FACTORS AFFECTING THE DIGESTIBILITY OF CALCIUM IN FEED INGREDIENTS AND REQUIREMENTS FOR DIGESTIBLE CALCIUM BY PIGS

BY

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DISSERTATION

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ABSTRACT: Eight experiments were conducted to understand factors that may affect the digestibility of Ca in diets fed to pigs and also to determine the requirement of Ca for the finishing pig. The first experiment was conducted to determine the apparent total tract digestibility (ATTD) and the standardized total tract digestibility (STTD) of Ca in feed ingredients of animal origin without and with microbial phytase. Results from Exp. 1 indicated that if no phytase was used, the ATTD and STTD of Ca in poultry byproduct meal were greater ($P < 0.05$) than in meat and bone meal and meat meal, but values for poultry meal were not different meat and bone meal, meat meal, and poultry byproduct meal. However, if phytase was added to the diets, no differences in ATTD or STTD of Ca among ingredients were observed (interaction $P < 0.05$). There was no effect of microbial phytase on ATTD or STTD of Ca in the 4 ingredients. The second and third experiments were conducted to determine if particle size of calcium carbonate influences the STTD of Ca or growth performance. Results from these 2 experiments indicated that particle size did not influence STTD of Ca or growth performance. The STTD of Ca was 74.15 ± 3.24%, 78.45 ± 2.71%, 74.13 ± 2.93%, and 76.24 ± 2.66% for diets containing Ca carbonate ground to an average particle size of 200, 500, 700, or 1125 µm. The fourth experiment was conducted to determine the effect of supplementing diets fed to growing pigs with fat sources that differ in their concentrations of saturated and unsaturated fatty acids on the ATTD of Ca. Results indicated that the ATTD of Ca was greater ($P < 0.05$) for pigs fed diets containing soybean oil, corn oil, palm oil, or tallow than for pigs fed a basal diet without the addition of fat or a diet containing choice white grease. The fifth experiment was conducted to determine if increasing concentrations of sodium chloride (NaCl) affect the ATTD or retention of Ca and P in diets fed to growing pigs. Results indicated that increasing dietary inclusion of NaCl above 0.4% reduced ($P < 0.05$) Ca intake, Ca absorbed, ATTD of Ca, and

retention of Ca. The concentrations of Ca in feces and urine were greater ($P < 0.05$) if phytase was not included in the diet; but Ca absorption, ATTD of Ca, and retention of Ca were increased ($P < 0.05$) by addition of microbial phytase. The sixth experiment was conducted to determine the effect on the digestibility of Ca in calcium carbonate of including sucrose or cornstarch in diets for growing pigs. Results indicated that the ATTD of Ca was not affected by inclusion of increasing concentrations of sucrose in the diets. Likewise, no effects of sucrose inclusion were observed for feed intake, fecal output, fecal Ca concentration, Ca intake, absorbed Ca, urine Ca, or Ca retention. Sucrose inclusion tended to affect fecal calcium output (g/d; $P = 0.066$) and fecal output (g/d; $P = 0.081$) quadratically with the greatest values being calculated for diets containing 20 or 40% sucrose. The seventh experiment was conducted to test the hypothesis that precipitation of Ca from calcium carbonate in diets based on cornstarch may be different from that of diets based on corn, and to determine the influence of phytase on the digestibility of Ca in both types of diets. Results from this experiment indicated that phytate in corn decreases ($P < 0.05$) the ATTD of Ca, but phytase had no effect on the ATTD of Ca regardless of the concentration of fiber in the diet. The final experiment was conducted to determine the digestible Ca requirement for pigs from 100 to 130 kg. Results from the experiment support the current requirements for Ca and STTD P, and feeding Ca at levels greater than the requirements (0.46% total Ca; 0.29% STTD Ca) is detrimental to growth performance of pigs. Additional research is needed to determine the STTD Ca requirements if different concentrations of phytase are included in the diet.

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

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Insufficient dietary Ca is detrimental to pig welfare and profitability of swine units. A deficiency or excess of Ca may result in lameness or broken bones and a reduction in growth performance, both leading to profit losses for swine producers (Crenshaw, 2001). In recent years, P has been highlighted as a research area not only because of the animal welfare concerns, but also because of the environmental consequences of P excretion in the feces (Ruddy et al., 2006). Phosphorus metabolism is, however, directly related to dietary Ca levels (Crenshaw, 2001); formulating for specific concentrations of P without accurate Ca formulation will limit the potential to meet the dietary needs of Ca and P for the pigs. Recommendations for dietary P are now available on a digestible P basis; whereas, Ca is only available on a total Ca basis (NRC, 2012). Previous research in our laboratory has been conducted to determine the digestibility of Ca in inorganic (Gonzalez-Vega et al., 2014; Gonzalez-Vega et al., 2015a) and plant-based ingredients (Gonzalez-Vega et al., 2013),; however, feed ingredients of animal origin have not been evaluated on a digestible Ca basis with the exception of meat and bone meal (Sulabo et al., 2013) and fish meal (Gonzalez-Vega et al., 2015b). Once the digestibility of Ca has been established in these ingredients, the industry can move towards formulating diets and making recommendations for Ca requirements based on a digestible Ca basis.

Because of the relatively low cost of Ca from limestone and other inorganic ingredients, little work has been reported with regard to Ca. This is especially true when it comes to evaluating the inclusion of other dietary ingredients and how those ingredients may affect the absorption of Ca. To better understand the current knowledge on Ca metabolism in pigs, the second chapter of this dissertation is a review of the literature on the digestibility of Ca in feed

ingredients and the Ca requirements of growing pigs. The aim of Chapter 3 is to further the knowledge of digestibility in animal feed ingredients and the impact of including microbial phytase on animal ingredients, which do not contain phytate. The 4th chapter describes research to evaluate the impact of varying the particle size of calcium carbonate on the digestibility and growth performance of weanling pigs. In Chapter 5, the effects of fat sources with varying levels of saturation on the digestibility of Ca are determined. The 6th chapter describes the significance of the recent increase of dietary Na and Cl recommendations, and potential changes to Ca metabolism that these may elicit. In Chapter 7, the digestibility of Ca in cornstarch and sucrose diets is being tested to evaluate the impact of including those ingredients in experimental diets for estimating Ca digestibility. In Chapter 8, changes in Ca digestibility are evaluated in two diets containing different levels of phytate bound P and to test the hypothesis that Ca digestibility in a diet that does not contain phytate bound P may be different from a diet containing phytate bound P. The digestible Ca requirement for pigs at 100 kg BW is determined in Chapter 9. The overall objective of this dissertation is to evaluate factors that may influence the digestibility of Ca and to establish the digestible Ca requirement in 100 to 135 kg pigs, thereby furthering the current knowledge of Ca metabolism in pigs and more accurately meeting the nutritional needs of the pig.

LITERATURE CITED

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. Pages 187-212 in Swine Nutrition. 2nd ed. A. J. Lewis, and L. L. Southern, ed. CRC Press, Boca Raton, FL.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of Ca and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816.

Gonzalez-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126-142.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93:2255-2264.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015b. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Scie. 93:4808-4818.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Ruddy, B. C., D. L. Lorenz, and D. K. Mueller. 2006. County-level estimates of nutrient inputs to the land surface of the conterminous United States, 1982–2001: U.S. Geological Survey Scientific Investigations Report 2006-5012.

Sulabo, R. C., and H. H. Stein. 2013. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. J. Anim. Sci. 91:1285-1294.

CHAPTER 2

DIGESTIBILITY OF CALCIUM IN FEED INGREDIENTS AND REQUIREMENTS OF CALCIUM BY GROWING PIGS: REVIEW OF LITERATURE

Concerns for maintaining adequate animal welfare, providing cost effective rations; and reducing nutrient excretion, which contributes to environmental pollution, have been the driving forces behind accurate formulation of diets with adequate inclusions of the macro-minerals Ca and P. Macro-minerals, by definition, are those that are supplied in the diet at levels greater than 100 ppm (Ewing and Charlton, 2007). Calcium and P are 2 of the most abundant minerals in the body, and total mineral concentration in the body is 3 to 5% (Hendriks and Moughan, 1993). Correct inclusions of Ca and P in the diets are, therefore, imperative for proper development and maintenance of the growing pig.

Physiological roles of Ca include muscle contraction, nerve impulses, enzyme activation, and skeletal structure (Crenshaw, 2001). Physiological roles of P include energy metabolism, acid-base homeostasis, bone mineralization, enzyme activation by addition or removal of phosphate groups, nucleic acid synthesis, membrane structure, and bone mineralization (NRC, 2012). If Ca is absent or limiting in the diet, swine may exhibit abnormal bone formation, rickets, weak bones susceptible to breaking, growth depression, fatigue, rachitic rosaries on the ribs, or death (Crenshaw, 2001; NRC, 2012). In contrast, if diets have excess Ca, pigs may exhibit symptoms of growth depression, Ca accumulation, P reabsorption from the bone, and kidney stones (Crenshaw, 2001; NRC, 2012). If P is absent or limiting in the diet, swine may exhibit growth depression, demineralization of the bone, or even death. Excess P appears to primarily be lost in the urine (Stein et al., 2011). A Ca or P deficiency may be due to inadequate supply of one

of these minerals, inadequate dietary ratio between Ca and P, or improper hormonal regulation (Crenshaw, 2001).

The whole body concentrations of Ca and P are approximately 0.8% and 0.5% (Hendriks and Moughan, 1993), respectively. Both Ca and P are primarily located in the skeleton. It is believed that 96 to 99% of Ca and 60 to 80% of P are located in the bones in the form of hydroxyappetite (Crenshaw, 2001). The composition of bone ash is 36 to 39% Ca and 17 to 19% P. The remainder of the Ca and P is located in blood and soft tissues. The normal concentrations of Ca and P in blood serum are 10.56 to 11.85 mg/100ml and 8.22 to 10.90 mg/100ml (Miller et al., 1962; Nimmo et al., 1981), respectively. The amounts of Ca and P in visceral organs of a pig at approximately 18 kg BW are 0.40 g and 8.3 g, respectively. The Ca and P in the whole empty body of a 18 kg pig are 186.5 and 151.3 g, respectively (Pettey et al., 2015). With such low levels in the soft tissues and the highly regulated homeostasis of Ca and P in the serum, bones are the common standards for evaluating Ca and P status because both Ca and P are most abundant in bone and most susceptible to changes due to diet modifications.

ECOLOGICAL AND ECONOMIC CONSEQUENCES OF NUTRIENT EXCRETION OF CA AND P

Minimizing nutrient excretion, particularly P, is important worldwide to reduce the overload of soil nutrients. In the Midwest region of the United States, farmers can apply manure directly to the field as a source of fertilizer. They are limited, however, by the concentration of P in the soil and in the manure. Restrictions also dictate the proximity to local streams and the weather conditions under which the manure can be applied (USEPA, 2003)

In modern animal production, it is more efficient to raise animals in larger, more concentrated operations. However, these large operations generate more manure in a smaller area. In the United States, it is estimated that an inventory of approximately 67 million pigs is maintained (USDA, 2009) and those pigs excrete 1.85×10^9 kg of P per year (Ruddy et al., 2006). To prevent excess application of nutrients to the soils, producers must transport the manure further distances, increasing costs to the producer.

If P concentrations are saturated in the soil, extra P has a greater chance of migrating to local streams, ponds, and ditches (Smith et al., 1999). Two factors known to limit plant growth in terrestrial and aquatic environments are P and N (Howarth et al., 2011). Eutrophication refers to the process of increasing nutrient density in the water, which will result in increased algae biomass, depleted oxygen levels, and significant shifts in animal and plant populations (Howarth et al., 2011). Nutrient overload was the cause of the hypoxic zone known as the "Dead Sea" located in the Gulf of Mexico (Diaz and Rosenberg, 2008). Agriculture has contributed to nutrient overload in terrestrial regions that has resulted in a "runoff" of nutrients into local waters. To alleviate these ecological challenges and reduce transportation costs, diets that reduce nutrient excretion from animals need to be formulated.

CONCEPTS OF BIOAVAILABILITY, DIGESTIBILITY, AND RETENTION

Bioavailability

Bioavailability is the proportion of an ingested nutrient that is absorbed and metabolized by the animal (Ammerman, 1995; Stein et al., 2007). Bioavailability is usually indirectly measured using a particular response criterion and expressed as the relative bioavailability (**RBV**). A commonly used and well digested ingredient (e.g., limestone or calcium carbonate for

Ca) is usually used as the standard. To determine RBV, the slope ratio method is employed as described by Littell et al. (1997). Relative bioavailability estimates provide insight into the differences within an experiment among sources of a specific nutrient. However, these values cannot be accurately converted to an expression of digestibility (Baker et al., 2012). Because RBV are limited to reference comparisons and cannot be directly calculated, digestibility values are preferred (NRC, 2012).

Digestibility

Digestibility refers to the percentage of a nutrient that is absorbed from the intestinal tract and which is available for metabolism by the animal (Stein et al., 2007). In animal nutrition, digestibility can be determined at different locations of the gastrointestinal tract. Digestibility can refer to the duodenal (González-Vega, 2014), ileal, or total tract digestibility (Stein et al., 2011), and the location of interest is based on the type of nutrient being evaluated. For Ca and P, it is assumed that negligible amounts of digestion occur after the small intestine and no net secretion of endogenous Ca or P takes place in the large intestine (González-Vega, 2014). Therefore, total tract digestibility is an appropriate measure for determining digestibility for these minerals. Total tract digestibility can be expressed as apparent total tract digestibility (**ATTD**), standardized total tract digestibility (**STTD**), or true total tract digestibility (**TTTD**; González-Vega et al., 2013) Values for ATTD express the difference between nutrient intake and nutrient excretion and may be calculated by the following equation for Ca (adapted from Petersen and Stein, 2006):

$$\text{ATTD of Ca (\%)} = [(Ca_{intake} - Ca_{feces}) / Ca_{intake}] \times 100, \quad [1]$$

where Ca_{intake} is the daily intake of Ca (g) and Ca_{feces} is the daily Ca output (g).

If endogenous losses of a nutrient exist, a more accurate expression of digestion is represented by the STTD or TTTD values (González-Vega et al., 2013). Endogenous losses may be diet specific or non-specific. The non-specific endogenous losses are also called basal losses and include losses of Ca from enzymes, saliva, mucus, and sloughed cells (González-Vega et al., 2013). The basal endogenous losses are secreted regardless of the type of diet that is fed and is believed to be constant among diets. Basal endogenous losses may be calculated as the Ca lost from pigs fed a Ca-free diet. Therefore, a Ca-free diet must be included in the experiments so that basal endogenous losses of Ca can be computed. Basal endogenous losses of Ca (**ECaL** mg/kg of DMI) can be calculated by the following equation (adapted from Petersen and Stein, 2006):

$$\text{Basal ECaL} = [(Ca_{feces} / Feed_{intake}) \times 1{,}000 \times 1{,}000], \quad [2]$$

where $Feed_{intake}$ represents the intake of feed on a DM basis and Ca_{feces} is the fecal output of Ca (g/kg of DM). Once the Basal ECaL has been determined, it can be included in the equation for the STTD of Ca as indicated below:

$$\text{STTD Ca (\%)} = [Ca_{intake} - (Ca_{feces} - \text{ECaL})/Ca_{intake}] \times 100, \quad [3]$$

Nutrient digestibility can also be expressed as TTTD, which corrects for both basal and diet specific endogenous losses. To make this correction, the regression procedure is applied (Fan et al., 2001; González-Vega et al., 2013). Dietary Ca intake is regressed against the response parameter of interest. The linear portion is then extrapolated back to the Y-axis. The negative Y intercept of the regression equation is the total endogenous losses and the slope of the line is the TTTD (Fan et al., 2001; Kil et al., 2010; González-Vega et al., 2013). Alternatively, TTTD can also be calculated from the following equation (Petersen and Stein, 2006):

$$TTTD\ (\%) = [Ca_{intake} - (Ca_{feces} - \text{total ECaL}) / Ca_{intake}] \times 100, \quad [4]$$

Basal endogenous losses of Ca have been measured using a Ca-free diet and the estimated total endogenous losses of Ca were 0.160g/kg of DMI for a canola meal diet in the absence of phytase and 0.189 in a canola meal diet in the presence of 1500 units/kg of supplemental phytase (González-Vega et al., 2013). Because pigs have endogenous losses of Ca, ATTD is not always an accurate expression of the digestibility of Ca. In contrast, values for STTD or TTTD may be more accurate because values for STTD or TTTD of Ca in individual feed ingredients are additive in mixed diets.

Retention

An additional measure of Ca absorption is retention of the nutrient. The following equations can be used to calculate the retention of Ca retained when expressed as grams per day or as a percentage, respectively (adapted from Petersen and Stein, 2006):

$$Ca_R\ (g/d) = [(Ca_{intake} - Ca_{feces} - Ca_{urine})] \times 100, \quad [6]$$

$$Ca_R\ (\%) = [(Ca_{intake} - Ca_{feces} - Ca_{urine}) / Ca_{intake}] \times 100, \quad [5]$$

where Ca_R is the Ca retained and Ca_{urine} is the total Ca excreted in the urine (grams).

REQUIREMENT FOR CALCIUM

Currently, a set of recommendations are provided by the NRC (2012) outlining the Ca and P requirements of swine (Table 2.1). Although improved from previous recommendations, more work is required before Ca recommendations are provided on a digestible basis and a ratio of STTD Ca:STTD P can be given. Values for STTD P in feed ingredients are in the current NRC (2012), but values for Ca are listed on a total basis only. To continue moving towards more accurate diet formulation, which provides reduced feed costs and nutrient excretion, it is

imperative that Ca requirements be formulated on the basis of STTD of Ca. Some work has already been completed determining the STTD of Ca in inorganic Ca ingredients (González-Vega et al., 2015a).

An individual animal's requirement for Ca may be based on its ability to maximize lean tissue growth, bone development, or other factors (Crenshaw, 2001). The requirement for maximal bone growth is greater than that to optimize soft tissue growth. Additionally, the Ca requirement for young growing swine may differ according to sex (NRC, 2012). It has been demonstrated that intact males have a greater requirement for Ca than gilts and castrated males and the Ca requirements are greater in gilts than in barrows (Table 2.2).

CALCIUM TO PHOSPHORUS RATIO

The NRC (2012) utilized a modelling approach to estimate the requirement for STTD P. The calculation started with calculating the level of P needed for maximizing whole-body P retention. It was assumed that 1) growth performance was optimized when P was offered at 85% of the requirement; 2) the marginal efficiency was 77% for the utilization of intake of STTD P for whole body P retention; 3) basal endogenous losses of P is 190 mg/kg of DMI 4) urine P losses are 7 mg/kg of BW/d at a minimum; and 4) whole-body P mass can be directly computed from body protein (**BP**). The total Ca requirements were then calculated from the STTD P requirements. The NRC (2012) has designated a ratio of 2.15:1 for the Ca:STTD P requirements or a ratio between 1.00:1.00 and 1.25:1.00 for total Ca: total P.

$$\text{STTD P requirements (g/d)} = 0.85 \times [(\text{maximum whole body P retention})/ 0.77 + (0.19 \times \text{DMI}) + (0.007 \times \text{BW})], \quad [6]$$

$$\text{Body P mass (g)} = 1.1613 + 26.012 \times \text{BP} + 0.2299 \times (\text{BP})^2 \quad [7]$$

The importance of the Ca to P ratio has been long evaluated and is still not fully understood. It appears that under Ca or P deficiency, the ratio is more important than if adequate levels of Ca and P are provided in the diet (Hayes, 1976; Peo, 1991; Crenshaw, 2001). It has long been reported that wide Ca:P ratios are detrimental to pigs and other species.

CALCIUM ABSORPTION

In pigs, Ca may be absorbed via passive or via active transport (Bronner, 2003). Passive absorption is thought to be the primary route used by the pig when dietary Ca supply is at or above the requirement; whereas, active absorption is the primary route when pigs are supplied low levels of dietary Ca.

Passive Absorption

Passive absorption is also referred to as passive diffusion or para-cellular absorption. This occurs primarily in the jejunum and ileum sections of the small intestine and is dependent on the concentration of Ca in the intestinal lumen and the electrochemical gradient across the epithelium (Gropper et al., 2009). It is imperative that this pathway is regulated to allow selective permeability. Using the passive transport pathway, Ca is passively moved from the lumen of the small intestinal space through tight junctions between the enterocytes (Gropper et al., 2009). Passive absorption is increased with increased solubility of Ca in the distal small intestine and increased by the amount of time the chyme resides in these segments of the intestine (Gropper et al., 2009).

Active Transport

Active absorption is also referred to as trans-cellular absorption as it passes through the cell. Active transport of Ca occurs primarily in the proximal small intestine and is a saturable process requiring energy and a Ca-binding protein for transport (Gropper et al., 2009). Calcium enters the lumen in the form of Ca salts. The reduction of pH in the stomach causes the Ca to solubilize. The free Ca enters the enterocyte by Ca channels located in the brush border membrane (Bronner, 2003). The Ca channels are referred to as transient reception potential vanilloids (**TRPV**). In the intestines, TRPV6 is present, whereas TRPV5 is located in the kidneys (Christakos, 2012). Upon absorption into the intestinal lumen of the enterocytes, Ca binds to the Ca-binding proteins. The Ca-binding proteins move Ca across the enterocyte (Gropper et al., 2009). Calcium exits the cell on the basolateral membrane by active transport using a Ca-Na pump or ATPase (Bronner, 2003), an enzyme that releases ATP and allows Ca to exit from the cell (Gropper et al., 2009).

PHOSPHORUS ABSORPTION

Phosphorus in feed is in the form of inorganic and organic mixtures. Organic P is poorly hydrolyzed by phosphatases in the intestines, and consequently, inorganic phosphate is the primary form of P absorbed (Food and Nutrition Board, 1997). Phosphorus absorption occurs in the duodenum and jejunum of the small intestine (Gropper et al., 2009). Similar to the absorption of Ca, P absorption occurs by facilitated diffusion or by an active transport pathway. The organically bound phosphate in the lumen is hydrolyzed by phospholipase and alkaline phosphatases. The phosphate molecule can diffuse across the enterocyte by entering the brush border membrane and exit via the basolateral membrane where it can complex as organic

phosphate or hydrogen can be added (Gropper et al., 2009). Alternatively, phosphate can be actively transported across the enterocyte by a carrier protein. Active absorption using 1, 25-dihydroxyvitamin D_3 is important when dietary intake of P is low; however, facilitated diffusion is otherwise the predominant route of absorption (Gropper et al., 2009). Even so, the efficiency of P absorption is fairly consistent over a broad range of dietary intakes of P (Food and Nutrition Board, 1997). Phosphorus excretion is primarily regulated by the kidneys (Stein et al., 2011). The parathyroid hormone (**PTH**) is the hormone secreted by the chief cells in the parathyroid gland, and this hormone is responsible for clearing P levels, allowing for P to either be reabsorbed into the body or removed through the urine (Food and Nutrition Board, 1997).

ENDOCRINE REGULATION OF CALCIUM HOMEOSTASIS

The 3 primary hormones that are responsible for maintaining Ca homeostasis are PTH, Vitamin D, and calcitonin (Gropper et al., 2009). The PTH is produced and secreted by the chief cells located in the parathyroid gland (Gropper et al., 2009). Secretion of PTH results in an increase in serum Ca levels, but a reduction in P levels. The primary regulation of PTH is a conformational change in the Ca sensing receptor, which indirectly inhibits PTH secretion from the parathyroid gland (Gropper et al., 2009).

Vitamin D in the active form is referred to as calcitriol. The overall effect of calcitriol is to increase levels of Ca in plasma (Gropper et al., 2009). In kidneys, calcitriol is synthesized from 25-OH vitamin D by the enzyme 1-hydroxylase, and this process is stimulated by PTH. Calcitriol causes reabsorption of Ca in the kidneys using calbindin D28k, and in the intestines, calcitriol increases calbindin D9k synthesis (Gropper et al., 2009).

Calcitonin is also important for Ca homeostasis. It is produced by the parafollicular cells in the thyroid gland and has the primary function of reducing serum Ca concentrations (Gropper et al., 2009). It accomplishes this by 1) inhibiting Ca absorption in the gastrointestinal tract, 2) inhibiting the action of osteoclasts in the bone, 3) excreting Ca in the urine, and 4) reabsorbing P in the kidneys (Gropper et al., 2009). Calcitonin also inhibits bone resorption by osteoblasts (Gropper et al., 2009). Its primary function is to maintain the skeleton when Ca demands increase such as in growth and lactation (Wimalawansa, 1996).

DIETARY SOURCES OF CALCIUM AND PHOSPHORUS

Dietary sources of Ca and P for pigs include ingredients of animal origin, plant origin, and inorganic supplements. Ingredients of animal origin include meat and bone meal, meat meal, milk products, fish meal, feather meal, and blood products. Using animal ingredients is advantageous as they usually are highly digestible (Kim and Easter, 2001; Gottlob et al., 2006), palatable (Hansen et al., 1993; Ermer et al., 1994), immune supporting, and free from anti-nutritional factors (Liener, 1981; Li et al., 1991; Anderson and Wolf, 1995). Animal proteins contain concentrations of Ca between 0.02 and 10.94% and P between 0.28 and 5.26% (Table 2.3).

Plant ingredients also serve as dietary sources of Ca and P. Plant ingredients include cereal grains and co-products of cereal grains, and oilseed meals. Most plant ingredients are, however, poor sources of Ca. Plant sources contain between 0.02 and 1.70% Ca, and between 0.12 and 1.28% P (Table 2.4; NRC, 2012). Calcium in plant sources has been generally believed to be highly digestible (González-Vega et al., 2014). In contrast, P is poorly digested in plant ingredients because it is usually bound to phytate (Cheryan, 1980).

Inorganic supplements can contribute a significant portion of the dietary Ca and P. Most dietary Ca is supplied by limestone, mono-calcium phosphate, or di-calcium phosphate. Calcium and P supplements contain between 16.9 and 38.5% Ca and between 17.7 and 21.5% P (Table 2.5; NRC, 2012).

Phytate

Phytic acid (myo-inositol-hexakisphosphate; **IP6**), is the primary storage form of P in plants. Phytic acid is utilized by the seed during germination; it is synthesized after flowering and continues through development until desiccation (Bohn et al., 2008). The chelated form of phytic acid is referred to as phytate (Cheryan, 1980). Within the plant, IP6 is stored within protein storage vacuoles within the aleurone cell layer or within the embryo of the seed. There are 6 phosphate groups esterified to the inositol ring in IP6. With this configuration, the negatively charged IP6 molecule can bind to 12 protons or 6 Ca^{2+} cations (Cheryan, 1980). With a pH range of 0.5 to 10.5, the confirmation of the phytic acid molecule is 1 phosphate in the axial position and the remaining 5 in the equatorial position. The conformation of the molecule changes as the pH increases beyond this range (Cheryan, 1980). Metabolic features of the phytic acid molecule include 1) chelating abilities, or ability to bind to metals; 2) donating and accepting phosphate groups; and 3) anti-oxidizing properties (Cheryan, 1980).

Phytic acid is present in many of the plant feed ingredients that are provided in diets for pigs. Concentrations of phytate can be low, as with sorghum (0.18% phytate P), or can be relatively high, as in canola meal (0.65% phytate P; NRC, 2012). In plants, phytate is often observed as a salt mixture with Mg and K (Onyango et al., 2009).

Phytic acid has an affinity for Fe, Zn, Ca, Mg, and Mn. Phytate also reduces the solubility of Ca, Mg, Fe, Zn, Cu, Mn, Mo, and Co by forming complexes, which results in reduced

availability of these minerals to the animal (Erdman, 1979; Torre et al., 1991; Anderson and Wolf, 1995). The bioavailability of minerals is dependent on many factors including pH, mineral concentration, phytate concentration, and the interactions between phytate and the nutrients, but ingested minerals attached to phytate cannot be absorbed (Cheryan, 1980; Torre et al., 1991). The phytate-bound P is excreted by the animal, but to compensate for this, inorganic P is usually added to the diets (Wilt and Carlson, 2005).

DIET MODIFICATIONS TO RELEASE PHOSPHORUS FROM PHYTIC ACID

Phytate bound minerals are unavailable to humans, pigs, and poultry because these species lack the ability to sufficiently synthesize endogenous phytase (Selle and Ravindran, 2008). On average, swine diets contain phytate at a concentration of approximately 1% (Graf, 1983).

The requirements of Ca and P for pigs differ by age, sex, and production stage (NRC, 2012); therefore, it is important to feed to the nutrient requirements of the pig so that excretion of nutrients is minimized. Feed processing techniques such as fermenting, heating, and extruding the feed, reduce phytate bound P and increase the digestibility of nutrients (Lee et al., 2011; Merriman et al., 2011; Rojas and Stein, 2012).

Phytase

One possible way to reduce excretion of minerals from animals has been by using the enzyme phytase. Phytase is the common name for the molecule myo-inositol hexakisphosphate phosphohydrolase, an enzyme that hydrolyzes the phytate molecule, freeing P for absorption and yielding inositol and ortho-phosphates (Nelson et al., 1968; Lolas et al., 1976; Reddy et al., 1982).

Phytase is produced by yeasts, fungi, and bacteria (Patwardhan, 1937). Phytases can also be classified as 3-phytase, 5-phytase, or 6-phytase. The numerical prefix indicates the position on the phytate molecule where the phytase attaches. Microbial phytases are better at releasing P from soybean meal than *Aspergillus niger*-based phytases (Rodriguez et al., 1999) and more stable than other phytases (Matsui et al., 2000). Phytases, however, differ in their optimum pH environments (Simons et al., 1990; Nakamura et al., 2000) and in their heat stability.

Within the gastrointestinal tract, the pH varies because of gastric secretions. The pH ranges in the small intestine are 3.6 to 6.2 in the duodenum, 5.5 to 6.8 in the jejunum, and 7.0 to 7.4 in the ileum (Braud et al., 1976). The pH in the stomach is 3.5 to 4.4 and the pH in the large intestine is 6.0 to 6.4 (Merchant et al., 2011).

Phytase is used extensively in plant-based diets fed to poultry and swine to increase digestion of P and other nutrients otherwise chelated to the phytate molecule. Adding exogenous phytase to the diet is an attractive approach to increase mineral utilization by animals because it is a relatively inexpensive and labor-free process compared with other methods. Phytase may be reported as phytase units per kilogram of feed (**FTU**). The FTU describes the amount of phytase necessary to hydrolyze 1 µmol of P each minute from 150 µmol/L of sodium phytate at 37º C at a pH of 5.5 (Ravindran et al., 2001). The use of microbial phytase has eliminated the need to use excess P to fulfill the P requirement in pigs (Jendza et al., 2005; Zeng et al., 2014). By feeding low levels of P in combination with phytase, the amount of P excreted is also reduced (Jendza et al., 2005; Almeida and Stein, 2010).

Addition of microbial phytase to diets may also improve energy utilization, CP digestibility, and mineral metabolism (Zeng et al., 2014). Phytase supplementation increases total tract digestibility of Ca, P, Na, K, Mg, and Zn (Zeng et al., 2014) and retention of Ca, P, Cu, Zn, and Mn (Lan et al., 2002).

CALCIUM AND PHYTASE

Calcium precipitates phytate through the formation of insoluble Ca-phytate complexes (Nelson and Kirby, 1987). Including phytase in the diet hydrolyzes phytate and thereby prevents the binding of Ca, which increases the digestibility of Ca (Table 2.6; González-Vega et al., 2014). The concentration of Ca and the ratio of Ca to P may also influence the effectiveness of phytase. Addition of phytase to the diet is more effective at releasing P at deficient Ca levels than at the recommended requirements (Lei et al., 1994; Sebastian et al., 1996). Greater ratios of Ca to P reduce the efficacy of phytase (Qian et al., 1996). Phytase not only improves the digestibility of Ca in plant ingredients that contain phytate, but can also improve the digestibility of Ca in ingredients that do not contain phytate, such as fish meal (González-Vega et al., 2015b).

EFFECTS OF PARTICLE SIZE ON CA DIGESTIBILITY

A reduction in particle size has been associated with improved nutrient digestibility in swine due to increased surface area (Owsley et al., 1981; Giesemann et al., 1990; Healy et al., 1994), but reduction of particle size results in increased feed processing costs, and lesions and ulcers in the stomach may increase if particle size of corn is reduced to 400 µm (Wondra et al., 1995). However, very little is known about implications of various particle sizes of limestone in

swine diets. Ross et al. (1984) evaluated the effect of particle sizes in various inorganic Ca sources in 15-kg pigs and observed only differences in the relative bioavailability among sources of Ca, but no effect of particle size on the relative bioavailability in the pig.

Few data are available on the effects of various particle sizes of calcium carbonate in swine diets. Particle size of calcium carbonate has been studied extensively in poultry and results have indicated that particle size of calcium carbonate is important for egg shell formation, Ca retention, and bone mineral content (Roland, 1986; Rao et al., 1992; Zhang and Coon, 1997; de Araujo et al., 2011). For laying hens, it is recommended to use a mixture of particle sizes that includes particle sizes of 1 mm or greater as particle size is directly related to retention time in the gizzard (de Araujo et al., 2011). With increased particle size, an increase in retention time is observed, which not only increases solubility, but also provides a Ca reserve for egg shell formation (Zhang and Coon, 1997). In broiler chicks, coarse particle size reduces Ca retention in the intestines and decreases mineralization of bone (Guinotte et al. 1991; Guinotte et al. 1995). However, in ruminants, particle size of calcium carbonate has no effect on the digestibility of Ca (Matsushima et al., 1955), and in rats, reducing the particle size of calcium carbonate from 18.5 to 13.0 μm had no effect on balance of Ca, bone mineral content, or bone mechanical properties (Shahnazari et al., 2009).

DIETARY CATION-ANION BALANCE

The dietary cation-anion balance (**DCAB**) quantifies the difference of the cation and anions involved in regulation of the osmotic pressure and maintenance of the acid-base status of the body. It has also been referred to as the dietary electrolyte balance and the dietary cation anion difference. The cations, or positively charged ions include Na, Ca, Mg, and K. The anions,

or negatively charged ions, include Cl, P, and S (Wall et al., 1992). The DCAB is not a physiological status, but rather an equation that uses the principle of the theory of Strong Ion Difference proposed by Stewart (1981). By the strong ion difference, the concentration of the H ions and bicarbonate in the blood is dependent on the sum of strong ions in body fluids. The sum of the cations must equal the sum of the anions to maintain the electro-neutrality essential for physiological systems (Stewart, 1981).

Sodium is important for the central nervous system and movement across cell membranes via Na-K pumps. Chloride molecules are important in the formation of HCl and as a component of bile. Potassium has functions in the excitation of neuromuscular components. Sulfur is a component of enzymes and amino acids and has functions in protein structure and carbohydrate metabolism (NRC, 2012). Each of these electrolytes influence the proton concentration in the body. For example, when either a Na or a K ion is absorbed in the gastrointestinal tract, a H ion is released. However, when a Cl ion is absorbed, a bicarbonate ion is released (Stutz, 1992). Therefore, any changes in dietary concentrations of Na, K, and Cl may change the acid-base status in pigs.

The DCAB has been quantified using different variations of the general equation. One of the most common equations (Golz and Crenshaw, 1991; Patience and Chaplin, 1997; Budde and Crenshaw, 2003) used in the determination of the DCAB is the following:

$$DCAB = mEq\ (Na^{+} + K^{+}) - (Cl^{-}) / kg\ of\ DMI \quad [8]$$

This equation includes only the monovalent ions, but an equation that includes S in the negative term of the equation has also been proposed (Popplewell et al., 1993; Baker et al., 1998; Cooper et al., 2000). This equation may need to be considered if high concentrations of DDGS are fed.

$$DCAB = mEq\ (Na^+ + K^+) - (Cl^- + SO_4^-) / kg\ of\ DMI \quad [9]$$

Other equations have included additional ions such as Ca, Mg, and P (Patience and Chaplin, 1997).

$$DCAB = mEq\ (Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + P^- + SO_4^-) / kg\ of\ DMI \quad [10]$$

Limited work has been conducted in pigs evaluating the effects of different DCAB on mineral metabolism and conflicting results have been reported. No impact of electrolytes on Ca excretion and retention (Golz and Crensaw, 1990; Golz and Crenshaw, 1991) or bone mineral content (Budde and Crenshaw, 2003) has been reported. However, a reduction in Ca balance has also been observed when pigs consumed low DCAB diets (Patience and Chaplin, 1997).

CALCIUM AND SODIUM CHLORIDE

In the newest addition of the NRC (2012), Na and Cl requirements were increased. The Na requirement was increased to 0.40, 0.35 and 0.28% and the Cl requirement was increased to 0.50, 0.45, and 0.32% for pigs from 5 to 7 kg, 7 to 11 kg, and 11 to 25 kg of BW, respectively. In the previous edition (NRC, 1998), the Na requirement was 0.20, 0.15, and 0.10% and the Cl requirement was 0.20, 0.15, and 0.08% for pigs 5 to 10 kg, 10 to 20 kg, and 20 to 50 kg of BW. This dietary recommendation increase was supported by data demonstrating an improvement in N digestibility, growth, and feed efficiency (Mahan et al., 1999), and, when given a choice, barrows prefer higher levels of salt during the early weeks post weaning (Monegue et al., 2011).

It is unknown if increasing dietary salt influences the digestibility of Ca and P, or if modifying the dietary salt concentration influences the efficacy of supplemental exogenous phytase in pigs. In humans, however, addition of dietary salt may increase the amount of Ca

excreted in the urine (Sellmeyer et al., 2002) or the reabsorption of Ca in the kidneys being directly related to the reabsorption of Na in the kidney, and as resorption of Na decreases with a high salt diet, so does the resorption of Ca (Sellmeyer et al., 2002).

CALCIUM AND FAT

Because fat influences the transit time of digesta, it was hypothesized that increasing dietary fat levels may extend the amount of time ingredients are retained in the gastrointestinal tract and allow for increased phytate degradation (Soares et al., 2012). However, experiments comparing diets supplemented with plant oils (e.g., soybean oil) and un-supplemented diets have failed to demonstrate any effects of soybean oil on the digestibility of Ca (Steiner et al., 2006; Gonzalez-Vega et al., 2015b).

In humans, increasing Ca intake reduces the digestibility of fat (Bendsen et al., 2008) and elevated levels of dietary Ca and vitamin D increase energy loss by fecal excretion because of formation of insoluble Ca soaps if Ca and fat form indigestible complexes (Soares et al., 2012). However, diets used in the human experiments contained fats with a higher degree of saturation than the diets used in the experiments with pigs.

Sources of fat vary considerably in the relative concentrations of saturated, monounsaturated, and polyunsaturated fatty acids (Boyle and Long, 2006). Oils extracted from corn and soybeans have low concentrations of saturated fats (13 to 15% of total fatty acids) and greater concentrations of monounsaturated (24 to 25% of total fatty acids) and polyunsaturated fatty acids (61 to 62%; Boyle and Long, 2006). In contrast, beef tallow and palm oil contain more saturated fats (51 to 52% of total fatty acids) and more monounsaturated fatty acids (39 to 44% of total fatty acids), but less polyunsaturated fatty acids (4 to 10%; Boyle and Long, 2006).

It is, therefore, possible that saturated fats have a greater negative effect on Ca digestibility than unsaturated fat, but this hypothesis does not appear to have been tested.

CALCIUM AND SUCROSE

Synthetic diets have been used in research to determine the digestibility of particular nutrients such as Ca. To evaluate the digestibility of Ca from individual ingredients, it is preferred that the individual ingredient provides nearly all of the dietary Ca (direct procedure). Therefore, in experiments evaluating Ca digestibility, soybean meal is often replaced by potato protein isolate, which contains very little Ca (González-Vega et al., 2014) and other ingredients, such as sucrose and cornstarch are included as sources of energy. Sucrose is a disaccharide containing a glucose and a fructose unit. Sucrose is often included in these synthetic diets (Sulabo and Stein, 2013; González-Vega et al., 2014) because it is devoid of Ca and provides a palatable ingredient in swine diets.

It is unknown how inclusions of synthetic ingredients like sucrose influence the digestibility of Ca and P in pigs. In humans, glucose may increase the transit time and increase Ca absorption (Griessen et al., 1989). Others have reported an increase in urine excretion of Ca and P when sucrose levels are increased in diets consumed by humans (Holl and Allen, 1987). In rats, Ca absorption was reduced by the inclusion of fructose; however, P absorption was not impacted (Douard et al., 2010). There are, however, no data demonstrating possible effects of fructose or glucose on Ca absorption in pigs.

CALCIUM IN SYNTHETIC AND NATURAL DIETS

Using synthetic diets may reduce the digestibility and growth in animals compared to counterparts consuming a more natural-based diet (González-Vega et al., 2015b). Furthermore, the ATTD of Ca and STTD of Ca are greater in corn-based diets compared with cornstarch-based diets (González-Vega et al., 2015b).

It has been hypothesized that differences in Ca digestibility among diet types may be due to differences in fiber content of the diets (González-Vega et al., 2015b). Fiber increases passage rate in the gastrointestinal tract (Bueno et al., 1981; Hillemeier, 1995), which allows for mixing between contents in the lumen and gastric secretions, increasing interactions between nutrients and epithelial cells, and increasing nutrient absorption (Vander et al., 2001). Because of the lower fiber content of cornstarch based synthetic diets, a precipitation of Ca may occur, resulting in complexes between Ca and either P, phytate, or other charged ions. There are, however, no data demonstrating the effects of diet ingredient composition on the precipitation of Ca in the intestinal tract.

CALCIUM REQUIREMENT

The requirement for Ca should preferably be expressed as STTD of Ca (NRC, 2012) but at this point, no such values are available. Determining a single requirement for the dietary level of STTD Ca is difficult as the requirement may differ depending upon inclusion of dietary P, age, stage of production, and the ratio between STTD of Ca and STTD of P. Furthermore, these factors are not easy to study. In a typical requirement study, the design of the experiment is a titration of the particular nutrient in question, with all other factors maintained across treatments. However, in this case, that would lead to wide ratios between Ca and P at either end of the curve.

Alternatively, the Ca:P ratio can be maintained across treatments, but this fails to maintain a constant P concentration across dietary treatments. It is possible that the Ca requirement depends on the desired P concentration in the diet. Because of the environmental push towards low P fecal emissions, swine producers in various regions have moved towards a diet that is marginally deficient in P; however, other regions may not have those same pressures to reduce fecal output, and consequently, they may feed higher concentrations of dietary P. It is possible that the amount of Ca may differ in those 2 situations, and that dietary Ca recommendations are lower in marginally P-deficient diets. For these reasons, a requirement study would ideally include a titration of Ca at multiple levels of P. This will result in a large number of dietary treatments and a large number of pigs are needed to meet the objective.

CONCLUSIONS

Calcium is located primarily in the bone tissue of pigs, but is a highly regulated nutrient that serves many functions in the body beyond providing a rigid structure to support the body. Bone tissue serves as a reservoir for Ca, allowing the body to cope with a transient inadequate dietary supply of Ca. However, inadequate dietary Ca may lead to welfare concerns and also has negative consequences on growth performance from too little or too much dietary inclusion; consequently, it is important that Ca be provided at the appropriate requirement for the animal. The current recommendations for Ca, however, are provided on a total basis and have been derived from a modelling approach. Future recommendations preferably should be made available in terms of digestible Ca and should also provide an optimum ratio between STTD Ca and STTD P. The digestibility of Ca in inorganic supplements and some plant ingredients have been determined; however, the digestibility of Ca needs to be examined for the remaining feed

ingredients, such as animal proteins which may provide a significant portion of the dietary Ca. Like other minerals, Ca is known to interact with other molecules, and for this reason, nutritionists must be mindful of how dietary ingredients may alter the digestibility of Ca, which may, as a result, impact growth performance. Furthermore, more work is needed to determine the effect of including dietary components like sucrose, fat, fiber, and exogenous enzymes on the digestibility of Ca. Phytase has been demonstrated to affect Ca digestibility, particularly when diets contain ingredients rich in phytate. More work is needed to determine how phytase impacts the digestibility of Ca and the release of Ca observed from each ingredient.

LITERATURE CITED

Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968-2977.

Ammerman, C. B. 1995. Methods for estimation of mineral bioavailability. Pages 83-94 in Bioavailability of Nutrients for Animals. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, San Diego, CA.

Anderson, R. L., and W. J. Wolf. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J. Nutr. 125:581S-588S.

Baker, L. A., D. R. Topliff, D. W. Freeman, R. G. Teeter, and B. Stoecker. 1998. The comparison of two forms of sodium and potassium and chloride versus sulfur in the dietary cation-anion difference equation: effects on acid-base status and mineral balance in sedentary horses. Proc. Equine Nutrition and Physiology Society Annual Symposium. 389-395.

Baker, S. R., B. G. Kim, and H. H. Stein. 2012. Comparisons of values for standardized total tract digesbility and bioavailability of phosphorus in dicalcium phosphate and distillers dried grains with solubles fed to growing pigs. J. Anim. Sci. 91:203-210.

Bendsen, N. T., A. L Hother, S. K. Jensen, J. K. Lorenzen, and A. Astrup. 2008. Effect of dairy calcium on fecal fat excretion: a randomized crossover trial. Int. J. Obesity. 32:1816-1824.

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396-2403.

Bohn, L., A. S. Meyer, and S. K. Rasmussen. 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J. Zhejiang Univ. Sci. B. 9:165-191.

Boyle, M., and S. Long. 2006. Personal Nutrition. 8th ed. San Francisco, CA. Cengage Learning p. 123-147.

Braude, R., R. J. Fulford, and A. G. Low. 1976. Studies on digestion and absorption in the intestines of growing pigs. Measurements of the flow of digesta and pH. Br. J. Nutr. 36:497-510.

Bronner, F. 2003. Mechanisms of intestinal calcium absorption. J. Cell. Biochem. 88:387-393.

Budde, R. A., and T. D. Crenshaw. 2003. Chronic metabolic acid load induced by changes in dietary electrolyte balance increased chloride retention but did not compromise bone in growing swine. J. Anim. Sci. 81:197-208.

Bueno L., F. Praddaude, J. Fioramonti, and Y. Ruckebusch. 1981. Effect of dietary fiber on gastrointestinal motility and jejunal transit time in dogs.Gastroenterology 80:701–707.

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Critical Reviews in Food Sci. and Nutr. 13:297-335.

Christakos, S. 2012. Mechanisms of action of 1,25-dihydroxyvitamin D3 on intestinal calcium absorption. Rev. Endocr. Metab. Disord. 13:39-44.

Cooper, S. R., D. R. Topliff, D. W. Freeman, J. E. Breazile, and R. D. Geisert. 2000. Effect of dietary cation-anion difference on mineral balance, serum osteocalcin concentrations and growth in weanling horses. Equine Vet. Sci. 20:39-43.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. Pages 187-212 in Swine Nutrition. 2nd ed. A. J. Lewis, and L. L. Southern, ed. CRC Press, Washington, DC.

Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926-929.

de Araujo, J. A., J. H. V. da Silva, F. G. P. Costa, J. M. B. de Sousa, P. E. N. Givisiez, and N. K. Sakomura. 2011. Effect of the levels of calcium and particle size of limestone on laying hens. Brasileira de Zootecnia v.40, n.5, p.997-1005.

Douard, V., A. Asgerally, Y. Sabbagh, S. Sugiura, S. A. Shapses, D. Casirola, and R. P. Ferraris. 2010. Dietary fructose inhibits intestinal calcium absorption and induces Vitamin D insufficiency in CKD. J. Am. Soc. Nephrol. 21:261-271.

Erdman, J. W. Jr. 1979. Oilseed phytates: nutritional implications. J. Am. Oil Chem. Soc. 56:736-741.

Ermer, P. M., P. S. Miller, and A. J. Lewis. 1994. Diet preference and meal patterns of weanling pigs offered diets containing either spray-dried porcine plasma or dried skim milk. J. Anim. Sci. 72:1548-1554.

Ewing W., and S. J. Charlton. The Minerals Directory. 2nd ed. Context Products Ltd; Leicestershire, United Kingdom: 2007. Calcium; pp. 5a-5f.pp. 19a

Fan, M. Z., T. Archbold, W. C. Sauer, D. Lackeyram, T. Rideout, Y. Gao, C. F. M de Lange, and R. R. Hacker. 2001. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. J. Nutr. 131:2388-2396.

Food and Nutrition Board. 1997. DRI Dietary Reference Intake for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. National Academy Press, Washington, D. C. p. 146-189.

Giesemann, M. A., A. J. Lewis, J. D. Hancock, and E. R. Peo, Jr. 1990. Effect of particle size of corn and grain sorghum on growth and digestibility by growing pigs. J. Anim. Sci. 68(Suppl. 1):104 (Abstr.)

Golz, D. I., and T. D. Crenshaw. 1990. Interrelationship of dietary sodium, potassium, and chloride on growth in young swine. J. Anim. Sci. 68:2736-2747.

Golz, D. I., and T. D. Crenshaw. 1991. The effect of dietary potassium and chloride on cation-anion balance on swine. J. Anim. Sci. 69:2504-2515.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of Ca and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126-142.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93:2255-2264.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015b. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Scie. 93:4808-4818.

Gottlob, R. O., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. L. Nelssen, C. W.

Hastad, and D. A. Knabe. 2006. AA and energy digestibility of protein sources for growing pigs. J. Anim. Sci. 84:1396-1402.

Graf, E. 1983. Application of phytic acid determination in soybean. J. Am. Oil Chem. Soc. 60:1861-1867.

Griessen, M., P. V. Speich, F. Infante, P. Bartholdi, B. Cochet, A Donath, B. Courvoisier, and J. P. Bonjour. 1989. Effect of absorbable and nonabsorbable sugars on calcium absorption in humans. Gastroenterology 96:769-775.

Gropper, S. S., J. L. Smith, and J. L. Groff. 2009. Macrominerals. Pages 429-457 in Advanced nutrition and human metabolism. 5th ed.Wadsworth, Cengage Learning, Belmont, CA.

Guinotte, F., Y. Nys, and F. de Monredon.1991. The effects of particle size and origin of calcium carbonate on performance and ossification characteristics in broiler chicks. Poul. Sci. 70**:**1908-1920.

Guinotte, F., J. Gautron, Y. Nys, and A. Soumarmon. 1995. Calcium solubilisation and retention in the gastrointestinal tract in chicks (*Gallus domesticus*) as a function of gastric acid secretion inhibition and of calcium carbonate particle size. Br. J. Nutr. 73:125-139.

Hansen, J. A., J. L. Nelssen, R. D. Goodband, and T. L. Weeden. 1993. Evaluation of animal protein supplements in diets of early-weaned pigs. J. Anim. Sci. 71:1853-1862.

Hayes, V.W. 1976. Phosphorus in Swine Nutrition. Published by Grants-in-Aid Committee, National Feed Ingredients Association, 1 Corporate Place, Suite 360, West Des Moines, IA 50265, U.S.A.

Healy, B. J., J. D. Hancock, G. A. Kennedy, P. J. Bramel-Cox, K. C. Behnke, and R. H. Hines. 1994. Optimum particle size of corn and hard and soft sorghum for nursery pigs. J. Anim. Sci. 72:2227-2236.

Hendriks, W. H., and P. J. Moughan, 1993. Whole-body mineral composition of entire male and

female pigs depositing protein at maximal rates. Livest. Prod. Sci., 33:16 l-170.

Hillemeier, C. 1995. An overview of effects of dietary fiber on gastrointestinal transit. Pediatrics. 96:997-999.

Holl, M. G., and L. H. Allen. 1987. Sucrose ingestion, insulin response and mineral metabolism in humans. J. Nutr. 117: 1229-1233.

Howarth, R., F. Chan, D. J. Conley, J. Garnier, S. C. Doney, R. Marino, and G. Billen. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. Front. Ecol. Environ. 9:18-26

Jendza J. A., R. N. Dilger, S. A. Adedokun, J. S. Sands, and O. Adeola. 2005. *Escherichia coli* phytase improves growth performance of starter, grower, and finisher pigs fed phosphorus-deficient diets. J. Anim. Sci. 83:1882-1889.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959-2967.

Kim, S. W., and R. A. Easter. 2001. Nutritional value of fish meals in the diet for young pigs. J. Anim. Sci. 79:1829-1839.

Lan, G. Q., N. Abdullah, S. Jalaludin, and Y. W. Ho. 2002. Efficacy of supplementation of a phytase producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean meal diets. Poult. Sci. 81:1522-1532.

Lee, J. D., J, P. Holt, and K. W. Tudor. 2011. Nutrient digestibility and performance of weaned pigs fed soybean meal processed using fermentation or extrusion. J. Anim. Sci. 89(Suppl. 2):78 (Abstr.)

Lei, X. G., P. K. Ku, E. R. Millar, M. T. Yokoyama, and D. E. Ullrey. 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean diets of weanling pigs. J. Anim. Sci. 72:139-143.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. Klemm, R. D. Goodband. 1991. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062-4069.

Liener, I. E. 1981. Factors affecting the nutritional quality of soya products. J. Amer. Oil Chem. Soc. 3:406-415.

Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672-2683.

Lolas, G. M., N. Palamidis, and P. Markakis. 1976. The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. Cereal Chem. 53:867-871.

Mahan, D. C., T. D. Wiseman, E. M. Weaver, and L. E. Russell. 1999. Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3 week old weaned pigs. J. Anim. Sci. 77:3016-3021.

Matsui T., Y. Nakagawa, A. Tamura, C. Watanabe, K. Fujita, T. Najima, H. Yano. 2000. Efficacy of yeast phytase in improving phosphorus bioavailability in a corn- soybean-meal-based diet for growing pigs. J. Anim. Sci. 78:94-99.

Matsushima, J., T. W. Dowe, C. L. Comar, S. L. Hansard, and W. J. Visek. 1955. The effect of particle size on the utilization of bone meal and limestone by beef cattle. J. Anim. Sci. 14:1042-1049.

Merchant, H. A., E. L. McConnell, F. Liu, C. Ramaswamy, R. P. Kulkarni, A. W. Basit, and S. Murdan. 2011. Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea

pig, rabbit and pig, and implications for their use in drug development. Euro. J. Pharm. Sci. 42:3–10.

Merriman, L. A., J. P. Holt, and K. W. Tudor. 2011. Nutrient digestibility and performance of weaned pigs fed diets containing fermented soybean meal and phytase. J. Anim. Sci. 89(Suppl. 2):44 (Abstr.)

Miller, E. R., D. E. Ullrey, C. L. Zutaut, V. B. Baltzer, D. A. Schmidt, J. A. Hoefer, and R. W. Luecke. 1962. Calcium requirement of the baby pig. J. Nutr. 77:7-17.

Monegue, J. S., M. D. Lindemann, H. J. Montegue, and G. L. Crondwell. 2011. Growth performance and diet preference of nursery pigs fed varying levels of salt. J. Anim. Sci. 89(Suppl. 2):66-67 (Abstr.)

Nakamura, Y., H. Fukuhara, and K. Sano. 2000. Secreted phytase activities of yeasts. Biosci. Biotechnol. Biochem. 64:841-844.

Nelson, R. S., and L. K. Kirby. 1987. The calcium binding properties of natural phytate in chick diets. Nutr. Rep. Int. 35:949-956.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1968. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poult. Sci. 47:1842-1848.

Nimmo, R. D., Peo, Jr., E. R., Moser, B. D., and A. J. Lewis. 1981. Effect of level of dietary calcium-phosphorus during growth and gestation on performance, blood and bone parameters of swine. J. Anim. Sci. 52 (6):1330-1342.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous

amino acid losses from the intestinal tract of chickens. Br. Poult. Sci. 101:836-842.

Owsley, W. F., D. A. Knabe, and T. D. Tanksley, Jr. 1981. Effect of sorghum particle size on digestibility of nutrients at the terminal ileum and over the total digestive tract of growing-finishing pigs. J. Anim. Sci. 52:557-566.

Patience, J. F., and R. K. Chaplin. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75:2445-2452.

Patwardhan, V. N. 1937. The occurrence of a phytic-splitting enzyme of the intestines of albino rats. Biochem. J. 31:560-564.

Peo, E. R., Jr. 1991. Calcium, phosphorus, and vitamin D in swine nutrition. In: E. R. Miller, D. E. Ullrey, and A. J. Lewis, editors, Swine nutrition. Butterworth-Heinemann, Stoneham, MA. p. 165-182.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating true endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126-2132.

Pettey, L. A., G. L. Cromwell, Y. D. Jang, and M. D. Lindemann. 2015. Estimation of calcium and phosphorus content in growing and finishing pigs: whole empty body components and relative accretion rates. J. Anim. Sci. 93:158-167.

Popplewell, J. C., D. R. Topliff, D. W. Freeman, and J. E. Breazile. 1993. Effects of dietary cation-anion balance on acid base balance and blood parameters in anaerobically exercised horses. Equine Vet. Sci. 13:552-555.

Qian, H., E. T. Kornegray, and D. E. Conner, Jr. 1996. Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74:1288-1297.

Rao, K. S., D. A. Roland Sr., J. L. Adams, and W. M. Durboraw. 1992. Improved limestone retention in the gizzard of commercial leghorn hens. J. Appl. Poult. Res. 1:6-10.

Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies, and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80:338-344.

Reddy, N. R., M. D. Pierson, S. K. Sathe, and D. K. Salunkhe. 1982. Legume-based fermented foods: Their preparation and nutritional quality CRC Crit. Rev. Food Sci. Nutr. 17:335-370.

Rodriguez, E., Y. Han, and X. G. Lei. 1999. Cloning, sequencing, and expression of an Escherichia coli acid phosphatase/phytase gene (appA2) isolated from pig colon. Biochem. Biophys. Res. Commun. 257:117-123.

Rojas, O. J., and H. H. Stein. 2012. Digestibility of phosphorus by growing pigs of fermented and conventional soybean meal without and with microbial phytase. J. Anim. Sci. 90:1506-1512.

Roland D. A. 1986. Eggshell quality. IV: Oyster shell versus limestone and the importance of particle size or solubility of calcium source. Worlds Poul. Sci. J. 42:166-171.

Ross, R. D., G. L. Cromwell, and T. S. Stahly. 1984. Effects of source and particle size on the biological availability of calcium in calcium supplements for growing pigs. J. Anim. Sci. 59:125-134.

Ruddy, B. C., D. L. Lorenz, and D. K. Mueller. 2006. County-level estimates of nutrient inputs to the land surface of the conterminous United States, 1982–2001: U.S. Geological Survey Scientific Investigations Report 2006-5012.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci.113:99-122.

Sellmeyer, D. E., M. Schloetter, and A. Sebastian. 2002. Potassium citrate prevents increased urine calcium excretion and bone resorption induced by a high sodium chloride diet. J. Clin. Endocrinol. Metab. 87:2008-2012.

Shahnazari M., B. R. Martin, L. L. Legette, P. J. Lachcik, J. Welch, and C. M. Weaver. 2009. Diet calcium level but not calcium supplement particle size affects bone density and mechanical properties in ovariectomized rats. J. Nutr. 139:1308–1314.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Box, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broiler and pigs. Br. J. Nutr. 64:525-540.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 75:1519-1523.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 100:179-196.

Soares, M. J., L. L. Murhadi, A. V. Kurpad, W. L. Chan She Ping-Delfos, and L. S. Piers. 2012. Mechanistic roles for calcium and vitamin D in the regulation of body weight. Obes. Rev. 13:592-605.

Stein, H. H., B. Seve, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited Review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139-2144.

Steiner, T., R. Mosenthin, and R. Greiner. 2006. Influence of feeding level and dietary oil supplementation on apparent ileal and total tract digestibilities of phosphorus and calcium in pigs fed low phosphorus diets supplemented with microbial or wheat phytase. Can. J. Anim. Sci. 86:479-488.

Stewart, P. A. 1981. How to understand acid-base. A quantitative acid-vase primer for biology and medicine. Elsevier New Holland, New York, NY.

Stutz, W. A., D. L. Wall, D. R. Topliff, D. W. Freeman, W. B. Tucker, D. G. Wagner, and J. W. Breazile. 1992. Effects of dietary cation-anion balance on blood parameters and mineral metabolism in exercising horses. Oklahoma Agriculture Experiment Station. In: Animal Science Research Report 12:286-294.

Sulabo, R. C., and H. H. Stein. 2013. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. J. Anim. Sci. 91:1285-1294.

Torre, M., A. R. Rodriguez, and F. Saura-Calixto.1991. Effects of dietary fiber and phytic acid on mineral availability. Critical Reviews in Food Sci. Nutr.1:1-22.

U.S. Department of Agriculture. 2009. 2007 Census of agriculture: United States summary and state data. Vol 1. AC-07-A-51.

USEPA. 2003. National pollutant discharge and elimination system permit regulation and effluent guidelines and standards for concentrated animal feeding operations. Final Rule. Fed. Regist. 68:7176-7274.

Vander, A. J., D. Luciano, and J. Sherman. 2001. The digestion and absorption of food. In: A. J. Vander, D. Luciano, and J. Sherman, editors, Human physiology: the mechanisms of body function, 8th ed. McGraw-Hill Higher Education, Boston, MA. p. 553-591.

Wall, D. L., D. R. Topliff, D. W. Freeman, D. G. Wagner, J. W. Breazile, and W. A. Stutz. 1992. Effect of dietary cation-anion balance on urinary mineral excretion in exercised horses. Equine Vet. Sci. 12:168-171.

Wilt, H., and M. Carlson. 2005. Swine diet manipulation to minimize environmental impacts. Publication G2324 (2005), Outreach and Extension, University of Missouri, Columbia, Missouri.

Wimalawansa, S. J. 1996. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. Endocr. Rev. 17:533-585.

Wondra, K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757-763.

Zeng, Z. K., D. Wang, X. S. Piao, P. F. Li, H. Y. Zhang, C. X. Shi, and S. K. Yu. 2014. Effects of adding super dose phytase to the phosphorus-deficient diets of young pigs on growth performance, bone quality, minerals, and amino acids digestibilities. Asian-Australas J. Anim. Sci. 27:237-246.

Zhang, B., and C. N. Coon. 1997. The relationship of calcium intake, source, size, solubility in vitro and in vivo, and gizzard limestone retention in laying hens. Poult. Sci. 76:1702-1706.

TABLES

Table 2.1. Calcium and phosphorus requirements according to body weight of pigs[1]

	Body weight range (kg)			
Item, g/d	25-50	50-75	75-100	100-135
Total Ca	9.87	12.43	13.14	12.80
STTD P	4.59	5.78	6.11	5.95
ATTD P	3.90	4.89	5.15	4.98
Total P	8.47	10.92	11.86	11.97

[1]Values reported from NRC (2012).

Table 2.2. Calcium and phosphorus requirements as affected by sex

Item, %	Body weight range (kg)[1]					
	50-75			75-100		
	Barrow	Gilt	Intact Male	Barrow	Gilt	Intact Male
Total Ca	0.56	0.61	0.64	0.50	0.56	0.61
STTD P	0.26	0.28	0.30	0.23	0.26	0.29
ATTD P	0.22	0.24	0.25	0.19	0.22	0.24
Total P	0.50	0.53	0.55	0.45	0.49	0.53

[1]Values reported from NRC (2012).

Table 2.3. Concentration (%), apparent total tract digestibility (ATTD, %), and standardized total tract digestibility (STTD, %) of Ca and P in animal ingredients[1]

	Calcium			Phosphorus		
Ingredient	Total	ATTD	STTD	Total	ATTD	STTD
Blood cells	0.02	-	-	0.34	80	93
Blood meal	0.05	-	-	0.21	37	88
Blood plasma	0.13	-	-	1.28	92	98
Egg, spray dried	0.29	-	-	0.69	50	55
Feather meal	0.41	-	-	0.28	74	89
Fish meal	4.28	73.1	76.2	2.93	79	81
Meat and bone meal	10.94	53-81[2]	-	5.26	68	70
Meat meal	6.37	-	-	3.16	82	86
Milk, whey powder	0.27	-	-	0.34	82	92
Milk, whey protein concentrate	0.63	-	-	0.38	82	92
Casein	0.2	-	-	0.68	87	98
Poultry byproduct	4.54	-	-	2.51	48	53
Poultry meal	2.82	-	-	1.94	49	62

[1]Values from NRC (2012).

[2]Values from Sulabo and Stein (2013).

Table 2.4. Concentration (%), apparent total tract digestibility (ATTD, %), and standardized total tract digestibility (STTD, %) of Ca and P plant ingredients[1]

	Calcium			Phosphorus			
Ingredient,	Total	ATTD	STTD	Total	ATTD	STTD	Phytate P
Alfalfa hay	1.46	-	-	0.26	-	-	-
Alfalfa meal	1.14	-	-	0.30	50	55	-
Bakery meal	0.13	-	-	0.25	-	-	-
Barley	0.06	-	-	0.35	45	39	0.22
Canola meal, solvent extracted	0.69	35[2]	49[2]	1.08	28	32	0.65
Corn, yellow dent	0.02	47[3]	-	0.26	26	34	0.21
Corn germ	0.02	-	-	1.27	33	37	1.07
Corn gluten meal	0.03	-	-	0.49	38	47	-
Cottonseed meal	0.25	-	-	0.98	31	36	-
Flaxseed	0.38	-	-	0.61	21	28	-
Oats	0.03	-	-	0.35	33	39	0.19
Peas, field peas	0.09	-	-	0.42	49	56	0.17

Table 2.4. (Cont.)

Potato protein concentrate	0.04[4]	-	-	0.16[4]	-	-	-
Rice	0.09	-	-	0.34	29	33	0.18
Rice bran	0.22	-	-	2.16	13	23	1.74
Rye	0.08	-	-	0.30	43	50	0.2
Safflower meal	0.34	-	-	0.75	-	-	-
Sesame meal	1.70	-	-	1.18	29	42	0.89
Sorghum	0.02	-	-	0.27	30	40	0.18
SBM, solvent extracted	0.35	49[3]	-	0.64	39	48	0.36
Soy protein isolate	0.17	-	-	0.75	39	48	-
Sunflower meal, solvent extracted	0.38	-	-	0.95	20	29	0.84
Triticale	0.04	-	-	0.33	50	56	0.21
Wheat, hard red	0.06	-	-	0.39	46	56	0.22

[1]Values from NRC (2012).

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

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

[4]Values from Merriman et al. (2016).

Table 2.5. Concentration (%), apparent total tract digestibility (ATTD, %), and standardized total tract digestibility (STTD, %) of Ca and P in inorganic supplements (as fed basis)[1]

	Ca	ATTD Ca	STTD Ca	P	ATTD P	STTD P
Calcium carbonate	38.5	58.0[3]	60.4[3]	0.02	-	-
Dicalcium phosphate	24.8	75.3[3]	77.8[3]	18.8	73.9	81.4
Monocalcium phosphate	16.9	82.8[3]	85.9[3]	21.5	82.8	88.3
Calcium sulfate	21.9	-	-	-	-	-
Limestone, ground	35.8	72.2[2]	77.7[2]	0.01	-	-
Phosphate, defluorinated	32.0	-	-	18.0	-	-
Phophate, monoammonium	0.35	-	-	24.2	-	-
Sodium phosphate, dibasic	-	-	-	21.2	-	-
Sodium phosphate, monobasic	0.09	-	-	24.7	86.7	93.8

[1]Values from NRC (2012).

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

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

Table 2.6. The apparent total tract digestibility (ATTD, %) and standardized total tract digestibility (STTD, %) of Ca without and with phytase added to the diet.

	Without phytase		With phytase	
Ingredient,	ATTD	STTD	ATTD	STTD
Canola meal[1]	42.96	47.93	64.19	69.18
Calcium carbonate[2,3]	57.98	60.43	70.62	73.07
Monocalcium phosphate[3]	82.76	85.86	83.24	86.34
Dicalcium phosphate[3]	75.29	77.80	76.39	78.90
Fish meal[4]	73.07	76.21	84.01	86.88
Meat and bone meal[5]	74.54	76.83	79.66	81.94
Meat meal[5]	74.61	76.97	83.25	85.75
Poultry meal[5]	80.74	82.41	74.31	76.06
Poultry byproduct meal[5]	85.35	87.76	83.51	86.66
Soybean meal[6]	46.7			

[1]González-Vega et al. (2013). [2]Merriman et al. (2016). [3]González-Vega et al. (2015a). [4]González-Vega et al. (2015b). [5]Merriman et al. (2016). [6]Bohlkle et al. (2005)

CHAPTER 3

EFFECTS OF MICROBIAL PHYTASE ON THE APPARENT AND STANDARDIZED TOTAL TRACT DIGESTIBILITY OF CALCIUM IN FEED INGREDIENTS OF ANIMAL ORIGIN FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to determine effects of microbial phytase on the apparent tract digestibility (**ATTD**) and the standardized total tract digestibility (**STTD**) of Ca in meat and bone meal (**MBM**), meat meal (**MM**), poultry by product meal (**PBPM**), and poultry meal (**PM**). Four corn-potato protein isolate-based diets were formulated to contain 0.70% Ca using MBM, MM, PBPM, or PM as the sole source of Ca. All diets also contained 0.33% STTD P with extra P being supplied by monosodium phosphate if needed. Four additional diets that were similar to the previous diets except that they contained 500 units of microbial phytase / kg and a Ca-free diet were also formulated. Growing barrows (n = 72; initial BW = 14.91 ± 0.19 kg) were allotted to a randomized complete block design with 9 dietary treatments and 8 replicate pigs per treatment. Experimental diets were provided for 12 d with the initial 5 d being the adaptation period. Total feces were collected for 5 d using the marker-to-marker approach. Values for ATTD of Ca in each ingredient were calculated using the direct procedure. The basal endogenous loss of Ca was calculated from pigs fed the Ca-free diet and this value was used to calculate the STTD of Ca in each ingredient. Results indicated that if no phytase was used, the ATTD and STTD of Ca in PBPM were greater ($P < 0.05$) than in MBM and MM, but values for PM were not different from any other ingredients. However, if phytase was added to the diets, no differences in ATTD or STTD of Ca among ingredients were observed (interaction $P < 0.05$). There was no effect of microbial phytase on ATTD or STTD of Ca in the 4 ingredients. If no

phytase was used, no differences among diets containing the 4 ingredients were observed for ATTD of P, but if phytase was added, the ATTD of P was greater ($P < 0.05$) in the diet containing PBPM compared with the diet containing MM. However, microbial phytase did not increase the ATTD of P in any diet. In conclusion, addition of microbial phytase did not affect the digestibility of Ca or P in ingredients of animal origin, and only small differences in the digestibility of Ca and P among the 4 ingredients were observed.

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

INTRODUCTION

Calcium is an important mineral not only for the synthesis of bone tissue, but also for other physiological functions in the body such as muscle contraction, transmission of nerve pulses, and enzyme activation (Crenshaw, 2001; Ewing and Charlton, 2007). Most plant ingredients have a relatively low concentration of Ca (NRC, 2012), and therefore, Ca needs to be supplemented to diets fed to pigs by adding inorganic Ca (e. g., calcium carbonate and calcium phosphates). Animal proteins such as meat and bone meal and poultry byproduct meals may also be included in the diets not only as a source of protein, but also as a source of Ca. To formulate diets, it is important to know the digestibility of the nutrients in the ingredients, but there are limited data for the apparent total tract digestibility (**ATTD**) and the standardized total tract digestibility (**STTD**) of Ca in ingredients of plant or animal origin. Values for ATTD and STTD of Ca in some plant ingredients have been reported (Bohlke et al., 2005; González-Vega et al., 2013), but to our knowledge, values for ATTD and STTD of Ca in feed ingredients of animal origin have not been reported with the exception that the ATTD of Ca in meat and bone meal,

ATTD of Ca in meat meal, ATTD of Ca in fish bones, and the ATTD and STTD of Ca in fish meal were recently published (Malde et al., 2010; Sulabo and Stein, 2013; González-Vega et al., 2015b).

Microbial phytase increases the ATTD and STTD of Ca in plant ingredients because they contain phytate (González-Vega et al., 2013). Although limestone does not contain phytate, Ca from limestone may be bound to the phytate in plant ingredients (Poulsen, 1995; Rodríguez et al., 2013); therefore, the ATTD of Ca in limestone is increased if microbial phytase is added to the diet (González-Vega et al., 2015a). Likewise, ATTD and STTD of Ca in fishmeal is improved by microbial phytase (González-Vega et al., 2015b), but effects of microbial phytase on ATTD and STTD of Ca in other ingredients of animal origin have not been reported. Therefore, the objectives of this experiment were to test the hypotheses that there are differences in the ATTD and STTD of Ca among animal sources of Ca and that inclusion of microbial phytase to the diets may increase the ATTD and STTD of Ca in phytate-containing diets in which Ca is provided by feed ingredients of animal origin.

MATERIALS AND METHODS

All animal practices and procedures detailed within this protocol were reviewed and approved by The Institutional Animal Care and Use Committee at the University of Illinois. Pigs used in the experiment were the offspring of G-Performer boars mated to Fertilis 25 females (Genetiporc, Alexandria, MN).

Animals and Housing

Seventy-two pigs with an average initial BW of 14.91 ± 0.19 kg were randomly allotted to blocks of 8 pigs based on initial BW. There were 9 experimental diets with 8 replicate pigs per

treatment. Pigs were housed individually in metabolism crates equipped with a slatted floor, a feeder, a nipple drinker, and a screen floor for total fecal collection. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding

The animal proteins used in the experiment were meat and bone meal (**MBM**), meat meal (**MM**), poultry byproduct meal (**PBPM**), and poultry meal (**PM**), and these ingredients were donated by Darling Ingredients Inc. (Irving, TX). Four corn-based diets containing the MBM, MM, PBM, or PM as the only source of Ca were formulated (Table 3.1). Four additional diets that were similar to the previous diets with the exception that 500 units (FTU) of microbial phytase (Quantum Blue; AB Vista, Marlborough, UK) / kg was added at the expense of corn, were also formulated. Potato protein isolate and monosodium phosphate were included to meet the AA and P requirements (NRC, 2012). A Ca-free diet was also formulated to determine basal endogenous losses of Ca. This diet contained corn, potato protein isolate, soybean oil, monosodium phosphate, synthetic AA, vitamins, and minerals.

Pigs were fed experimental diets for 12 d and feed was provided in the amount of 3 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg of $BW^{0.60}$; NRC, 2012). The daily allotment of feed was divided into 2 equal meals and provided at 0700 and 1700 h. Pigs were provided free access to water throughout the experiment. The initial 5 d were designated as the adaptation period to the diets and fecal samples were collected quantitatively from d 6 to 12 using the marker-to-marker approach (Adeola, 2001). On d 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection and on d 11, ferric oxide was added to the morning meal to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Orts collected during the collection

period were dried in a forced-air oven at 65ºC, and the weight was subtracted from the total feed intake.

Sample Analyses

Diets and ingredients were analyzed for DM using a drying oven at 135˚C for 2 h (method 930.15; AOAC Int., 2007), for ash (method 942.05; AOAC Int., 2007), and for N using the combustion procedure (method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Crude protein was calculated as N × 6.25. Diets were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom^{2000} Fiber Analyzer, Ankom Technology, Macedon, NY). Diets were analyzed for phytase activity by ELISA, using Quantiplate Kits for Quantum Blue® as supplied by Envirologix and using the Envirologix method AP181, Rev. 12-28-11, with some modifications (ESC Standard Analytical Method SAM099). Phytate-bound P was analyzed in the diet samples using a Foss NIR spectrometer with the phytate-P levels predicted using AUNIR calibration standards (Standard Analytical Method 120 at ESC). Ingredients were also analyzed for Ca and P by inductively coupled plasma (**ICP**) spectroscopy (method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (method 975.03 B(b); AOAC Int., 2007). Diets and ingredients were also analyzed for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL) and for acid hydrolyzed ether extract on an ANKOM XT10 fat extractor (method AM 5-04; AOAC Int., 2007).

Fecal samples were dried in a forced-air oven at 65°C and ground through a 1-mm screen using a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). Fecal samples were analyzed for DM, Ca, and P as explained for diets and ingredients.

Calculations and Statistical Analyses

The ATTD values for Ca and P in all diets were calculated according to standard procedures (Almeida and Stein, 2010; NRC, 2012). Basal endogenous losses of Ca (**ECaL,** mg/kg of DMI) were determined from pigs fed the Ca free diet according to the following equation (Almeida and Stein, 2010):

$$\text{Basal ECaL} = ([Ca_{feces}/F_{intake})] \times 1{,}000 \times 1{,}000),$$

where Ca_{feces} is the average daily fecal Ca output (g) and F_{intake} is the ADFI (g of DM) from d 6 to 11. The daily ECaL in pigs fed the Ca-containing diets was calculated by multiplying the calculated ECaL per kilogram DMI by the daily DMI of each pig. The STTD (%) of Ca was calculated for each ingredient by correcting the ATTD values by the basal ECaL (Almeida and Stein, 2010):

$$\text{STTD (\%)} = [Ca_{intake} - (Ca_{feces} - \text{basal ECaL})/Ca_{intake}] \times 100.$$

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). This model contained fixed effects of diet and phytase, and the random effect of block. To verify normality of data and to identify outliers, the UNIVARIATE procedure of SAS was employed. The experimental unit was the pig. Statistical significance were observed when $P < 0.05$ and tendencies were considered at $0.05 \leq P < 0.10$.

RESULTS

The concentrations of Ca were 8.28% in MBM, 8.12% in MM, 3.60% in PBPM, and 3.95% in PM (Table 3.2). Corn and potato protein contained 0.02% and 0.04% Ca, respectively. All diets without microbial phytase added to the formulation contained no detectable level of phytase (Table 3.3). Diets formulated to contain 500 phytase units per kg analyzed between 473

and 716 units of phytase / kg.

There were differences ($P < 0.05$) among diets for Ca intake, Ca in feces, Ca output, and basal ECaL, but no interactions between diets and phytase were observed for these parameters (Table 3.4). Phytase reduced ($P < 0.05$) the concentration of Ca in feces and tended ($P < 0.07$) to reduce daily intake of Ca.

When phytase was not included in the diet, absorption of Ca was greater ($P < 0.05$) for PM than for MBM, MM, and PBPM; and, if phytase was used, the absorption of Ca from PM was greater ($P < 0.05$) than from PBPM, but not greater than from MBM and MM (interaction, $P < 0.05$). The ATTD and the STTD of Ca in PBPM was greater ($P < 0.05$) than in MBM and MM if no phytase was used, but if phytase was included in the diets, no differences in ATTD or STTD among ingredients were observed (interaction, $P < 0.05$). Microbial phytase did not affect absorption of Ca, ATTD of Ca, or STTD of Ca.

Intake of P was greater ($P < 0.05$) if PM was included in the diet than if MBM, MM, or PBPM was used regardless of the level of phytase (Table 3.5). However, there were no differences among MBM, MM, or PBPM in P intake if no phytase was used, but if phytase was added to the diet, intake of P from PBPM was greater ($P < 0.05$) than from MM (interaction, $P < 0.05$). The concentration of P in feces was different ($P < 0.05$) among ingredients and was reduced ($P < 0.05$) if phytase was used. The amount of P absorbed was greater ($P < 0.05$) from PM compared with MM and MBM, regardless of the level of phytase in the diet. However, there were no differences among MBM, MM, and PBPM if no phytase was included in the diet, but if phytase was added to the diet, P absorption was greater ($P < 0.05$) from PBPM than from MM (interaction, $P < 0.05$). The ATTD of P was not different among MBM, MM, PBPM, and PM if phytase was not added to the diet, but if phytase was used, the ATTD of P in PBMP was greater

($P < 0.05$) than in MM, but not different from MBM or PM (interaction, $P = 0.053$)

DISCUSSION

Meat and bone meal, MM, PBPM, and PM are products from rendering of animals. Meat and bone meal is the rendered product that contains bone excluding hair, hoof, hide, or intestinal contents (AAFCO, 2011). It must contain at least 4.0% P and Ca cannot exceed 2.2 × P (AAFCO, 2011). Meat meal contains the rendered animal tissues, but excludes blood, hair, hoof, horn, hide, manure, and stomach and rumen contents; and the Ca level may not exceed the P level by a multiplication factor of 2.2, but the concentration of P can be less than 4.0% (AAFCO, 2011). Poultry meal contains flesh, skin, or other parts of the bird except feathers, heads, feet, and the gastrointestinal tract, and PBPM may include necks, feet, intestines, and undeveloped eggs, but not feathers (AAFCO, 2011).

There are limited data available for the digestibility of Ca and P in ingredients of animal origin. Data for ATTD of Ca have been reported for MBM and MM (Sulabo and Stein, 2013), but not for PBPM and PM. The ATTD of Ca in meat and bone meal in this experiment was consistent with previous observations indicating that ATTD of Ca in MBM is between 57 and 81% (Sulabo and Stein, 2013). The diet containing PM was formulated based on the NRC (2012) value for Ca (2.82%), but the amount of Ca analyzed in PM was actually 3.95%. For this reason, the PM diet contained more Ca than predicted, but the level of Ca in the diet does not affect the digestibility of Ca if it is between 0.33 and 1.07% (Stein et al., 2011). It is, therefore, unlikely that the concentration of Ca in the diet containing PM influenced the ATTD or the STTD of Ca.

To our knowledge, no values for ATTD or STTD of Ca in MM, PBPM, or PM have been previously reported. The values presented in this experiment are within the ranges previously

reported for MBM (Jongbloed and Kemme, 1990; Rodehutscord et al., 1997; King et al., 2005; Bünzen et al., 2009) and results indicate that all these ingredients have excellent digestibility of Ca. Based on these results and others (Malde et al., 2010; Sulabo and Stein, 2013; Gonzalez-Vega et al., 2015b), it appears that the ATTD and STTD of Ca in feed ingredients of animal origin are greater than in plant ingredients (Bohlke et al al., 2005; González-Vega et al., 2013) and also greater than in calcium carbonate (Stein et al., 2011; González-Vega et al., 2015a).

Basal endogenous losses of Ca in this experiment were calculated at 0.55 g/kg of DMI. Previous reports (González-Vega et al., 2013; 2015a; Merriman and Stein, 2016) for basal endogenous losses have been reported between 0.16 and 0.396 g/kg of DMI. We are uncertain why basal ECaL is so variable in pigs, but it may be affected by the source of Ca in the diet (Gonzalez-Vega et al., 2014) or affected by other ingredients included in the diet.

Results from a previous experiment with fish meal (González-Vega et al., 2015b) indicated that the digestibility of Ca is improved by the addition of microbial phytase if fishmeal is added to a corn based diet. It was hypothesized that the phytate in corn may chelate Ca from fishmeal and that phytase would release this Ca. However, phytase does not appear to affect the STTD or ATTD of Ca in the ingredients used in this experiment. Each of these ingredients contain calcium carbonate in the bones and previous reports demonstrated that phytase is effective at improving the digestibility of Ca in calcium carbonate (González-Vega et al., 2015a). However, due to a reduced inclusion of corn in diets used in this experiment compared with diets used in previous experiments (Gonzalez-Vega et al., 2015a, 2015b), the concentration of phytate in diets used in this experiment was less than 50% of the concentration in diets used in previous experiments. This may have resulted in less Ca being chelated to phytate, which may be the reason for the lack of an effect of phytase in the present experiment. It is also possible that the

calcium carbonate in the bones in the ingredients used in this experiment is less likely to be chelated to phytate because it is bound in the hydroxyapatite complex in the bones.

The ATTD of P for meat and bone meal in this experiment (76%) was within the range of values previously reported between 54 and 85% (Jongbloed and Kemme, 1990; Poulsen, 1995; Rodehutscord et al., 1997; King et al., 2005; Bünzen et al., 2009; Sulabo and Stein, 2013) although 0.15% monosodium phosphate was added to the MBM diet in this experiment. The value for the ATTD of P in this experiment (76%) for MM was also consistent with previously reported values for MM (74 to 80%; Sulabo and Stein, 2013) if phytase is not included in the diet. Values for ATTD of P in PM and PBPM have not been reported. The ATTD of P in other animal proteins such as feather meal (56 to 67%; Sulabo et al., 2013), fish meal (63%; Kim et al., 2014), whey powder (84%; Kim et al., 2012), spray dried plasma protein (91%; Almeida and Stein, 2011), porcine blood meal (76%; Almeida and Stein, 2011), and avian blood meal (58%; Almeida and Stein, 2011) have been reported. The ATTD of P in PM and PBPM in this experiment are within the range of values observed for other animal proteins.

In conclusion, ingredients of animal origin may contribute a majority of the pigs' dietary Ca, and that Ca is highly digestible by growing pigs. Results from this experiment indicated that the digestibility of Ca and P in meat and bone meal, meat meal, poultry byproduct meal, and poultry meal are not affected by the addition of microbial phytase at 500 units per kg. Further research is needed to determine if adding microbial phytase to the diet affects the digestibility of Ca or P if diets contain greater concentrations of phytate.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968–2977.

Almeida, F. N., and H. H. Stein. 2011. Standardized total tract digestibility of phosphorus in blood products fed to weanling pigs. Rev. Colomb. Cience. Pecu. 24:617-622.

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer, Jr., eds. AOAC Int., Gaithersburg, MD.

Association of American Feed Control Officials (AAFCO). 2011. Official publication. AAFCO, Oxford, IN.

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396-2403.

Bünzen, S., H. S. Rostagno, D. C. Lopes, P. C. Gomes, F. A. M. Hashimoto, L. R. Apolônio, and C. G. Borsatto. 2009. Apparent and true digestibility of phosphorus from animal origin feedstuffs for swine. Arq. Bras. Med. Vet. Zootec. 61:903–909.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 187-212.

Ewing, W., and S. J. Charlton. 2007. Calcium. In: The minerals directory. 2nd ed. Context Products Ltd, Leicestershire, UK. p. 5a-5f, and 19a.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of Ca and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816. doi:10.2527/jas2013-6410.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level, and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126-142. doi: 10.1080/1745039X.2014.892249.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93:2255-2264. doi: 10.2527/jas.2014-8215.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015b. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818. doi: 10.2527/jas.2015-8992.

Jongbloed, A. W., and P. A. Kemme. 1990. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement, and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. Neth. J. Agric. Sci. 38:567-575.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85 (Suppl. 2):112. (Abstr.)

Kim, B. G., J. W. Lee, and H. H. Stein. 2012. Energy concentration and phosphorus digestibility in whey powder, whey permeate, and low-ash whey permeate fed to weanling pigs. J. Anim. Sci. 90:289-295.

Kim, B. G., Y. Liu, and H. H. Stein. 2014. Energy concentration and phosphorus digestibility in yeast products produced from the ethanol industry, and in brewers' yeast, fish meal, and

soybean meal fed to growing pigs. J. Anim. Sci. 92:5476-5484. doi: 10.2527/jas.2013-7416.

King, R. H., J. Cauduro, and P. J. Eason. 2005. The apparent digestibility of phosphorus from a range of inorganic sources is less than 100% in pigs. In: Manipulating pig production. X. J. E. Paterson, editor, Austr. Pig Sci. Assoc., Werribee, Victoria, Australia. p. 156.

Malde, M. K., I. E. Graff, H. Siljander-Rasi, E. Venalainen, K. Julshamn, J. I. Pedersen, and J. Valaja. 2010. Fish bones—a highly available calcium source for growing pigs. J. Anim. Physiol. Anim. Nutr. 94:e66-76. doi: 10.1111/j.1439-0396.2009.00979.x.

Merriman, L. A., and H. H. Stein. 2016. Effect of particle size of Ca carbonate on apparent and standardized total tract digestibility and retention of calcium by growing pigs. J. Anim. Sci. Submitted.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Poulsen, H. D. 1995. Phosphorus digestibility in phosphates and meat and bone meal. Rep. no. 34. Natl. Inst. Anim. Sci., Foulum, Denmark.

Rodehutscord, M., M. Faust, and C. Hof. 1997. Digestibility of phosphorus in protein-rich ingredients for pig diets. Arch. Anim. Nutr. 50:201-211.

Rodríguez, D. A., R. C. Sulabo, J. C. González-Vega, and H. H. Stein. 2013. Energy concentration and phosphorus digestibility in canola, cottonseed, and sunflower products fed to growing pigs. Can. J. Anim. Sci. 93:493-503. doi:10.4141/CJAS2013-020.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but reduces digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139-2144. doi: 10.2527/jas.2010-3522.

Sulabo, R. C., and H. H. Stein. 2013. Digestibility of phosphorus and Ca in meat and bone meal fed to growing pigs. J. Anim. Sci. 91:1285-1294. doi: 10.2527/jas.2011-4632.

Sulabo, R. C., L. I. Chiba, F. N. Almeida, S. D. Brotzge, R. L. Payne, and H. H. Stein. 2013. Amino acid and phosphorus digestibility and concentration of digestible and metabolizable energy in hydrolyzed feather meal fed to growing pigs. J. Anim. Sci. 91:5829-5837. doi: 10.2527/jas.2013-6267.

TABLES

Table 3.1. Ingredient composition of experimental diets, as-fed basis[1]

Ingredient, %	Diet				
	Ca free	Meat and bone meal	Meat meal	Poultry byproduct meal	Poultry meal
Ground corn	80.00	40.00	40.00	44.15	47.65
Potato protein	12.00	12.00	12.00	5.50	0.00
Soybean oil	3.00	3.00	3.00	3.00	3.00
Test ingredient	-	8.50	8.40	15.25	24.50
Monosodium phosphate	0.98	0.15	0.38	0.35	0.01
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20
Cornstarch	3.42	35.75	35.62	31.15	24.24
Total	100.00	100.00	100.00	100.00	100.00

[1]Four additional diets that were similar to the 4 Ca containing diets with the exception that they contained 0.01% of microbial phytase (5,000 phytase units per g; Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) were also formulated. The phytase premix was included in these diets at the expense of cornstarch and provided 500 phytase units of phytase per kilogram complete feed.

Table 3.1. (Cont)

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

Table 3.2. Analyzed composition of ingredients, as-fed basis

	Dietary ingredient[1]						
Composition, %	Corn	Potato protein	MBM	MM	PBPM	PM	MSP
DM	89.70	92.23	95.93	97.27	96.71	96.57	99.99
Ash	1.68	0.56	25.11	24.8	12.98	13.65	91.50
Ca	0.02	0.04	8.28	8.12	3.60	3.95	0.03
P	0.23	0.16	4.00	2.96	2.20	2.30	27.89
CP	6.63	81.93	53.84	55.24	68.77	73.27	-
AEE[2]	3.51	0.37	12.98	13.05	13.90	11.99	-
GE	3,753	5,247	4,364	4,347	5,158	5,149	-

[1]MBM = meat meal; MM = meat meal; PBPM = poultry byproduct meal; PM = poultry meal; MSP = monosodium phosphate.

[2]AEE = acid hydrolyzed ether extract.

Table 3.3. Analyzed composition of experimental diets without and with microbial phytase, as-fed basis

	Diet[1]								
	Without phytase					With 500 units/kg of microbial phytase			
Item	Ca free	MBM	MM	PBPM	PM	MBM	MM	PBPM	PM
DM, %	87.48	88.93	89.49	89.46	89.72	88.69	89.07	89.37	90.21
CP, %	16.97	17.86	16.94	18.70	21.36	17.31	19.16	15.55	20.61
ADF, %	3.40	3.29	2.15	2.37	3.00	1.99	2.26	3.62	2.93
NDF, %	9.80	7.73	8.26	9.53	12.08	7.50	6.32	9.14	10.55
Ash, %	2.70	2.72	3.51	3.74	5.00	3.47	3.88	3.40	4.60
Ca, %	0.09	0.72	0.68	0.62	0.94	0.72	0.69	0.54	0.91
P, %	0.48	0.54	0.49	0.54	0.69	0.50	0.48	0.59	0.71
Phytase, FTU/kg	< 50	< 50	< 50	< 50	< 50	716	595	473	634
Phytate P	0.27	0.28	0.28	0.29	0.21	0.29	0.27	0.40	0.40

[1]MBM = meat and bone meal; MM = meat meal; PBPM = poultry byproduct meal, PM = poultry meal.

[2]FTU = phytase units.

Table 3.4. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca by pigs fed meat and bone meal, meat meal, poultry byproduct meal, or poultry meal without or with microbial phytase[1,2]

Item	Ca intake, g/d	Ca in feces, %	Ca output, g/d	Ca absorbed, g/d	ATTD of Ca, %	Endogenous Ca, g/d	STTD of Ca, %
Without phytase							
Meat and bone meal	4.89	2.88	1.27	3.62^{c}	74.54^{b}	0.33	76.83^{b}
Meat meal	4.72	2.55	1.18	3.54^{c}	74.61^{b}	0.34	76.97^{b}
Poultry byproduct meal	4.71	1.75	0.70	4.01^{bc}	85.34^{a}	0.32	87.76^{a}
Poultry meal	6.60	2.52	1.27	5.33^{a}	80.74^{ab}	0.35	82.41^{ab}
With phytase							
Meat and bone meal	4.91	2.39	1.02	3.89^{bc}	79.66^{ab}	0.33	81.94^{ab}
Meat meal	4.62	1.77	0.83	3.79^{bc}	83.25^{ab}	0.34	85.75^{ab}
Poultry byproduct meal	3.60	1.41	0.60	3.00^{c}	83.51^{ab}	0.33	86.66^{ab}
Poultry meal	6.41	2.76	1.62	4.79^{ab}	74.31^{b}	0.35	76.06^{b}
SEM	0.27	0.21	0.14	0.23	2.35	0.02	2.40
P-value							

Table 3.4. (Cont.)

Ingredient	< 0.01	< 0.01	< 0.01	< 0.01	0.010	0.580	0.004
Phytase	0.07	0.032	0.365	0.121	0.413	0.994	0.345
Ingredient × phytase	0.14	0.133	0.074	0.016	0.010	0.934	0.013

[ab]Means within a column not sharing a common superscript letter are different ($P < 0.05$).

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

[2]Phytase included at 500 phytase units per kilogram.

Table 3.5. Apparent total tract digestibility (ATTD) of P by pigs fed a corn-potato protein based diets containing meat and bone meal, meat meal, poultry byproduct meal, or poultry meal without or with microbial phytase[1,2]

Item	P intake, g/d	P in feces, %	P output, g/d	P absorbed, g/d	ATTD of P, %
Without phytase					
MBM	3.67[b]	1.91	0.89	2.78[bc]	76.00[b]
MM	3.40[bc]	1.67	0.80	2.60[c]	76.01[b]
PBPM	3.22[bc]	1.65	0.71	2.51[c]	78.30[ab]
PM	4.85[a]	1.80	0.97	3.88[a]	80.12[ab]
With phytase[3]					
MBM	3.28[bc]	1.43	0.64	2.63[bc]	80.48[ab]
MM	2.75[c]	1.35	0.65	2.09[c]	75.79[b]
PBPM	3.93[b]	1.20	0.56	3.37[ab]	85.99[a]
PM	5.00[a]	1.81	1.13	3.87[a]	77.11[ab]
SEM	0.20	0.10	0.08	0.17	2.05
P-value					
Ingredient	< 0.01	0.002	< 0.01	< 0.01	0.031

Table 3.5. (Cont.)

Phytase	0.738	< 0.01	0.094	0.691	0.128
Ingredient × phytase	0.006	0.062	0.064	0.002	0.053

[ab]Means within a column not sharing a common superscript are different ($P < 0.05$).

[1]MBM = meat and meal; MM = meat meal; PBPM = poultry byproduct meal, PM = poultry meal.

[2]Data are least squares means of 8 observations for all treatments.

[3]Phytase included at 500 phytase units per kilogram.

CHAPTER 4

EFFECT OF PARTICLE SIZE OF CA CARBONATE ON APPARENT AND STANDARDIZED TOTAL TRACT DIGESTIBILITY AND RETENTION OF CALCIUM BY GROWING PIGS

ABSTRACT: Two experiments were conducted to evaluate particle size of Ca carbonate for diets fed to growing pigs. Experiment 1 was conducted to determine apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and retention of Ca among diets containing Ca carbonate produced to different particle sizes. Experiment 2 was conducted to determine if growth performance of weanling pigs was affected by particle size of Ca carbonate. In Exp. 1, 4 diets based on corn and potato protein isolate were formulated to contain identical concentrations of Ca and P, but the Ca carbonate used in the diets were ground to 4 different particle sizes (200, 500, 700, and 1125 µm). A Ca-free diet was formulated to determine basal endogenous losses of Ca. In Exp. 2, 4 diets were based on corn and soybean meal and the only differences among diets was that each diet contained Ca carbonate ground to the 4 particle sizes used in Exp. 1. In Exp. 1 and 2, diets were formulated to contain 0.70% Ca and 0.33% STTD P. In Exp. 1, 40 barrows (initial BW: 15.42 ± 0.70 kg) were allotted to the 5 diets with 8 replicate pigs per diet using a randomized complete block design. In Exp. 2, 128 pigs with an initial BW of 9.61 ± 0.09 kg were randomly allotted to 4 experimental diets. Each diet was fed to 8 replicate pens with 4 pigs per pen for 21 d. Results of Exp. 1 indicated that basal endogenous losses of Ca were 0.329 g/kg of DMI and particle size did not influence ATTD, STTD, or retention of Ca. The ATTD of Ca was 69.98 ± 3.24%, 74.28 ± 2.71%, 69.96 ± 2.93%,

and 72.07 ± 2.66% and the STTD of Ca was 74.15 ± 3.24%, 78.45 ± 2.71%, 74.13 ± 2.93%, and 76.24 ± 2.66% for diets containing Ca carbonate ground to 200, 500, 700, or 1125 µm. Retention of Ca was 67.39 ± 3.08%, 70.40 ± 2.63%, 63.93 ± 2.79%, and 67.18 ± 2.16% for diets containing Ca carbonate ground to 200, 500, 700, or 1125 µm. The ATTD of P was 64.46 ± 1.71%, 66.78 ± 2.65%, 64.18 ± 2.98%, and 63.18 ± 1.69% and retention of P was 61.38 ± 1.39%, 63.83 ± 2.82%, 61.90 ± 2.76%, and 60.94 ± 1.53% for diets containing Ca carbonate ground to 200, 500, 700 or 1125 µm. Neither ATTD of P nor retention of P were influenced by the particle size of Ca. Results of Exp. 2 indicated that ADG, ADFI, and G:F were not impacted by the particle size of Ca carbonate. In conclusion, particle size of Ca carbonate did not affect ATTD of Ca, STTD of Ca, or retention of Ca, ATTD of P, retention of P, or growth performance of weaned pigs.

Key words: calcium, Ca carbonate, digestibility, particle size, pigs, retention

INTRODUCTION

A reduction in the particle size of feed ingredients has been associated with improved nutrient digestibility in swine due to increased surface area of the particles (Owsley et al., 1981; Giesemann et al., 1990; Healy et al., 1992; Wondra et al., 1995). However, data on effects of reducing the particle size of Ca carbonate in diets are limited. Particle size of Ca carbonate has been studied extensively in poultry and results have indicated that particle size of Ca carbonate is important for egg shell formation, Ca retention, and bone mineral content (Roland, 1986; Rao et al., 1992; Zhang and Coon, 1997; de Araujo et al., 2011). For laying hens, it is recommended to use particle sizes of 1.00 mm or greater as particle size is directly related to retention time in the gizzard (de Araujo, 2011). With increased particle size, an increase in retention time is observed, which not only increases solubility, but also provides a Ca reserve for egg shell formation

(Zhang and Coon, 1997). In broiler chicks, coarse particle size reduces Ca retention in the intestines and decreases mineralization of bone (Guinotte et al. 1991; Guinotte et al. 1995). However, in ruminants, particle size of Ca carbonate has no effect on the digestibility of Ca (Matsushima et al., 1955), and in rats, reducing the particle size of Ca carbonate from 18.5 to 13.0 µm had no effect on balance of Ca, bone mineral content, or bone mechanical properties (Shahnazari et al., 2009). In pigs, particle size of Ca carbonate does not affect the relative bioavailability of Ca (Ross et al., 1984). However, to our knowledge, the impact of particle size on the digestibility of Ca and on pig growth performance has not been reported. Therefore, the objective of this experiment was to determine if particle size of Ca carbonate influences the apparent total tract digestibility (**ATTD**) or the standardized total tract digestibility (**STTD**) of Ca or the growth performance of growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocols describing animal procedures for the 2 experiments. Pigs used in both experiments were the offspring of Line 359 boars mated to C46 females (Pig Improvement Company, Hendersonville, TN).

Experiment 1, Digestibility and Retention of Ca

Animals and Housing. Forty growing barrows (average initial BW of 15.42 ± 0.70 kg) were randomly allotted to 5 experimental diets. Each diet was fed to 8 replicate pigs using a randomized complete block design based on BW. Pigs were allotted to experimental diets using The Experimental Allotment Program (Kim and Lindemann, 2007). Pigs were individually housed in metabolism crates equipped with a feeder, a fully slatted floor, and pigs were allowed

free access to water via a nipple waterer. During collection of urine and feces, a screen, a urine pan, and a urine bucket were placed under each crate to allow for total collection of both urine and feces.

Diets and Feeding. Four diets based on corn and potato protein isolate were formulated to contain identical concentrations of Ca and P, but the Ca carbonate (Iowa Limestone Company, Alden, IA) used in these diets were ground to 4 different average particle sizes (200, 500, 700, or 1,125 µm). The same batches of all ingredients were used in all diets (Table 4.1). All diets contained the same amount of corn and potato protein isolate to keep the level of phytate constant among diets (Table 4.2). Diets were formulated to contain approximately 0.70% Ca and 0.33% STTD of P (Table 4.3). One additional diet that was similar to the other diets except that this diet contained no Ca carbonate was also formulated. This diet was considered calcium-free and used to estimate the basal endogenous losses of Ca.

Pigs were offered feed at 3 times the maintenance requirement for energy (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012) for the duration of the experiment. The amount of feed offered was recorded daily. Orts were weighed, dried in a forced air oven at 65ºC, and accounted for in the calculation for feed consumption. The initial 5 d was an adaptation period to the diets and fecal samples originating from the feed that was provided from d 6 to 11 were collected quantitatively using the marker-to-marker approach (Adeola, 2001). On d 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection and on d 11, ferric oxide was added to the morning meal to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Urine was collected every morning from d 6 to 11 and a 20% subsample was stored at -20°C after

collection. After each urine collection, 50 mL of 6*N* HCL was added to each empty urine bucket. The stored urine was thawed and mixed at the conclusion of the experiment.

Sample Analyses

Diets were analyzed for DM using a drying oven at 135ºC for 2 h (Method 930.15; AOAC Int., 2007) and for ash (Method 942.05; AOAC Int., 2007). Diets were also analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ), and CP was calculated as N × 6.25. Diets were analyzed for crude fat using ether extraction (Method 920.39 (A); AOAC Int., 2007), ADF (Method 973.18; AOAC Int., 2007), NDF (Holst, 1973), and crude fiber (Method 978.10; AOAC Int., 2007). Urine samples were thawed at room temperature, mixed, filtered, and a 10 mL subsample of each urine sample was collected. Urine and diet samples were analyzed for Ca and P by inductively coupled plasma (**ICP**) spectroscopy (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Fecal samples dried in a forced-air oven at 65°C. Dried fecal samples were ground using a 1-mm screen in a Wiley Mill (model 4; Thomas Scientific, Swedesboro, NJ) and analyzed for DM and for Ca and P as explained for diets.

Calculations and Statistical Analysis. The ATTD values of Ca and P were calculated according to standard procedures (NRC, 2012). The basal endogenous Ca losses (**ECaL** mg / kg of DMI) were determined from pigs fed the Ca-free diet according to the following equation (Almeida and Stein, 2010):

$$\text{Basal ECaL} = ([Ca_{feces}/F_{intake}] \times 1{,}000 \times 1{,}000),$$

where Ca_{feces} is the average daily fecal Ca output (g) and F_{intake} is the average daily feed intake (g)from d 6 to 11. The daily basal ECaL in pigs fed the Ca-containing diets was calculated by multiplying the calculated ECaL per kilogram DMI by the daily DMI of each pig.

By correcting ATTD values for the basal ECaL, the STTD (%) of Ca was calculated for each ingredient (Almeida and Stein, 2010):

$$STTD = [Ca_{intake} – (Ca_{feces} – \text{basal ECaL})/Ca_{intake}] \times 100.$$

Retention of Ca was calculated using the following equation (Petersen and Stein, 2006):

$$Ca_R = [(Ca_{intake} – Ca_{fecal} - Ca_{urine})/Ca_{intake}] \times 100,$$

where Ca_R is Ca retention (%), Ca_{fecal} is Ca output in the feces, and Ca_{urine} is the total Ca output in the urine (g). The retention of P was also calculated using this equation.

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model contained diet as fixed effect and block as the random effect. To test for normality and identify outliers, the UNIVARIATE procedure of SAS was used, but no outliers were identified. The experimental unit was the crate. Because Ca carbonate particle size was not evenly spaced among sources, the interactive matrix language procedure of SAS (PROC IML) was used to obtain appropriate coefficients, and polynomial contrasts were used for determination of linear and quadratic effects of Ca carbonate particle size. Statistical significance was observed when $P < 0.05$ and tendencies were considered at $0.05 \leq P < 0.10$.

Experiment 2, Growth Performance

Animals and Housing. One hundred twenty eight pigs with an average initial BW of 9.61 ± 1.00 kg were randomly allotted to 4 experimental diets. There were 4 pigs per pen and each experimental diet was fed to 8 replicate pens. Pigs were housed in an environmentally controlled

room and had free access to water via a nipple waterer throughout the experiment. All pens were equipped with fully slatted floors. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding. Four corn-soybean meal-based diets were formulated to contain identical concentrations of Ca and P, but the Ca carbonate used in the diets was ground to 4 different average particle sizes (200, 500, 700, and 1,125 µm). The 4 sources of Ca carbonate used in this experiment were from the same batches as those used in Exp. 1. All diets contained the same quantity of corn and soybean meal to keep the level of phytate constant (Table 4.4). Diets were formulated to contain 0.70% total Ca and 0.33% STTD P.

Pigs were allowed ad libitum access to feed throughout the experiment. Pigs were weighed at the beginning and at the conclusion of the 21-d experiment. The amount of feed offered to each pen was recorded, and at the end of the experiment, the amount of feed left in the feeder was weighed and used to calculate feed disappearance. All diets were analyzed for DM, ash, GE, CP, Ca, and P (Table 4.5) as explained for Exp. 1.

Statistical Analyses. Data were analyzed using the linear and quadratic statements as explained for Exp. 1. The pen was the experimental unit and the alpha value was 0.05.

RESULTS

Experiment 1

Feed intake and fecal output increased linearly ($P < 0.01$) as particle size increased (Table 4.6). Intake of Ca (g/d) also increased linearly and a tendency ($P = 0.10$) for a linear increase as particle size increased was observed for daily output of Ca. Urine Ca increased linearly as particle size increased ($P < 0.05$).

Basal endogenous losses of Ca were 0.329 g/kg of DMI. No linear or quadratic effects of particle size were observed for ATTD of Ca, STTD of Ca, or retention of Ca, but daily basal endogenous losses of Ca increased linearly ($P < 0.01$) as particle size of Ca carbonate increased.

Phosphorus intake increased linearly ($P < 0.01$) and quadratically ($P < 0.05$) and fecal P output increased (linear, $P < 0.05$) with increasing particle size of Ca carbonate, but no effect of particle size was observed for urine P output (Table 4.7). Absorption of P increased ($P < 0.05$) linearly and quadratically ($P < 0.05$) as particle size increased, but no effect of particle size on ATTD of P or retention of P was observed.

Experiment 2

The ADG, ADFI, and G:F by pigs fed experimental diets for 21 d were neither linearly nor quadratically affected by the particle size of Ca carbonate (Table 4.8).

DISCUSSION

Results of experiments using diets based on corn, potato protein isolate, monosodium phosphate, and Ca carbonate indicate that the ATTD of Ca is between 61 and 71% and the ATTD of P is between 47 and 61% (Stein et al., 2011; Gonzalez-Vega et al., 2015b). Values obtained in this experiment were slightly greater, but less corn was included in diets used in this experiment, which may have influenced results because corn contributes phytate to the diet and phytate may bind Ca and thus reduce digestibility.

Particle size of Ca carbonate did not influence the relative bioavailability of Ca in Ca carbonate if bone ash or bone breaking strength were used as the response criteria (Ross et al., 1984). However, we are not aware of any previous reports on the effects of particle size on the ATTD or STTD of Ca in Ca carbonate, but the present results indicated that pigs were able to

digest and absorb Ca from Ca carbonate with the same efficiency if the particle size is between 200 and 1,125 µm. As a consequence, retention of Ca is also not influenced by particle size of Ca carbonate. This observation agrees with data from ruminants (Matsushima et al., 1955) and rats (Shahnazari et al., 2009), but is different from data for poultry. Anatomical differences between chickens and pigs may explain why responses to particle size differ, because chickens have a crop and gizzard, and laying hens require the inclusion of larger particle size of Ca carbonate for a Ca reserve for overnight egg formation (Zhang and Coon, 1997).

Calcium from Ca carbonate is mostly absorbed in the stomach or proximal duodenum (Gonzalez-Vega et al., 2014). Secretions of HCl from the stomach solubilize Ca from Ca carbonate into the ionic form. Even if particle size reduces the absorption of Ca in the duodenum, the pig may still be able to absorb the Ca from Ca carbonate later in the gastrointestinal tract, and as a result, ATTD of Ca may not be reduced. Data from a study that compared site of absorption of Ca from different sources of Ca indicated that differences among Ca sources exist in terms of where in the gastrointestinal tract Ca is absorbed (Gonzalez-Vega et al., 2014). It is, therefore, possible that particle size of Ca from Ca carbonate also influence the site of absorption of Ca, but research to determine the site of absorption of Ca carbonate within the intestinal tract will have to be conducted to answer this question.

In this experiment, basal endogenous losses of Ca were 0.329 g/kg of DMI. In previous experiments, values for endogenous losses have been reported in the range between 0.123 and 0.396 g/kg of DMI (Gonzalez-Vega et al., 2013, 2014, 2015a,b**)**.

In Exp. 1, feed intake, and therefore also Ca and P intake and output, were impacted by the particle size of Ca carbonate. This was surprising because pigs were offered the same amount of feed each day. The differences in ADFI, therefore, reflect differences in orts collected from

the pigs, with increasing quantities being collected as the particle size of Ca carbonate was reduced. Although ATTD and STTD values were not different among diets, the differences in feed intake were unexpected; therefore, a second experiment was conducted to determine if particle size of Ca carbonate influences feed intake of pigs that are allowed free access to commercial diets. The observation that there were no effects of particle size of Ca carbonate on ADG, ADGI or G:F of pigs in the second experiment indicated that particle size does not affect feed intake in pigs fed corn-soybean meal based diets. It is, therefore, likely that the differences in feed intake observed among treatments in Exp. 1 are a result of the semi-synthetic diets used in that experiment.

CONCLUSIONS

Results from these experiments indicate that Ca carbonate can be included in swine diets at a broad range of particle sizes without impacting the digestibility of Ca or P when diets are sufficient in Ca and P. The retention of Ca and P are also not influenced by the particle size of Ca carbonate. Likewise, there is no influence of particle size of Ca carbonate on growth performance of weanling pigs.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968-2977.

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer, Jr., eds. AOAC Int., Gaithersburg, MD.

de Araujo, J. A., J. H. V. da Silva, F. G. P. Costa, J. M. B. de Sousa, P. E. N. Givisiez, and N. K. Sakomura. 2011. Effect of the levels of calcium and particle size of limestone on laying hens. R. Bras. Zootec. 40:997-1005.

Giesemann, M. A., A. J. Lewis, J. D. Hancock, and E. R. Peo, Jr. 1990. Effect of particle size of corn and grain sorghum on growth and digestibility by growing pigs. J. Anim. Sci. 68(Suppl. 1):104 (Abstr.)

González-Vega, J. C., C. L. Walk, Y. Liu and H. H. Stein. 2013. Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level, and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126-142.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract

digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015b. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93:2255-2264.

Guinotte, F., Y. Nys, and F. de Monredon. 1991. The effects of particle size and origin of Ca carbonate on performance and ossification characteristics in broiler chicks. Poult. Sci. 70:1908-1920.

Guinotte, F., J. Gautron, Y. Nys, and A. Soumarmon. 1995. Calcium solubilisation and retention in the gastrointestinal tract in chicks (*Gallus domesticus*) as a function of gastric acid secretion inhibition and of Ca carbonate particle size. Br. J. Nutr. 73:125–139.

Healy, B. J., J. D. Hancock, G. A. Kennedy, P. J. Bramel-Cox, K. C. Behnke, and R. H. Hines. 1994. Optimum particle size of corn and hard and soft sorghum for nursery pigs. J. Anim. Sci. 72:2227-2236.

Holst, D. O. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. J. Assoc. Off. Anal. Chem. 56:1352–1356.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85 (Suppl. 2):112. (Abstr.)

Matsushima, J., T. W. Dowe, C. L. Comar, S. L. Hansard, and W. J. Visek. 1955. The effect of particle size on the utilization of bone meal and limestone by beef cattle. J. Anim. Sci. 14:1042-1049.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Owsley, W. F., D. A. Knabe, and T. D. Tanksley, Jr. 1981. Effect of sorghum particle size on

digestibility of nutrients at the terminal ileum and over the total digestive tract of growing-finishing pigs. J. Anim. Sci. 52:557-566.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126-2132.

Rao, K. S., D. A. Roland Sr., J. L. Adams, and W. M. Durboraw. 1992. Improved limestone retention in the gizzard of commercial leghorn hens. J. Appl. Poult. Res. 1:6-10.

Roland D. A. 1986. Eggshell quality. IV: Oyster shell versus limestone and the importance of particle size or solubility of calcium source. World Poult. Sci. J. 42:166-171.

Ross, R. D., G. L. Cromwell, and T. S. Stahly. 1984. Effects of source and particle size on the biological availability of calcium in calcium supplements for growing pigs. J. Anim. Sci. 59:125-134.

Shahnazari M., B. R. Martin, L. L. Legette, P. J. Lachcik, J. Welch, and C. M. Weaver. 2009. Diet calcium level but not calcium supplement particle size affects bone density and mechanical properties in ovariectomized rats. J. Nutr. 139:1308-1314.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by Ca carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139-2144.

Wondra, K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757-763.

Zhang, B., and C. Coon. 1997. The relationship of calcium intake, source, size, solubility in vitro and in vivo, and gizzard limestone retention in laying hens. Poult. Sci. 76:1702-1706.

TABLES

Table 4.1. Analyzed composition of ingredients

Ingredient	Ingredient (%)			
	DM	Ash	Ca	P
Ground corn (Exp. 1)	85.5	1.7	< 0.01	0.19
Potato protein isolate (Exp. 1)	90.5	1.7	0.04	0.14
Ca carbonate[1]				
200 µm	99.9	93.8	37.43	< 0.01
500 µm	99.9	82.4	38.30	< 0.01
700 µm	99.9	87.6	38.18	< 0.01
1125 µm	99.9	86.4	38.44	< 0.01
Monosodium phosphate	99.5	91.5	0.03	27.89
Ground corn (Exp. 2)	86.4	1.3	0.01	0.24
Soybean meal (Exp. 2)	84.2	5.9	0.31	0.59

[1]Ground to an average of the particle size indicated.

Table 4.2. Ingredient composition of experimental diets, Exp. 1

Ingredient, %	Ca carbonate particle size[1], µm				Ca-free
	200	500	700	1,125	
Ground corn	75.60	75.60	75.60	75.60	77.30
Potato protein isolate	18.00	18.00	18.00	18.00	18.00
Soybean oil	3.00	3.00	3.00	3.00	3.00
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20	0.20
Monosodium phosphate	0.98	0.98	0.98	0.98	0.98
Ca carbonate	1.73	1.73	1.73	1.73	-
L-Lys HCl, 78% Lys	0.09	0.09	0.09	0.09	0.09

[1]Ground to an average of the particle size indicated.

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

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

	Ca carbonate particle size[1], µm				
Item, %	200	500	700	1,125	Ca-free
DM	87.05	87.06	87.13	87.20	86.62
CP	20.36	19.83	20.35	19.84	20.52
Crude fat	3.13	3.19	3.21	3.50	3.14
NDF	10.44	9.09	9.48	12.08	13.34
ADF	3.29	3.45	3.62	4.41	3.81
Crude fiber	2.09	2.12	2.21	2.17	2.14
Ash	3.83	3.93	3.80	3.98	2.16
Ca	0.68	0.70	0.82	0.67	0.02
P	0.47	0.50	0.53	0.48	0.44

[1]Ground to an average of the particle size indicated.

Table 4.4. Ingredient composition of experimental diets, Exp. 2

Ingredient, %	Ca carbonate particle size[1], µm			
	200	500	700	1,125
Ground corn	63.47	63.47	63.47	63.47
Soybean meal	30.00	30.00	30.00	30.00
Soybean oil	3.00	3.00	3.00	3.00
Salt	0.40	0.40	0.40	0.40
Vitamin mineral premix[2]	0.20	0.20	0.20	0.20
Monosodium phosphate	0.70	0.70	0.70	0.70
Ca carbonate	1.55	1.55	1.55	1.55
L-Lys HCl, 78% Lys	0.41	0.41	0.41	0.41
DL-Met	0.10	0.10	0.10	0.10
L-Thr	0.12	0.12	0.12	0.12

[1]Ground to an average of the particle size indicated.

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

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

	Ca carbonate particle size[1], µm			
Item	200	500	700	1,125
DM, %	88.17	88.04	87.71	87.75
CP, %	16.72	16.95	19.31	18.00
GE, kcal/kg	4,023	3,997	3,935	3,953
Ash, %	0.04	0.04	0.05	0.05
Ca, %	0.70	0.86	0.73	0.69
P, %	0.52	0.55	0.50	0.55

[1]Ground to an average of the particle size indicated.

Table 4.6. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca in diets containing Ca carbonate ground to different particle size[1]

	Ca carbonate particle size[2], µm					*P*-value	
Item	200	500	700	1,125	SEM	Linear	Quadratic
Feed intake, g of DM/d	529	572	639	677	34	<0.01	0.55
Fecal output, g of DM/d	60	59	70	75	4.52	<0.01	0.71
Ca in feces, %	2.07	1.97	2.09	1.97	0.15	0.73	0.93
Ca intake, g/d	4.17	4.52	5.04	5.34	0.27	<0.01	0.54
Ca feces, g/d	1.24	1.17	1.46	1.49	0.14	0.10	0.94
Ca in urine, g/d	0.29	0.36	0.52	0.48	0.07	0.02	0.24
Ca absorbed, g/d	2.93	3.34	3.57	3.85	0.25	0.01	0.55
ATTD, Ca, %	69.98	74.29	69.96	72.07	2.89	0.81	0.76
Endogenous[3] Ca, g/d	0.17	0.19	0.21	0.22	0.01	<0.01	0.55
STTD, Ca (%)	74.15	78.45	74.13	76.24	2.89	0.81	0.77
Ca retention, %	67.38	70.40	63.93	67.18	2.69	0.69	0.82

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

[2]Ground to an average of the particle size indicated.

[3]Basal endogenous losses were determined from pigs fed the Ca-free diet as 0.329 g/kg of DMI.

Table 4.7. Apparent total tract digestibility (ATTD) of P in diets containing Ca carbonate ground to different particle size[1]

	Ca carbonate particle size[2], µm					*P*-value	
Item	200	500	700	1,125	SEM	Linear	Quadratic
P intake, g/d	2.80	3.23	3.82	3.66	0.20	< 0.01	0.02
P in feces, %	1.62	1.75	1.77	1.40	0.09	0.57	0.24
P in feces, g/d	1.00	1.07	1.32	1.35	0.08	< 0.01	0.45
P in urine, g/d	0.08	0.10	0.10	0.08	0.03	0.91	0.60
ATTD, P (%)	64.46	66.78	64.18	63.18	2.33	0.55	0.56
P absorbed, g/d	1.80	2.15	2.50	2.31	0.16	0.02	0.04
P retention, %	61.39	63.83	61.90	60.93	2.23	0.74	0.50

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

[2]Ground to an average of the particle size indicated.

Table 4.8. Growth performance of pigs fed diets containing Ca carbonate ground to different particle size, Exp. 2[1]

	Ca carbonate particle size[2], µm					*P*-value	
Item	200	500	700	1,125	SEM	Linear	Quadratic
Initial BW, kg	9.61	9.62	9.62	9.61	0.03	0.98	0.85
Final BW, kg	19.52	19.40	19.45	19.58	0.05	0.86	0.75
ADFI, g/d	728	733	731	738	2.0	0.69	0.97
ADG, g/d	472	466	468	475	1.6	0.85	0.72
G:F	0.649	0.637	0.640	0.643	0.001	0.81	0.58

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

[2]Ground to an average of the particle size indicated.

CHAPTER 5

EFFECTS ON APPARENT TOTAL TRACT DIGESTIBILITY OF MINERALS WITH INCLUSION OF TALLOW, CHOICE WHITE GREASE, PALM OIL, CORN OIL, OR SOYBEAN OIL TO DIETS FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to determine the effect of supplementing diets fed to growing pigs with fat sources differing in their concentrations of fatty acids on the apparent total tract digestibility (ATTD) of Ca, P, Mg, Zn, Mn, Na, and K. A diet based on corn, potato protein isolate, and 7% sucrose was formulated. Five additional diets that were similar to the previous diet with the exception that sucrose was replaced by 7% with either tallow, choice white grease, palm oil, corn oil, or soybean oil, were also formulated. Diets were formulated to contain 0.70% Ca and 0.33% standardized total tract digestible P. Growing barrows (n = 60; initial BW = 15.99 ± 1.48 kg) were allotted to a randomized complete block design with 2 blocks of 30 pigs, 6 dietary treatments, and 10 replicate pigs per treatment. Experimental diets were provided for 12 d with the initial 5 d being the adaptation period. Total feces were collected for a 5 d collection period using the marker-to-marker approach. Data were analyzed using the MIXED procedure of SAS with the fixed effect of diet and the random effect of block. Digestibility of DM was greater ($P < 0.05$) in the diet containing soybean oil compared with the diet containing choice white grease with all other diets being intermediate. The ATTD of Ca was greater ($P < 0.05$) for pigs fed diets containing soybean oil, corn oil, palm oil, or tallow than for pigs fed the basal diet or the diet containing choice white grease. The ATTD of S was greater ($P < 0.05$) for the diet with soybean oil, corn oil, palm oil, or tallow than the diet supplemented with choice white grease. The ATTD of P was greater ($P < 0.05$) for diets containing soybean oil, corn oil, palm oil, or tallow compared with the basal diet or the diet containing choice white grease. The ATTD of Zn,

Mn, Na, and K were not different among dietary treatments. In conclusion, supplementation of a basal diet with tallow, palm oil, corn oil, or soybean oil may increase the ATTD of some macrominerals; whereas, choice white grease did not influence the ATTD of minerals. There was no evidence of any negative effects of the fat sources used in this experiment on the ATTD of any minerals.

Key words: calcium digestibility, choice white grease, vegetable oil, minerals, pigs

INTRODUCTION

Because dietary fat influences intestinal transit time, it was hypothesized that increasing dietary fat levels may extend the amount of time ingredients are retained in the gastrointestinal tract and allow for increased time for phytate degradation (Soares et al., 2012). However, experiments comparing diets supplemented with soybean oil and un-supplemented diets failed to demonstrate effects of dietary fat on the digestibility of Ca and P (Steiner et al., 2006; González-Vega et al., 2015).

In humans, increasing dietary Ca intake reduces the digestibility of fat (Bendsen et al., 2008) and elevated levels of dietary Ca and vitamin D may increase loss of energy by fecal excretion because of formation of indigestible complexes between Ca and fat (Soares et al., 2012). However, the diets used in the human experiments contained fats with a higher degree of saturation than those used in the experiments with pigs (Steiner et al., 2006; González-Vega et al., 2015).

Sources of fat vary considerably in the relative concentrations of saturated, monounsaturated, and polyunsaturated fatty acids (Boyle and Long, 2006). Oil extracted from corn and soybeans have low concentrations of saturated fats (13 to 15% of total fatty acids) and

greater concentrations of monounsaturated (24 to 25% of total fatty acids) and polyunsaturated fatty acids (61 to 62%; Boyle and Long, 2006). In contrast, beef tallow and palm oil contain more saturated fats (51 to 52% of total fatty acids) and more monounsaturated (39 to 44% of total fatty acids), but less polyunsaturated fatty acids (4 to 10%; Boyle and Long, 2006).

To our knowledge, no experiments have been conducted to evaluate the impact of different sources of dietary fat on the digestibility of Ca and other minerals by pigs; however, based on the research with pigs and humans we hypothesized that fats with greater concentrations of saturated fatty acids may have a greater negative impact on Ca digestibility than more unsaturated fatty acids. Therefore, the objective of this experiment was to test the hypothesis that the apparent total tract digestibility (**ATTD**) of Ca and other minerals is influenced by the source of fat that is included in the diet.

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 C-46 females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

Sixty pigs with an average initial BW of 15.99 ± 1.48 kg were randomly allotted to 6 diets with 10 replicate pigs per treatment. There were 2 blocks of 30 pigs with 5 replicate pigs in each block. Pigs were housed individually in metabolism crates that were equipped with a slatted floor, a feeder, a nipple drinker, and a screen floor, which allowed for total fecal collection. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding

The soybean oil and choice white grease used in this experiment (Table 5.1) were purchased from the University of Illinois Feed Mill (Champaign, IL). The tallow, palm oil, and corn oil were purchased from Soapers Choice (Des Plaines, IL). A basal diet based on corn and potato protein isolate that included 7% sucrose was formulated. Five additional diets were formulated by adding 7% of either tallow, choice white grease, palm oil, corn oil, or soybean oil at the expense of sucrose (Tables 5.2 and 5.3). All diets were formulated to meet or exceed requirements for AA, Ca, and P. Diets were formulated to contain 0.70% Ca and 0.33% standardized total tract digestible P. The source of Ca for these diets was calcium carbonate (ILC Resources, Alden, IA).

Pigs were fed each diet for 12 d and they were provided feed at a level equal to 3 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The daily allotment of feed was divided into 2 equal meals and provided at 0700 and 1600 h. Pigs were provided free access to water throughout the experiment. The initial 5 d was the adaptation period to the diets and fecal samples were collected quantitatively from d 6 to 12 using the marker-to-marker approach (Adeola, 2001). On d 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection and on d 11, a different indigestible marker (ferric oxide) was added to the morning meal to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. The orts that were collected during the collection period were dried in a forced-air oven at 65ºC, and the weight was subtracted from the total feed intake.

Sample Analyses

Diets were analyzed for DM using a drying oven at 135˚C for 2 h (Method 930.15; AOAC Int., 2007) and for ash (Method 942.05; AOAC Int., 2007). Diets were also analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Crude protein was calculated as N × 6.25. Diets were also analyzed for ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Mineral analyses were conducted on diets by inductively coupled plasma (**ICP**) spectroscopy (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC Int., 2007). Diets were also analyzed for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL). Diets were analyzed for acid hydrolyzed ether extract content (method AM 5-04; AOAC Int., 2007) and for ether extract without acid hydrolysis using an ANKOM XT10 fat extractor (method AM 5-04; AOAC Int., 2007) with the exception that the acid hydrolysis step was not performed.

The 5 sources of fat were analyzed for fatty acid profile (Method 969.33; AOAC Int., 2007), ether extract (Method 996.06; AOAC Int., 2007), insolubles (Method CA 3a-46; AOAC Int., 2007), unsaponifiables (Method Ca 6a-40; AOAC Int., 2007), moisture (Method Ca 2c-25; AOAC Int., 2007), free fatty acid (Method Ca 5a-40; AOAC Int., 2007a), peroxide value (Method 965.33, AOAC Int., 2007), and total fatty acid profile (Methods 966.06, AOAC Int., 2007).

Fecal samples were dried in a forced-air oven at 65°C and ground through a 1-mm screen using a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). Fecal samples were analyzed for DM, minerals, acid hydrolyzed ether extract, and ether extract as explained for diets.

Calculations and Statistical Analyses

The ATTD values for DM, Ca, P, Mg, K, Na, S, Zn, Mn, ether extract, and acid hydrolyzed ether extract were calculated according to standard procedures (Almeida and Stein, 2010; NRC, 2012) using the direct method. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). This model contained diet as a fixed effect and block as a random effect. To test for normality and to identify outliers, the UNIVARIATE procedure of SAS was used. The experimental unit was the pig. Statistical significance was observed when $P < 0.05$ and tendencies were considered at $0.05 \leq P < 0.10$.

RESULTS

The ether extract in the fat sources was between 86.58% and 99.83% (Table 5.1), and moisture was between 0.53 and 2.89%. The insoluble fat was between 0.08 and 0.76%. Unsaponifiables were between 0.27 and 0.77 among fat sources. Total fatty acids were between 85.79 and 94.09% and free fatty acids were between 0.21 and 11.29%. The peroxide value of the fat sources was between 5.51 and 45.25.

Palmitic acid concentration was 23.51, 23.09, and 41.51% of total fats in tallow, choice white grease, and palm oil, but only 11.67 and 10.90% in corn oil and soybean oil. Linoleic acid was 3.00, 12.24, and 8.21% of total fats in tallow, choice white grease, and palm oil, but 54.82 and 50.70% in corn and soybean oil, respectively.

Analyzed dietary concentrations of minerals was consistent among diets except for Ca where the basal diet analyzed 1.00% Ca (Table 5.3) even though identical concentrations of Ca were formulated among diets.

Digestibility of DM was greater ($P < 0.05$) for pigs fed the diet containing soybean oil than the diet containing choice white grease with all remaining diets being intermediate (Table 5.4). The ATTD of Ca and P were greater ($P < 0.05$) for pigs fed diets containing soybean oil, corn oil, palm oil, or tallow than pigs fed the basal diet or in the diet with choice white grease. There was a tendency ($P = 0.057$) for the ATTD of Mg to be greater in diets containing soybean oil or corn oil compared with diets containing tallow or choice white grease. There was no difference in the digestibility of Na among dietary treatments. The ATTD of S was greater ($P < 0.05$) for pigs fed the soybean oil, corn oil, palm oil, or tallow supplemented diets than in the basal diet or the diet supplemented with choice white grease. The ATTD of K, Mn, and Zn were not different among dietary treatments (Table 5.5).

The ATTD of ether extract was greater ($P < 0.01$) in diets containing soybean oil, corn oil, or palm oil than in the diet containing choice white grease, but not different from the diet containing tallow (Table 5.6). The ATTD of ether extract in the basal diet was less than in all other diets ($P < 0.01$). The ATTD of acid hydrolyzed ether extract was greater ($P < 0.01$) in the diet containing soybean oil than in the diet containing choice white grease with diets containing tallow, palm oil, or corn oil being intermediate, but the ATTD of acid hydrolyzed ether extract in the basal diet was less ($P < 0.01$) than in all other diets. Regardless of extraction method, fecal fat was greater ($P < 0.01$) in diets containing choice white grease or palm oil than in diets containing soybean oil or corn oil.

DISCUSSION

The concentrations of C16:0, C18:0, C18:1, and C18:2 fatty acids in tallow (Cera et al., 1988), choice white grease (Li et al., 1990), palm oil (Cater et al., 1997), corn oil (Cera et al.,

1988), and soybean oil (Li et al., 1990) were consistent with values previously reported, with greater concentrations of saturated fatty acids in tallow, choice white grease, and palm oil, but greater concentrations of unsaturated fatty acids in corn oil and soybean oil. The fat sources used in this experiment also contained different combinations of short-chain, medium-chain, long-chain, and very long chain fatty acids; therefore, if differences in fatty acid composition modified the digestibility of Ca or fat, then this would have been observed by this experiment.

The ATTD of Ca in these diets reflected the ATTD of Ca in calcium carbonate because calcium carbonate was the only source of Ca in the diets. The ATTD of Ca in these diets are comparable to values previously reported between 60 and 74% in diets containing calcium carbonate and soybean oil (Stein et al., 2011; González-Vega et al., 2015; Merriman et al., 2016).

With the exception of choice white grease, the ATTD of Ca was not negatively influenced by fat. This indicates that complexes between Ca and fat in the gastrointestinal tract may not result in a reduction in the digestibility of fat or Ca and it is, therefore, not likely that Ca influences the digestibility of energy. In this experiment, fat analyses were conducted by ether extraction only and by ether extraction preceded by an acid hydrolysis step. The acid hydrolysis step breaks the bonds between fat and Ca, and a better estimation of fat content is expected (Stoldt, 1952). Because it has been suggested that soaps between Ca and fat are excreted in the feces, samples were subjected to both methods to determine if such Ca fat complexes could be quantified. However, the values for ATTD of AEE and EE were almost identical, further indicating that complexes between Ca and fat are not excreted in the feces. These results are also in agreement with previous observations with pigs, indicating that there is no influence on the digestibility of Ca when fat is added to the diet at adequate concentrations of Ca (Steiner et al., 2006; González-Vega et al., 2015).

Results from human data (Davies et al., 2000; Zemel et al., 2000; 2001; 2002; Heaney, 2003; Lorenzen et al., 2007; Bendsen et al., 2008;) have indicated that increasing the concentrations of dietary Ca may result in formation of insoluble complexes between Ca and fat leading to excretion of Ca and fat in the feces and, because of the loss of fat, weight loss may follow. However, results have been inconclusive, and some data indicate that only Ca in diary products, not Ca from inorganic supplements, may be responsible for this observation (Lorenzen et al., 2007). Results from the present experiment indicated that at adequate concentrations of Ca and using calcium carbonate as the source of Ca, the digestibility of fat and Ca do not differ among different fat sources. Results from human data have reported greater concentrations of Ca in the feces with greater inclusion levels of dietary Ca (Bendsen et al., 2008). This observation may be only a result of greater concentrations of dietary Ca, and may be unrelated to formation of soaps between Ca and fat because the digestibility in the present experiment was not affected. It is also possible that the source of Ca in this experiment, calcium carbonate, may be less likely to form Ca soaps than other sources of Ca.

The ATTD of minerals varied among minerals. The ATTD of Na and S were high but consistent with values previously reported for Na (Sauer et al., 2008) and S (Song et al., 2013). The ATTD of Mg, Zn, and Mn were much lower than that of other minerals but the ATTD of Mg was comparable to previous reports (Kemme et al., 1997); whereas, values for the ATTD of Mn and Zn were slightly lower than previously observed (Liu et al., 2014). The ATTD of K was only slightly higher than values previously reported (Sauer et al., 2008).

The ATTD of Ca, P, EE, and S were lower in the diet containing choice white grease compared with the diets containing corn oil or soybean oil. The peroxide value for choice white grease was more than 40 mEq/kg, and it has been indicated that growth performance was

reduced if peroxide values were greater than 40 mEq/kg (DeRouchey et al., 2004). The peroxide value is an estimate of the concentration of hydroperoxides, and provides an estimate of the quality of the fat (DeRouchey et al., 2004). Previous results have demonstrated a reduction in growth performance as a result of lipid oxidation with choice white grease included at 6% of the diet (DeRouchey et al., 2004). Oxidation of fat in the present experiment also resulted in a decrease in the intake of fat compared with other fat supplemented diets. Furthermore, the choice white grease had greater concentrations of free fatty acids than the other fat ingredients, and this also indicates that the choice white grease that was used in this experiment had been oxidized. It was apparently the quality of the choice white grease, not the type of fat that caused the negative effects on the digestibility of nutrients by pigs fed the diet containing choice white grease.

Pigs fed the basal diet had greater feed intake than pigs fed fat supplemented diets. This was consistent with previous observations (DeRouchey et al., 2004). This was expected because the caloric density of the basal diet was less than that of the fat supplemented diets and pigs were limit fed at 3 times the maintenance energy requirement. A reduction of feed from the fat supplemented diets was, therefore, caused by the greater concentration of ME in the diets containing fat compared with the basal diet.

The low digestibility of fat in the basal diet is consistent with data from previous reports (Kil et al., 2010), and indicates that the low digestibility in fat is observed because of the relatively high contribution of endogenous fat to the total output of fat if low levels of fat are included in the diet. In addition, intact fat from plant ingredient has lower ATTD than extracted, supplemented fat (Kil et al., 2010). However, the ATTD of fat in the diets containing supplemented fat is consistent with results from previous experiments indicating that the ATTD

of fat was 89% for corn oil, 84% for choice white grease, and 82% for tallow by pigs (Cera et al., 1988).

In conclusion, results of this experiment failed to support the hypothesis that fat will bind Ca in the intestinal tract and thereby reduce the digestibility of both fat and Ca and under the conditions of this experiment, the degree of saturation of the dietary fat did not affect the digestibility of Ca. The implication of this observation is that there is no need to reduce the inclusion of fat in diets for pigs to avoid creating a reduction in digestibility of Ca. Likewise, it appears that the energetic contribution of fat is not reduced by the inclusion of calcium carbonate in diets fed to pigs. Results also indicated that Ca soaps may not always formed in the intestinal tract of pigs.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968-2977.

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer, Jr., eds. AOAC Int., Gaithersburg, MD.

Bendsen, N. T., A. L. Hother, S. K. Jensen, J. K. Lorenzen, and A. Astrup. 2008. Effect of dairy calcium on fecal fat excretion: a randomized crossover trial. Int. J. Obesity. 32:1816-1824.

Boyle, M., and S. Long. 2006. Personal Nutrition. 8th ed. San Francisco, CA. Cengage Learning p. 123-147.

Cater, N. B., H. J. Heller, and M. A. Denke. 1997. Comparison of the effects of medium-chain triacylglycerols, palm oil, and high oleic acid sunflower oil on plasma triacylglycerol fatty acids and lipid and lipoprotein concentrations in humans. Am. J. Clin. Nutr. 65:41-45.

Cera, K. R., D. C. Mahan, and G. A. Reinhart. 1988. Weekly digestibilities of diets supplemented with corn oil, lard or tallow by weanling swine. J. Anim. Sci. 66:1430-1437.

Davies, K. M., R. P. Heaney, R. R. Recker, J. M. Lappe, M. J. Barger-Lux, K. Rafferty, and S. Hinders. 2000. Calcium intake and body weight. J. Clin. Endocrinol. Metab. 85:4635-4638.

DeRouchey, J. M., J. D. Hancock, R. H. Hines, C. A. Maloney, D. J. Lee, H. Cao, D. W. Dean, and J. S. Park. 2004. Effects of rancidity and free fatty acids in choice white grease on growth performance and nutrient digestibility in weanling pigs. J. Anim. Sci. 82:2937-2944.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818.

Heaney, R. P. 2003. Normalizing calcium intake: projected population effects for body weight. J. Nutr. 133:268S-270S.

Holst, D. O. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. J. Assoc. Off. Anal. Chem. 56:1352–1356.

Kemme, P.A., A.W. Jongbloed, Z. Mroz, J. Kogut, A.C. Beynen. 1997. Digestibility of nutrients in growing-finishing pigs is affected by Aspergillus niger phytase, phytate and lactic acid levels. 1. Apparent ileal digestibility of amino acids. Livest. Prod. Sci. 58:107-117.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959-2967.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85 (Suppl. 2):112. Abstr.

Li, D. F., R. C. Thaler, J. L. Nelssen, D. L. Harmon, G. L. Allee, and T. L. Weeden. 1990. Effect

of fat sources and combination on starter pig performance, nutrient digestibility and intestinal morphology. J. Anim. Sci. 68:3694-3704.

Liu, Y., Y. L. Ma, J. M. Zhao, M. Vazquez-Anon, and H. H. Stein. 2014. Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. J. Anim. Sci. 92:3407-3415.

Lorenzen, J. K., S. Nielsen, J. J. Holst, I. Tetens, J. F. Rehfeld, and A. Astrup. 2007. Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake. Am. J. Clin. Nutr. 85:678-687.

Merriman, L. A., and H. H. Stein. 2016. Effect of particle size of calcium carbonate on apparent and standardizd total tract digestibility and retention of calcium by growing pigs. J. Anim. Sci. Submitted.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Sauer, W., M. Cervantes, J. Yanez, B. Araiza, and G. Muroch. 2008. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. Livest. Sci. 122:162-168.

Soares, M. J., L. L. Murhadi, A. V. Kurpad, W. L. Chan She Ping-Delfos, and L. S. Piers. 2012. Mechanistic roles for calcium and vitamin D in the regulation of body weight. Obesity Rev. 13:592-605.

Song, R., C. Chen, L. Wang, L. J. Johnston, B. J. Kerr, T. E. Weber, and G. C. Shurson. 2013. High sulfur content in corn dried distillers grains with solubles protects against oxidized lipids by increasing sulfur-containing antioxidants in nursery pigs. J. Anim. Sci. 91:2715 -2728.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the

apparent total tract digestibility of calcium, but reduces digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139-2144.

Steiner, T., R. Mosenthin, and R. Greiner. 2006. Influence of feeding level and dietary oil supplementation on apparent ileal and total tract digestibilities of phosphorus and calcium in pigs fed low phosphorus diets supplemented with microbial or wheat phytase. Can. J. Anim. Sci. 86:479-488.

Stoldt, W. 1952. Vorschlag zur Vereinheitlichung der Fettbestimmung in Leibensmitteln. F. Seif. Anst. 54: 206-207.

Zemel, M. B., H. Shi, B. Greer, D. Dirienzo, and P. C. Zemel. 2000. Regulation of adiposity by dietary calcium. FASEB J. 14:1132-1138.

Zemel, M. B. 2001. Effects of calcium-fortified breakfast cereal on adiposity in a transgenic mouse model of obesity. FASEB J. 15:A598

Zemel, M. B., W. Thompson, P. Zemel, A. M. Nocton, A. Milstead, K. Morris, and P. Campbell. 2002. Dietary calcium and dairy products accelerate weight and fat loss during energy restriction in obese adults. Am. J. Clin. Nutr. 70(Suppl):342-343.

TABLES

Table 5.1. Analyzed composition of fat sources[1]

	Ingredient				
Item, %	Tallow	Choice white grease	Palm oil	Corn oil	Soybean oil
Ether extract	86.58	98.99	96.25	99.83	97.21
Moisture	0.98	0.93	1.60	0.53	2.89
Insolubles	0.15	0.76	0.23	0.08	0.09
Unsaponifiables	0.27	0.61	0.24	0.77	0.51
Total fatty acids	85.79	92.39	92.82	94.31	94.09
Free fatty acids	0.36	13.98	0.38	0.21	0.22
Peroxide value	2.53	45.25	11.29	5.51	24.75
Fatty acid profile (expressed as percent of total fat)					
Myristic (14:0)	3.12	1.53	0.90	0.04	0.08

Table 5.1. (Cont.)

Myristoleic (9c-14:1)	0.69	0.12	0.00	0.00	0.00
C15:0	0.51	0.13	0.06	0.01	0.02
Palmitic (16:0)	23.51	23.09	41.51	11.67	10.90
Palmitoleic (9c-16:1)	2.69	2.21	0.15	0.11	0.09
Margaric (17:0)	1.44	0.50	0.12	0.07	0.10
Stearic (18:0)	15.19	13.29	4.36	1.80	4.60
Oleic (9c-18:1)	35.32	36.77	41.90	27.76	22.38
Vaccenic (11c-18:1)	1.34	2.51	0.69	0.59	1.42
Linoleic (18:2n6)	3.00	12.24	8.21	54.82	50.70
Linolenic (18:3n3)	0.21	0.51	0.16	0.91	6.65
Arachidic (20:0)	0.12	0.22	0.39	0.41	0.38
Gonodic (20:1n9)	0.25	0.88	0.17	0.26	0.21

Table 5.1. (Cont.)

Behenoic (22:0)	0.02	0.04	0.07	0.14	0.38
Erucic [22:1n9]	0.00	0.05	0.00	0.00	0.00
Lignoceric (24:0)	0.00	0.03	0.08	0.17	0.14

[1]Nervonic (24:1n9), DHA (22:6n3), Clupanodonic (22:5n3), EPA (20:5n3), 3n-Arachidonic (20:4n3), Arachidonic [20:4n6], Homo-a-linolenic(20:3n3), Stearidonic (18:4n3), and Elaidic (9t-18:1) were also analyzed, but were not detected in the samples.

Table 5.2. Ingredient composition of experimental diets, as-fed basis

Ingredient, %	Basal	Source of fat				
		Tallow	Choice white grease	Palm oil	Soybean oil	Corn oil
Corn	71.59	71.59	71.59	71.59	71.59	71.59
Potato protein isolate	18.00	18.00	18.00	18.00	18.00	18.00
Sucrose	7.00	-	-	-	-	-
Source of fat	-	7.00	7.00	7.00	7.00	7.00
Calcium carbonate	1.73	1.73	1.73	1.73	1.73	1.73
Monosodium phosphate	0.98	0.98	0.98	0.98	0.98	0.98
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
L-Lys HCl, 78% Lys	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20

Table 5.2. (Cont.)

Total	100.00	100.00	100.00	100.00	100.00	100.00

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

Table 5.3. Analyzed composition of experimental diets, as-fed basis[1,2]

	Diet					
Ingredient,	Basal	Tallow	Choice white grease	Palm oil	Corn oil	Soybean oil
DM, %	88.14	89.62	89.41	89.44	88.03	89.62
Ash, %	4.40	4.01	4.57	4.43	4.42	4.28
GE, %	3,940	4,275	4,360	4,457	4,460	3,798
CP, %	20.87	20.80	22.41	19.94	21.48	21.13
AEE[3], %	2.37	9.66	9.07	9.48	8.94	9.03
Ether extract, %	1.95	8.96	8.97	9.20	9.12	9.27
NDF	7.87	7.55	8.21	7.73	8.20	7.82
ADF	5.09	5.37	4.56	4.57	4.65	4.51
Ca, %	1.00	0.80	0.85	0.76	0.78	0.77

Table 5.3 (Cont.)

P, %	0.50	0.47	0.50	0.50	0.51	0.49
Na, %	0.37	0.40	0.37	0.36	0.41	0.38
K, %	0.25	0.22	0.26	0.25	0.24	0.25
Mg, ppm	662	574	693	686	659	658
Zn, ppm	79	100	143	83	93	128
Fe, ppm	174	163	154	163	162	162
Cu, ppm	13	12	17	25	10	12
Mn, ppm	69	84	59	60	52	31
S, %	0.28	0.30	0.30	0.28	0.30	0.28

[1]All diets were formulated to contain 1.40% Lys, 0.45% Met, 1.06% Thr, and 0.25% Trp.

[2]The basal diet was formulated to contain 3,399 kcal of ME per kg; the diets containing tallow, palm oil, choice white grease, corn oil, and soybean oil were formulated to contain 3,695, 3,715, 3,585, 3,747, and 3,747 kcal ME per kg (as fed basis), respectively.

[3]AEE = acid hydrolyzed ether extract.

Table 5.4. Apparent total tract digestibility (ATTD) of dry matter and macro-minerals in diets containing different fat sources

Item	Diet							
	Basal	Tallow	CWG[2]	Palm oil	Corn oil	Soybean oil	SEM	*P* Value
Intake, g/d	783[a]	644[c]	655[bc]	751[ab]	643[c]	680[bc]	38	0.023
Fecal output g DM /d	70[a]	54[bc]	63[ab]	62[abc]	53[bc]	51[c]	4	0.017
ATTD DM, %	89.79[bc]	90.68[ab]	89.25[c]	90.88[ab]	90.73[ab]	91.59[a]	0.49	0.006
Ca intake, g/d	6.27[a]	5.15[c]	5.24[bc]	6.01[ab]	5.15[c]	5.44[bc]	0.30	0.023
Ca output, g/d	3.10[a]	1.84[c]	2.43[b]	2.04[bc]	1.72[c]	1.55[c]	0.22	< 0.001
ATTD Ca, %	50.69[b]	65.13[a]	54.07[b]	66.07[a]	71.20[a]	71.24[a]	3.05	< 0.001
P intake, g/d	3.92[a]	3.22c	3.28[bc]	3.75[ab]	3.22[c]	3.4[bc]	0.19	0.023
P output, g/d	1.86[a]	1.21[c]	1.53[b]	1.46[bc]	1.30[bc]	1.26[c]	0.10	< 0.001
ATTD P, %	52.06[b]	62.00[a]	53.52[b]	61.06[a]	59.80[a]	62.98[a]	2.10	0.001
Mg intake, g/d	5.09[a]	4.18[c]	4.26[bc]	4.88[ab]	4.18[c]	4.42[bc]	2.46	0.023
Mg output, g/d	3.78[a]	3.07[bc]	3.47[ab]	3.57[ab]	2.90[c]	2.82[c]	2.19	0.004
ATTD Mg, %	25.62	24.73	23.53	26.94	30.91	35.37	3.44	0.057
Na intake, g/d	3.13[a]	2.57[c]	2.62[bc]	3.00[ab]	2.57[c]	2.72[bc]	0.15	0.023

Table 5.4. (Cont.)

Na output, g/d	0.30[a]	0.24[bc]	0.24[abc]	0.28[ab]	0.23[bc]	0.22[c]	0.02	0.049
ATTD Na, %	90.40	90.99	90.66	90.69	91.30	91.95	0.53	0.346
S intake, g/d	2.35[a]	1.93[c]	1.97[bc]	2.25[ab]	1.93[c]	2.04[bc]	0.11	0.023
S output, g/d	0.43[a]	0.32[b]	0.37[ab]	0.36[ab]	0.31[b]	0.32[b]	0.02	0.005
ATTD S, %	81.70[b]	83.75[a]	81.31[b]	83.98[a]	84.03[a]	83.40[a]	0.73	0.011
K intake, g/d	1.96[a]	1.61[c]	1.64[bc]	1.88[ab]	1.61[c]	1.70[bc]	0.09	0.023
K output, g/d	0.44	0.41	0.45	0.43	0.42	0.35	0.04	0.419
ATTD K, %	76.68	74.25	72.60	77.37	73.89	79.17	1.81	0.116

[a-c]Values within a row sharing a common superscript are not statistically different.

[1]Data represent least squares means of 10 observations per treatment.

[2]CWG = choice white grease.

Table 5.5. Apparent total tract digestibility (ATTD) of micro-minerals in diets containing different fat sources[1]

Item	Diet							
	Basal	Tallow	CWG[2]	Palm oil	Corn oil	Soybean oil	SEM	*P* Value
Mn intake, ppm/d	470.02[a]	386.16[c]	393.15[bc]	450.43[ab]	386.06[c]	408.13[bc]	22.67	0.023
Mn output, ppm/d	399.86[a]	308.32[b]	350.71[ab]	354.91[ab]	301.23[b]	328.94[b]	24.61	0.024
ATTD Mn, %	14.70	20.59	10.96	21.43	22.49	19.38	4.14	0.206
Zn intake, ppm/d	783.34[a]	843.60[c]	655.25[bc]	750.71[ab]	643.43[c]	680.21[bc]	37.78	0.023
Zn output, ppm/d	670.82	566.99	621.38	647.67	532.39	592.82	43.99	0.158
Zn ATTD, %	13.51	11.14	11.74	13.68	17.00	12.29	4.94	0.618

[a-c]Values within a row sharing a common superscript are not statistically different.

[1]Data represent least squares means of 10 observations per treatment.

[2]CWG = choice white grease.

Table 5.6. Apparent total tract digestibility (ATTD) of ether extract (EE) and acid hydrolyzed ether extract (AEE) in diets containing different fat sources[1]

Item	Diet							
	Basal	Tallow	CWG[2]	Palm oil	Corn oil	Soybean oil	SEM	*P* Value
EE intake, g/d	15.28^{d}	57.67^{bc}	53.80^{c}	69.07^{a}	58.68^{bc}	63.06^{ab}	3.12	< 0.001
EE output, g/d	10.59^{ab}	10.42^{abc}	11.97^{a}	11.49^{a}	9.02^{bc}	8.64^{c}	0.77	0.020
ATTD of EE, %	29.62^{c}	81.59^{ab}	77.77^{b}	83.23^{a}	84.59^{a}	86.15^{a}	2.02	< 0.001
AEE intake, g/d	18.57^{c}	62.17^{b}	58.78^{b}	71.17^{a}	57.52^{b}	61.42^{b}	3.23	< 0.001
AEE output, g/d	11.02^{abc}	11.68^{ab}	12.27^{a}	12.16^{a}	9.79^{bc}	9.04^{c}	0.80	0.011
ATTD of AEE, %	39.75^{c}	81.17^{ab}	79.05^{b}	82.77^{ab}	82.84^{ab}	85.17^{a}	1.67	< 0.001

[a-c]Values within a row sharing a common superscript are not statistically different.

[1]Data represent least squares means of 10 observations per treatment.

[2]CWG = choice white grease.

CHAPTER 6

EFFECTS OF SODIUM CHLORIDE AND MICROBIAL PHYTASE ON CALCIUM DIGESTIBILITY IN DIETS FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to determine if increasing concentrations of sodium chloride (**NaCl**) affects the apparent total tract digestibility (ATTD) or retention of Ca and P in diets fed to growing pigs. Four diets based on corn, canola meal, and calcium carbonate were formulated with sodium chloride included at 0.2, 0.4, 0.6, or 0.8% of the diet. An additional 4 diets that were identical to the previous 4 diets except that they contained 500 units/kg of microbial phytase were also formulated. Diets were formulated to contain 0.70% Ca and 0.33% standardized total tract digestible P. Eighty pigs with an average initial BW of 16.10 ± 1.82 kg were randomly allotted to 2 blocks of 40 pigs, 8 diets, and 10 replicate pigs per treatment. Experimental diets were provided for 12 d with the initial 5 d being the adaptation period and total feces were collected for a 5 d collection period using the marker-to-marker approach. Increasing dietary inclusion of NaCl above 0.4% resulted in a reduction ($P < 0.05$) in Ca intake, Ca absorbed, ATTD of Ca, and retention of Ca. The concentration of Ca in feces and urine was reduced ($P < 0.05$) if phytase was included in the diet, but Ca absorption, ATTD of Ca, and retention of Ca increased ($P < 0.05$) by addition of microbial phytase. If phytase was included in the diet, ATTD of P was greater ($P < 0.05$) in pigs fed the diet containing 0.20% NaCl than the diet containing 0.40% NaCl, but if phytase was not added, diets containing 0.20 or 0.40% NaCl were not different (interaction; $P < 0.05$). However, the ATTD of P in the diet containing 0.20 or 0.80% NaCl was less ($P < 0.05$) than in the diet containing 0.60% NaCl. The Na in feces, ATTD of Na, and Na in urine increased ($P < 0.05$) with increasing concentration of NaCl in the diet,

regardless of phytase inclusion. The ATTD of Cl also increased ($P < 0.05$) with increasing concentration of NaCl, regardless of phytase inclusion. In conclusion, addition of NaCl improved the digestibility of Na and Cl, but adding more than 0.60% NaCl to diets fed to weanling pigs reduced the ATTD of Ca. The ATTD of Ca and P increased with the addition of microbial phytase, but inclusion of 0.8% NaCl reduced ATTD of P if phytase was not added to the diet; whereas, the effects of microbial phytase on ATTD of Ca was not influenced by dietary NaCl concentration.

Key words: calcium digestibility, pigs, phosphorus digestibility, sodium chloride

INTRODUCTION

The Na requirement for weanling pigs was recently increased to 0.40, 0.35 and 0.28%, and the Cl requirement was increased to 0.50, 0.45, and 0.32% for pigs from 5 to 7 kg, 7 to 11 kg, and 11 to 25 kg BW, respectively (NRC, 2012). Previously, the Na requirement was 0.20, 0.15, and 0.10% and the Cl requirement was 0.20, 0.15, and 0.08% for pigs from 5 to 10 kg, 10 to 20 kg, and 20 to 50 kg BW (NRC, 1998). The change in Na requirement was supported by data demonstrating an improvement in N digestibility, growth performance, and feed efficiency if dietary concentrations of NaCl were increased (Mahan et al., 1999), and, if given a choice, pigs prefer to eat diets with greater concentrations of NaCl during the early weeks post weaning (Monegue et al., 2011).

Previous work in poultry and swine indicated that dietary phytate may increase the excretion of Na, and that the addition of microbial phytase may reduce that excretion (Cowieson et al., 2004; Ravindran et al., 2006; Selle et al, 2009; Zeng et al., 2014). It has also been documented that Ca digestibility is improved by the addition of microbial phytase (Almeida and

Stein, 2010; González-Vega et al., 2013; Merriman et al., 2016). In poultry, fecal Ca concentration is influenced not only by phytase but also by the concentration of Na in the diet (Goodgame et al., 2011).

It is, however, unknown if increasing dietary NaCl inclusion influences the digestibility of Ca and P or if modifying the amount of dietary NaCl influences the efficacy of supplemental exogenous phytase in diets fed to pigs. In humans, addition of dietary NaCl may increase the amount of Ca excreted in the urine (Sellmeyer et al., 2002) because reabsorption of Ca in the kidneys is directly related to the reabsorption of Na in the kidney; and, as resorption of Na decreases if NaCl is provided in the diet, so does the reabsorption of Ca (Sellmeyer et al., 2002).

To our knowledge, no experiments have been conducted to evaluate the effect of increasing dietary NaCl inclusion on the digestibility of Ca and P by pigs or on the efficacy of phytase. Therefore, the first objective of this experiment was to test the hypothesis that dietary NaCl influences the apparent total tract digestibility (**ATTD**) of Ca and P in pigs and the second objective was to determine if NaCl inclusion changes the response to microbial phytase in the diet.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the animal practices and procedures detailed within this protocol. Pigs used in the experiment were the offspring of Line 359 boars and C-46 females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

Eighty pigs with an average initial BW of 16.10 ± 1.82 kg were randomly allotted to 2 blocks of 40 pigs, 8 diets, and 10 replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a slatted floor, a feeder, a nipple drinker, and a screen floor that allowed for total fecal collection. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding

Four diets based on corn, canola meal, calcium carbonate, and monosodium phosphate were formulated with NaCl included at 0.2, 0.4, 0.6, or 0.8% of the diet (Tables 6.1 and 6.2). Four additional diets that were identical to the previous 4 diets except that they contained microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) at 500 units per kg were also formulated. Diets were formulated to contain 0.70% Ca and 0.33% standardized total tract digestible P. The majority of Ca in the experimental diets was supplied by calcium carbonate.

Pigs were fed each diet for 12 d and feed was provided in an amount equal to 3 times the daily maintenance requirement for energy (i.e., 3 × 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The daily allotment of feed was divided into 2 equal meals and provided at 0700 and 1600 h. Pigs had free access to water throughout the experiment. The initial 5 d was the adaptation period to the diets. Fecal samples were collected quantitatively from d 6 to 12 using the marker-to-marker approach (Adeola, 2001). On d 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection and on d 11, ferric oxide was added to the morning meal to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Orts that were collected during the collection period were dried in a

forced-air oven at 65ºC and the weight was subtracted from the total feed intake. All excreted urine was collected. Urine volume was recorded and a 20% sample was stored at -20ºC. At the conclusion of the experiment, each daily aliquot was combined and subsampled.

Sample Analysis

Diets were analyzed for DM using a drying oven at 135°C for 2 h (Method 930.15; AOAC Int., 2007). Diets were also analyzed for Na using flame emission photometry (Method 956.01; AOAC Int., 2006), Cl using manual titration (Method 915.01, 943.01; AOAC Int., 2007), and for Ca and P by inductively coupled plasma-optical emission spectroscopy (**ICP-OES**; Method 985.01 A, B, and D; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Diet samples were analyzed for GE using an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) and for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. Diets were also analyzed for ash (Method 942.05; AOAC Int., 2007), and for acid-hydrolyzed ether extract using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on an ANKOM XT10 fat extractor (method AM 5-04; AOAC Int., 2007). Diets were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Diets were analyzed for phytase activity by ELISA, using Quantiplate Kits for Quantum Blue® as supplied by Envirologix and using the Envirologix method AP181, Rev. 12-28-11, with some modifications (ESC Standard Analytical Method SAM099). Phytate-bound P was analyzed in the diet samples using a Foss NIR spectrometer with the phytate-P levels predicted using AUNIR calibration standards (Standard Analytical Method 120 at ESC).

Fecal samples were dried in a forced-air oven at 65°C and then ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen. Dry matter and ash concentrations of the feces were analyzed using the same methods as used to analyze the diets. Urine samples were thawed at room temperature, mixed, filtered, and a 10mL subsample of each urine sample was collected. Urine and fecal samples were analyzed for Ca, P, Na, and Cl as described for the diets.

Corn, canola meal, cornstarch, calcium carbonate, and monosodium phosphate were analyzed for DM, ash, Ca, and P, and corn, canola meal, and cornstarch were also analyzed for GE (Table 6.3).

Calculations and Statistical Analyses

Absorption of Ca, P, Na, and Cl was calculated as output of the nutrient subtracted from intake. The ATTD values of Ca, P, Na, and Cl were calculated according to standard procedures (Almeida and Stein, 2010; NRC, 2012):

$$\text{ATTD of Ca (\%)} = [(Ca_{intake} - Ca_{feces}) / Ca_{intake}] \times 100,$$

where Ca_{intake} is the daily intake of Ca (g) and Ca_{feces} is the daily Ca output (g) during each experimental period.

Retention of Ca, P, and Na for each pig was calculation using the following equation (Petersen and Stein, 2006):

$$\text{Retention of Ca (\%)} = [(Ca_{intake} - Ca_{feces} - Ca_{urine}) / Ca_{intake}] \times 100,$$

where Ca_{urine} is the daily output of Ca in the urine.

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model contained the fixed effects of NaCl level, phytase inclusion, and the interaction between NaCl and phytase as well as the random effect of

replicate. To test for normality and identify outliers, the UNIVARIATE procedure of SAS was used. If the interaction term was not significant, linear and quadratic contrast statements were used to test the main effect of NaCl inclusion level. Statistical significance was observed when $P < 0.05$ and tendencies were considered at $0.05 \leq P < 0.10$.

RESULTS

All pigs remained healthy during the experiments and consumed their respective diets without problems. Diet analyses confirmed that diets formulated to contain no microbial phytase did not contain phytase and those expected to contain phytase, contained a minimum 499 units of phytase per kg.

For all Ca responses, the interaction between NaCl and phytase was not significant, and the interaction term was, therefore, removed from the model and only main effects are reported. Linear ($P < 0.05$) and quadratic ($P < 0.05$) effects of NaCl inclusion were observed for Ca intake, Ca absorbed, ATTD of Ca, and retention of Ca (Table 6.4). Increasing dietary inclusion of NaCl above 0.4% resulted in a reduction in Ca intake, Ca absorbed, ATTD of Ca, and retention of Ca. There was no effect of NaCl inclusion on Ca in feces or Ca in urine. The Ca in feces and urine was greater ($P < 0.05$) when phytase was not included in the diet, and as a consequence, Ca absorption, ATTD of Ca, and retention of Ca were all improved ($P < 0.05$) by the addition of microbial phytase (Table 6.5).

Interactions between dietary NaCl and phytase were observed for P intake, P absorbed, ATTD of P, and P retention (Table 6.6). If phytase was not included in the diet, P intake was greater by pigs fed the diet containing 0.60% NaCl compared with pigs fed all other diets, but if phytase was added, no differences among diets were observed (interaction; $P < 0.05$). Pigs fed

diets without phytase had greater ($P < 0.05$) P output than pigs fed diets containing 500 units per kg of phytase. When phytase was not included in the diet, P absorption was greater ($P < 0.05$) by pigs fed the diet containing 0.60% NaCl compared with pigs fed all other diets, but if phytase was included, P absorption was less in the diet containing 0.40% NaCl compared with pigs fed the other diets (interaction; $P < 0.05$). If phytase was included in the diet, ATTD of P was greater in pigs fed the diet containing 0.20% NaCl than the diet containing 0.40% NaCl, but if phytase was not added, diets containing 0.20 or 0.40% NaCl were not different (interaction; $P < 0.05$). However, the ATTD of P in the diets containing 0.20 or 0.80% NaCl was less ($P < 0.05$) than the ATTD of P in the diet containing 0.60% NaCl. The concentration of P in urine was greater ($P < 0.05$) if phytase was added to the diet than if no phytase was added, but the concentration of NaCl did not affect P in urine. Retention of P was greater in the diet containing 0.60% NaCl than in the diet containing 0.80% NaCl if no phytase was used, but if phytase was added, retention of P was greater by pigs fed the diet containing 0.20% NaCl than by pigs fed the diet containing 0.60% NaCl (interaction, $P < 0.05$).

Intake and absorption of Na increased ($P < 0.05$) if NaCl was added to the diet (Table 6.7), but without phytase, the increase was greater than if phytase was added (interaction; $P < 0.05$). The interaction was not significant for Na in feces, ATTD of Na, Na in urine, and retention of urine. The Na in feces, ATTD of Na, and Na in urine increased ($P < 0.05$) with increasing levels of NaCl, regardless of phytase level.

Intake of Cl and Cl absorbed increased ($P < 0.05$) if NaCl increased in the diet, but a greater increase was observed if phytase was not included than if phytase was used (interaction; $P < 0.05$). The ATTD of Cl increased ($P < 0.05$) with increasing level of NaCl, regardless of

phytase concentration. Chloride was not detected in the urine; therefore, retention was not computed. As a consequence, Cl retention is believed to be equal to Cl absorption.

DISCUSSION

In this experiment, canola meal and corn were included as they contain relatively high concentrations of phytate. Canola meal contains 0.65% phytate P and corn contains 0.21% phytate P (NRC, 2012). Concentrations of phytate P in these corn and canola meal diets are approximately 0.40%, and greater than in diets based on corn and soybean meal because soybean meal contains less phytate-bound P than canola meal. It is, therefore, believed that a greater response to added microbial phytase is obtained in diets based on canola meal than in diets based on soybean meal.

The negative influence on ATTD of Ca that was observed as NaCl increased may be a result of Na and Ca interactions in the proximal and distal tubules of the kidneys (Peacock, 1988; Brunette et al., 1992). In humans, addition of NaCl improves the absorption of Ca from the intestines (Meyer et al., 1976), and it was hypothesized that this was necessary to overcome the increase in excretion of Ca in the urine.

The reason microbial phytase increases the ATTD and retention of Ca is most likely that at the low pH in the stomach, phytate may dissociate or solubilize (Woyengo et al., 2010), and the dissociated phytate can then bind to the Ca from calcium carbonate in the small intestine, where pH is more basic (Stein et al., 2011; González-Vega et al., 2015; Merriman et al., 2016). Calcium, like other divalent cations, has a high affinity for binding to the phytate molecule, and

because Ca is the primary cation mineral in pig diets, phytate has a greater probability of interacting with Ca than with other cations (Woyengo et al., 2010).

The values for the digestibility of P were consistent with previous reports (Maison et al., 2015; Merriman et al., 2015; 2016), and demonstrated that P from phytate containing plant ingredients is not very well digested by the pig. It has been demonstrated that addition of microbial phytase improves the digestibility of P (Almeida and Stein, 2012; Almeida et al., 2013; Merriman et al., 2016) because phytase removes P from the phytate molecule by a hydrolysis reaction. Results from the current experiment are in agreement with those observations. The fact that P excretion in feces was greater by pigs fed diets containing no phytase indicates that the P was bound to the phytate molecule and not available for absorption by the pig,, but when phytase was included in the diet, some of the phytate-P bonds were hydrolyzed increasing the ATTD of P and reducing P concentration in the feces.

Because pigs fed diets containing microbial phytase had greater absorption and lower fecal output of P, those pigs were absorbing P at greater concentrations than required. As a result, more P was eliminated in the urine by pigs fed diets containing phytase, which is also in agreement with previous research (Stein et al., 2006).

The interactions between NaCl and phytase observed for the ATTD and retention of P occurred because with no phytase, the ATTD and retention of P decreased at 0.80% compared with 0.60% of dietary NaCl, but not if phytase was added. The ATTD of P at 0.20% NaCl was also greater than at 0.40% NaCl if phytase was added to the diet, but not if phytase was not added. These observations indicated that there were no clear effects of NaCl on the ATTD of P.

The digestibility of Na was high regardless of dietary treatment, which is in agreement with values previously reported (Budde and Crenshaw, 2003; Sauer et al., 2009; Merriman et al., 2016). Fecal excretion of Na, therefore, is very low. It is not surprising that the ATTD of Na was not affected by addition of microbial phytase because excess Na was eliminated in the urine. In fact, the urine Na concentrations were more than twice that of fecal Na concentrations, indicating that there is little regulation of Na absorption from the intestines and that most regulation occurs in the kidneys.

The increased ATTD of Na and Cl with increasing dietary concentrations of NaCl may be explained by the lower relative contributions of Na and Cl from endogenous losses at greater concentrations of dietary NaCl as has been demonstrated for Ca (González-Vega et al., 2013), P (Fan et al., 2001; Petersen and Stein, 2006), fat (Kil et al., 2010), and AA (Fan and Sauer, 1995; Moter and Stein, 2004). It is, therefore, possible that if the standardized or true digestibility of Na and Cl were calculated, no effects of dietary concentrations of NaCl would be observed.

It has been hypothesized that phytate does not affect the solubility of Na in the stomach, but that Na concentrations are increased in the jejunum when phytate is added to the diet (Woyengo et al., 2010). The greater concentrations of Na in the jejunum are thought to be a result of increased endogenous secretions from mucin production as a buffering system to address the change in pH that results from phytate and AA interactions (Woyengo et al., 2010). It was hypothesized that phytic acid binds to pepsinogen, which has basic AA side chains, and this reduces the activity of pepsin, causing a lower secretion of pepsin and HCl (Woyengo et al., 2010) Also, sodium bicarbonate is secreted by the pancreatic duct cells to increase the pH in the small intestine; therefore, any diets that influence the pH in the gastrointestinal tract may change the endogenous secretions of Na. We did not observe any differences in the digestibility of Na

because of the addition of microbial phytase at 500 units per kg. It is possible that the 500 units per kg of phytase was not enough to completely hydrolyze the large quantity of phytate molecules provided in the canola meal diet that was in this experiment; however, the urine Na losses were less from pigs fed the diets with phytase, which indicates that the increased absorption of Ca and P that was observed if phytase was used may have increased the need for Na to be retained in the body. The reason for this increased Na need may be related to increased metabolism of Ca and P, which requires Na, as well as increased Na used for bone tissue synthesis.

Based on these observations, very little Cl is excreted in the feces or urine; therefore, almost all dietary Cl is retained in the body. This is consistent with previous observations for fecal excretions of Cl (Budde and Crenshaw, 2003); however, others have observed Cl excretion in the urine similar to Na (Budde and Crenshaw, 2003). These observations indicate that Cl, like Na, is mainly regulated by the kidney.

Previous reports have indicated that electrolyte balance may reduce the digestibility of Ca when pigs consume diets with a low cation anion balance (Patience and Chaplin; 1997). In this experiment, it is unlikely that electrolyte balance influenced the ATTD or retention of Ca as all diets were between 225 and 275 mEq / kg of DMI.

In conclusion, addition of NaCl had no effect on the efficacy of phytase on the digestibility of Ca when a microbial phytase was included at 500 units per kg. Addition of NaCl improved the digestibility of Na and Cl; however, addition of more than 0.60% NaCl to diets fed to weanling pigs reduced the ATTD of Ca, and therefore, this is not recommended for weanling pigs. Phytase improved the digestibility of Ca and P but high levels of NaCl may reduce the efficacy of phytase. Based on the results of this experiment, NaCl should be included at 0.40% of

the diet for pigs between 11 and 25 kg of BW, which supports the increase in requirements for Na and Cl described in the NRC (2012).

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968-2977.

Almeida, F. N., and H. H. Stein. 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs. J. Anim. Sci. 90:1262-1269.

Almeida, F. N., R. C. Sulabo, and H. H. Stein. 2013. Effects of a novel bacterial phytase expressed in*Aspergillus oryzae* on digestibility of calcium and phosphorus in diets fed to weanling or growing pigs. J. Anim. Sci. Biotechnol. 4:8.

AOAC. 2006. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2007. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Brunette, M. G., J. Mailloux, and D. Lajeunesse. 1992. Calcium transport through the luminal membrane of the distal tubule. I. Interrelationship with sodium. Kidney Int. 41:281-288.

Budde, R. A., and T. D. Crenshaw. 2003. Chronic metabolic acid load induced changes in dietary electrolyte increased chloride retention did not compromise bone in growing swine. J. Anim. Sci. 81:197-208.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101-108.

Fan, M. Z., T. Archbold, W. C. Sauer, D. Lackeyram, T. Rideout, Y. Gao, C. F. de Lange, and R.

R. Hacker. 2001. Novel methodology allows simulatenous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. J. Nutr. 131:2388-2396.

Fan, M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal AA digestibility in pigs: Effect of dietary AA level. J. Anim. Sci. 72:2851-2859.

Fan, M. Z. and W. C. Sauer. 1995. Determination of apparent ileal amino acid digestibility in peas for pigs with the direct, difference, and regression methods. Livest. Prod. Sci. 44:61-72.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2015. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818.

Goodgame, S. D., F. J. Mussini, C. Lu, C. D. Bradley and P. W. Waldroup. 2011. Effect of phytase on the sodium requirement of starting broilers 1. Sodium bicarbonate as primary sodium source. Int. J. Poult. Sci. 10:251-256.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959-2967.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal

allotment. J. Anim. Sci. 85 (Suppl. 2):112. (Abstr.)

Mahan, D. C., T. D. Wiseman, E. M. Weaver, and L. E. Russell. 1999. Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3 week old weaned pigs. J. Anim. Sci. 77:3016-3021.

Maison, T., Y. Liu, and H. H. Stein. 2015. Apparent and standardized total tract digestibility by growing pigs of phosphorus in canola meal from North America and 00-rapeseed meal and 00-rapeseed expellers from Europe without and with microbial phytase. J. Anim. Sci. 93:3494-3502.

Merriman, L. A., and H. H. Stein. 2015. Effect of particle size in calcium carbonate on apparent and standardized total tract digestibility and retention of calcium by growing pigs. J. Anim. Sci. 93:(Suppl. 2):52. (Abstr.)

Merriman, L. A., C. L. Walk, and H. H. Stein. 2016. Effect of fat sources on ATTD of minerals. J. Anim. Sci. 94(Suppl. 2):104. (Abstr.)

Meyer, W. J., I. Transbol, F. C. Bartter, and C. Delea. 1976. Control of calcium absorption: effect of sodium chloride loading and depletion. Metab. Clin. Exp. 25:989-993.

Monegue, J. S., M. D. Lindemann, H. J. Montague, and G. L. Crondwell. 2011. Growth performance and diet preference of nursery pigs fed varying levels of salt. J. Anim. Sci. 89(Suppl. 2):66-67 (Abstr.)

Moter V., and H. H. Stein. 2004. Effect of feed intake on endogenous losses and amino acid and energy digestibility by growing pigs. J. Anim. Sci. 82:3518-3525.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Patience, J. F., and R. K. Chaplin. 1997. The relationship among dietary undetermined anion, acid-base balance, and nutrient metabolism in swine. J. Anim. Sci. 75:2445–2452.

Peacock, M. 1988. Renal excretion of calcium. In: Nordin BEC (ed.) Calcium in Human Biology, Springer-Verlag, New York, New York, U. S. A. p. 93-123.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126-2132.

Ravindran, V., P. C. H. Morel, G. C. Partridge, M. Hruby and J. S. Sands. 2006. Influence of an E. coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85:82-89.

Sauer, W., M. Cervantes, J. Yanez, B. Araiza, G. Murdoch, A. Morales, and R. T. Zijlstra. 2009. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. Livest. Sci. 122:162-168.

Selle, P. H., V. Ravindran and G. C. Partridge. 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilization, mineral retention and growth performance in wheat-based broiler diets. Anim. Feed Sci. Tech. 153:303-313.

Sellmeyer, D. E., M. Schloetter, and A. Sebastian. 2002. Potassium citrate prevents increased urine calcium excretion and bone resorption induced by a high sodium chloride diet. J. Clin. Endocrinol. Metab. 87:2008-2012.

Stein, H. H., M. G. Boersma, and C. Pedersen. 2006. Apparent and true total tract digestibility of phosphorus in field peas (*Pisum sativum L.*) by growing pigs. Can. J. Anim. Sci. 86:523–525.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but reduces digestibility of phosphorus by growing pigs. J Anim Sci. 89:2139-2144.

Woyengo, T. A., O. Adeola, C. C. Udenigwe, and C. M. Nyachoti. 2010. Gastro-intestinal digesta pH, pepsin activity and soluble mineral concentration responses to supplemental phytic acid and phytase in piglets. Livest. Sci. 134:91-93.

Zeng, Z. K., D. Wang, X. S. Piao, P. F. Li, H. Y. Zhang, C. W. Shi, and S. K. Yu. 2014. Effects of adding super dose phytase to the phosphorus deficient diets of young pigs on growth performance, bone quality, mnerals, and amino acids digestibilities. Asian Australas. J. Anim. Sci. 27:237-246.

TABLES

Table 6.1. Ingredient composition of experimental diets without microbial phytase, as-fed basis

Ingredient, %	0 units per kg phytase				500 units per kg phytase			
NaCl (%):	0.20	0.40	0.60	0.80	0.20	0.40	0.60	0.80
Corn	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Canola meal	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Cornstarch	4.36	4.16	3.96	3.76	4.34	4.14	3.94	3.74
Calcium carbonate	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Monosodium phosphate	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium chloride	0.20	0.40	0.60	0.80	0.20	0.40	0.60	0.80
DL-Met	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06
L-Lys HCl, 78% Lys	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65

Table 6.1. (Cont.)

Vitamin mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Phytase premix[2]	-	-	-	-	0.01	0.01	0.01	0.01

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

[2]Phytase was included at 0.01% of microbial phytase (5,000 phytase units per g; Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK). The phytase premix was included in these diets at the expense of cornstarch and provided 500 phytase units of phytase per kilogram complete feed.

Table 6.2. Analyzed composition of experimental diets, as-fed basis

Item	0 units phytase per kg				500 units phytase per kg			
NaCl (%):	0.20	0.40	0.60	0.80	0.20	0.40	0.60	0.80
DM, %	88.13	88.17	88.26	88.27	87.74	88.23	88.48	87.79
Ash, %	4.74	5.05	4.81	5.29	4.84	5.07	5.25	5.69
GE, kcal / kg	4,072	4,054	4,031	4,037	4,059	4,034	4,007	4,034
AEE[1], %	5.65	5.93	5.27	5.70	5.29	5.32	5.85	6.13
CP, %	19.50	19.65	18.56	20.11	19.74	19.01	18.70	20.32
NDF, %	12.59	12.28	11.61	12.45	11.92	12.18	11.48	12.23
ADF, %	7.88	8.13	7.94	7.86	7.89	7.87	7.85	8.14
Ca, %	0.84	0.84	0.76	0.71	0.81	0.89	0.86	0.71
P, %	0.70	0.67	0.72	0.64	0.73	0.67	0.68	0.68
Na, %	0.21	0.26	0.39	0.47	0.27	0.29	0.33	0.48

Table 6.2. (Cont.)

Cl, %	0.27	0.42	0.53	0.72	0.32	0.43	0.53	0.67
DCAD[2], mEq/kg	253	230	259	238	266	242	228	258
Phytate P	0.40	0.40	0.40	0.40	0.39	0.40	0.40	0.40
Phytase, FTU[3] / kg	BDL[4]	BDL	BDL	BDL	499	551	792	550

[1]AEE = acid hydrolyzed ether extract.

[2]DCAD = dietary cation anion difference = (Na + K) – Cl.

[3]FTU = phytase units per kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[4]BDL = below detectable limits.

Table 6.3. Nutrient analyses of ingredients

	Item				
Ingredient	DM, %	Ash, %	Ca, %	P, %	GE, kcal/kg
Corn	85.91	1.32	0.04	0.23	3,802
Canola meal	88.22	7.11	0.61	1.04	4,254
Cornstarch	99.94	0.15	BDL[1]	0.01	3,629
Calcium carbonate	99.94	91.31	38.18	< 0.01	-
Monosodium phosphate	99.50	91.50	0.03	27.89	-

[1]BDL = below detectable limits.

Table 6.4. Apparent total tract digestibility (ATTD) and retention of Ca as affected by NaCl[1,2]

	NaCl, %				SEM	*P* value	
Ingredient	0.20	0.40	0.60	0.80		linear	quadratic
Ca intake, g/d	8.41	8.78	8.68	7.54	0.26	< 0.001	< 0.001
Ca in feces, g/d	2.28	2.06	2.24	2.31	0.14	0.563	0.176
Ca absorbed, g/d	6.13	6.73	6.43	5.23	0.21	< 0.001	< 0.001
ATTD Ca, %	73.11	76.57	74.02	69.51	1.4	0.019	0.002
Ca urine, mg/d	160	150	202	166	35	0.647	0.707
Ca retention, %	71.25	74.91	71.65	67.25	1.47	0.008	0.002

[1]The NaCl × phytase term was not significant; therefore, only main effects are report for Ca.

[2]Values are least squares means.

Table 6.5. Apparent total tract digestibility (ATTD) and retention of Ca.

Ingredient	Phytase			
	0 FTU[1,2,3]/kg	500 FTU/kg	SEM	*P* value
Ca intake, g/d	8.27	8.44	0.25	0.248
Ca in feces, g/d	2.53[a]	1.92[b]	0.12	< 0.001
Ca absorbed, g/d	5.74[b]	6.52[a]	0.18	< 0.001
ATTD Ca, %	69.33[b]	77.28[a]	1.16	< 0.001
Ca urine, mg/d	224[a]	115[b]	25	0.003
Ca retention, %	66.62[b]	75.91[a]	1.18	< 0.001

[a-b]means within a row not sharing a common superscript are different ($P < 0.05$). [1]FTU = phytase units.

[2]The NaCl × phytase term was not significant; therefore, only main effects are report for Ca.

[3]Values are least squares means.

Table 6.6. Apparent total tract digestibility (ATTD) and retention of P

	0 FTU[1,2]/kg				500 FTU/kg					*P* value		
Ingredient	0.20	0.40	0.60	0.80	0.20	0.40	0.60	0.80	SEM	NaCl	Phytase	NaCl × Phytase
P intake, g/d	7.2^{bc}	6.9^{cd}	7.8^{a}	6.8^{cd}	7.3^{b}	6.7^{d}	7.1^{bcd}	7.2^{bc}	0.2	< 0.001	0.523	0.008
P in feces, g/d	3.3	2.8	3.1	3.2	2.1	2.4	2.2	2.4	0.2	0.801	< 0.001	0.181
P absorbed, g/d	3.9^{cd}	4.0^{cd}	4.8^{ab}	3.6^{d}	5.2^{a}	4.3^{bc}	4.9^{a}	4.8^{a}	0.2	0.001	< 0.001	0.002
ATTD P, %	54.6^{ef}	58.6^{def}	61.0^{cde}	52.9^{f}	72.4^{a}	64.0^{bcd}	69.0^{ab}	67.1^{abc}	2.4	0.167	< 0.001	0.040
P urine, mg/d	372	345	375	577	1,109	1,049	1,833	903	0.2	0.292	< 0.001	0.129
P retention, %	49.5^{ab}	53.8^{ab}	56.2^{a}	44.2^{b}	57.6^{a}	47.8^{ab}	43.5^{b}	54.5^{ab}	4.0	0.728	0.983	0.014

[a-f]means within a row not sharing a common superscript are different ($P < 0.05$).

[1]FTU = phytase units.

[2]Values are least squares means.

Table 6.7. Balance and apparent total tract digestibility (ATTD) of Na and Cl in pigs fed experimental diets

	0 FTU[1,2]/kg				500 FTU/kg							
Ingredient	0.20	0.40	0.60	0.80	0.20	0.40	0.60	0.80	SEM	NaCl	Phytase	NaCl × Phytase
Na intake, g/d	2.1[f]	2.7[e]	4.2[b]	5.0[a]	2.7[e]	2.9[d]	3.5[c]	5.1[a]	0.1	< 0.001	0.293	< 0.001
Na in feces, g/d	0.4	0.5	0.5	0.5	0.3	0.4	0.4	0.5	0.0	0.007	0.529	0.755
Na absorbed, g/d	1.8[f]	2.2[e]	3.8[b]	4.5[a]	2.3[de]	2.5[d]	3.1[c]	4.6[a]	0.1	< 0.001	0.172	< 0.001
ATTD Na, %	82.8	83.3	89.1	90.5	87.7	85.0	88.2	90.3	1.3	< 0.001	0.119	0.097
Na urine, g/d	1.0	1.3	1.9	2.4	0.9	1.3	1.9	1.8	0.5	< 0.001	0.337	0.322
Na retention, %	36.4[bc]	33.6[bc]	45.8[ab]	42.3[abc]	53.6[a]	39.3[bc]	32.2[c]	54.5[a]	5.2	0.044	0.103	0.008
Cl intake, g/d	2.8[f]	4.3[d]	5.8[c]	7.6[a]	3.2[e]	4.3[d]	5.6[c]	7.1[b]	0.2	< 0.001	0.521	0.002
Cl in feces, g/d	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.003	0.095	0.250
Cl absorbed, g/d	2.7[f]	4.2[d]	5.7[c]	7.4[a]	3.1[e]	4.2[d]	5.4[c]	6.9[b]	0.2	< 0.001	0.386	0.001

Table 6.7. (Cont.)

ATTD Cl, %	96.1	97.2	97.7	98.2	96.8	97.1	97.4	97.3	0.4	0.004	0.525	0.251

[a-f]means within a row not sharing a common superscript are different ($P < 0.05$).

[1]FTU = phytase units.

[2]Values are least squares means.

CHAPTER 7

EFFECT OF SUCROSE ON APPARENT TOTAL TRACT DIGESTIBILITY OF CALCIUM AND PHOSPHORUS

ABSTRACT: An experiment was conducted to determine the effect on the digestibility of Ca in calcium carbonate of including sucrose or cornstarch in diets for growing pigs. Four diets based on corn and potato protein isolate were formulated to contain 0.70% Ca and 0.33% standardized total tract digestible (STTD) P. Experimental diets had identical composition except that sixty percent of the diets was supplied by cornstarch and sucrose in the following proportions: 60:0, 40:20, 20:40, or 0:60. Forty barrows (average initial BW = 17.95 ± 1.86 kg) were allotted to the 4 experimental diets based on initial BW. Pigs were fed experimental diets for 12 d with the initial 5 d as the adaptation period, and pigs were fed 3 times the daily maintenance energy requirement. Total feces were collected for 5 d using the marker to marker approach. Total urine was collected for determination of retention of Ca and P. Data were analyzed using the MIXED procedure of SAS with the fixed effect of diet and the random effect of block with polynomial contrasts to determine linear and quadratic effects. Results indicated that the apparent total tract digestibility (ATTD) of Ca was not linearly or quadratically affected by inclusion of increasing concentrations of sucrose in the diets. Likewise, no linear or quadratic effects of sucrose inclusion were observed for feed intake, fecal output, fecal Ca concentration, Ca intake, absorbed Ca, urine Ca, or Ca retention. Sucrose inclusion tended to affect fecal calcium output (g/d; P = 0.066) and fecal output (g/d; P = 0.081) quadratically with the greatest values being calculated for diets containing 20 or 40% sucrose. There was a tendency for the ATTD (P = 0.058) and the STTD (P = 0.055) of P to increase quadratically with increasing sucrose inclusion with diets

containing 20 or 40% sucrose tending to have greater ATTD and STTD of P than diets containing 0 or 60% sucrose. Quadratic effects were not observed for P intake, P in feces (g/d), absorbed P, or P retention. However, P in urine was greater for pigs fed diets containing 20 or 40% sucrose than for pigs fed diets containing 0 or 60% sucrose (quadratic, $P < 0.05$). In contrast, P in feces (%) was less ($P < 0.05$) for pigs fed the diets containing 20 or 40% sucrose compared with pigs fed diets containing 0 or 60% sucrose. In conclusion, with the exception for tendencies for small improvement in digestibility of P with the addition of 20 or 40% sucrose in diets fed to growing pigs, there appeared to be little difference between cornstarch and sucrose in terms of effects on Ca and P balances and retention.

Key words: calcium digestibility, cornstarch, phosphorus digestibility, pigs, sucrose

INTRODUCTION

Synthetic diets have been used in research to determine the digestibility of nutrients such as Ca (González-Vega et al., 2014) and P (Petersen and Stein, 2006). To evaluate the digestibility of Ca from individual ingredients, it is preferred that the ingredient of interest provide all the dietary Ca (i.e., direct procedure). Therefore, in experiments evaluating Ca digestibility, soybean meal is often replaced by potato protein isolate (González-Vega et al., 2014) and other ingredients, such as sucrose and cornstarch, because these ingredients do not contain Ca. Sucrose is a disaccharide containing glucose and fructose units. Sucrose is often included in these synthetic diets (Sulabo and Stein, 2013; González-Vega et al., 2014) because it is devoid of Ca and provides a palatable ingredient in swine diets.

It is unknown how inclusions of synthetic ingredients like sucrose influence the digestibility of Ca and P in pigs, but in humans, glucose increases transit time and increases Ca

absorption (Griessen et al., 1989). An increase in urine excretion of Ca and P when sucrose levels are increased has also been observed in humans (Holl and Allen, 1987). In rats, Ca absorption was reduced by the inclusion of fructose; however, P absorption was not impacted (Douard et al., 2010).

To our knowledge, no experiments have been conducted in pigs that have evaluated the impact of increasing sucrose levels on the digestibility of Ca; therefore, the objective of this experiment was to test the hypothesis that there are differences in the apparent total tract digestibility (**ATTD**) of Ca and P if different concentrations of sucrose and cornstarch are used.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the animal procedures for the experiment. Pigs used in the experiment were the offspring of Line 359 boars mated to C46 females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

Forty pigs with an average initial BW of 17.95 ± 1.86 kg were randomly allotted to 4 diets with 10 replicate pigs per treatment. Pigs were housed individually in metabolism crates that were equipped with a slatted floor, a feeder, a nipple drinker, and a screen floor for total fecal collection. A urine pan was installed below the screen floor to drain urine into urine collection buckets. Pigs were allotted to experimental diets using The Experimental Animal Allotment Program (Kim and Lindemann, 2007).

Diets and Feeding

Four diets based on corn, potato protein isolate, cornstarch, and sucrose were formulated. Sixty percent of the total diet was supplied by either cornstarch, sucrose, or a combination of cornstarch and sucrose. The concentrations of cornstarch and sucrose in the 4 diets were 60:0, 40:20, 20:40, or 0:60 (Table 7.1). All diets were formulated to meet or exceed requirements for AA, Ca, and P for pigs from 11 to 25 kg BW (NRC, 2012). Diets were formulated to contain approximately 0.70% Ca and 0.33% digestible P. The source of Ca for all diets was calcium carbonate and P was supplied by monosodium phosphate.

Pigs were fed each diet for 12 d and were fed 3 times the estimated daily maintenance energy requirement (i.e., 3 × 197 kcal of ME/kg of $BW^{0.60}$; NRC, 2012). The daily allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h. Pigs had free access to water throughout the experiment. The initial 5 d served as the adaptation period to the diets and fecal samples were collected quantitatively from d 6 to 12 using the marker-to-marker approach (Adeola, 2001). On d 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection and on d 11, ferric oxide was added to the morning meal to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Orts that were collected during the collection period were dried in a forced-air oven at 65ºC, and the weight was subtracted from total feed intake. The ATTD values of Ca and P were calculated according to standard procedures (NRC, 2012).

Sample Analysis

Diets (Table 7.2) and ingredients (Table 7.3) were analyzed for DM using a drying oven at 135°C for 2 h (Method 930.15; AOAC Int., 2007), and for Ca and P by inductively coupled plasma-optical emission spectroscopy (**ICP-OES**; Method 985.01 A, B, and D; AOAC Int.,

2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Ingredient and diet samples were also analyzed for GE using an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) and for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. Diets and ingredients were analyzed for ash (Method 942.05; AOAC Int., 2007), and diets were analyzed for acid-hydrolyzed ether extract using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on an ANKOM XT10 fat extractor (method AM 5-04; AOAC Int., 2007). Diets were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom2000 Fiber Analyzer, Ankom Technology, Macedon, NY).

Fecal samples were dried in a forced-air oven at 65°C and then ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen. Fecal samples were analyzed for DM, as described for diets. Urine samples were thawed at room temperature, mixed, filtered, and a 10mL subsample of each urine sample was collected. Urine and fecal samples were analyzed for Ca and P using the same methods as described for diets and ingredients.

Calculations and statistical analyses

Absorption of Ca and P was calculated as output of the nutrient subtracted from intake, both reported in grams per day. The ATTD of Ca and P was calculated by the following equation (adapted from Petersen and Stein, 2006):

$$\text{ATTD of Ca (\%)} = [(Ca_{intake} - Ca_{feces}) / Ca_{intake}] \times 100,$$

where Ca_{intake} is the daily intake of Ca (grams) and Ca_{feces} is the daily Ca output (grams) during each experimental period.

For P, the standardized total tract digestibility (**STTD**) was calculated using the following equation:

$$\text{STTD P (\%)} = [P_{intake} - (P_{feces} - EPL)/P_{intake}] \times 100,$$

where EPL is the basal endogenous loss of P. For this experiment, the value of 190g/kg DMI (NRC, 2012) was used for EPL.

The retention of Ca and P for each pig was calculated according to the following equation (Petersen and Stein, 2006):

$$\text{ATTD of Ca (\%)} = [(Ca_{intake} - Ca_{feces} - Ca_{urine}) / Ca_{intake}] \times 100,$$

where Ca_{urine} is the daily output of Ca from d 6 to 11.

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). This model contained diet as a fixed effect and replicate as a random effect. Polynomial contrasts were used for determination of linear and quadratic effects of sucrose level. To test for normality and identify outliers, the UNIVARIATE procedure of SAS was used. The experimental unit was the pig. Statistical significance were observed when $P < 0.05$ and tendencies were considered at $0.05 \leq P < 0.10$.

RESULTS

No linear or quadratic effects of sucrose inclusion were observed for feed intake, fecal Ca (%), Ca intake, absorbed Ca, urine Ca, ATTD of Ca, or Ca retention (Table 7.4). Sucrose inclusion tended to affect fecal Ca output (g/d; $P = 0.066$) and total fecal output ($P = 0.081$) quadratically with the greatest values obtained for diets containing 20 or 40% sucrose and the least values for diets containing 0 or 60% sucrose.

No linear effects were observed for any of the parameters for P digestibility (Table 7.5). Quadratic effects were not observed for P intake, P in feces, absorbed P, or P retention; however, quadratic effects ($P < 0.05$) were observed for P output in feces (g/d) with the greatest concentrations observed if 60% cornstarch or 60% sucrose were used. In contrast, P in urine was greater for the 2 diets containing a combination of cornstarch and sucrose compared with diets containing either cornstarch or sucrose (quadratic, $P < 0.05$). Sucrose inclusion tended to affect the ATTD of P (quadratic, $P = 0.058$) and the STTD of P (quadratic, $P = 0.055$) with the values for diets containing 20 or 40% sucrose tending to be greater than values for diets containing 0 or 60% sucrose.

DISCUSSION

Feed intake was less than previously reported for pigs between 15 and 20 kg of BW (González-Vega et al., 2013). Because these pigs were limit-fed at 3 times maintenance energy requirement, this reduction in feed intake is a result of feed refusal.

Most of the dietary P originated from monosodium phosphate and the dietary Ca was provided by calcium carbonate; therefore, the ATTD and STTD values for P represent the digestibility of P in monosodium phosphate and the ATTD of Ca represents the ATTD of Ca in calcium carbonate. Previous data for monosodium phosphate indicate that the ATTD of P is 91.88% and the true total tract digestibility of P is 98.20% (Petersen and Stein, 2006). Results from the present experiment are in agreement with the previous data. The ATTD of Ca in calcium carbonate has been reported between 62 and 74% (González-Vega et al., 2015; Merriman and Stein, 2016), and the current values are close to the previous values.

Data obtained in humans have indicated that sucrose increases urinary excretion of Ca and P (Holl and Allen, 1987). In the present experiment, excretion of P in urine was increased as sucrose was added at 20 or 40% in the diet, thus confirming the result by Holl and Allen (1987) although sucrose did not influence Ca in urine in our experiment.

The lack of an effect of sucrose on ATTD of Ca contradicts the data of Duoard et al. (2010) which indicated that dietary fructose reduces Ca absorption; however, because we added sucrose, and not fructose as a monosaccharide, it is possible that there was not enough free fructose in the intestinal lumen to impact Ca absorption. The tendency for a small quadratic effect of sucrose on the ATTD and STTD of P likely had no practical implication.

Previous data comparing the inclusion of cornstarch or corn germ in diets fed to pigs indicated that a reduction in the ATTD of Ca and P were observed when cornstarch was used (González-Vega et al., 2015). Corn germ contains fiber, which may influence the digestibility of Ca and P because fermentation of fiber reduces intestinal pH. Neither cornstarch nor sucrose contain fiber; therefore, it was expected that intestinal pH would not be affected by the change from cornstarch to sucrose. The observation that ATTD of Ca was not impacted by the concentration of sucrose in the diet confirmed this hypothesis.

The quadratic response observed in P may be due to the pH effect of the diet. In a study with dairy cattle, rumen pH was constant when a dose of cornstarch was included, but rumen pH was reduced after a sucrose dose, becoming more acidic when sucrose was included (Oba et al., 2015). Because a low pH is necessary to solubilize the Ca from calcium carbonate, it is possible that cornstarch diets may not allow for the necessary pH adjustment for Ca to solubilize, and ultimately be taken up and utilized by the body.

The practical implication of the results from this study is that the digestibility and retention of Ca is not affected by the concentration of sucrose in the diets; therefore, inclusion of sucrose should not necessarily be avoided when synthetic diets are formulated for pigs.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer, Jr., eds. AOAC Int., Gaithersburg, MD.

Douard, V., A. Asgerally, Y. Sabbagh, S. Sugiura, S. A. Shapses, D. Casirola, and R. P. Ferraris. 2010. Dietary fructose inhibits intestinal calcium absorption and induced vitamin D insufficiency in CKD. J. Am. Soc. Nephrol. 21:261-271.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of calcium and true total tract digestibility of Ca in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126-142.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818.

Griessen, M., P. V. Speich, F. Infante, P. Bartholdi, B. Cochet, A. Donath, B. Courvoisier, and J. P. Bonjour. 1989. Effect of absorbable and nonabsorbable sugars on calcium absorption in humans. Gastroenterol. 96:769-775.

Holl, M. G., and L. H. Allen. 1987. Sucrose ingestion, insulin response and mineral metabolism in humans. J. Nutr. 117:1229-1233.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85 (Suppl. 2):112. (Abstr.)

Merriman, L. A., and H. H. Stein. 2016. Effect of particle size of calcium carbonate on apparent and standardizd total tract digestibility and retention of calcium by growing pigs. J. Anim. Sci. Submitted.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Oba, M., J. L. Mewis, and Z. Zhining. 2015. Effects of ruminal doses of sucrose, lactose, and corn starch on ruminal fermentation and expression of genes in ruminal epithelial cells. J. Dairy Sci. 98:586-594.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126-2132.

Sanderson, P. 1986. A new method of analysis of feedingstuffs for the determination of crude oils and fats. Pages 77–81 in Recent Advances in Animal Nutrition. W. Haresign and D. J. A. Cole, ed. Butterworths, London.

Sulabo, R. C., and H. H. Stein. 2013. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. J. Anim. Sci. 91:1285-1294.

TABLES

Table 7.1. Ingredient composition of experimental diets, as-fed basis

	Percentage cornstarch: percentage sucrose			
Ingredient, %	60:0	40:20	20:40	0:60
Corn	10.38	10.38	10.38	10.38
Potato protein isolate	23.00	23.00	23.00	23.00
Sucrose	-	20.00	40.00	60.00
Cornstarch	60.00	40.00	20.00	-
Calcium carbonate	1.77	1.77	1.77	1.77
Soybean oil	3.00	3.00	3.00	3.00
Monosodium phosphate	1.15	1.15	1.15	1.15
Sodium chloride	0.40	0.40	0.40	0.40
L-Lys HCl, 78% Lys	0.10	0.10	0.10	0.10
Vitamin mineral premix[1]	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-

Table 7.1. (Cont.)

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 7.2. Analyzed composition of experimental diets, as-fed basis

	Percentage cornstarch: percentage sucrose			
Ingredient, %	60:0	40:20	20:40	0:60
DM	94.06	95.27	96.60	97.86
Ash	2.90	3.08	2.68	3.07
GE, kcal / kg	4,043	4,076	4,189	4,217
NDF	2.89	2.30	1.48	1.48
ADF	1.65	1.62	1.46	1.45
AEE[1]	3.21	3.09	3.12	2.35
CP	20.02	19.58	19.34	19.52
Ca	0.72	0.69	0.67	0.73
P	0.39	0.36	0.38	0.36

[1]AEE = acid hydrolyzed ether extract.

Table 7.3. Analyzed composition of ingredients, as is basis

Ingredient, %	DM	Ash	CP	Ca	P	GE
Corn	88.76	1.62	6.63	0.13	0.23	3,753
Potato protein isolate	91.85	0.53	91.93	0.04	0.15	5,247
Sucrose	99.95	0.13	0.38	0.02	0.01	3,909
Cornstarch	88.17	0.15	0.51	BDL[1]	0.01	3,629
Calcium carbonate	99.94	91.31	-	38.18	< 0.01	-
Monosodium phosphate	99.50	91.50	-	0.03	27.89	-

[1]BDL = below detectable limits.

Table 7.4. Calcium balance, apparent total tract digestibility (ATTD), and retention of Ca by pigs fed experimental diets

Ingredient,	Percentage cornstarch: percentage sucrose[1]				SEM	*P* value	
	60:0	40:20	20:40	0:60		Linear	Quadratic
Feed intake, g of DM/d	450	508	540	507	37	0.226	0.234
Ca intake, g/d	3.45	4.17	3.75	3.78	0.28	0.641	0.224
Fecal output, g of DM/d	13.05	16.99	15.19	14.86	1.44	0.485	0.081
Ca in feces, %	6.78	8.22	7.84	7.52	0.82	0.613	0.300
Ca feces, g/d	0.95	1.52	1.30	1.21	0.19	0.468	0.066
Ca in urine, g/d	0.04	0.06	0.06	0.04	0.01	0.765	0.150
Ca absorbed, g/d	2.49	2.65	2.44	2.57	0.25	0.982	0.939
ATTD, Ca, %	71.90	63.87	64.96	67.44	4.37	0.440	0.158
Ca retention, %	70.67	62.51	63.44	66.39	4.40	0.455	0.137

[1]Data are least squares means of 10 observations for all treatments except the diet containing 20% cornstarch and 40% sucrose, which had 8 observations.

Table 7.5. Phosphorus balance, apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and retention of P by pigs fed experimental diets

	Percentage cornstarch: percentage sucrose[1]					*P* Value	
Ingredient,	60:0	40:20	20:40	0:60	SEM	Linear	Quadratic
P intake, g/d	1.87	1.93	2.13	1.86	0.14	0.766	0.279
P in feces, %	1.06	0.70	0.71	0.93	0.09	0.347	0.003
P in feces, g/d	0.15	0.13	0.11	0.14	0.02	0.608	0.151
P in urine, g/d	0.45	0.62	0.57	0.43	0.05	0.640	0.003
ATTD, P, %	91.86	93.48	94.48	91.76	1.10	0.888	0.058
P absorbed, g/d	1.72	1.80	2.02	1.72	0.14	0.711	0.201
P retention, %	67.66	61.15	64.77	66.98	4.39	0.455	0.137
STTD, P[2], %	94.03	95.52	96.45	93.93	1.01	0.887	0.055

[1]Data are least squared means of 10 observations for all treatments except the diet containing 20% cornstarch and 40% sucrose, which had 8 observations.

[2]STTD of P was computed assuming the basal endogenous loss of P being of 190g/kg DMI (NRC, 2012).

CHAPTER 8

EFFECTS OF PHYTASE ON BALANCE AND DIGESTIBILITY OF CA AND P AND ON DIGESTA PH AND ASH CONCENTRATION OF DIGESTA IN DIETS WITHOUT OR WITH PHYTATE-BOUND P FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to test the hypothesis that digestibility of Ca from calcium carbonate in diets based on cornstarch may be different from that of diets based on corn, and to determine the influence of phytase on the digestibility of Ca in both types of diets. Growing barrows (n = 40; initial BW = 17.99 ± 1.42 kg) were randomly allotted to 4 diets. Each diet was fed to 10 replicate pigs. One diet was based on corn and potato protein isolate and another diet was based on cornstarch and potato protein isolate. Two additional diets that were similar to these 2 diets with the exception that microbial phytase was included at 0.01% of the diet to provide 500 units per kg were also formulated. Diets were formulated to contain 0.70% Ca and 0.33% digestible P. The source of Ca in all diets was calcium carbonate. The P was supplied by monosodium phosphate in the cornstarch based diet and corn and monosodium phosphate in the corn based diet. Experimental diets were provided for 12 d with the initial 5 d being the adaptation period. Total feces were collected for 5 d using the marker-to-marker approach. Data were analyzed using the MIXED procedure of SAS. The ATTD of Ca was lower ($P < 0.05$) in the corn based diets compared with the cornstarch based diets. Concentrations of Ca in the urine were greater ($P < 0.05$) in the corn based diets compared with the cornstarch based diets. The ATTD of P in the corn based diets was greater ($P < 0.05$) when phytase was added to the diet, but addition of phytase did not influence the ATTD of P in the cornstarch based diets regardless of phytase inclusion (interaction; $P < 0.05$). In conclusion, corn negatively

influenced the digestibility of Ca and P, and microbial phytase improved P digestibility, but not Ca digestibility in the corn based diets.

Key words: phytase, synthetic diets, calcium digestibility, pigs

INTRODUCTION

Using synthetic diets may reduce the digestibility of Ca compared with corn-based diets (González-Vega et al., 2015). It has been hypothesized that differences in Ca digestibility among diet types may be due to differences in fiber content of the diet (González-Vega et al., 2015) because fiber increases the movement in the gastrointestinal tract (Bueno et al., 1981; Hillemeier, 1995). This movement allows for the mixing between contents in the lumen and gastric secretions, increasing interactions between nutrients and epithelial cells, and increasing nutrient absorption (Vander et al., 2001). Fiber also reduces the pH of the digesta, which may affect the solubility of Ca (Canh et al., 1998; Moeser and van Kempen, 2002).

Corn also contains phytate, which binds to cations such as Ca, and may reduce the digestibility of those minerals. Phytase has improved the digestibility of Ca in diets that contain phytate from plant ingredients (González-Vega et al., 2015).

To our knowledge, no experiments have been conducted in pigs to evaluate diet type and phytase inclusion on the apparent total tract digestibility of Ca and P, pH of contents of the digesta from the stomach, duodenum, jenum, and ileum, and the concentrations of ash in the digesta of pigs. Therefore, the objective of this experiment was to determine the ATTD of Ca in cornstarch based diets and corn based diets, and to determine the influence of phytase on the digestibility of Ca in both types of diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the animal practices and procedures that were used. Pigs used in the experiment were the offspring of Line 359 boars and C-46 females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

Forty pigs with an average initial BW of 17.99 ± 1.42 kg were randomly allotted to 4 diets with 10 replicate pigs per treatment. Pigs were housed individually in metabolism crates that were equipped with a slatted floor, a feeder, a nipple drinker, and a screen floor that allowed for total collection of feces. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets.

Diets and Feeding

Two diets were based on corn and potato protein isolate or cornstarch and potato protein isolate (Table 8.1). Two additional diets that were similar to the previous 2 diets were formulated with the exception that microbial phytase was included at 0.01% of the diet to provide 500 phytase units per kg (Quantum Blue; AB Vista, Marlborough, UK). Diets were formulated to contain 0.70% Ca and 0.33% digestible P. The source of Ca in all diets was calcium carbonate and most of the digestible P was supplied by monosodium phosphate.

Pigs were fed each diet for 12 d and feed was offered at 3 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The daily allotments of feed were divided into 2 equal meals and provided at 0700 and 1600 h. Pigs had free access to water throughout the experiment. The initial 5 d of the experiment was the adaptation period to the

diets, and fecal samples were collected quantitatively from d 6 to 12 using the marker-to-marker approach (Adeola, 2001). On d 6, an indigestible marker (indigo carmine) was added to the morning meal to mark the beginning of fecal collection and on d 11, an indigestible marker (ferric oxide) was added to the morning meal to mark the conclusion of fecal collection. Fecal samples were stored at -20°C immediately after collection. Orts were collected during the collection period and dried in a forced-air oven at 65ºC, and the weight was subtracted from the total feed intake. Urine was collected from d 6 to 11. Before each daily collection period, 50 ml of 6*N* HCl was added to the urine pale and used as a preservative. Total daily quanitities of urine was recorded and a 20% representative sample was retained. At the conclusion of the experiment, each daily aliquot was combined and further subsampled.

Sample Analysis

Diets were analyzed for DM (Table 8.2) using a drying oven at 135°C for 2 h (Method 930.15; AOAC Int., 2007), and for Ca and P by inductively coupled plasma-optical emission spectroscopy (**ICP-OES**; Method 985.01 A, B, and D; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Diets were analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. Diet samples were analyzed for ash (Method 942.05; AOAC Int., 2007), and for acid-hydrolyzed ether extract using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on an ANKOM XT10 fat extractor (method AM 5-04; AOAC Int., 2007). Diets were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Diets were analyzed for phytase activity by ELISA, using Quantiplate Kits for

Quantum Blue® as supplied by Envirologix and using the Envirologix method AP181, Rev. 12-28-11, with some modifications (ESC Standard Analytical Method SAM099). Phytate-bound P was analyzed in the diet samples using a Foss NIR spectrometer with the phytate-P levels predicted using AUNIR calibration standards (Standard Analytical Method 120 at ESC).

Corn, potato protein, cornstarch, calcium carbonate, and monosodium phosphate were analyzed for DM, ash, Ca, and P as described for diets (Table 8.3). Fecal samples were dried in a forced-air oven at 65°C and then ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen. Fecal samples were analyzed for DM, Ca, and P as described for diets. Urine samples were thawed at room temperature, mixed, filtered, and a 10 mL subsample of each urine sample analyzed for Ca and P using the same methods as described for the diets.

At the conclusion of the experiment, each pig was euthanized by captive bolt. Digesta from the stomach, duodenum, jejunum, and ileum was collected. The digesta from each of these areas was incubated for 1 h at 37ºC in a shaking water bath to simulate motility in the gastrointestinal tract. The aqueous solution portion was removed. The precipitate material was subsequently ashed in a muffle oven as described for diets.

Calculations and Statistical Analyses

Absorption of Ca and P was calculated as output of the nutrient subtracted from intake. The ATTD values for Ca and P were calculated according to standard procedures (Almeida and Stein, 2010; NRC, 2012):

$$\text{ATTD of Ca (\%)} = [(Ca_{intake} - Ca_{feces}) / Ca_{intake}] \times 100,$$

where Ca_{intake} is the daily intake of Ca (grams) and Ca_{feces} is the daily Ca output (grams) during each experimental period.

Retention of Ca and P for each pig was calculation using the following equation (Petersen and Stein, 2006):

$$\text{Retention of Ca (\%)} = [(Ca_{intake} - Ca_{feces} - Ca_{urine}) / Ca_{intake}] \times 100,$$

where Ca_{urine} is the daily output of Ca in the urine.

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). This model contained diet type and phytase inclusion as fixed effects and replicate as a random effect. To test for normality and identify outliers, the UNIVARIATE procedure of SAS was used. The experimental unit was the pig. Statistical significance was observed when $P < 0.05$ and tendencies were considered at $0.05 \leq P < 0.10$.

RESULTS AND DISCUSSION

One pig that was fed the cornstarch diet with phytase died during the experiment. All results for that treatment had only 9 replicate pigs; whereas, all other diets had 10 replicate pigs. Feed intake and fecal output were greater ($P < 0.05$) by pigs fed the corn diet compared with pigs fed the cornstarch diets. Although the metabolizable energy concentration of the diet was greater in the cornstarch diet by approximately 400 kcal/kg, the observed difference in feed intake was mostly a result of feed refusal by pigs fed the cornstarch diets (Table 8.4). Intake by pigs fed the corn based diets was consistent with previously reported values (González-Vega et al., 2015; Merriman et al., 2016).

There was no interaction between diet type and phytase inclusion in the diet for any of the Ca responses, so only main effects are discussed. Because of lower feed intake of cornstarch based diets, the intake of Ca and P of those diets was also lower ($P < 0.05$) than of the corn based diets. The concentration of Ca in feces was greater ($P < 0.05$) in the corn based diets compared

with the cornstarch based diets, which resulted in lower ($P < 0.05$) absorption of Ca from the corn based diets. Because the corn based diets contained fiber and phytate, the Ca likely formed complexes with phytate which were excreted in the feces. This observation is consistent with other high fiber and phytate containing diets (Guggenbuhl et al., 2012). There was, however, no effect of phytase on the ATTD of Ca in the corn diet, which is contrary to other findings (González-Vega et al., 2015). The ATTD of Ca was lower ($P < 0.05$) in the corn based diets compared with the cornstarch based diets. Concentrations of Ca in the urine were greater ($P < 0.05$) from pigs fed the corn based diets compared with pigs fed the cornstarch based diets. This resulted in reduced ($P < 0.05$) retention of Ca by pigs fed the corn based diets compared with pigs fed the cornstarch based diets. The greater concentration of Ca in the urine of pigs fed the corn based diets indicates that these diets were formulated above the requirement for Ca; however, the concentrations of Ca were similar among all dietary treatments. Despite the reduced ATTD of Ca in the corn based diets, pigs fed these diets had greater daily absorption of Ca because of the greater daily feed intake, which likely is the reason for the greater output of Ca in urine. It was surprising that phytase did not affect the ATTD of Ca as has previously been observed (Almeida and Stein, 2010; González-Vega et al., 2015), but if diets exceed the requirement for Ca by the pigs, it is possible that additional Ca released by phytase is excreted and thus Ca digestibility is reduced (Lei et al., 1994).

The concentration of P in the feces was greater ($P < 0.05$) by pigs fed the corn based diets compared with pigs fed the cornstarch based diets if phytase was not added to the diet, but, if phytase was added, there was no difference between the corn based diets and the cornstarch based diets (interaction; $P < 0.05$). Therefore, P absorption was greater ($P < 0.05$) by pigs fed the corn based diets when phytase was added compared with absorption if phytase was not added to

the corn based diet, but no differences were observed in the cornstarch diets, regardless of phytase inclusion (interaction; $P < 0.05$). The ATTD of P in the corn based diets was greater ($P < 0.05$) when phytase was added to the diet, but addition of phytase did not influence the ATTD of P in the cornstarch based diets (interaction; $P < 0.05$). All P in the cornstarch based diets originated from monosodium phosphate; therefore, the values for ATTD of P represent the digestibility of P in monosodium phosphate and the values from this experiment are in agreement with previous reports (Petersen and Stein, 2006). The ATTD values for P in the corn based diets are a combination of the ATTD of P in corn and P in monosodium phosphate, and because P in corn is bound to phytate, the ATTD of P is expected to be low, as previously observed (Almeida and Stein, 2010; 2012).

The concentration of P in urine from pigs fed the corn diet without phytase was less ($P < 0.05$) than if pigs were fed the corn based diets with phytase, but addition of phytase did not affect the concentrations of P in urine from pigs fed the cornstarch based diets. This indicated that the cornstarch based diets both without and with phytase and the corn based diet with phytase may have contained more digestible P than required by the pig.

The pH of the contents in the stomach, duodenum, jejunum, or ileum, and in the feces directly after slaughter was not affected by dietary inclusion of microbial phtyase (Table 8.5). There was a trend for pH in the duodenum ($P = 0.084$) and jejunum ($P = 0.088$) to be greater in the cornstarch based diets compared with corn based diets. In the ileal and fecal samples, pH was greater ($P < 0.05$) in samples from the cornstarch based diets compared with the corn based diets. The differences in pH between corn based diets and cornstarch based diets may be a result of greater concentrations of VFA in the intestinal tract, which reduces the pH in digesta and fecal samples (Canh et al., 1998; Moeser and van Kempen, 2002)

After digesta samples were subjected to a shaking water bath, the precipitate and liquid portions of those samples were separated, if possible. There were no differences in the pH of the digesta from the stomach, duodenum, jejunum, or ileum in the precipitate (Table 8.6). However, the pH in the liquid phase of the duodenum, jejunum, and ileum was greater ($P < 0.05$) in samples from pigs fed the cornstarch based diets compared with pigs fed the corn based diets. In the jejunum, pH was less ($P < 0.05$) in the corn based diet compared with the cornstarch based diets, but if phytase was included in the diet, diet type did not influence the pH (interaction; $P < 0.05$).

These observations indicated that it is unlikely that Ca precipitates in the small intestine of pigs fed the cornstarch based diets compared with pigs fed the corn based diets because if that was the case, the pH in the liquid portion would have been reduced in pigs fed the cornstarch based diets. It is possible that the in vitro model used in this experiment to indicate precipitation in the intestines is inadequate to predict in vivo conditions; nevertheless, the observations that pH in the liquid portion was not affected by type of diet and that concentrations of ash in intestinal contents were not changed indicate that cornstarch based diets do not impair Ca digestibility.

The concentration of ash in contents from the stomach and duodenum was not affected by diet type or phytase (Table 8.7). The concentration of ash was greater ($P < 0.05$) in the corn based diet if phytase was not added than if phytase was added, but the concentration of ash was not different among the cornstarch based diets at 0 or 500 units of phytase / kg. This indicated that minerals are bound to the phytate molecule in the corn based diets and that the addition of phytase cleaves those bounds. Based on the results from this experiment, the greater concentration of ash was likely a result of greater concentrations of P because P digestibility was

less in the corn diet without phytase; whereas, ATTD of Ca was not affected. Concentration of ash in the ileum was similar to the values observed for the jejunum, however, because of greater variability among those samples, significant differences among samples were not detected.

In conclusion, the type of diet needs to be considered when formulating diets for Ca and P. Improvements in the digestibility of Ca were not achieved by phytase inclusion in this experiment as have been observed in other experiments. More research is warranted to determine the types of diet that may affect Ca digestibility by pigs. Phytase is effective in improving the digestibility of P in diets that contain phytate-bound P but does not improve the digestibility of P in diets without phytate.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 903-916.

Almeida, F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968-2977.

Almeida, F. N., and H. H. Stein. 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs. J. Anim. Sci. 90:1262-1269.

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer, Jr., eds. AOAC Int., Gaithersburg, MD.

Bueno, L., F. Praddaude, J. Fioramonti, and Y. Ruckebusch. 1981. Effect of dietary fiber on gastrointestinal motility and jejunal transit time in dogs. Gastroenterol. 80:701-707.

Canh, T. T., A. L. Sutton, A. J. Aarnink, M. W. Verstegen, J. W. Schrama, and G. C. Bakker. 1998. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. J. Anim. Sci. 76:1887-1895.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2015. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818.

Guggenbuhl, P., Y. Wache, C. Simoes Nunes, and F. Fru. 2012. Comparative effects of three phytases on the phosphorus and calcium use in the weaned piglet. J. Anim. Sci. 90:95-97.

Hillemeier, C. 1995. An overview of effects of dietary fiber on gastrointestinal transit. Pediatrics. 96:997-999.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85 (Suppl. 2):112. (Abstr.)

Lei, X. G., P. K. Ku, E. R. Miller, M. T. Yokoyama, and D. E. Ullrey. 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. J. Anim. Sci. 72:139-143.

Merriman, L. A., C. L. Walk, and H. H. Stein. 2016. Effect of fat sources on ATTD of minerals. J. Anim. Sci. 94(Suppl. 2):104. (Abstr.)

Moeser, A. J., and T. T. T. G. van Kempen. 2002. Dietary fibre level and enzyme inclusion affect nutrient digestibility and excreta characteristics in grower pigs. J. Sci. Food Agric. 82:1606-1613.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126-2132.

Sanderson, P. 1986. A new method of analysis of feedingstuffs for the determination of crude oils and fats. Pages 77–81 in Recent Advances in Animal Nutrition. W. Haresign and D. J. A. Cole, ed. Butterworths, London.

Vander, A. J., D. Luciano, and J. Sherman. 2001. The digestion and absorption of food. In: A. J. Vander, D. Luciano, and J. Sherman, editors, Human physiology: the mechanisms of body function, 8th ed. McGraw-Hill Higher Education, Boston, MA. p. 553-591.

TABLES

Table 8.1. Ingredient composition of experimental diets, as-fed basis

	Corn diets		Cornstarch diets	
Ingredient, %	0 FTU/kg	500 FTU/kg	0 FTU/kg	500 FTU/kg
Corn	76.65	75.64	-	-
Cornstarch	-	-	58.42	58.41
Sucrose	-	-	10.00	10.00
Phytase[2]	-	0.01	-	0.01
Potato protein isolate	18.00	18.00	25.00	25.00
Soybean oil	3.00	3.00	3.00	3.00
Calcium carbonate	1.75	1.75	1.75	1.75
Monosodium phosphate	1.00	1.00	1.20	1.20
Salt	0.40	0.40	0.40	0.40
Vitamin mineral premix[1]	0.20	0.20	0.20	0.20

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-

Table 8.1. (Cont.)

pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

[2]Phytase was included at 0.01% of microbial phytase (5,000 phytase units per g; Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK). The phytase premix was included in these diets at the expense of corn or cornstarch and provided 500 phytase units of phytase per kilogram complete feed.

Table 8.2. Analyzed composition of experimental diets, as-fed basis

	Corn diets		Cornstarch diets	
Ingredient	0 FTU	500 FTU	0 FTU	500 FTU
DM, %	91.10	91.22	95.52	94.71
Ash, %	3.02	3.53	3.14	3.40
CP, %	19.20	20.18	20.48	20.91
NDF, %	6.10	6.22	0.54	0.66
ADF, %	2.41	2.42	1.07	0.89
AEE[1], %	5.76	5.62	3.98	4.04
Ca, %	0.50	0.47	0.49	0.44
P, %	0.43	0.51	0.26	0.31
Phytate P, %	0.38	0.37	-	-
Phytase activity, FTU[2]	BDL[3]	571	BDL	375

[1]AEE = acid hydrolyzed ether extract.

[2]FTU = phytase units per kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[3]BDL = below detectable limits.

Table 8.3. Nutrient analyses of ingredients

	Item			
Ingredient	DM, %	Ash, %	Ca, %	P, %
Corn	85.91	1.32	0.04	0.23
Potato protein	92.23	0.56	0.04	0.16
Cornstarch	99.94	0.15	BDL[1]	0.01
Calcium carbonate	99.94	91.31	38.18	< 0.01
Monosodium phosphate	99.50	91.50	0.03	27.89

[1]BDL = below detectable limits.

Table 8.4. Balance and apparent total tract digestibility (ATTD) of Ca and P by pigs fed experimental diets[1]

Item	Corn diets		Cornstarch diets			*P* value		
Phytase Units (FTU/kg):	0	500	0	500	SEM	Diet type	Phytase	Diet type × phytase
ADFI, g of DM	637	677	394	414	36	< 0.001	0.403	0.776
Feces, g of DM/d	49	48	9	8	2	< 0.001	0.700	0.898
Ca intake, g/d	3.46	3.49	2.10	1.91	0.19	< 0.001	0.646	0.555
Ca in feces, g/d	1.68	1.69	0.79	0.54	0.17	< 0.001	0.480	0.451
Ca absorbed, g/d	1.79	1.80	1.33	1.37	0.19	0.025	0.865	0.934
ATTD of Ca, %	50.52	51.90	63.28	71.25	6.4	0.016	0.466	0.607
Ca in urine, mg/d	2.5	2.4	0.4	0.7	0.5	< 0.001	0.858	0.617
Retention of Ca, %	50.50	51.88	63.28	71.24	6.3	0.016	0.467	0.607

Table 8.4. (Cont.)

P intake, g/d	3.01	3.64	1.12	1.36	0.17	< 0.001	0.016	0.256
P in feces, g/d	1.05[a]	0.65[b]	0.07[c]	0.08[c]	0.05	< 0.001	< 0.001	< 0.001
P absorbed, g/d	1.96[a]	2.99[b]	1.05[c]	1.28[c]	0.15	< 0.001	< 0.001	0.010
ATTD of P, %	65.18[c]	81.83[b]	93.63[a]	93.57[a]	1.60	< 0.001	< 0.001	< 0.001
P in urine, g/d	1.08[c]	3.02[a]	2.17[b]	2.69[ab]	0.29	0.174	< 0.001	0.015
Retention of P, %	57.29	65.06	55.69	52.23	4.0	0.048	0.534	0.113

[1]Values are least squares means.

Table 8.5. The pH of contents from the stomach, duodenum, jejunum, and ileum, and in feces directly after slaughter[1]

pH	Corn diets		Cornstarch diets		SEM	*P* value		
Phytase Units (FTU/kg):	0	500	0	500		Diet type	Phytase	Diet type × phytase
Stomach	3.18	3.56	3.83	3.59	0.29	0.266	0.780	0.276
Duodenum	4.89	4.91	5.59	5.32	0.31	0.084	0.805	0.746
Jejunum	6.13	6.22	6.81	6.29	0.21	0.088	0.310	0.156
Ileum	6.59	6.61	6.86	7.11	0.10	< 0.001	0.213	0.303
Fecal	5.94	5.80	6.35	6.35	0.20	0.018	0.720	0.712

[1]Values are least squares means.

Table 8.6. The pH in the precipitate and liquid portions of digesta in the stomach, duodenum, jejunum, and ileum[1]

pH	Corn diets		Cornstarch diets		SEM	*P* value		
Phytase Units (FTU/kg):	0	500	0	500		Diet type	Phytase	Diet type × phytase
Liquid phase								
Stomach	2.84	3.21	3.41	3.03	0.25	0.631	0.604	0.060
Duodenum	4.22	4.41	5.53	4.86	0.44	0.010	0.455	0.188
Jejunum	5.40[b]	6.01[ab]	6.54[a]	5.84[ab]	0.28	0.083	0.874	0.028
Ileum	5.40	6.37	6.75	6.52	0.19	0.035	0.294	0.091
Precipitate								
Stomach	3.14	3.29	3.41	2.91	0.27	0.838	0.499	0.232
Duodenum	4.36	4.49	5.30	4.41	0.30	0.152	0.201	0.091
Jejunum	5.58	5.77	5.91	5.53	0.30	0.899	0.798	0.424

Table 8.6. (Cont.)

Ileum	5.34	6.19	6.05	6.49	0.37	0.289	0.180	0.661

[1]Values are least squares means.

Table 8.7. The concentrations of ash (%) in digesta from the stomach, duodenum, jejunum, and ileum

Ash, %	Corn diets		Cornstarch diets		SEM	*P* value		
Phytase Units (FTU/kg):	0	500	0	500		Diet type	Phytase	Diet type × phytase
Stomach	0.46	0.63	1.00	0.78	2.54	0.419	0.354	0.406
Duodenum	1.09	0.95	1.10	0.95	0.13	0.829	0.488	0.821
Jejunum	1.39[a]	1.02[b]	0.84[b]	0.91[b]	0.10	0.002	0.1878	0.020
Ileum	1.30	1.00	0.91	1.01	0.30	0.389	0.643	0.352

CHAPTER 9

REQUIREMENT FOR DIGESTIBLE CALCIUM BY 100 TO 130 KG PIGS AT DIFFERENT DIETARY CONCENTRATIONS OF PHOSPHORUS BY GROWTH PERFORMANCE AND BONE ASH CONCENTRATION

ABSTRACT: An experiment was conducted to determine the digestible Ca requirement by pigs from 100 to 130 kg. Ninety pigs (average initial BW = 99.89 ± 3.34 kg) were randomly allotted to 15 experimental diets. Each diet was fed to 6 replicate pens using a randomized complete block design. Fifteen corn and soybean meal-based diets were formulated and all diets had the same concentrations of phytate and Na. Diets were formulated using a 3 × 5 factorial design with diets containing 0.11, 0.21, or 0.31% standardized total tract digestible (STTD) P and 0.12, 0.29, 0.46, 0.61, or 0.78% total Ca (0.08, 0.18, 0.29, 0.38, or 0.49% STTD Ca). The P concentrations ranged from 48 to 152% of the STTD P requirement and the Ca concentrations ranged from 27 to 173% of the total Ca requirement. Experimental diets were fed for 28 d and pigs were individually housed. Pig and feeder weights were recorded at the beginning and at the conclusion of the experiment to calculate ADFI, ADG, and G:F. On d 28, all pigs were euthanized and the right femur was extracted. Ash, Ca, and P concentrations were determined from the de-fatted, dried femurs. Results indicated that as dietary concentrations of STTD Ca increased, the ADFI and ADG decreased (main effects of Ca, $P < 0.05$), regardless of the dietary concentration of P. Models to predict ADFI [ADFI = 3.6782 – 1.2722 × STTD Ca (%); $P = 0.001$] and ADG [ADG = 1.2141 – 0.6230 × STTD Ca (%); $P = 0.008$] were dependent only on the concentration of dietary STTD Ca, but not on the STTD of P. There were no effects of STTD Ca or STTD P on G:F indicating that the negative effects of STTD Ca on ADG was a result of reduced ADFI. Linear increases were observed for bone ash, bone Ca, and bone P as dietary

concentrations of STTD Ca increased for all concentrations of STTD P but the increase was greater at the highest concentration of STTD P than at lower levels (interaction, $P < 0.001$). In conclusion, results from the experiment support the current requirements for Ca and STTD P, and feeding Ca at levels greater than the requirements (0.46% total Ca; 0.29% STTD Ca) is detrimental to growth performance of pigs.

Key words: bone ash, calcium, digestible calcium, pigs, phosphorus, requirements

INTRODUCTION

Inclusion of Ca and P in diets fed to pigs needs to be adequate to optimize growth performance and at the same time minimize nutrient excretion from pigs. It is believed that diets are most accurately formulated by using values for standardized total tract digestibility (**STTD**) of Ca and P to meet the requirement for these minerals at each stage of production (NRC, 2012). The current NRC (2012) provides requirements for P on a STTD P basis; however, the requirements for Ca were estimated as total Ca due to a lack of data for digestibility of Ca in feed ingredients. The STTD P requirements were estimated based on a modeling approach and the requirement for Ca was obtained using a fixed ratio of 2.15 to 1 between total Ca and STTD P (NRC, 2012).

Recently, values for digestibility of Ca have been reported in inorganic supplements (González-Vega et al., 2014; González-Vega et al., 2015b), plant ingredients (Bohlke et al., 2005; González-Vega et al., 2013), and ingredients of animal origin (Kim et al., 2012; Sulabo and Stein, 2013; González-Vega et al., 2015a; Merriman et al., 2016). It is now possible to formulate diets using values for STTD of Ca in feed ingredients; however, there are no current estimates for the requirement of STTD Ca by pigs with a BW of 100 to 130 kg.

To determine requirements for digestible Ca, it is necessary to determine the concentrations that result in optimum growth performance of the pigs or the greatest bone mineral concentration. Because P influences both growth performance and bone ash concentration, the influence of P on Ca requirements also must be considered; therefore, the objective of the present experiment was to determine the requirement for STTD Ca by 100 to 130 kg pigs fed 3 dietary concentrations of STTD P by determining the quantities of STTD Ca that are needed to maximize ADG, ADFI, G:F, bone ash, bone Ca, and bone P at each level of STTD P.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol describing animal procedures for the experiment. Pigs used in the experiment were the offspring of Line 359 boars mated to C46 females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

Ninety barrows (average initial BW of 99.89 ± 3.34 kg) were randomly allotted to a randomized complete block design with 15 experimental diets. Each diet was fed to 6 replicate pigs per diet. Pigs were allotted to experimental diets using The Experimental Animal Allotment Program (Kim and Lindemann, 2007). Pens had fully slatted floors and pigs had free access to water via a nipple waterer and feed from an individual feeder. Pigs were housed in environmentally controlled buildings.

Diets and Feeding

Fifteen corn-soybean meal based diets were formulated to contain different Ca and P concentrations, but to keep the concentration of phytate constant, all diets contained the same amount of corn and soybean meal (Tables 9.1, 9.2, and 9.3). Diets were formulated using a 3 × 5 factorial design with diets containing 0.11, 0.21, or 0.31% STTD and 0.12, 0.29, 0.46, 0.61, or 0.78% total Ca (0.08, 0.18, 0.29, 0.38, or 0.49% STTD Ca). Concentrations of STTD P ranged from 48 to 152% of the requirement (NRC, 2012) and the Ca concentrations ranged from 27 to 173% of the Ca requirement (NRC, 2012). All diets had equal concentrations of Na, which was accomplished by reducing the sodium bicarbonate inclusion as the concentration of monosodium phosphate increased in diets to provide increasing concentrations of dietary P.

Pigs were provided ad libitum access to experimental diets for 28 d. Pigs were weighed at the beginning and at the conclusion of the experiment. Feeder weights were also recorded on these days to calculate feed consumption and ADFI, ADG, and G:F were calculated from these data.

Sample Analyses

On the last day of the experiment, barrows were euthanized at the Meats Science Laboratory at the University of Illinois. The right femur from each barrow was removed, cleaned, and stored at -20ºC. Bones were broken and soaked in petroleum ether under a chemical hood to remove marrow and fat for 72 h and then were dried overnight at 130ºC.

Diets were analyzed for DM using a drying oven at 135ºC for 2 h (Method 930.15; AOAC Int., 2007) and diets and bones were analyzed for ash (Method 942.05; AOAC Int., 2007). Diets were also analyzed for N using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc.,

Mt. Laurel, NJ), and CP was calculated as N × 6.25. Diets were analyzed for crude fat using ether extraction (Method 920.39 (A); AOAC Int., 2007), ADF (Method 973.18; AOAC Int., 2007), and NDF (Holst, 1973). Diets and bone ash samples were analyzed for Ca and P by inductively coupled plasma (**ICP**) spectroscopy (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007].

Statistical Analyses

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

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

where Y was the dependent variable, a was the intercept, b, c, d, e, and f were the coefficients, and STTD Ca and STTD P were the percentage concentrations of dietary STTD Ca and STTD P.

RESULTS AND DISCUSSION

All pigs remained healthy throughout the experiment and consumed their respective diets without apparent problems. Interactions between STTD Ca and STTD P were not significant for ADG, ADFI, or G:F (Table 9.4). For ADG and ADFI, the reduced model contained only STTD Ca and excluded all terms containing STTD P as well as the quadratic term for STTD Ca. Intake was greatest by pigs consuming the least concentration of STTD Ca, and a marked reduction ($P < 0.01$) in intake occurred as dietary concentrations of STTD Ca increased. Based on these results, it appears that if pigs are fed below the requirement for dietary Ca, they will increase ADFI, which results the greatest ADG. In contrast, if dietary Ca is above the requirement, pigs will reduce ADFI, which results in a suppression of ADG.

The current dietary requirements (NRC, 2012) for pigs from 100 to 125 kg BW are 0.46% total Ca and 0.21% STTD P. Using the digestibility coefficients for STTD of Ca in corn, soybean meal, and calcium carbonate, the STTD of Ca in the diet containing 0.46% total Ca was calculated to be 0.29% STTD Ca. The greatest ADG was achieved by pigs fed diets at concentrations equal to or less 0.29% STTD Ca and 0.21% STTD P. Addition of either Ca or P above those concentrations have previously resulted in a reduction in growth performance (Reinhart and Mahan, 1986; Hall et al, 1991; NRC, 2012). The results from this experiment are in agreement with the previous observations, and indicate that the current requirements for STTD Ca and STTD P are the maximum dietary concentrations that should be fed to pigs at 100 to 130 kg BW. If Ca and P are added above the requirement, growth performance will be reduced.

Neither Ca nor P could be used to predict G:F, indicating that the efficiency of feed utilization for gain was not modified by dietary Ca or P concentration. Instead, it appears that the growth potential was limited by pigs fed diets with the higher concentrations of STTD Ca

because of reduced ADFI. This is different from pigs between 25 to 50 kg BW, where G:F can be predicted using linear and quadratic coefficients for dietary Ca and P (González-Vega et al., 2016b). Likewise for pigs from 11 to 25 kg BW, G:F was quadratically influenced by the dietary concentrations of STTD Ca (González-Vega et al., 2016a). It is possible that the reason G:F was not influenced by dietary Ca and P in the current experiment is that older pigs have a greater capacity to regulate the composition of body gain than younger pigs.

In contrast to results for ADG and ADFI, results from the current experiment indicated that the concentrations of STTD Ca needed to maximize bone ash, bone Ca, and bone P may be greater that the current requirement (NRC, 2012), and concentrations of Ca and P needed to maximize bone ash are also greater than that needed to maximize growth performance. These observations are in agreement with previous observations (Nimmo et al., 1980; Kornegay et al., 1981; Maxson and Mahan, 1983; González-Vega et al., 2016a; 2016b). The implications of these observations are that pigs can utilize dietary Ca and P to synthesize bone tissue at levels above that required to maximize growth performance.

Growth response curves typically have a positive linear relationship to increased concentrations of the limiting nutrient being tested if nutrient intake is below the requirement. This experiment and other experiments evaluating concentrations of dietary Ca below the requirement have failed to demonstrate such a response for Ca (González-Vega et al., 2016a; 2016b). The reason for this observation may be that pigs used in the current experiment were provided diets containing adequate concentrations of Ca and P prior to the initiation of the experiment; therefore, these pigs may have had a sufficient amount of stored Ca and P in the bones to manage the deficiencies subjected to them during this experiment. However, the

outcome may have been different had the deficiencies been carried out through the entire growth period of the pig; further research is needed to verify this hypothesis.

Significant interactions ($P < 0.05$) were observed between STTD Ca and STTD P for bone ash, bone Ca, and bone P (Table 9.5). However, due to the positive linear nature of the predicted equations, a predicted maximum was not obtained. Bone ash (Figure 9.1), bone Ca (Figure 9.2), and bone P (Figure 9.3) increased ($P < 0.05$) as dietary concentrations of Ca and P increased; and the greatest bone ash, bone Ca, and bone P concentrations were observed at the greatest level of STTD Ca and STTD P. In previous experiments (González-Vega et al., 2016a; 2016b) bone responses were curvilinear and bone responses plateaued at the greatest levels of Ca. This was not the case in the present study. Despite the detrimental impact on growth performance, the pig continued to deposit Ca and P within the bone as Ca or P or both Ca and P in the diets increased.

In the diets used in this experiment, the ratio between STTD Ca and STTD P was between 0.26:1 and 4.45:1. In pigs at 25 to 50 kg of BW, the ratio was optimized between 1.16:1 and 1.43:1 for ADG and G:F (González-Vega et al., 2016b). It has been reported that Ca:P ratios are important when dietary concentrations of P are limiting, but may be of less importance if adequate concentrations of dietary P are provided (Crenshaw, 2001). Ironically, the ratio between Ca and P in the bone was close to 2.1:1.0 for all dietary treatments. These ratios are consistent with previous reports (Gutzwiller et al., 2014; González Vega et al., 2016b).

In conclusion, results from the current experiment indicated that adding dietary STTD Ca above 0.29% STTD Ca and above 0.21% STTD P is detrimental to growth performance for pigs from 100 to 130 kg of BW. Dietary Ca above the requirement results in reduced ADFI and therefore, reduced ADG; whereas, efficiency of growth was not changed. These observations

indicated that the current dietary requirements (NRC, 2012) for total Ca and STTD P derived from mathematical equations are accurate for the finishing pig; however, Ca and P must be included above the requirements to maximize bone ash and concentrations of bone Ca and bone P. Feeding concentrations of dietary Ca and P needed to maximize bone ash will result in reduced growth performance of the pigs.

LITERATURE CITED

AOAC. 2007. Official Methods of Analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:485-494.

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396-2403.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 187-212.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of Ca and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807-4816.

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126-142.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93:4808-4818.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015b. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93:2255-2264.

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2016a. Requirements for digestible Ca by growing pigs. J. Anim. Sci. Sci. 94 (Suppl. 2):101. (Abstr.).

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2016b. Digestible calcium requirements for 25 to 50 kg pigs. J. Anim. Sci. 94 (Suppl. 2):101. (Abstr.).

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

Hall, D. D., G. L. Cromwell, and T. S. Stahly. 1991. Effects of dietary calcium, phosphorus, calcium: phosphorus ratio and vitamin K on performance, bone strength, and blood clotting status of pigs. J. Anim. Sci. 69:646-655.

Holst, D. O. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. J. Assoc. Off. Anal. Chem. 56:1352–1356.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85(Suppl. 2):112. (Abstr.).

Kim, B. G., J. W. Lee, and H. H. Stein. 2012. Energy concentration and phosphorus digestibility in whey powder, whey permeate, and low-ash whey permeate fed to weanling pigs. J. Anim. Sci. 90:289-295. doi: 10.2527/jas.2011-4145.

Kornegay, E. T., H. R. Thomas, and J. L. Baker. 1981. Phosphorous in swine. II Influence of dietary calcium and phosphorous levels and growth rate on serum minerals, soundness scores and bone development in barrows, gilts and boars. J. Anim. Sci. 52:1049-1059.

Maxson, P. F., and D. C. Mahan. 1983. Dietary calcium and phosphorus levels for growing swine from 18 to 57 kilograms body weight. J. Anim. Sci. 54:1124-1134.

Merriman, L. A., C. L. Walk, and H. H. Stein. 2016. Effect of microbial phytase on apparent and standardized total tract digestibility of calcium in feed ingredients of animal origin. J. Anim. Sci. 94(Suppl. 2):110 (Abstr.).

Nimmo, R. D., E. R. Peo, Jr., B. D. Moser, P. J. Cunningham, D. G. Olson, and T. D. Crenshaw. 1980. Effect of various levels of dietary calcium and phosphorus on performance, blood and bone parameters in growing boars. J. Anim. Sci. 51:100-111.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Reinhart, G. A., and D. C. Mahan. 1986. Effect of various calcium:phosphorus ratios at low and high dietary phosphorus for starter, grower, and finisher swine. J. Anim. Sci. 63:457-466.

Sulabo, R. C., and H. H. Stein. 2013. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. J. Anim. Sci. 91:1285-1294.

FIGURES

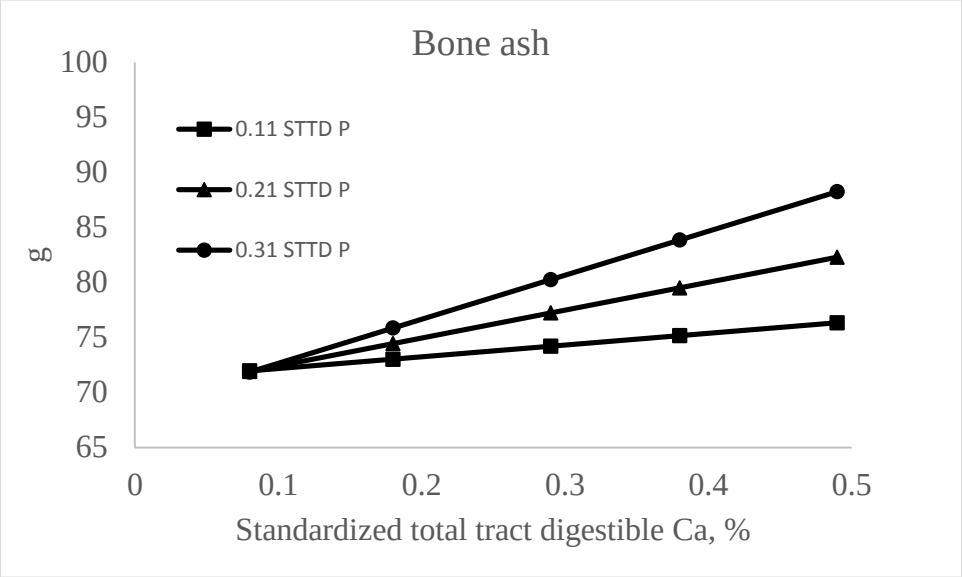

Figure 9.1. Predicted values, based on the interaction between STTD Ca and STTD P (*P* = 0.049), for bone ash (grams) in pigs fed diets containing from 0.08 to 0.49% standardized total tract digestible (STTD) Ca and from 0.11 to 0.31% STTD P. All responses were linear; therefore, no maximum values were estimated.

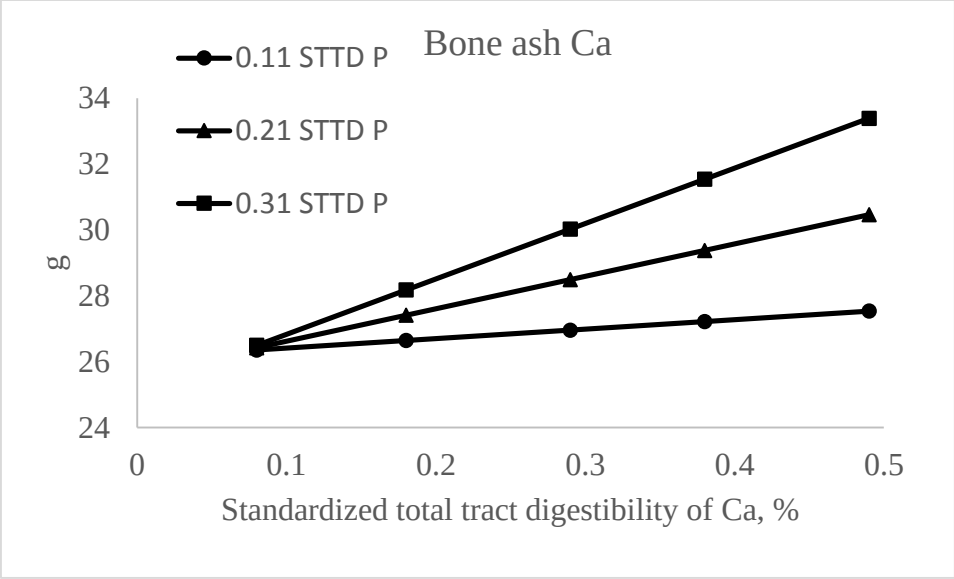

Figure 9.2. Predicted values, based on the interaction between STTD Ca and STTD P (*P* = 0.049), for bone ash Ca (grams) in pigs fed diets containing from 0.08 to 0.49% standardized total tract digestible (STTD) Ca and from 0.11 to 0.31% STTD P. All responses were linear; therefore, no maximum values were estimated.

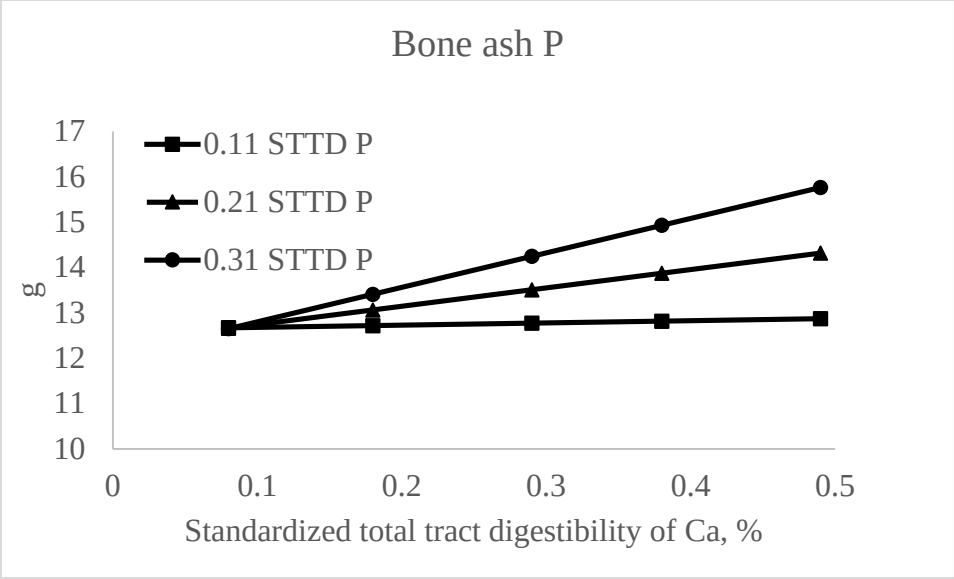

Figure 9.3. Predicted values, based on the interaction between STTD Ca and STTD P (*P* = 0.049), for bone ash P (grams) in pigs fed diets containing from 0.08 to 0.49% standardized total tract digestible (STTD) Ca and from 0.11 to 0.31% STTD P. All responses were linear; therefore, no maximum values were estimated.

TABLES

Table 9.1. Ingredient composition and nutrient analysis of experimental diets containing 0.11% standardized total tract digestible (STTD) P, as-fed basis

	0.11% STTD P				
Total Ca, %	0.12	0.29	0.46	0.61	0.78
STTD Ca, %	0.08	0.18	0.29	0.38	0.49
STTD Ca: STTD P	0.73:1.00	1.64:1.00	2.64:1.00	3.45:1.00	4.45:1.00
Item					
Corn, %	65.00	65.00	65.00	65.00	65.00
Soybean meal, %	20.00	20.00	20.00	20.00	20.00
Cornstarch, %	13.55	12.31	10.95	9.80	8.56
Soybean oil, %	-	0.80	1.70	2.45	3.25
Calcium carbonate, %	-	0.44	0.90	1.30	1.74
Sodium bicarbonate, %	1.05	1.05	1.05	1.05	1.05
Sodium chloride, %	0.20	0.20	0.20	0.20	0.20
Vitamin mineral premix[1], %	0.20	0.20	0.20	0.20	0.20

Table 9.1. (Cont.)

Analyzed composition					
CP, %	14.49	15.55	14.68	14.81	14.29
AEE2, %	2.57	2.21	3.14	3.48	4.63
ADF, %	2.76	2.65	3.01	3.43	3.97
NDF, %	6.46	5.68	6.69	5.88	7.22
DM, %	90.56	90.53	90.41	90.60	90.26
Ash, %	2.46	2.75	3.03	3.46	3.94
Ca, %	0.14	0.25	0.39	0.62	0.71
P, %	0.27	0.26	0.27	0.29	0.29
Phytate P, %	0.10	0.10	0.13	0.15	0.16
Calculated composition					
NE, kcal/kg	2,629	2,629	2,629	2,629	2,629
Indispensable SID3					
AA, %					
Arg	0.86	0.86	0.86	0.86	0.86

Table 9.1. (Cont.)

His	0.36	0.36	0.36	0.36	0.36
Ile	0.53	0.53	0.53	0.53	0.53
Leu	1.18	1.18	1.18	1.18	1.18
Lys	0.65	0.65	0.65	0.65	0.65
Met	0.22	0.22	0.22	0.22	0.22
Phe	0.64	0.64	0.64	0.64	0.64
Thr	0.55	0.55	0.55	0.55	0.55
Trp	0.15	0.15	0.15	0.15	0.15
Val	0.59	0.59	0.59	0.59	0.59
Na, %	0.37	0.37	0.37	0.37	0.37
Cl, %	0.15	0.15	0.15	0.15	0.15
Total Ca:total P	0.43:1.00	1.04:1.00	1.64:1.00	2.18:1.00	2.79:1.00
Total Ca:STTD P	1.09:1.00	2.64:1.00	4.18:1.00	5.55:1.00	7.09:1.00
STTD Ca:STTD P	0.73:1.00	1.64:1.00	2.64:1.00	3.45:1.00	4.45:1.00

Table 9.1. (Cont.)

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

[2]AEE = acid hydrolyzed ether extract.

[3]SID = standardized ileal digestibility.

Table 9.2. Ingredient composition and nutrient analysis of experimental diets containing 0.21% standardized total tract digestible (STTD) P, as-fed basis

	0.21% STTD P				
Total Ca, %	0.12	0.29	0.46	0.61	0.78
STTD Ca, %	0.08	0.18	0.29	0.38	0.49
STTD Ca: STTD P	0.38:1.00	0.86:1.00	1.38:1.00	1.81:1.00	2.33:1.00
Item					
Corn, %	65.00	65.00	65.00	65.00	65.00
Soybean meal, %	20.00	20.00	20.00	20.00	20.00
Cornstarch, %	13.23	11.93	10.63	9.48	8.19
Soybean oil, %	0.20	1.05	1.90	2.65	3.50
Calcium carbonate, %	-	0.45	0.90	1.30	1.74
Sodium bicarbonate, %	0.72	0.72	0.72	0.72	0.72
Monosodium phosphate, %	0.45	0.45	0.45	0.45	0.45
Sodium chloride, %	0.20	0.20	0.20	0.20	0.20
Vitamin mineral premix[1], %	0.20	0.20	0.20	0.20	0.20

Table 9.2. (Cont.)

Analyzed composition					
CP, %	15.32	13.73	15.50	12.99	15.23
AEE2, %	2.17	3.17	3.37	3.64	4.46
ADF, %	3.29	3.46	3.46	3.46	3.38
NDF, %	7.7	6.53	8.64	8.18	10.26
DM, %	90.71	90.94	90.84	90.69	90.36
Ash, %	2.79	3.74	3.54	3.16	3.74
Ca, %	0.09	0.29	0.51	0.51	0.65
P, %	0.34	0.39	0.43	0.39	0.39
Phytate P, %	0.10	0.10	0.10	0.15	0.16
Calculated composition					
NE, kcal/kg	2,629	2,629	2,629	2,629	2,629
Indispensable SID3 AA, %					
Arg	0.86	0.86	0.86	0.86	0.86

Table 9.2. (Cont.)

His	0.36	0.36	0.36	0.36	0.36
Ile	0.53	0.53	0.53	0.53	0.53
Leu	1.18	1.18	1.18	1.18	1.18
Lys	0.65	0.65	0.65	0.65	0.65
Met	0.22	0.22	0.22	0.22	0.22
Phe	0.64	0.64	0.64	0.64	0.64
Thr	0.55	0.55	0.55	0.55	0.55
Trp	0.15	0.15	0.15	0.15	0.15
Val	0.59	0.59	0.59	0.59	0.59
Na, %	0.37	0.37	0.37	0.37	0.37
Cl, %	0.15	0.15	0.15	0.15	0.15
Total Ca:total P	0.31:1.00	0.74:1.00	1.18:1.00	1.56:1.00	2.00:1.00
Total Ca:STTD P	0.57:1.00	1.38:1.00	2.19:1.00	2.90:1.00	3.71:1.00
STTD Ca:STTD P	0.38:1.00	0.86:1.00	1.38:1.00	1.81:1.00	2.33:1.00

Table 9.2. (Cont.)

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

[2]AEE = acid hydrolyzed ether extract.

[3]SID = standardized ileal digestibility.

Table 9.3. Ingredient composition and nutrient analysis of experimental diets containing 0.31% standardized total tract digestible (STTD) P, as-fed basis

	0.31% STTD P				
Total Ca, %	0.12	0.29	0.46	0.61	0.78
STTD Ca, %	0.08	0.18	0.29	0.38	0.49
STTD Ca: STTD P	0.26:1.00	0.58:1.00	0.94:1.00	1.23:1.00	1.58:1.00
Item					
Corn, %	65.00	65.00	65.00	65.00	65.00
Soybean meal, %	20.00	20.00	20.00	20.00	20.00
Cornstarch, %	12.85	11.55	10.25	9.12	7.81
Soybean oil, %	0.45	1.30	2.15	2.90	3.75
Calcium carbonate, %	-	0.45	0.90	1.28	1.74
Sodium bicarbonate, %	0.40	0.40	0.40	0.40	0.40
Monosodium phosphate, %	0.90	0.90	0.90	0.90	0.90
Sodium chloride, %	0.20	0.20	0.20	0.20	0.20
Vitamin mineral premix[1], %	0.20	0.20	0.20	0.20	0.20

Table 9.3. (Cont.)

Analyzed composition					
CP, %	14.70	15.02	14.54	14.41	14.37
AEE2, %	2.15	2.81	3.29	3.78	4.21
ADF, %	3.09	3.47	3.49	3.61	3.65
NDF, %	8.09	8.14	8.97	9.65	9.98
DM, %	90.75	91.10	90.94	90.40	90.57
Ash, %	3.20	3.50	3.57	3.96	4.12
Ca, %	0.13	0.27	0.50	0.72	0.95
P, %	0.50	0.45	0.51	0.54	0.52
Phytate P, %	0.10	0.11	0.14	0.14	0.16
Calculated composition					
NE, kcal/kg	2,629	2,629	2,629	2,629	2,629
Indispensable SID3 AA, %					
Arg	0.86	0.86	0.86	0.86	0.86

Table 9.3. (Cont.)

His	0.36	0.36	0.36	0.36	0.36
Ile	0.53	0.53	0.53	0.53	0.53
Leu	1.18	1.18	1.18	1.18	1.18
Lys	0.65	0.65	0.65	0.65	0.65
Met	0.22	0.22	0.22	0.22	0.22
Phe	0.64	0.64	0.64	0.64	0.64
Thr	0.55	0.55	0.55	0.55	0.55
Trp	0.15	0.15	0.15	0.15	0.15
Val	0.59	0.59	0.59	0.59	0.59
Na, %	0.37	0.37	0.37	0.37	0.37
Cl, %	0.15	0.15	0.15	0.15	0.15
Total Ca:total P	0.24:1.00	0.58:1.00	0.92:1.00	1.22:1.00	1.56:1.00
Total Ca:STTD P	0.39:1.00	0.94:1.00	1.48:1.00	1.97:1.00	2.52:1.00
STTD Ca:STTD P	0.26:1.00	0.58:1.00	0.94:1.00	1.23:1.00	1.58:1.00

Table 9.3. (Cont.)

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

[2]AEE = acid hydrolyzed ether extract.

[3]SID = standardized ileal digestibility.

Table 9.4. Growth performance of pigs fed experimental diets with varying concentrations of standardized total tract digestible (STTD) Ca and P for 28 d

	Total Ca (STTD Ca), %				
Item	0.12	0.29	0.46	0.61	0.78
STTD Ca, %:	(0.08)	(0.18)	(0.29)	(0.38)	(0.49)
Initial BW, kg					
0.11% STTD P	98.83	100.28	101.75	97.62	99.23
0.21 % STTD P	99.42	98.38	101.07	98.47	100.45
0.31 % STTD P	101.13	99.15	99.25	102.60	100.72
ADG, kg[1,2]					
0.11% STTD P	1.21	1.14	1.17	0.89	0.83
0.21 % STTD P	1.16	1.20	1.17	1.15	0.96
0.31 % STTD P	1.11	1.10	1.08	1.00	1.10

Table 9.4. (Cont.)

ADFI, kg[3,4]					
0.11% STTD P	3.70	3.29	3.27	3.16	2.88
0.21 % STTD P	3.72	3.58	3.31	3.32	3.16
0.31 % STTD P	3.46	3.32	3.27	3.16	3.16
GF, d1-28[5,6]					
0.11% STTD P	0.33	0.35	0.37	0.28	0.29
0.21 % STTD P	0.32	0.34	0.36	0.35	0.31
0.31 % STTD P	0.33	0.33	0.34	0.32	0.35
Final BW, kg[7,8]					
0.11% STTD P	132.68	132.33	134.48	122.42	122.52
0.21 % STTD P	132.02	131.85	133.75	130.77	127.32
0.31 % STTD P	132.33	129.90	129.62	130.57	131.47

Table 9.4. (Cont.)

[1]Results indicated that ADG from d 1 to 28 at different combinations of STTD Ca and STTD P can be described by the following model: 1.2141 – 0.6230 × STTD Ca (P = 0.008).

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

[3]Results indicated that ADFI from d 1 to 28 at different concentrations of STTD Ca can be described by the following model: 3.6782 – 1.2722 × STTD Ca (P = 0.001).

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

[5]Results indicated that G:F from d 1 to 28 could not be predicted using STTD Ca or STTD P.

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

[7]Results indicated that final BW at different combinations of STTD Ca and STTD P can be described by the following model: 140.4729 – 42.9212 × STTD Ca – 30.3919 × STTD P + 140.2884 STTD Ca × STTD P (P = 0.006).

[8]Standard error of the within treatment least squares means = 2.87.

Table 9.5. Least squares means for bone ash, bone Ca, and bone P in pigs fed diets containing from 0.12 to 0.78% standardized total tract digestible (STTD) Ca and from 0.11 to 0.31% STTD P

	Total Ca (STTD Ca), %				
Item	0.12	0.29	0.46	0.61	0.78
STTD Ca, %:	(0.08)	(0.18)	(0.29)	(0.38)	(0.49)
Bone ash[1,2], g					
0.11% STTD P	73.32	72.94	73.99	70.62	76.08
0.21% STTD P	69.04	72.42	84.26	83.32	83.81
0.31% STTD P	73.87	75.43	78.18	81.16	87.68
Bone Ca[3,4], g					
0.11% STTD P	26.98	27.27	25.41	25.01	28.14
0.21% STTD P	25.47	27.15	30.79	31.37	31.20
0.31% STTD P	27.26	27.83	28.98	30.63	33.03
Bone P[5,6], g					
0.11% STTD P	12.86	13.06	12.06	11.84	13.03
0.21% STTD P	12.19	13.06	14.52	14.98	14.65

Table 9.5. (Cont.)

0.31% STTD P	13.00	13.24	13.71	14.47	15.58
Ca:P in bone					
0.11% STTD P	2.10:1.00	2.09:1.00	2.11:1.00	2.11:1.00	2.16:1.00
0.21% STTD P	2.09:1.00	2.08:1.00	2.12:1.00	2.10:1.00	2.13:1.00
0.31% STTD P	2.10:1.00	2.10:1.00	2.11:1.00	2.12:1.00	2.12:1.00

[1]Results indicated that bone ash at different combinations of STTD Ca and STTD P can be described by the following model: 72.4527 – 5.4437 × STTD Ca – 12.2622 × STTD P + 146.6110 × STTD Ca × STTD P ($P < 0.001$).

[2]Standard error of the within treatments least squares means = 3.31.

[3]Results indicated that bone Ca at different combinations of STTD Ca and STTD P can be described by the following model: 26.6645 – 4.7992 × STTD Ca – 4.8919 × STTD P + 69.7578 × STTD Ca × STTD P ($P < 0.001$).

[4]Standard error of the within treatments least squares means = 1.31.

[5]Results indicated that bone P at different combinations of STTD Ca and STTD P can be described by the following model: 12.9522 – 3.4175 × STTD Ca – 2.9441 × STTD P + 35.5359 × STTD Ca × STTD P ($P < 0.001$).

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

GENERAL CONCLUSIONS

Historically, the requirements for Ca by pigs have been expressed on a total Ca basis. Until recently, values for the apparent total tract digestibility (**ATTD**) and standardized total tract digestibility (**STTD**) of Ca were not published; therefore, were unavailable to use for formulation of swine diets. Most dietary Ca is supplied by inorganic supplements and by feed ingredients of animal origin. Although inorganic supplements are relatively inexpensive, accurate formulation of Ca and P is essential because inadequate quantities or proportions are detrimental to growth performance and P metabolism; therefore, it is important to not only determine the digestibility of feed ingredients, but also other factors or ingredients that may alter the digestion of these 2 minerals.

Ingredients of animal origin may contribute a significant portion of the dietary Ca, and for that reason it was important to determine the digestibility of Ca in those ingredients. Based on the results from the experiment, the digestibility of Ca was not equal among all feed ingredients of animal origin. Results from the experiment also indicated that although ingredients of animal origin do not contain phytate, Ca from ingredients of animal origin may bind to the phytate in plant ingredients. Also responses to supplemental phytase were not equal among different feed ingredients of animal origin; therefore, use of STTD values of Ca is necessary if diets are to be accurately formulated for the Ca requirement of the pig.

There are ingredients provided in the diets to pigs that may influence the digestibility of Ca even though they themselves do not contain Ca. It was important to understand how these ingredients impact Ca digestibility not only for practical reasons, but also to accurately determine ATTD and STTD of Ca in different feed ingredients. Effects of NaCl, fat, sugars, and fiber on

ATTD of Ca were evaluated. Adding NaCl above 0.40% of the diet reduced the ATTD of Ca in corn and canola meal based diets, and therefore, it is recommended to include NaCl at 0.40% of the diet, which supported the increase in Na and Cl requirements provided in the newest edition of NRC. Inclusion of extracted fat increased the ATTD of Ca from a basal diet with no extracted fat, however fat quality may impact ATTD of Ca, and use of choice white grease that has been oxidized may result in ATTD values of Ca that are less than values resulting from the use of other fats. There was no difference in the ATTD of Ca between diets containing cornstarch or sucrose; therefore, either of those ingredients can be included in research diets without concern of their effect on Ca. The presence of phytate in a corn based diet reduced the ATTD of Ca compared with a diet containing no phytate and based on cornstarch. Although phytase was able to liberate some of the Ca bound to phytate, phytase could not completely ameliorate the effects of phytate in the diet as the ATTD of Ca in the cornstarch based diets were greater than in the corn based diet with 500 units/kg of microbial phytase. The phytase may have been saturated with the high levels of phytate in this diet; therefore, more units of microbial phytase may be advantageous in this circumstance.

For 100 to 130 kg pigs, the results supported the current requirements for total Ca and for STTD P. In our experiment, the total Ca requirement at 0.46% was equal to a STTD Ca value of 0.29% using calcium carbonate as the source of Ca. There was a negative effect of adding Ca or P above the requirement, but Ca deficient diets were not detrimental to growth performance; therefore, the STTD Ca:STTD P ratio to optimize growth performance may be no greater than 2.19:1.00. In contrast, the optimum dietary Ca and P concentrations needed to maximize concentration of bone ash, bone Ca, and bone P could not be determined in the experiment, which indicates that finishing pigs may deposit large quantities of Ca and P in bone ash. The

slopes of these lines were greater with increasing dietary STTD P, indicating that the pig was able to continually deposit more Ca and P and that P is the limiting factor for deposition of bone. Results from this experiment also validate earlier work in our laboratory indicating that the requirements of Ca for bone ash synthesis are greater than the requirements of Ca for growth performance.

In conclusion, results from these experiments indicated that the digestibility of Ca varies among different dietary sources of Ca; therefore, diets fed to pigs should be formulated based on values for STTD Ca to optimize growth performance and P metabolism. Also, other dietary components such as quality of fat, fiber concentration, and NaCl may influence the amount of Ca that can be utilized by the pig.