27
P, %	0.65	0.63	0.66	0.70	0.70	0.75	0.77	0.79	0.81	0.78
Phytate[4], %	0.50	0.57	0.50	0.64	0.60	0.43	0.57	0.53	0.64	0.67

Table 4.3. (Cont.)

Phytate-bound P, %	0.14	0.16	0.14	0.18	0.17	0.12	0.16	0.15	0.18	0.19
Non-phytate P[5], %	0.51	0.47	0.52	0.52	0.53	0.63	0.61	0.64	0.63	0.59
Na, %	0.47	0.45	0.47	0.47	0.50	0.47	0.50	0.48	0.46	0.46
K, %	0.91	0.93	0.88	0.95	0.92	0.92	0.95	0.93	0.95	0.91
Cl, %	0.31	0.34	0.32	0.32	0.33	0.30	0.33	0.31	0.30	0.32
DCAD[6], mEq/kg	349	339	341	360	358	355	369	359	358	347
Total Ca: total P	0.33:1	0.71:1	1.11:1	1.51:1	1.89:1	0.30:1	0.63:1	0.99:1	1.34:1	1.68:1
Total Ca: STTD P	0.50:1	1.07:1	1.67:1	2.26:1	2.83:1	0.42:1	0.90:1	1.40:1	1.90:1	2.38:1
STTD Ca: STTD P	0.33:1	0.69:1	1.05:1	1.40:1	1.76:1	0.28:1	0.58:1	0.88:1	1.18:1	1.48:1

[1]All diets were formulated to have the following quantities of NE (kcal/kg), CP (%), AA (expressed as standardized ileal digestible AA; %), and minerals (%): NE, 2,520; CP, 19.30; Arg, 1.20; His, 0.47; Ile, 0.72; Leu, 1.42; Lys, 1.23; Met, 0.40; Phe, 0.84; Thr, 0.73; Trp, 0.22; Val, 0.78; Na, 0.47; Cl, 0.33; and K, 0.90.

[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, 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.

[3]AEE = acid ether extract.

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

[5]Non-phytate P was calculated as the difference between total P and phytate-bound P. [6]DCAD = dietary cation-anion difference. The DCAD was calculated as Na + K – Cl.

Table 4.4. Gene-specific primer sets

Target[1]	Forward	Reverse	Length	Source
Transcellular absorption and transport of Ca				
ATP2B1	5'-GGGCGGGCAGGTCATT-3'	5'-CCGCCGGGAGAAGATCA-3'	86	Vigors et al., 2014
TRPV6	5'-CCAGACAGAGGACCCTAACAAG-3'	5'GTGAGAAACAGCTCAAAGGTGCTA-3'	82	Vigors et al., 2014
S100G	5'-CGCAACAGTCCCATTTAAGGA-3'	5'-TCAGCAGAGACATGGGTGGTT-3'	72	Vigors et al., 2014
Paracellular absorption of Ca				
OCLN	5'-TCCTGGGTGTGATGGTGTTC-3'	5'-CGTAGAGTCCAGTCACCGCA-3'	145	Hu et al., 2013
ZO1	5'AAGCCCTAAGTTCAATCACAATCT-3'	5'-ATCAAACTCAGGAGGCGGC-3'	130	Hu et al., 2013
CLDN1	5'-AGAAGATGCGGATGGCTGTC-3'	5'-CCCAGAAGGCAGAGAGAAGC-3'	193	Hu et al., 2013
Internal control genes				
β-ACTIN	5'-GGATGCAGAAGGAGATCACG-3'	5'-ATCTGCTGGAAGGTGGACAG-3'	150	Lackeyram et al., 2010
GAPDH	5'-TTCGTCAAGCTCATTTCCTGGTA-3'	5'-TCCTCGCGTGCTCTTGCT-3'	130	Vigors et al., 2014
HMBS	5'-CTGAACAAAGGTGCCAAGAACA-3'	5'-GCCCCGCAGACCAGTTAGT-3'	74	Vigors et al., 2014

[1]*ATP2B1*= ATPase, Ca2+ transporting, plasma membrane-1; *TRPV6*= transient receptor potential cation channel, subfamily V, member 6; *S100G*= S100 calcium binding protein G; *OCLN*= Occludin; *ZO1* = Zonula occludens-1; *CLDN1*= Claudin-1; *ACTB*= β-actin; *GAPDH*= glyceraldehyde 3-phosphate dehydrogenase; *HMBS*= hydroxymethylbilane synthase.

Table 4.5. Least squares means for growth performance of pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and STTD P for 21 d[1]

	STTD Ca, %				
Item	0.14	0.29	0.44	0.59	0.74
Initial BW, kg[2]					
0.16% STTD P	11.13	10.98	11.21	11.06	11.02
0.33% STTD P	10.89	10.98	11.01	11.17	11.09
0.42% STTD P	11.11	11.12	11.12	11.14	10.95
0.50% STTD P	11.15	11.09	11.05	11.19	11.08
Final BW, kg[3,4]					
0.16% STTD P	20.87	20.91	20.77	19.36	18.11
0.33% STTD P	22.07	23.78	23.79	23.47	22.61
0.42% STTD P	20.69	24.50	23.67	24.00	23.47
0.50% STTD P	20.04	22.80	23.71	24.27	24.01
ADG, g[5,6]					
0.16% STTD P	465	473	455	395	337
0.33% STTD P	530	609	621	586	548
0.42% STTD P	451	637	598	612	596
0.50% STTD P	413	557	599	622	615
ADFI, g[7,8]					
0.16% STTD P	848	849	864	808	791
0.33% STTD P	871	948	931	933	914
0.42% STTD P	763	978	895	958	928

Table 4.5. (Cont.)

0.50% STTD P	752	869	925	940	936
G:F, g:g[9,10]					
0.16% STTD P	0.549	0.556	0.527	0.488	0.425
0.33% STTD P	0.610	0.645	0.656	0.628	0.601
0.42% STTD P	0.592	0.654	0.671	0.640	0.643
0.50% STTD P	0.549	0.643	0.649	0.663	0.658

[1]Data are least square means of 8 observations with the exception of diet 0.44 % STTD Ca and 0.33% STTD P for ADG (n= 7).

[2]Standard error of the within treatment least squares means = 0.51.

[3]Results indicated that final BW from d 1 to 21 at different combinations of STTD Ca and STTD P could be described by the following model: $15.900 + 6.279 \times Ca - 18.447 \times Ca^2 + 30.788 \times P - 55.129 \times P^2 + 32.103 \times Ca \times P$ ($P < 0.001$).

[4]Standard error of the within treatment least squares means = 0.80.

[5]Results indicated that ADG from d 1 to 21 at different combinations of STTD Ca and STTD P could be described by the following model: $217.656 + 302.483 \times Ca - 895.665 \times Ca^2 + 1{,}564.314 \times P - 2{,}828.675 \times P^2 + 1{,}574.542 \times Ca \times P$ ($P < 0.001$).

[6]Standard error of the within treatment least squares means = 0.02.

[7]Results indicated that ADFI from d 1 to 21 at different combinations of STTD Ca and STTD P could be described by the following model: $678.646 + 323.053 \times Ca - 717.966 \times Ca^2 + 919.235 \times P - 1{,}968.795 \times P^2 + 1{,}190.694 \times Ca \times P$ ($P < 0.001$).

[8]Standard error of the within treatment least squares means = 0.04.

[9]Results indicated that G:F from d 1 to 21 at different combinations of STTD Ca and STTD P could be described by the following model: $0.389 + 0.084 \times Ca - 0.529 \times Ca^2 + 1.161 \times P - 1.930 \times P^2 + 1.070 \times Ca \times P$ ($P < 0.001$).

[10]Standard error of the within treatment least squares means = 0.01.

Table 4.6. Least squares means for concentration (g per femur) and percentage of bone ash, bone Ca, and bone P in pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and STTD P for 21 d[1]

	STTD Ca, %				
Item	0.14	0.29	0.44	0.59	0.74
Bone ash, g per femur[2,3]					
0.16% STTD P	5.97	5.85	6.52	5.63	6.24
0.33% STTD P	7.08	10.63	11.20	11.12	10.76
0.42% STTD P	7.29	10.49	11.42	13.97	12.75
0.50% STTD P	8.13	9.89	11.84	13.44	14.19
Bone Ca, g per femur[4,5]					
0.16% STTD P	2.20	2.17	2.46	2.12	2.39
0.33% STTD P	2.61	4.01	4.35	4.27	4.12
0.42% STTD P	2.73	3.96	4.38	5.46	4.95
0.50% STTD P	3.03	3.76	4.46	5.18	5.50
Bone P, g per femur[6,7]					
0.16% STTD P	1.06	1.03	1.13	0.98	1.08
0.33% STTD P	1.29	1.95	2.08	2.00	1.92
0.42% STTD P	1.35	1.92	2.13	2.59	2.30
0.50% STTD P	1.49	1.83	2.15	2.45	2.60
Bone ash, %[8,9]					
0.16% STTD P	39.85	40.38	42.05	40.12	43.68
0.33% STTD P	43.37	48.08	48.95	49.25	48.63

Table 4.6. (Cont.)

0.42% STTD P	42.93	48.49	49.95	51.92	51.47
0.50% STTD P	45.12	47.95	49.92	52.58	53.12
Bone Ca, %[10,11]					
0.16% STTD P	37.11	36.98	37.67	37.82	38.12
0.33% STTD P	36.79	37.73	38.80	38.43	38.32
0.42% STTD P	37.41	37.68	38.33	38.92	38.87
0.50% STTD P	37.20	38.10	37.61	38.51	38.62
Bone P, %[12,13]					
0.16% STTD P	17.78	17.64	17.38	17.58	17.27
0.33% STTD P	18.25	18.33	18.62	17.96	17.86
0.42% STTD P	18.49	18.25	18.63	18.49	18.08
0.50% STTD P	18.34	18.55	18.17	18.22	18.29
Ca:P in bone[14]					
0.16% STTD P	2.09	2.09	2.17	2.15	2.21
0.33% STTD P	2.01	2.06	2.08	2.14	2.15
0.42% STTD P	2.02	2.06	2.06	2.10	2.15
0.50% STTD P	2.03	2.05	2.07	2.11	2.11

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

[2]Results indicated that bone ash in g per femur at different combinations of STTD Ca and STTD P could be described by the following model: $12.833 – 66.524 \times Ca + 68.290 \times Ca^2 – 54.933 \times P + 80.864 \times P^2 + 531.751 \times Ca \times P – 555.413 \times Ca^2 \times P – 727.143 \times Ca \times P^2 + 807.115 \times Ca^2 \times P^2$ ($P < 0.001$).

[3]Standard error of the within treatment least squares means = 0.54.

Table 4.6. (Cont.)

[4]Results indicated that bone Ca in g per femur at different combinations of STTD Ca and STTD P could be described by the following model: $5.159 – 27.616 \times Ca + 28.575 \times Ca^2 – 23.865 \times P + 35.365 \times P^2 + 222.193 \times Ca \times P – 232.677 \times Ca^2 \times P – 307.380 \times Ca \times P^2 + 340.908 \times Ca^2 \times P^2$ ($P < 0.001$).

[5]Standard error of the within treatment least squares means = 0.23.

[6]Results indicated that bone P in g per femur at different combinations of STTD Ca and STTD P could be described by the following model: $2.453 – 13.627 \times Ca + 14.197 \times Ca^2 – 11.094 \times P + 16.525 \times P^2 + 108.129 \times Ca \times P – 114.866 \times Ca^2 \times P – 149.264 \times Ca \times P^2 + 167.696 \times Ca^2 \times P^2$ ($P < 0.001$).

[7]Standard error of the within treatment least squares means = 0.10.

[8]Results indicated that percentage of bone ash at different combinations of STTD Ca and STTD P could be described by the following model: $48.455 – 100.190 \times Ca + 115.437 \times Ca^2 – 66.172 \times P + 104.184 \times P^2 + 780.228 \times Ca \times P – 865.953 \times Ca^2 \times P – 1{,}055.175 \times Ca \times P^2 + 1{,}217.046 \times Ca^2 \times P^2$ ($P < 0.001$).

[9]Standard error of the within treatment least squares means = 0.82.

[10]Results indicated that percentage of bone Ca at different combinations of STTD Ca and STTD P could be described by the following model: $36.347 + 2.282 \times Ca + 1.556 \times P$ ($P < 0.001$).

[11]Standard error of the within treatment least squares means = 0.57.

[12] Results indicated that percentage of bone P at different combinations of STTD Ca and STTD P could be described by the following model: $16.524 – 0.553 \times Ca + 9.110 \times P – 10.270 \times P^2$ ($P < 0.001$).

[13]Standard error of the within treatment least squares means = 0.26.

[14]Standard error of the within treatment least squares means = 0.02.

Table 4.7. Least squares means for concentration of Ca and P in plasma of pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and STTD P for 21 d[1]

	STTD Ca, %				
Item	0.14	0.29	0.44	0.59	0.74
Plasma Ca, mg/dL[2,3]					
0.16% STTD P	11.13	12.07	13.78	13.65	15.42
0.33% STTD P	9.37	11.68	12.35	12.96	13.47
0.42% STTD P	8.07	11.33	11.67	12.50	13.20
0.50% STTD P	9.40	11.03	12.07	11.78	12.14
Plasma P, mg/dL [4,5]					
0.16% STTD P	8.17	8.36	8.85	9.08	9.75
0.33% STTD P	12.78	14.67	14.26	12.18	12.48
0.42% STTD P	13.73	14.16	15.28	15.21	14.11
0.50% STTD P	13.51	14.66	15.05	14.59	16.29

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

[2]Results indicated that plasma Ca concentration at different combinations of STTD Ca and STTD P could be described by the following model: $9.816 + 14.764 \times Ca - 9.848 \times Ca^2 - 5.804 \times P$ ($P < 0.001$).

[3]Standard error of the within treatment least squares means = 0.42.

[4]Results indicated that plasma P concentration at different combinations of STTD Ca and STTD P could be described by the following model: $-3.803 + 7.365 \times Ca + 10.279 \times Ca^2 + 81.692 \times P - 98.511 \times P^2 - 127.912 \times Ca^2 \times P + 199.572 \times Ca^2 \times P^2$ ($P < 0.001$).

[5]Standard error of the within treatment least squares means = 0.59.

Table 4.8. Least squares means (log_2-backtransformed) for gene expression in the duodenum of pigs fed diets containing different concentrations of standardized total tract digestible (STTD) Ca and STTD P for 21 d[1]

	STTD Ca, %				
Item	0.14	0.29	0.44	0.59	0.74
TRPV6[2,3]					
0.16% STTD P	0.461	0.478	0.438	0.447	0.425
0.33% STTD P	0.526	0.632	0.587	0.479	0.466
0.42% STTD P	0.717	0.522	0.539	0.474	0.585
0.50% STTD P	0.749	0.633	0.601	0.519	0.431
S100G[4,5]					
0.16% STTD P	1.175	1.126	0.968	1.050	1.041
0.33% STTD P	1.182	1.175	1.208	0.983	1.091
0.42% STTD P	1.117	1.116	1.106	0.955	1.109
0.50% STTD P	1.207	1.017	1.112	1.053	1.162
ATP2B1[6,7]					
0.16% STTD P	0.426	0.394	0.388	0.409	0.390
0.33% STTD P	0.381	0.410	0.435	0.399	0.467
0.42% STTD P	0.422	0.389	0.376	0.412	0.419
0.50% STTD P	0.435	0.389	0.374	0.422	0.468
OCLN[8,9]					
0.16% STTD P	0.550	0.518	0.470	0.490	0.475
0.33% STTD P	0.526	0.523	0.521	0.483	0.510

Table 4.8. (Cont.)

0.42% STTD P	0.548	0.511	0.548	0.471	0.505
0.50% STTD P	0.580	0.512	0.502	0.488	0.515
ZO1[10,11]					
0.16% STTD P	0.534	0.542	0.504	0.510	0.486
0.33% STTD P	0.535	0.521	0.501	0.500	0.458
0.42% STTD P	0.511	0.508	0.537	0.493	0.491
0.50% STTD P	0.525	0.497	0.448	0.468	0.519
CLDN1[12,13]					
0.16% STTD P	0.656	0.338	0.338	0.323	0.588
0.33% STTD P	0.338	0.495	0.290	0.287	0.160
0.42% STTD P	0.460	0.304	0.470	0.385	0.298
0.50% STTD P	0.349	0.522	0.313	0.314	0.359

[1]Data are least square means of 6, 7, or 8 observations.

[2]Results indicated that expression of *TRPV6* at different combinations of STTD Ca and STTD P could be described by the following model (log_2): –0.680 – 0.596 × Ca ($P < 0.05$).

[3]Standard error of the within treatment least squares means = 0.09.

[4]Results indicated that expression of *S100G* at different combinations of STTD Ca and STTD P could be described by the following model (log_2): 0.259 – 0.245 × Ca ($P < 0.05$).

[5]Standard error of the within treatment least squares means = 0.10.

[6]Results indicated that expression of *ATP2B1* could not be predicted using STTD Ca or STTD P.

[7]Standard error of the within treatment least squares means = 0.04.

[8]Results indicated that expression of *OCLN* at different combinations of STTD Ca and STTD P could be described by the following model (log_2): –0.846 – 0.256 × Ca ($P < 0.05$).

Table 4.8. (Cont.)

[9]Standard error of the within treatment least squares means = 0.04.

[10]Results indicated that expression of *ZO1* at different combinations of STTD Ca and STTD P could be described by the following model (log_2): –0.916 – 0.195 × Ca ($P < 0.05$).

[11]Standard error of the within treatment least squares means = 0.03.

[12]Results indicated that expression of *CLDN1* could not be predicted using STTD Ca or STTD P.

[13]Standard error of the within treatment least squares means = 0.10.

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CHAPTER 5: CONCLUSIONS

Results from this work indicate a detrimental effect of increasing concentrations of dietary Ca on growth performance of pigs fed diets in which the concentration of digestible P is at or below the requirement. This effect is ameliorated if dietary P is included above the requirement and G:F may even be increased, indicating that the negative impact of increased dietary Ca on growth performance may be a result of formation of complexes between Ca and P in the gastrointestinal tract, which prevents appropriate absorption of P.

The ratio between standardized total tract digestible (**STTD**) Ca and STTD P needed to maximize growth performance is less than the ratio needed to maximize bone ash, which indicates an ability by pigs to use Ca and P to synthesize bone tissue after the requirement for growth performance has been met. If STTD P is supplied in diets at adequate concentrations, the STTD Ca:STTD P requirement indicated by growth performance decreases as the pig gets older. In contrast, the requirement for STTD Ca:STTD P to maximize bone ash increases as the pig gets older. If STTD P is at the requirement, growth performance is maximized at a STTD Ca:STTD P ratio that is less than 1.40:1 and 1.25:1 in pigs from 11 to 25 kg and 50 to 85 kg, respectively, whereas bone ash is maximized at STTD Ca:STTD P ratios of 1.66:1 and 2.03:1, respectively. For 11- to 25-kg and 50- to 85-kg pigs, if STTD P is included in diets below or above the requirement, dietary STTD Ca also needs to be included at concentrations below and above the requirement, respectively, because the ratio between STTD Ca and STTD P is more important than the absolute values.

From this work, it was also concluded that increasing concentrations of STTD Ca increases plasma Ca and downregulates the expression of genes related to transcellular

absorption of Ca, as well as genes related to tight junction proteins, indicating an increase in Ca absorption via the paracellular pathway at high dietary Ca concentrations.

Overall, it is concluded that diets fed to growing terminal pigs need to be formulated to meet a ratio between STTD Ca and STTD P that is around 1.35:1 for 11- to 25-kg pigs and 1.23:1 for 50- to 85-kg pigs. However, pigs that are intended to be used in the breeding herd may require diets with greater concentration of STTD Ca and STTD P and a ratio between STTD Ca and STTD P that is greater than the ratios required to maximize growth to ensure maximum bone mineralization.