ASPECTS OF CALCIUM DIGESTIBILITY IN PIGS

BY

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THESIS

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ABSTRACT

Two experiments were conducted to determine endogenous losses of Ca and determine the effects of phytase, phytate, source of dietary Ca, and Ca level on Ca digestibility. The first objective of Exp. 1 was to test the hypothesis that endogenous Ca is lost from the gastrointestinal tract of growing pigs, and that values for true total tract digestibility (**TTTD**) of Ca are different from values for apparent total tract digestibility (**ATTD**) of Ca. The second objective was to determine the effect of microbial phytase on ATTD and TTTD of Ca in canola meal. Forty eight growing barrows (initial BW: 16.7 ± 2.5 kg) were allotted to a randomized complete block design with 8 dietary treatments and 6 pigs per treatment. Diets were formulated to contain 0.08, 0.16, 0.24, or 0.32% Ca from canola meal and 0 or 1,500 units per kg of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK). Feces and urine samples were collected from d 6 to d 11. Total endogenous losses of Ca were determined using the regression procedure. Results indicate that the estimated total endogenous losses of Ca were 0.160 and 0.189 g/kg DMI for canola meal without and with microbial phytase, respectively, and these values were not different. The ATTD of Ca increased ($P < 0.05$) if dietary Ca increased; however, the TTTD of Ca was not affected by dietary Ca. The ATTD and TTTD of Ca increased ($P < 0.01$) if phytase was added to the diets. The first objective of Exp. 2 was to test the hypothesis that standardized duodenal digestibility (**SDD**), standardized ileal digestibility (**SID**), and standardized total tract digestibility (**STTD**) of Ca in calcium carbonate and Vistacal are not different at 2 different Ca levels, and that phytate affects digestibility of Ca in these 2 ingredients to the same degree. The second objective was to determine where in the intestinal tract Ca absorption takes place and if measurable quantities of basal endogenous Ca are lost in the stomach, small intestine, or large intestine. Nine growing pigs (initial BW: 23.8 ± 1.3 kg) were

surgically equipped with a T-cannula in the duodenum and another cannula in the distal ileum and were allotted to a 9 × 6 incomplete Latin square design with 9 diets and 6 periods. Diets contained calcium carbonate or Vistacal as the sole source of Ca, 0 or 1% phytate, and 0.4 or 0.8% Ca. A Ca-free diet was also formulated and used to measure endogenous losses of Ca. Fecal, ileal, and duodenal samples were collected on d 5 and 6, 7 and 8, and 9 and 10, respectively. Results indicated that duodenal endogenous losses of Ca (1.03 g/kg of DMI) were greater ($P < 0.05$) than ileal (0.42 g/kg of DMI) and total tract endogenous losses of Ca (0.67 g/kg of DMI). Standardized digestibility of Ca was not affected by level of phytate, but decreased ($P < 0.05$) as Ca level increased in Vistacal diets; standardized digestibility of Ca did not decrease as Ca increased if calcium carbonate was the source of Ca (interaction, $P < 0.05$). The SDD, SID, and STTD of Ca were not different if calcium carbonate was the source of Ca. However, the SID and STTD of Ca in Vistacal was greater ($P < 0.05$) than the SDD of Ca, but no differences were observed between SID and STTD of Ca (interaction, $P < 0.05$). The SDD, SID, and STTD of Ca in calcium carbonate were greater ($P < 0.05$) than in Vistacal. It was concluded that endogenous Ca is lost from the intestinal tract of pigs. As a consequence, values for STTD or TTTD of Ca need to be determined in feed ingredients fed to pigs. For formulation of diets fed to pigs, Ca source, dietary Ca level, and inclusion of microbial phytase to the diets are factors to consider because they influence Ca digestibility.

Keywords: calcium, digestibility, endogenous losses, phytase, phytate, pig

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CHAPTER 1

INTRODUCTION

For formulation of diets for pigs, it is necessary to consider an appropriate Ca:P ratio for an adequate absorption and utilization of both minerals. Using standardized digestibility values rather than apparent digestibility values to formulate diets may be more accurate for nutrients that have endogenous losses in the intestinal tract because these values are additive in mixed diets (Stein et al., 2005). It has been documented that endogenous P is lost in the intestines of pigs and values for the standardized total tract digestibility (**STTD**) of P in feed ingredients have been reported (Petersen and Stein, 2006; Almeida and Stein, 2010; NRC, 2012). However, only apparent total tract digestibility (**ATTD**) values of Ca have been reported in pigs (Bohlke et al., 2005; Stein et al., 2006, 2008, and 2011). Standardized total tract digestibility of a nutrient is calculated by correcting values for ATTD for basal endogenous losses (Almeida and Stein, 2010), but values for basal endogenous losses of Ca for pigs have not been reported. There are different methods to estimate endogenous losses of a nutrient such as regression analysis (Fan et al., 2001), use of a nutrient free-diet (Petersen and Stein, 2006; Almeida and Stein, 2010), or using radioactively-labelled or stable isotopes (Visek et al., 1953). Therefore, the first objective of this thesis is to determine if there is a measurable endogenous intestinal loss of Ca from pigs, and also to determine if measurable quantities of basal endogenous Ca are lost in the stomach, the small intestine, or the large intestine.

The type of collection that is needed to conduct a digestibility study depends on the place that the nutrient is absorbed and if there are endogenous secretions (e.g., ileal collection for AA digestibility and total tract collection for P digestibility; NRC, 2012). Although most Ca is absorbed in the small intestine (Moore and Tyler, 1955a; b; Partridge, 1978; Liu et al., 2000),

there are indications that Ca may also be absorbed in the colon under some circumstances (Liu et al., 2000). However, type of diet may influence the specific region where Ca is absorbed (Partridge, 1978). Therefore, the second objective of this thesis is to determine if Ca absorption takes place only in the small intestine or if absorption also occurs in the stomach or the large intestine. It will then be determined which type of collection is most accurate in Ca digestibility studies with pigs.

Phosphorus bound to phytate is the main form of P that is present in plant ingredients. Therefore, plant ingredients have low bio-availability of P due to a lack of phytase secretion by pigs (Kies, 2005). During the last 2 decades, inclusion of microbial phytase in swine diets has improved P digestibility (Cromwell et al., 1995; Selle and Ravindran, 2008; Almeida and Stein, 2010; Kerr et al., 2010). However, it has been reported that a wide Ca:P ratio reduces the efficacy of microbial phytase (Liu et al., 2000). This reduction in efficacy of phytase may be due to formation of insoluble calcium-phytate complexes, or Ca-P complexes, which not only affect the efficacy of phytase, but also the digestibility of P (Lei et al., 1994; Lantzsch et al., 1995) and Ca (Wise, 1983). Therefore, the third objective of this thesis is to elucidate the interactions among Ca, P, phytase, and phytate in pigs.

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CHAPTER 2

DIGESTIBILITY AND METABOLISM OF CALCIUM IN PIGS: LITERATURE REVIEW

INTRODUCTION

Although minerals are required in small quantities in diets for pigs, inorganic elements are essential for normal growth and reproduction in pigs. The concentration of minerals in the animal body is 2 to 5% depending on the species, and the skeleton system contains the largest amounts of minerals (Gillespie, 1987). Calcium is the most abundant mineral in the body followed by P (Kellems and Church, 1998). The most abundant minerals in bone ash are Ca (36-39%) and P (17-19%; Crenshaw, 2001). Minerals are also present in soft tissue, blood, body fluids, and some secretions, and they are involved in many biochemical reactions in the body (Gillespie, 1987; Kellems and Church, 1998). Between 96 and 99% of the total Ca in the body and 60-80% of total P in the body are stored in bone tissue (Crenshaw, 2001). Calcium and P are important for the formation and maintenance of bones and teeth, but these minerals are also important for physiological functions such as muscle contraction, transmission of nerve impulses, enzyme activation, metabolic reactions, protein synthesis, maintenance of osmotic and acid-base balances, components in membranes, and other functions (Crenshaw, 2001; Ewing and Charlton, 2007).

Calcium and P are considered macrominerals because they are required at levels greater than 100 ppm in the diets (Ewing and Charlton, 2007). However, the Ca:P ratio must be

controlled by the inclusion of each mineral because an interaction exists between Ca and P, which may influence absorption of both minerals (Crenshaw, 2001; Ewing and Charlton, 2007). Excess or deficiency in one of these minerals causes problems in the utilization of the other. For grain-soybean meal diets, the total Ca:total P ratio should be between 1:1 and 1.25:1 (NRC, 2012). The Ca and P in cereal grains, oil seed meals, and many other plant ingredients have low bio-availability, but the bio-availability of Ca is relatively high in inorganic sources such as limestone, calcium carbonate, and calcium phosphates. Plant ingredients have low bio-availability of P because some of the P in these ingredients is bound to phytate. Phytase is the enzyme that is needed to release the P bound to phytate; however, this enzyme is not secreted by pigs. Phytate-bound P is, therefore, not absorbed by pigs, but is excreted in their feces, which may contribute to pollution and environmental problems (Knowlton et al., 2004). Inclusion of microbial phytase in swine diets results in release of some of the P that is bound to phytate and improves P digestibility and reduces the excretion of P from the pigs (Cromwell et al., 1995; Almeida and Stein, 2010; Kerr et al., 2010). However, high levels of Ca in swine diets form complexes between Ca and phytate in the intestinal tract. These insoluble calcium-phytate complexes reduce the effect of phytase and affect digestibility of P (Lei et al., 1994; Lantzsch et al., 1995) and Ca (Wise, 1983). There is, however, limited information about the mechanisms that regulate Ca digestibility and the role of phytate in the digestibility of Ca is not well understood.

SOURCES OF DIETARY CALCIUM

Most of the Ca in swine diets originates from inorganic sources because the concentration of Ca in most cereal grains is relatively low. However, there is limited information about the

digestibility of Ca in both organic and inorganic sources of Ca when fed to pigs. In a typical corn-soybean meal diet for a 40 kg pig, the Ca contribution from corn and soybean meal is around 1 g per kilogram of diet; whereas, approximately 5 g Ca per kilogram diet is supplied by limestone and calcium phosphates (NRC, 1998).

Inorganic sources of Ca contain between 17.0 and 38.5% Ca (Table 2.1) and plant based feed ingredients contain between 0 and 1% Ca (Table 2.2). Feed ingredients of animal origin contain more Ca than plant-based feed ingredients, but usually less than in the inorganic sources of Ca (Table 2.3). Ground limestone, calcium carbonate, and calcium phosphates are the most common Ca supplements in swine diets. If calcium carbonate is used as the standard, the relative bioavailability of Ca in limestone, aragonite, gypsum, marble dust, and oyster shell is between 93 and 102%, and in dolomitic limestone, the relative bioavailability is between 51 and 78% (Ross et al., 1984). The low relative bioavailability of Ca in dolomitic limestone may be due to the high concentration of Mg, which may reduce the absorption of Ca because Ca and Mg use a common transport mechanism (Ross et al., 1984). Particle size did not influence the relative bioavailability of Ca in these ingredients (Ross et al., 1984).

Relative bioavailability is a comparative measure and does not always reflect the real percentage of Ca that is absorbed by the animal. Absorption of Ca may also vary with age of the animal (Hansard et al., 1961), but that is not always the case (Baker, 2011). Therefore, nutrient digestibility may be measured to more accurately estimate the availability of nutrients fed to pigs (Stein et al., 2007; 2011). The advantage of determining nutrient digestibility rather than the relative bio-availability is that the proportion of the nutrient that is absorbed by the animal is determined, and the quantity of the nutrient that is excreted from the animal may also be determined.

DIGESTIBILITY OF CALCIUM

Digestibility of nutrients is an estimate of availability if all the nutrients that disappear from the intestinal tract are absorbed (Stein et al., 2007) and if all the absorbed nutrients are available for the animal (Ammerman, 1995). However, digestibility of nutrients can be defined as apparent, standardized, or true digestibility (Stein et al., 2007). To calculate standardized or true digestibility, it is necessary to measure endogenous losses, but that is not needed when values for apparent digestibility are calculated. A portion of the endogenous loss is excreted in response to the presence of DM in the intestinal tract and is independent of the diet. This portion is known as the basal endogenous loss. However, another portion of the endogenous loss is excreted in response to characteristics in the diet or feed ingredient and is known as the specific endogenous loss. Both basal and specific endogenous losses contribute to the total endogenous losses and are used to calculate the true digestibility. However, basal endogenous losses are sometimes easier to measure than total endogenous losses, and basal endogenous losses are used to calculate standardized digestibility (Stein et al., 2007; Almeida and Stein, 2010). One of the disadvantages of using values for apparent digestibility is that these values are not always additive in mixed diets, but for standardized digestibility, values are additive and can be used in formulation of mixed diets (Stein et al., 2005).

Digestibility can be expressed as duodenal, ileal or total tract digestibility. The digestibility of AA is expressed as ileal digestibility (Stein et al., 2007) and the digestibility of P may be expressed as ileal digestibility or as total tract digestibility because there is no net absorption or secretion of P in the lower gut (Bohlke et al., 2005). However, for practical reasons, total tract digestibility of P is usually determined, and values for the apparent total tract

digestibility (**ATTD**) and standardized total tract digestibility (**STTD**) of P have been determined in several ingredients (Almeida and Stein, 2010 and 2012).

Endogenous losses of minerals originate from saliva (Tryon and Bibby, 1966), cells, and pancreatic and bile secretions secreted into the digestive tract (Vitti and Da Silva Filho, 2010). Several methods have been used to measure endogenous losses of P (Fernández, 1995; Fan et al., 2001; Petersen and Stein, 2006; Bünzen et al., 2008). However, basal endogenous ileal or total tract losses of Ca have not been reported for pigs although it has been reported that there are endogenous losses of Ca in cattle (Visek et al., 1953; Hansard et al., 1957; Martz et al., 1999).

Basal endogenous losses of P have been measured using a P-free diet and an average value of approximately 0.2 g/kg of DMI has been reported in growing pigs (Petersen and Stein, 2006; Stein et al., 2006; Widmer et al., 2007; Almeida and Stein, 2010). A specific nutrient-free diet may be used to measure basal endogenous losses of P or AA (Petersen and Stein, 2006; Stein et al., 2007), but this approach has not been used to estimate the basal endogenous losses of Ca in pigs. If there is a linear relationship between graded levels of a nutrient in the diet and the nutrient output in feces, total endogenous losses of the nutrient can be calculated as the y-intercept of the linear regression after extrapolation back to zero input of the nutrient, and true digestibility of the nutrient is represented by the slope of the regression line (Fan et al., 2001; Kil et al., 2010).

The STTD of P has been reported for some feed ingredients (Petersen and Stein, 2006; Stein et al., 2006; Widmer et al., 2007; Almeida and Stein, 2010; 2012), but for Ca, no estimates of STTD are available. The ATTD of Ca in corn, soybean meal, and calcium carbonate (Bohlke et al., 2005; Stein et al., 2011) and in some diets (Kemme et al., 1997; Stein et al., 2006; 2008; Malde et al., 2010) has been reported. To be able to convert these values to STTD or true total

tract digestibility (TTTD) of Ca, there is a need for determining basal or total endogenous losses of Ca.

The Calcium-Phytate Complex

The majority of the P in plant ingredients is bound to phytate, and 1/3 of dietary Ca may also be bound to this molecule, which results in a limited availability of both minerals (Selle et al., 2009). The presence of phytate in typical swine diets is approximately 10 g per kg of diet (Selle et al., 2009); therefore, phytate significantly affects the digestibility of Ca and P. Inclusion of phytase to swine diets increases the digestibility of Ca and P (Brady et al., 2002; Liao et al., 2006; Poulsen et al., 2010), but increased levels of Ca in the diets may reduce the efficacy of phytase (Lei et al., 1994; Lantzsch et al., 1995; Brady et al., 2002; Selle et al., 2009). There are 3 mechanisms that may explain the negative effect of extra Ca in the diet on the efficacy of phytase (Liu et al., 1998). One possible mechanism is the formation of insoluble Ca-phytate complexes in the small intestine (Wise, 1983; Fisher, 1992; Selle et al., 2009). Another possible mechanism is that high concentration of Ca increases pH in the intestinal content, which reduces the efficacy of phytase (Sandberg et al., 1993; Selle et al., 2009). The third possible mechanism is that extra Ca in the diet may compete for the active site of phytase and thereby reduce the efficacy of phytase in hydrolyzing phytate (Wise, 1983; Pointillart et al., 1985; Qian et al., 1996). More research is needed to elucidate the interactions among Ca, phytate, and phytase and to determine the amount of Ca that is needed in the diets.

ABSORPTION OF CALCIUM

Most dietary Ca is absorbed in the small intestine (Partridge, 1978; Liu et al., 2000), but results of some studies have indicated that Ca may also be absorbed in the colon (Liu et al.,

2000). The specific region in the small intestine where Ca is absorbed is not well defined because although the absorption of Ca is greater in the proximal fourth of the small intestine than in the remaining part (Moore and Tyler, 1955a; b), the type of diet that is fed may affect the location in the intestine where Ca is absorbed (Partridge, 1978).

Before Ca is absorbed, Ca bound to other dietary compounds needs to be released in a soluble or ionic form. Gastric acids and pH-dependent enzymes assist in releasing this Ca. The presence of Ca-binding proteins in the enterocytes and the pH of the duodenum and jejunum explain the efficacy of Ca absorption in the proximal small intestine (Allen, 1982).

Calcium is absorbed in the intestine by both nonsaturable paracellular (diffusion) and saturable transcellular (active transport) mechanisms (Bronner, 1987). The saturable mechanism occurs mainly in the proximal small intestine (duodenum and upper jejunum; Bronner, 1987). This mechanism is influenced by the amount of Ca in the diet and is most active when Ca intake is low relative to the requirement for Ca (Bronner, 1987). Paracellular absorption occurs along the small intestine between the mucosal cells, and although the nonsaturable mechanism is independent of the diet, the amount of Ca moved by this mechanism will be greater if Ca intake is relatively high compared with the requirement (Bronner, 1987).

The saturable transcellular mechanism in the small intestine absorbs Ca following 3 steps (Bronner, 1998; R & D Systems Inc., 2007). First, Ca in the lumen enters the enterocyte by channel proteins that are located in the membrane. These channels are open if the luminal Ca concentration is low and the number of channels increase in response to vitamin D. Calcium is then transported through the enterocyte by Ca-binding proteins such as calbindins, which are regulated by vitamin D. Finally, the Ca transported by the calbindins is delivered to a Ca-Na exchanger, which exchanges 3 ions of Na for 1 ion of Ca, or to a Ca-ATPase, which pumps Ca

out of the cell. Both the Ca-Na exchanger and the Ca-ATPase are located on the basolateral membrane of the enterocyte. Vitamin D plays an important role in the expression of genes for the channel proteins and also in the expression of the Ca-ATPase (Bronner, 1998; R & D Systems Inc., 2007).

Increasing the Ca:P ratio (Liu et al., 1998) and inclusion of phytase in swine diets (Lantzsch et al., 1995; Almeida and Stein, 2010; Poulsen et al., 2010) increase apparent Ca absorption, but increasing Ca levels relative to the requirement does not affect the ATTD of Ca (Stein et al., 2011). Absorption of Ca may be reduced by oxalates that may bind Ca (Weaver et al., 1987). Sulfates may also reduce Ca absorption, which may be important to consider if ingredients such as corn distillers dried grains with solubles, that are high in S, are included in the diet (Pineda et al., 2008). Magnesium may directly affect Ca absorption because Ca and Mg use a common transport mechanism, which explains the low bio-availability of Ca in dolomitic limestone (Ross et al., 1984). Also, high levels of Sr in the diets may reduce the absorption of Ca in broiler chicks (Corradino et al., 1971). Calcium absorption may be increased if antibiotics are supplied in diets fed to chickens (Migicovsky et al., 1951) and to rats (Heggeness, 1959). Likewise, some fibers (Weaver et al., 2010), some AA such as Lys and Arg (Wasserman et al., 1956), and prebiotics increase absorption of Ca (Scholz-Ahrens et al., 2007).

METABOLISM OF CALCIUM

Between 30 and 70% of the Ca in the diet is usually absorbed, and 65 to 95% of this Ca is retained by the pig (Kornegay, 1985; Stein et al., 2011). Retained Ca is mainly used for development and maintenance of the skeleton. However, small amounts of Ca are also used for blood clotting, enzyme activation, muscle contraction, and other functions (Soares, 1995; Taylor

and Bushinsky, 2009). After Ca is absorbed, it circulates in body fluids. Approximately 50% of the Ca in serum is free as ionized calcium, 45% is bound to albumin or globulins, and the remaining 5% is present in other forms (Bringhurst and Leder, 2006). At normal plasma levels of Ca, 50% of Ca that circulates through the bones is deposited, which replaces Ca from degraded bone tissue (Bronner and Stein, 1992). Of the Ca that circulates in kidney, approximately 50% is filtered in the renal tubules, and approximately 70% of this Ca is recirculated; whereas, the rest is excreted in the urine (Vitti et al., 2010). However, the extend of the excretion of Ca depends on the level of Ca and P in the diet.

Bone

Bone is composed of organic and inorganic components. Approximately one third of the weight of bone consists of organic material such as collagen and glycosaminoglycans. This fraction is important for maintaining the structure of bone because these components provide resilience and toughness to the bones (Frandson et al., 2009). The other two thirds of bone weight is composed of inorganic material, such as Ca and P salts (Frandson et al., 2009). The Ca:P ratio in bones is approximately 2.2:1 (Crenshaw, 2001). Eighty percent of this inorganic fraction consists of calcium phosphate, which is mainly present as hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$], and the other 20% is mainly calcium carbonate and magnesium phosphate (Frandson et al., 2009). The inorganic fraction provides hardness and rigidity to the bones (Frandson et al., 2009). Deposition of Ca and P in bones is interdependent, which means that both minerals have to be present for deposition to occur (Crenshaw, 2001).

Bone tissue is continuously degraded and synthesized and osteoclasts, osteoblasts, and osteocytes are involved in this process. Osteoblasts are bone cells responsible for synthesis of the bones, osteoclasts are bone cells responsible for resorption of the bones, and osteocytes are

mature cells that are derived from the osteoblasts (Frandson et al., 2009; Veum, 2010). Osteocytes are involved in the deposition and resorption of bone tissue, and are also responsible for the transduction of signals involved in these processes (Burger et al., 1995). The activity of these cells is regulated by parathyroid hormone (**PTH**) and calcitonin, which are secreted by the parathyroid gland and by the thyroid gland, respectively, depending on the concentration of Ca in plasma (Frandson et al., 2009). When concentrations of Ca in plasma are lower than normal, PTH is secreted, which increases the resorption of Ca from bones by increasing the activity of osteoclasts and inhibiting the activity of osteoblasts. Parathyroid hormone also acts in the kidneys by stimulating Ca recycling and inhibiting phosphate recycling. The enzyme 1α-hydroxylase, which activates vitamin D to 1,25-dihydroxycholecalciferol, is also stimulated, which results in increased Ca absorption from the intestines (Costanzo, 2006), because 1,25-dihydroxycholecalciferol may enhance the synthesis of calcium binding proteins in the enterocyte (Kumar, 1995).

When concentrations of Ca in plasma are greater than normal, calcitonin is secreted, which inhibits osteoclastic activity and reduces Ca release from bone (Costanzo, 2006). Excretion of Ca via the kidneys is also increased, and the uptake of Ca by cells is increased (Crenshaw, 2001).

Renal Ca Metabolism

The kidneys play an important role in the regulation of Ca by filtering, reabsorbing, and excreting the Ca in blood to maintain stable Ca levels in plasma (Taylor and Bushinsky, 2009). Calcium is absorbed regardless of the dietary Ca and P concentration, but if Ca is absorbed in excess of the requirement, excesses are excreted in the urine (Stein et al., 2006; 2011). The Ca:P ratio plays an important role in the urine excretion of these minerals. If the Ca:P ratio is greater

than that needed for bone tissue synthesis, Ca excretion in the urine is increased due to the low availability of P (Helander et al., 1996; Stein et al., 2006). However, if diets with a low Ca:P ratio is fed, most of the P is excreted in the urine due to a lack of Ca for bone tissue synthesis (Stein et al., 2011). Although the body can regulate the levels of Ca in plasma, prolonged consumption of diets with excess Ca or deficiency of Ca can cause serious problems such as kidney stones or bone diseases (Taylor and Bushinsky, 2009).

Not all the Ca in the blood is recycled by the kidneys because Ca that is bound to albumin is not filtered by the glomerulus (R & D Systems Inc., 2007). When Ca is filtered by the glomerulus, approximately 60% of the Ca is resorbed in the proximal tubule, 10 to 15% in the ascending loop of Henle, and the remaining 15 to 25% in the distal convoluted tubule and collecting duct (Kumar, 1995).

CONCLUSION

Calcium is needed for bone and teeth formation and for many other physiological functions in the body. Plasma Ca levels are mainly regulated by PTH and calcitonin that have major effects on Ca metabolism in intestines, bones, and kidneys. For the retention of Ca and P in the body, both minerals need to be available in bone cells, because a low concentration of one mineral will cause the excretion of the other in the urine. Most of the Ca needs of pigs fed commercial diets is usually supplemented because of the low concentration of Ca in most feed ingredients of plant origin. The supplemented Ca can be from an organic or an inorganic source, and to formulate diets, it is important to consider the digestibility of Ca in these sources. More information about Ca digestibility in ingredients included in diets fed to pigs is needed to improve the utilization of both Ca and P by the animals. Also, because most ingredients from

plant origin contain phytic acid, the effect of phytic acid on Ca digestibility needs to be clarified. However, to determine if apparent, standardized, or true digestibility values are most accurate, endogenous losses of Ca from the gastrointestinal tract of the pig need to be determined, and additivity of digestibility values from individual ingredients used in mixed diets need to be confirmed. To determine if ileal or total tract digestibility values most accurately describe the absorption of Ca, the site of absorption also needs to be described.

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Tables

Table 2.1. Concentration of Ca and P in inorganic sources of Ca

Source	Ca, %	P, %
Calcium carbonate[1]	38.5	0.02
Calcium chloride[2]	36.0[4], 27.0[5]	-
Calcium sulphate[2]	29.0[4], 23.0[5]	-
Dolomite limestone[2]	22.0	-
Ground limestone[1]	35.8	0.01
Monocalcium phosphate[1]	16.9	21.5
Dicalcium phosphate[1]	24.8	18.8
Tricalcium phosphate[1]	34.2	17.7

[1]NRC, 2012.

[2]Sauvant et al., 2004.

[4]Anhydrous form.

[5]Dihydrate form.

Table 2.2. Calcium, P, and phytate concentration in common feed ingredients of plant origin

Source	Ca, %	Total P, %	Phytate,%	Phytate P, %	Non-phytate P[8], %
Canola meal	0.56[5] - 0.69[4]	0.81[5] - 1.08[4]	-	0.54[5] - 0.65[4]	0.27[5] – 0.43[4]
Corn	0.01[2] - 0.02[4]	0.25[2,5] - 0.26[4]	0.64[2]	0.18[2, 7] – 0.21[4]	0.05[4] - 0.07[2]
Corn germ	0.02[2] - 0.04[5]	0.51[5] – 1.41[2]	3.80[2]	1.07[2, 7]	0.33[2]
Corn germ meal	0.03[4]	0.9[4]	-	-	-
Corn gluten feed	0.09[4]	0.78[4]	-	0.62[4]	0.16[4]
Corn gluten meal	0.03[4, 5]	0.47[5] - 0.49[4]	-	0.41[5]	0.06[5]
DDGS[1]	0.02[2] - 0.12[4]	0.73[4] - 0.85[2]	0.91[2]	0.26[2, 4, 7]	0.47[4] - 0.59[2]
Cottonseed meal	0.25[4] - 0.43[5]	0.98[4] - 1.03[5]	-	0.59[5]	0.44[5]
Field peas	0.09[4] - 1.10[6]	0.40[3, 6] – 0.42[4]	0.73[3]	0.17[4]	0.25[4]
HP-DDG[1]	0.01[2] – 0.02[4]	0.36[4] - 0.39[2]	0.41[2]	0.11[2, 4, 7]	0.25[4] - 0.28[2]
Soybean meal	0.31[5] - 0.33[4]	0.63[5] - 0.71[4]	-	0.38[4] - 0.39[5]	0.24[5] – 0.33[4]
Sunflower meal	0.35[5] - 0.39[4]	1.03[5] -1.16[4]	-	0.69[5] – 0.89[4]	0.27[4] - 0.34[5]
Wheat	0.05[5] – 0.06[4]	0.32[5] – 0.39[4]	-	0.21[5] – 0.22[4]	0.11[5] – 0.17[4]
Wheat germ	0.09[5]	0.88[5]	-	0.58[5]	0.30[5]

[1]DDGS = distillers dried grains with solubles; HP-DDG = high protein distillers dried grain.
[2]Almeida and Stein, 2012.

[3]Helander et al., 1996.

[4]NRC, 2012.

[5]Rostagno et al., 2011.

[6]Sauvant et al., 2004.

[7]Calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

Table 2.3. Calcium and P concentration in animal feed ingredients

Source	Ca, %	P, %
Blood meal	0.05[1] - 0.23[2]	0.21[1] - 0.22[2]
Blood plasma	0.13[1] - 0.19[2]	0.45[2] – 1.28[1]
Fish meal	4.28[1], 4.70[2] – 5.88[2]	2.41[2] - 2.89[2], 2.93[1]
Meat meal	6.37[1]	3.16[1]
Meat and bone meal	7.40[2] – 14.21[2], 10.94[1]	3.70[2] – 7.11[2], 5.26[1]
Milk skimmed dried	1.21[2] – 1.27[1]	0.75[2] – 1.06[1]
Milk whey dried	0.62[1] - 0.75[2]	0.68[2] – 0.69[1]
Milk whey permeate dried	0.86[2]	0.66[2]
Poultry by-product meal	4.34[2] - 4.54[1]	2.51[1] – 2.54[2]

[1]NRC, 2012.

[2]Rostagno et al., 2011.

Table 2.4. Availability and digestibility of Ca

Sources	Relative bioavailability, %	ATTD, %
Aragonite	93.0[1] -102.0[1]	-
Gypsum	99.0[1]	-
Limestone	99.0[1]	-
Dolomitic limestone	78.0[1]	-
Marble dust	98.0[1]	-
Oyster shells, ground	98.0[1]	-
Ground corn	-	49.6[2]
Soybean meal	-	46.7[2]
Calcium carbonate	-	60.9 - 70.9[3]

[1]Ross et al., 1984.

[2]Bohlke et al., 2005.

[3]Stein et al., 2011.

CHAPTER 3

DETERMINATION OF ENDOGENOUS INTESTINAL LOSSES OF CALCIUM AND APPARENT AND TRUE TOTAL TRACT DIGESTIBILITY OF CALCIUM IN CANOLA MEAL FED TO GROWING PIGS

ABSTRACT

An experiment was conducted to test the hypothesis that endogenous Ca is lost from the gastrointestinal tract of growing pigs. The objective was to determine endogenous losses of Ca, the apparent total tract digestibility of Ca (ATTD), and the true total tract digestibility (TTTD) of Ca in canola meal without and with added microbial phytase. The second objective was to determine the balance of Ca in pigs fed diets based on canola meal without or with microbial phytase. Forty eight growing barrows (average initial BW: 16.7 ± 2.5 kg) were allotted to a randomized complete block design with 8 dietary treatments and 6 pigs per treatment. Diets were based on sucrose, cornstarch, potato protein isolate, corn gluten meal, and canola meal. Diets were formulated to contain 0.08, 0.16, 0.24, or 0.32% Ca from canola meal. All diets were formulated with 0 or 1,500 units per kg of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK) and contained 0.32% standardized total tract digestible P. Feces and urine samples were collected from d 6 to d 11. Total endogenous losses of Ca were determined using the regression procedure. Results indicated that ATTD of Ca and Ca retention increased ($P < 0.05$) if dietary Ca increased and also increased ($P < 0.01$) if phytase was added to the diets. The estimated total endogenous loss of Ca was 0.160 and 0.189 g/kg DMI for canola

meal without and with microbial phytase, respectively, and these values were not different. The TTTD of Ca increased ($P < 0.01$) if phytase was used, but was not affected by the level of dietary Ca. As dietary Ca increased, the amount of Ca absorbed and retained increased ($P < 0.01$) to a greater extent if phytase was used than if no phytase was included in the diet (interaction, $P < 0.05$). Fecal P excretion increased ($P < 0.01$) as dietary Ca increased, but was reduced ($P < 0.01$) by the use of phytase. The ATTD of P decreased ($P < 0.01$) with increasing dietary Ca to a lesser extent if phytase was used than if no phytase was used (interaction, $P < 0.01$). In conclusion, endogenous Ca was lost from the gastrointestinal tract of growing pigs. Therefore, values for TTTD of Ca differed from values for ATTD of Ca. Values for ATTD of Ca were influenced by level of dietary Ca, but that was not the case for values for TTTD of Ca. The ATTD of P decreased as dietary Ca increased. Microbial phytase increased Ca and P digestibility and Ca retention in pigs fed diets based on canola meal, but did not influence the endogenous losses of Ca.

Key words: apparent digestibility, calcium, endogenous losses, phytase, pigs, true digestibility

INTRODUCTION

When diets are formulated for swine, it is more accurate to use values for standardized nutrient digestibility than values for apparent nutrient digestibility because values for standardized digestibility are additive in mixed diets (Stein et al., 2005). Values for the standardized total tract digestibility (**STTD**) of P in pigs have been reported (Petersen and Stein, 2006; Almeida and Stein, 2010), but only apparent total tract digestibility (**ATTD**) values have been reported for Ca (Bohlke et al., 2005; Stein et al., 2006, 2008, and 2011). Standardized total tract digestibility of a nutrient is calculated by correcting the ATTD for basal endogenous losses; whereas, true total tract digestibility (**TTTD**) of a nutrient is calculated by correcting ATTD by

total endogenous losses (Stein et al., 2007). Basal endogenous losses may be estimated by using a nutrient free-diet (Petersen and Stein, 2006; Stein et al., 2007); whereas, total endogenous losses may be estimated using a regression procedure (Fan et al., 2001) or by using radioactively labelled isotopes (Visek et al., 1953). There are endogenous losses of Ca in cattle (Visek et al., 1953; Hansard et al., 1957; Martz et al., 1999), and in chickens (Cowieson et al., 2004; Liu et al., 2012), but limited data has been published on endogenous losses of Ca in pigs (Besançon and Guéguen, 1969; Fernández; 1995). However, to our knowledge there are no data for the effects of the level of feed intake on endogenous losses of Ca and it is not known if microbial phytase impacts endogenous losses of Ca in pigs. The present experiment was conducted to test the hypothesis that endogenous Ca is lost from the gastrointestinal tract of growing pigs, and that values for TTTD of Ca, therefore, are different from values for ATTD of Ca. The objective was to determine the ATTD and TTTD of Ca in canola meal without and with added microbial phytase. A second objective was to determine the balance of Ca in pigs fed different levels of canola meal without or with microbial phytase.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment.

Animals and Housing

Forty eight growing pigs with an average initial BW of 16.7 ± 2.5 kg were used. Pigs were the offspring of G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN). Pigs were housed individually in metabolism cages that were equipped with a slatted floor, a feeder, and a nipple drinker. A screen floor for fecal collection and a tray for urine collection

were placed under each cage. Animals were allotted to a randomized complete block design with 8 dietary treatments and 6 pigs per treatment. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used for the allotment.

Diets and Feeding

Eight diets were formulated to contain 0.32% STTD of P. Monosodium phosphate was used as the source of inorganic P. Diets were based on sucrose, cornstarch, potato protein isolate, corn gluten meal, vitamins, minerals, and canola meal (Table 3.1). All minerals except Ca were included at recommended levels (NRC, 1998). Four diets containing 12.3, 24.7, 37.0, or 50.0% canola meal were formulated to contain 0.08, 0.16, 0.24, or 0.32% Ca, respectively (Tables 3.2 and 3.3). Canola meal was used in this experiment because it is one of the few ingredients that contain both phytate and appreciable amounts of Ca. Four additional diets that were similar to the previous 4 diets with the exception that they also contained 1,500 units per kilogram of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK) were also formulated. Canola meal and microbial phytase were included in these diets at the expense of cornstarch. Canola meal provided all Ca in the diets.

Experimental diets were fed for 12 d. Pigs were fed 3 times the daily maintenance energy requirement (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998), and the daily allotments were divided into 2 equal meals that were provided at 0700 and 1700 h. The initial 5 d was an adaptation period to the diets. On d 6, an indigestible marker (ferric oxide) was added to the morning meal to mark the beginning of fecal collection and on d 11, Indigo carmine was added to the morning meal to mark the conclusion of fecal collection. Feces were collected quantitatively using the marker-to-marker approach (Adeola, 2001). Urine collection started on d 6 and ceased on d 11. Fecal samples and 20% of the collected urine 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. Pigs had free access to water throughout the experiment.

Sample Analysis

Before analysis, fecal samples were dried in a forced-air oven at 65ºC and ground through a 2-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). Urine samples were thawed at room temperature and a subsample of 10 mL was collected. Potato protein isolate, corn gluten meal, canola meal, monosodium phosphate, feces, and urine samples were analyzed for Ca and P by inductively coupled plasma spectroscopy (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC Int., 2007), and diets were analyzed for Ca by an atomic absorption spectrophotometer procedure (Method 968.08; AOAC Int., 2007) after wet digestion sample preparation (Method 935.13; AOAC Int., 2007) and the concentration of P in the diets was analyzed using a colorimetric procedure (Method 931.01; AOAC Int., 2007). Potato protein isolate, corn gluten meal, canola meal, monosodium phosphate, diets, and fecal samples were also analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007), and ingredients and diets were analyzed for ash (Method 942.05; AOAC Int., 2007). Potato protein isolate, corn gluten meal, canola meal, and diets were analyzed for phytase activity (Engelen et al., 2001), and for phytic acid (Megazyme method; AB Vista Feed Ingredients, Ystrad Mynach, UK). These samples were also analyzed for GE using an adiabatic bomb calorimeter (Model 6300, Parr Instruments, Moline, IL) with benzoic acid as the standard for calibration and for CP using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25.

Potato protein isolate, corn gluten meal, canola meal, and diets were also analyzed for acid-hydrolyzed ether extract (**AEE**) using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Potato protein isolate, corn gluten meal, and canola meal were also analyzed for ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Five g of each diet and 15 mL of distilled water were mixed and filtered, and diet pH was measured in the solution with a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA).

Calculations and Statistical Analysis

The concentration of phytate-bound P in potato protein isolate, corn gluten meal, canola meal, and diets was calculated as 28.2% of phytate (Tran and Sauvant, 2004) and the concentration of non-phytate bound P was calculated as the difference between total P and phytate-bound P. Values for ATTD (%) of Ca were calculated according to the following equation (Petersen and Stein, 2006):

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

where ATTD is the apparent tract total digestibility (%) of Ca, Ca_{intake} is the total Ca-intake (g) and Ca_{fecal} is the total fecal Ca output (g). The ATTD of P was calculated using the same procedure.

Total endogenous losses of Ca were estimated using the regression procedure (Fan et al., 2001). The dependent variable, apparent total tract digested Ca (Ca_D) expressed as g/kg DMI, was regressed against the independent variable, dietary Ca intake (g/kg DM). Separate regressions were conducted using the 4 diets without microbial phytase and for the 4 diets with

microbial phytase. Because there was a linear relationship between the graded levels of Ca intake and the digested Ca, the following equation was used (Akinmusire and Adeola, 2009):

$$Ca_D = (TTTD \times Ca_{intake}) - ECaL \quad [2]$$

The slope of the regression line represents the estimate of TTTD of Ca, and ECaL is the negative Y-intercept and represents the estimate for total endogenous loss of Ca (g/kg DMI). The TTTD of Ca in each diet was also calculated by correcting the ATTD of Ca for total endogenous losses of Ca according to Eq. 3 (Stein et al., 2007).

$$TTTD\ (\%) = ATTD + [(ECaL_{total}/Ca_{diet}) \times 100], \quad [3]$$

where Ca_{diet} is the concentration of Ca in the diet (g/kg DM).

Retention of Ca was calculated using Eq. 4 (Petersen and Stein, 2006):

$$Ca_R = \{[Ca_{intake} - (Ca_{fecal} + Ca_{urine})]/Ca_{intake}\} \times 100, \quad [4]$$

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

Endogenous losses of Ca expressed in g per d were calculating by using the average of the 2 estimates of endogenous losses obtained from the linear regression and it was multiplied by DMI expressed in kg per d for each pig. For the partitioning of Ca output, dietary Ca in feces was calculated by subtracting endogenous losses of Ca from total fecal output of Ca. Dietary Ca absorbed was calculated by subtracting dietary Ca in feces from Ca intake, and dietary Ca retained was calculated by subtracting Ca in urine and endogenous losses of Ca from dietary Ca absorbed.

Data were analyzed as a 4 × 2 factorial using the Proc MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure was used to identify outliers and outliers were identified as values that deviated from the treatment mean by more than 3 times the

interquartile range (Devore and Peck, 1993). Four pigs were identified as outliers and removed from the data set (one pig was fed 0.08% Ca without phytase, another pig was fed 0.08% Ca with phytase, one pig was fed 0.16% Ca with phytase, and one pig was fed 0.24% Ca with phytase). In the model, Ca level, phytase, and the interaction between Ca level and phytase were the fixed effects; whereas, replicate was considered the random effect. The pig was the experimental unit for all analyses. The LSMEANS procedure was used to calculate the mean values for the treatments and an alpha level of 0.05 was used to assess differences among means.

Linear effects of Ca intake on apparent total tract digested Ca in canola meal diets without and with phytase were determined using orthogonal CONTRAST statements. The REG procedure was used to estimate the Y-intercept and the slope to determine endogenous losses of Ca and the TTTD of Ca, respectively. Intercepts and slopes obtained for the diets without microbial phytase were compared with values obtained for diets that contained microbial phytase using the 95% confidence interval derived from the SE of the respective regression coefficients (Dilger and Adeola, 2006). Linear and quadratic effects of the proportion of endogenous Ca in the fecal output of pigs fed canola meal either without or with microbial phytase at different levels of Ca intake were determined using orthogonal CONTRAST statements. Linear and quadratic analyses were analyzed using the PROC REG procedure of SAS when linear and quadratic effects were significant.

RESULTS

The analyzed concentrations of Ca in the diets were between 0.04 and 0.07% greater than expected (Table 3.4), but the expected differences among diets were obtained. This was true for the diets without microbial phytase and for the diets with phytase. The analyzed concentrations

of total P in the diets were up to 0.06% greater than expected and the values for non-phytate P in the diets were between 0.01% less and 0.08% greater than expected. These differences were assumed not to affect the results of the experiment.

All pigs consumed their diets and remained healthy during the experiment. Feed intake, Ca intake, urine Ca output, and endogenous Ca increased ($P < 0.01$) by increasing Ca level in the diets, and were not affected by inclusion of phytase in the diets (Table 3.5). The ATTD of Ca and the Ca retention expressed as percentage of Ca intake increased ($P < 0.05$) by increasing Ca level in the diets, and also were greater ($P < 0.01$) when phytase was added to the diets than if no phytase was used. Therefore, Ca excretion expressed as percentage of intake decreased ($P < 0.01$) by increasing dietary Ca level and also was less ($P < 0.01$) when phytase was added to the diets than if no phytase was used. The TTTD of Ca was greater ($P < 0.01$) for diets containing phytase than for diets with no phytase, but was not affected by dietary Ca level. Calcium output in feces and excretion of Ca in grams per d increased ($P < 0.01$) with dietary Ca to a greater extent if no phytase was added to the diet than if phytase was used (interaction, $P < 0.05$). In contrast, absorbed Ca and retention of Ca in grams per d increased ($P < 0.01$) with dietary Ca to a greater extent if phytase was added to the diet than if no phytase was used (interaction, $P < 0.05$).

The estimated endogenous losses of Ca for canola meal without phytase and canola meal with phytase were 0.160 and 0.189 g/kg DMI, respectively, and these values were not different (Table 3.6). The estimated average TTTD of Ca in canola meal without phytase (46.6%) was less ($P < 0.05$) than in canola meal with microbial phytase (70.3%).

The proportion of endogenous Ca in the fecal output of pigs fed canola meal without phytase and pigs fed canola meal with phytase decreased quadratically ($P < 0.01$) and linearly (P

< 0.01), respectively, as dietary Ca levels increased (Figure 3.1). Dietary Ca excreted in feces increased ($P < 0.01$) with dietary Ca intake to a greater extent if no phytase was added to the diets than if phytase was added (interaction, $P < 0.05$; Table 3.7). Therefore, dietary Ca absorbed and dietary Ca retained increased ($P < 0.01$) with dietary Ca intake to a greater extent if phytase was added to the diets than if phytase was not added (interaction, $P < 0.05$).

Phosphorus intake, P excretion in grams per d and as percentage of intake, and P retention in grams per d increased ($P < 0.01$) by increasing Ca level in the diets, but P retention as percentage of intake decreased ($P < 0.01$) by increasing Ca level in the diets. However, P intake, P excretion, and P retention either in grams per d or as percentage of intake were not affected by addition of microbial phytase to the diets (Table 3.8). Phosphorus output in feces increased ($P < 0.01$) with increasing levels of dietary Ca to a greater extent if no phytase was added to the diet than if phytase was used (interaction, $P < 0.01$). Phosphorus excreted in the urine was not affected by increasing dietary Ca, but P excreted in urine and absorbed P increased more with increasing dietary Ca intake when phytase was added to the diets than if no phytase was used (interaction, $P < 0.05$). The ATTD of P decreased ($P < 0.01$) as dietary Ca increased to a lesser extent if phytase was added to the diets than if phytase was not added (interaction, $P < 0.01$); therefore, microbial phytase increased ($P < 0.01$) the ATTD of P.

DISCUSSION

The concentration of Ca, total P, and phytate-bound P in canola meal used in this experiment is in agreement with the concentration reported by NRC (2012) and by Rodríguez et al. (2012), but less than the concentration reported by Maison and Stein (2012). The concentration of non-phytate P was kept constant in the diets by decreasing the amount of

monosodium phosphate as the inclusion of canola meal increased assuming the ATTD of P in monosodium phosphate is 92% (Petersen and Stein, 2006), and assuming the ATTD of P in canola meal is 32% (Sauvant et al., 2004). The low ATTD of P in canola meal is mainly due to the high concentration of phytate, which binds most of the P and reduces P digestibility (Rodríguez et al., 2012). Therefore, increasing the concentration of canola meal in the diet was expected to reduce the ATTD of P, which was also observed. It is possible that the ATTD of P was negatively affected as the level of Ca in the diets increased because Ca may form Ca-P complexes in the small intestine and thereby reduce P digestibility (Clark, 1969; Stein et al., 2011). There may also be an indirect effect of dietary Ca on P digestibility because vitamin D is activated at low Ca concentration, which may enhance both Ca and P absorption (Berner, 1997), and therefore, increase P digestibility at low Ca concentrations.

The values of 0.11 – 0.13 grams of endogenous losses of Ca per d that were obtained in the present experiment, are less than the value of 1.49 grams per d reported by Besançon and Guéguen (1969) and the value of 0.55 grams per d reported by Fernández (1995). However, the latter values were obtained using isotope dilution; whereas, our values were calculated using the regression procedure. Endogenous losses of P may be different if different methods are used to estimate the losses (Dilger and Adeola, 2006; Almeida and Stein, 2010) and it is possible, this is also the case for Ca. Nevertheless, results indicate that there is a measurable endogenous loss of Ca from pigs. Possible sources of Ca of endogenous origin may be saliva (Tryon and Bibby, 1966), epithelial intestinal cells (Bronner, 1997; Frandson et al., 2009), gastric juice (Trautmann and Kirchhof, 1937; Moore and Tyler, 1955), pancreatic juice (Gamble and McIver, 1928; Partridge et al., 1982; Bronner, 1997), bile (Sullivan et al., 1981; Bronner, 1997), and intestinal secretions (Moore and Tyler, 1955; Bronner, 1997). The observation that the proportion of

endogenous losses of Ca in feces decreased as Ca intake increased demonstrates that principles for endogenous Ca excretion are similar to principles for excretion of endogenous P, which also decreases as P intake increases (Fan et al., 2001). The increased endogenous loss of Ca associated with increased dietary Ca has also been observed in ruminants (Vitti et al., 2010). It is, however, possible that this increase may be due to an increased intake of anti-nutritional factors and fiber because Ca was increased by increasing the concentration of canola meal in the diet, and both anti-nutritional factors and fiber may influence endogenous losses of Ca (Cowieson et al., 2004) and P (Fang et al., 2007). It is, therefore, not possible to determine if the increase in endogenous loss of Ca, that was observed as dietary Ca increased, is a direct effect of increased Ca in the diet or a result of the increased concentration of canola meal. To our knowledge, the effect of phytase on the endogenous loss of Ca in pigs has not been reported, but the observation that the addition of phytase to the diets did not influence the endogenous loss of Ca is in agreement with observations in chickens (Cowieson et al., 2004; Pirgozliev et al., 2009).

To our knowledge, TTTD values for Ca in canola meal have not been reported, but it was demonstrated in the present experiment that TTTD values for Ca are different from ATTD values because of the loss of endogenous Ca from the gastrointestinal tract. The increase in the ATTD of Ca that was observed as dietary Ca increased, is a result of the reduced contribution of endogenous Ca to total Ca output in the feces as Ca intake increased. However, because endogenous Ca is subtracted from Ca output as values for TTTD of Ca are calculated, TTTD values are unaffected by the level of dietary Ca, which indicates that the only reason for the increase in ATTD of Ca, that was observed as Ca intake increased, is the reduced contribution of endogenous Ca to total Ca output. This observation indicates that digestibility of Ca in pigs and excretion of endogenous Ca follow the principles observed for AA (Mosenthin et al., 2000) and

P (Fan et al., 2001). We are not aware of any other data illustrating this principle for Ca in pigs. Because TTTD values for Ca were not influenced by the level of dietary Ca, these values are expected to be additive in mixed diets.

The observation that phytase supplementation increases ATTD of Ca is in agreement with previous data (Guggenbuhl et al., 2007; Almeida and Stein, 2010; Poulsen et al., 2010), but to our knowledge, the effect of phytase on TTTD of Ca has not been reported. The positive effect of phytase on the ATTD and TTTD of Ca in canola meal is believed to be a result of the hydrolysis of phytate esters, which reduces the ability of phytate to chelate Ca (Selle et al., 2009).

Calcium may be absorbed from the intestinal tract by diffusion and by active transport (Bronner, 1987; Veum, 2010) and, although active transport is down-regulated as dietary Ca intake increases, Ca absorption may not be affected because more Ca is absorbed by diffusion if the concentration of Ca in the diet is increased (Bronner, 1987; Stein et al., 2011). This is likely the reason, TTTD of Ca did not change as Ca intake increased. This observation indicates that intestinal regulation of Ca retention plays a minor role in Ca homeostasis. However, for Ca to be retained, both Ca and P have to be available (Crenshaw, 2001), but the fact that Ca retention increased as dietary Ca increased indicates that sufficient P was available to increase bone synthesis as dietary Ca increased. Likewise, more Ca was retained when phytase was used because phytase increased the amount of both Ca and P absorbed. This observation is also in agreement with previous data (Poulsen et al., 2010).

The negative effect of dietary Ca on the ATTD of P is likely a result of formation of Ca-P complexes in the intestinal tract (Selle et al., 2009; Stein et al., 2011). The positive effect of phytase on P digestibility is in agreement with results from experiments in which pigs were fed

diets containing canola meal (Akinmusire and Adeola, 2009) or diets based on barley and canola meal (Sauer et al., 2003).The increase in P digestibility is due to the release of P from phytate. Microbial phytase may also increase P retention (Sauer et al., 2003), but that was not observed in this experiment, which is likely a result of a lack of Ca for bone synthesis. For P to be retained in bone tissue, both Ca and P need to be available (Crenshaw, 2001; Stein et al., 2006) and because of the lack of Ca, the increased P absorption associated with use of phytase, resulted in increased P excretion in the urine. Thus, as is the case for Ca, the major regulation of P homeostasis appears to be at the renal level; whereas, regulation at the intestinal level seems to be of minor importance.

In conclusion, endogenous Ca was lost from the gastrointestinal tract of pigs, but inclusion of phytase to the diets did not influence the endogenous loss of Ca. Because of the endogenous loss of Ca, values for ATTD were influenced by dietary Ca level, but that was not the case for values for TTTD. As a consequence, TTTD values for Ca were expected to be additive in mixed diets. Phosphorus digestibility was negatively affected by the levels of dietary Ca. The ATTD of both Ca and P and Ca retention was increased if microbial phytase was added to diets that were deficient in Ca. The present results indicated that both Ca and P homeostasis in the ranges studied were mainly regulated at the renal level and that regulation at the intestinal level played only a minor role.

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Tables

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

	Ingredient			
Item	Potato protein isolate	Corn gluten meal	Canola meal	Monosodium phosphate
GE, kcal/kg	5,268	5,018	4,258	-
DM, %	91.19	90.86	89.65	98.99
CP, %	80.75	59.15	37.69	-
Ash, %	0.48	4.62	7.57	90.61
AEE,[1] %	0.50	4.58	3.27	-
ADF, %	3.60	6.44	19.21	-
NDF, %	1.12	9.25	33.47	-
Ca, %	0.03	0.02	0.66	0.08
P, %	0.12	0.54	1.00	29.69
Phytase,[2] FTU/kg	52	<50	<50	-
Phytic acid, %	0.33	1.64	2.58	-
Phytate-bound P,[3] %	0.09	0.46	0.73	-
Non-phytate P,[4] %	0.03	0.08	0.27	-

[1]AEE = acid-hydrolyzed ether extract.

[2]FTU = phytase units.

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

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

Table 3.2. Ingredient composition of experimental diets, as-fed basis[1]

	Ca from canola meal, %			
Ingredient, %	0.08	0.16	0.24	0.32
Canola meal	12.33	24.66	37.00	50.00
Corn gluten meal	7.50	5.00	2.50	-
Cornstarch	42.48	35.71	28.90	21.42
Sucrose	20.00	20.00	20.00	20.00
Potato protein isolate	10.00	8.00	6.00	4.00
Soybean oil	3.00	3.00	3.00	3.00
Monosodium phosphate	1.08	1.00	0.92	0.84
L-Lys HCL	0.34	0.24	0.13	0.03
DL-Met	0.02	-	-	-
L-Trp	0.03	0.01	-	-
Potassium carbonate	0.30	0.20	0.10	-
Magnesium oxide	0.08	0.05	0.03	-
Sodium chloride	0.29	0.33	0.37	0.41
Solka floc[2]	2.25	1.50	0.75	-
Vitamin mineral premix[3]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00

[1]Four additional diets that were similar to these diets with the exception that 1,500 units per kilogram of microbial phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK) was included in the diets at the expense of cornstarch were also formulated.

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

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 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 as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.3. Analyzed composition of experimental diets, as-fed basis

	Diet							
	Ca from canola meal, %				Ca from canola meal + phytase,[2] %			
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32
GE, kcal/kg	4,156	4,167	4,176	4,198	4,187	4,156	4,183	4,212
DM, %	92.82	91.68	91.82	91.06	92.01	92.07	91.96	91.45
CP, %	18.48	20.23	21.94	23.71	19.26	20.97	22.47	23.05
Ash, %	2.74	3.46	4.46	5.04	2.90	3.68	4.44	5.38
AEE,[1] %	2.91	4.36	4.64	4.97	4.10	4.11	4.56	5.11
Ca, %	0.15	0.21	0.29	0.36	0.15	0.21	0.29	0.38
P, %	0.51	0.60	0.63	0.75	0.50	0.57	0.63	0.76
Phytase,[3] FTU/kg	<50	<50	<50	<50	1,990	1,300	1,440	1,290
Phytic acid, %	0.65	0.88	1.14	1.40	0.53	0.85	0.93	1.29
Phytate-bound P,[4] %	0.18	0.25	0.32	0.40	0.15	0.24	0.26	0.36
Non-phytate P,[5] %	0.33	0.35	0.31	0.35	0.35	0.33	0.37	0.40
pH	5.30	5.10	5.05	5.04	5.21	5.21	5.09	5.09

[1]AEE = acid-hydrolyzed ether extract.

[2]These diets contained 1,500 units of phytase (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK).

[3]FTU = phytase units.

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

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

Table 3.4. Analyzed and calculated concentrations of Ca and P in experimental diets (as-fed basis)

	Diet							
	Ca from canola meal, %				Ca from canola meal + phytase,[1] %			
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32
Ca concentration, %								
Calculated value	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32
Analyzed value	0.15	0.21	0.29	0.36	0.15	0.21	0.29	0.38
Total P concentration, %								
Calculated value	0.45	0.54	0.63	0.73	0.45	0.54	0.63	0.73
Analyzed value	0.51	0.60	0.63	0.75	0.50	0.57	0.63	0.76
Non-phytate P concentration,[2] %								
Calculated value	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Analyzed value	0.33	0.35	0.31	0.35	0.35	0.33	0.37	0.40

[1]Each diet contained 1,500 units of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]Calculated by subtracting phytate P (i.e., 28.2% of phytate; Tran and Sauvant, 2004) from total P concentration.

Table 3.5. Calcium balance, apparent total tract digestibility (ATTD), and true total tract digestibility (TTTD) of Ca for pigs fed canola meal without or with microbial phytase at different levels of Ca

	Canola meal without phytase				Canola meal with phytase[1]					*P*-value		
Item Ca, %:	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32	SEM	Ca level	Phytase	Ca level × phytase
Feed intake, g/d	679	723	734	786	702	711	727	765	38	<0.01	0.716	0.571
Ca intake, g/d	0.52	1.16	1.76	2.52	0.57	1.14	1.76	2.45	0.09	<0.01	0.728	0.709
Fecal Ca output, g/d	0.35	0.76	1.07	1.45	0.30	0.46	0.60	0.89	0.09	<0.01	<0.01	0.012
Urine Ca output, mg/d	127	123	160	205	123	135	137	182	24	<0.01	0.521	0.817
Absorbed Ca, g/d	0.18	0.40	0.69	1.07	0.26	0.67	1.16	1.56	0.07	<0.01	<0.01	0.012
Ca retention, g/d	0.06	0.27	0.53	0.86	0.14	0.54	1.03	1.37	0.07	<0.01	<0.01	<0.01
Ca retention, %	9.75	24.15	30.52	34.77	24.89	45.56	58.35	56.90	5.47	<0.01	<0.01	0.733
Ca excretion, g/d	0.48	0.88	1.23	1.65	0.42	0.59	0.73	1.08	0.10	<0.01	<0.01	0.017
Ca excretion, %	90.25	75.85	69.48	65.23	75.11	54.44	41.65	43.10	5.47	<0.01	<0.01	0.733
ATTD of Ca, %	33.71	34.65	39.60	42.96	45.89	57.30	65.91	64.19	4.94	0.030	<0.01	0.560
Endogenous Ca,[2] g/d	0.11	0.12	0.12	0.13	0.11	0.11	0.12	0.12	0.006	<0.01	0.758	0.704

Table 3.5. (Cont.)

TTTD of Ca,[3] %	53.95	44.65	46.28	47.93	65.96	67.34	72.59	69.18	4.94	0.862	<0.01	0.548

[1]Each diet contained 1,500 units of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]Endogenous Ca was calculated by multiplying 0.160 and 0.189 g by daily DMI (kg) for pigs fed canola meal without phytase and canola meal with phytase, respectively.

[3]TTTD of Ca was calculated by correcting the ATTD values for the average (0.175 g/kg DMI) of the endogenous losses of Ca estimated in the linear regression.

Table 3.6. Regression of apparent total tract digested Ca (g/kg DMI) on dietary Ca intake (g/kg DM)[1]

Item	Regression equation	SE of the slope	SE of the intercept	R^2	Estimated TTTD[2] of Ca, %	Estimated ECaL,[2] g/kg DMI
Canola meal	y = 0.4661x - 0.1598	0.0428	0.1045	0.85	46.6[b]	0.160
Canola meal + phytase	y = 0.7026x - 0.1892	0.0438	0.1047	0.92	70.3[a]	0.189

[a,b] Means within a column with no common superscript are different ($P < 0.05$).

[1]Regression analyses of apparent total tract digested Ca on dietary Ca intake was linear ($P < 0.01$).

[2]TTTD = True total tract digestibility; ECaL = endogenous losses of Ca.

Table 3.7. Partitioning of dietary Ca from pigs fed canola meal without or with microbial phytase at different levels of Ca intake

	Canola meal without phytase				Canola meal with phytase[1]					*P*-value		
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32	SEM	Ca level	Phytase	Ca level × phytase
Ca intake, g/d	0.52	1.16	1.76	2.52	0.57	1.14	1.75	2.45	0.09	<0.01	0.728	0.709
Dietary Ca in feces,[2] g/d	0.24	0.64	0.95	1.32	0.19	0.35	0.48	0.77	0.08	<0.01	<0.01	0.012
Dietary Ca absorbed,[3] g/d	0.29	0.51	0.81	1.20	0.37	0.79	1.27	1.68	0.07	<0.01	<0.01	0.016
Dietary Ca retained,[4] g/d	0.06	0.27	0.53	0.87	0.14	0.54	1.03	1.37	0.07	<0.01	<0.01	<0.01

[1]Each diet contained 1,500 units of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]Dietary Ca in feces was calculated by subtracting endogenous losses of Ca from total fecal output of Ca.

[3]Dietary Ca absorbed was calculated by subtracting dietary Ca in feces from Ca intake.

[4]Dietary Ca retained was calculated by subtracting Ca in urine and endogenous losses of Ca from dietary Ca absorbed.

Table 3.8. Phosphorus balance and apparent total tract digestibility (ATTD) of P for pigs fed canola meal without or with microbial phytase at different levels of Ca

	Canola meal without phytase				Canola meal with phytase[1]					P-value		
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32	SEM	Ca level	Phytase	Ca level × phytase
P intake, g/d	3.44	4.34	4.63	5.89	3.52	4.05	4.63	5.81	0.24	<0.01	0.302	0.443
Fecal P output, g/d	0.78	1.64	2.26	3.03	0.50	0.80	1.36	1.71	0.15	<0.01	<0.01	<0.01
Urine P output, g/d	1.24	0.96	0.91	0.88	1.62	1.61	1.63	1.90	0.18	0.071	<0.01	<0.01
Absorbed P, g/d	2.68	2.70	2.36	2.87	3.02	3.24	3.25	4.11	0.18	<0.01	<0.01	0.010
P retention, g/d	1.45	1.73	1.45	1.98	1.39	1.62	1.64	2.21	0.11	<0.01	0.452	0.325
P retention, %	41.11	40.31	31.89	34.49	40.27	40.07	35.57	38.09	2.50	<0.01	0.304	0.579
P excretion, g/d	2.02	2.60	3.17	3.91	2.11	2.42	2.97	3.61	0.24	<0.01	0.138	0.524
P excretion, %	58.89	59.69	68.11	65.51	59.73	59.93	64.43	61.91	2.50	<0.01	0.304	0.579
ATTD of P, %	77.06	62.39	51.25	49.07	85.56	79.71	70.53	70.67	1.92	<0.01	<0.01	<0.01

[1]Each diet contained 1,500 units of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

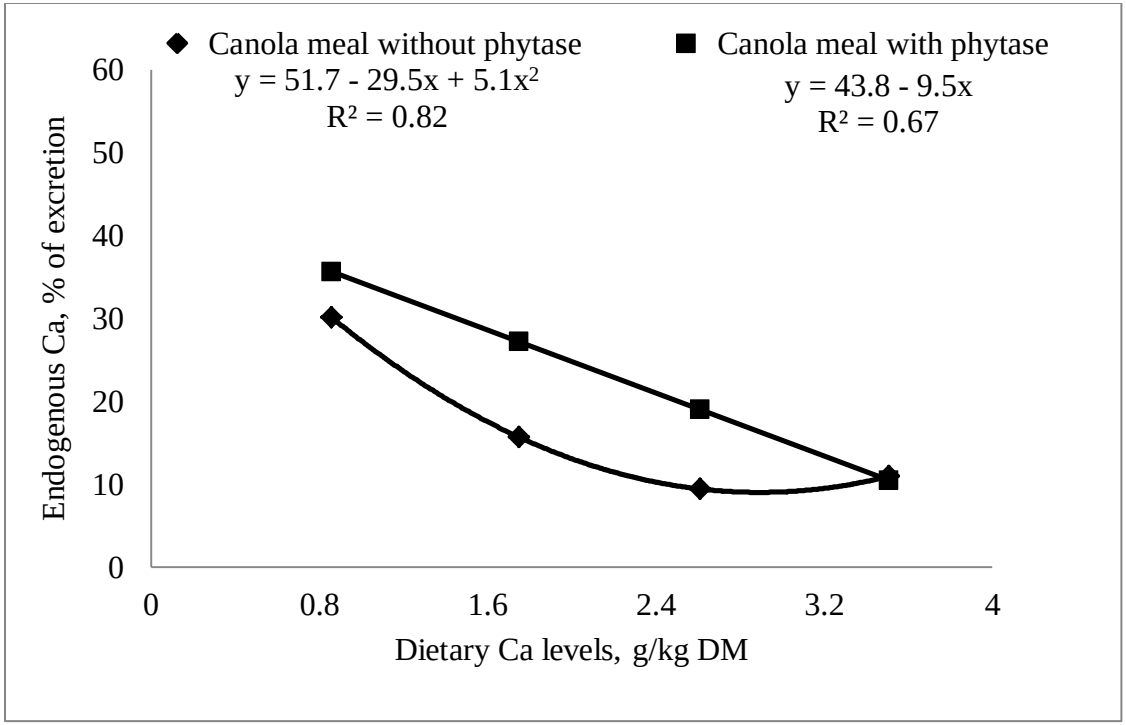

Figure 3.1. Endogenous Ca in fecal output of pigs fed canola meal without (quadratic, $P < 0.01$) or with microbial phytase (linear, $P < 0.01$) at different levels of dietary Ca.

CHAPTER 4

DETERMINATION OF THE SITE OF NET-ABSORPTION OF CALCIUM FROM THE INTESTINAL TRACT OF GROWING PIGS

ABSTRACT

An experiment was conducted to test the hypothesis that apparent and standardized digestibility of Ca in calcium carbonate and Vistacal are not different regardless of the level of dietary Ca, and that phytate affects digestibility of Ca in these 2 ingredients to the same degree. The objectives were to determine where in the intestinal tract Ca absorption takes place and if measurable quantities of basal endogenous Ca are lost in the stomach, small intestine, or large intestine. Nine growing pigs (initial BW: 23.8 ± 1.3 kg) were cannulated in the duodenum and in the distal ileum and were allotted to a 9 × 6 incomplete Latin square design with 9 diets and 6 periods. Diets contained calcium carbonate or Vistacal as the sole source of Ca, 0 or 1% phytate, and 0.4 or 0.8% Ca. A Ca-free diet was also formulated and used to measure endogenous losses of Ca. Fecal, ileal, and duodenal samples were collected on d 5 and 6, 7 and 8, and 9 and 10, respectively. Duodenal endogenous losses of Ca were greater ($P < 0.01$) than ileal and total tract endogenous losses of Ca, but ileal and total tract losses were not different. Apparent and standardized digestibility of Ca were not affected by level of phytate, but standardized digestibility of Ca decreased ($P < 0.05$) as Ca level increased in Vistacal diets, which was not the case if calcium carbonate was the source of Ca (interaction, $P < 0.05$). In contrast, apparent digestibility of Ca was not affected by dietary Ca level. The apparent (ADD) and standardized duodenal digestibility (SDD), the apparent (AID) and standardized ileal digestibility (SID), and

the apparent (ATTD) and standardized total tract digestibility (STTD) of Ca were not different if calcium carbonate was the source of dietary Ca. However, the AID and ATTD, and the SID and STTD of Ca in Vistacal were greater ($P < 0.01$) than the ADD and SDD of Ca, but no differences were observed between AID and SID, and between SID and STTD of Ca (interaction, $P < 0.05$). The ADD, AID, ATTD, SDD, SID, and STTD of Ca in calcium carbonate were greater ($P < 0.01$) than in Vistacal. In conclusion, duodenal endogenous Ca losses were greater than ileal and total tract endogenous losses of Ca. Apparent and standardized digestibility of Ca were not affected by level of phytate, but standardized digestibility of Ca was affected by dietary Ca level if Vistacal was the source of Ca, but not if calcium carbonate was used. However, apparent digestibility of Ca was not affected by dietary Ca level. Calcium from calcium carbonate was mostly absorbed before the duodenum, but Ca from Vistacal was mostly absorbed in the jejunum and ileum.

Key words: calcium, endogenous losses, phytate, pigs, standardized digestibility

INTRODUCTION

There is a relatively low concentration of Ca in most feed ingredients produced from plants, and Ca, therefore, has to be supplemented to diets fed to swine. This is accomplished by adding inorganic Ca, such as calcium carbonate, or organic Ca, such as *Lithothamnium calcareum*, to the diet (Melo and Moura, 2009). Although calcium carbonate is the most commonly used source of Ca in swine diets, *Lithothamnium calcareum* may also be used (Fialho et al., 1992). However, the digestibility of Ca in this source of Ca has not been reported.

Increasing dietary concentrations of Ca from calcium carbonate does not affect apparent digestibility of Ca, but it reduces the digestibility of P (Stein et al., 2011). It is, however, not

known if the dietary concentration of *Lithothamnium calcareum* influences Ca absorption. Phytate is an anti-nutritional factor present in plant ingredients. Phytate not only binds P, but also may bind Ca, zinc, copper (Santos, 2012), protein, starch, and fatty acids (Kies, 2005), which reduces the absorption of these nutrients. However, it is not known how phytate affects digestibility of Ca from calcium carbonate and from *Lithothamnium calcareum* added at different concentrations to swine diets.

The majority of Ca is absorbed in the small intestine of pigs (Moore and Tyler, 1955a, b; Partridge, 1978; Liu et al., 2000; Schröder and Breves, 2006), but it is not known if Ca also may be absorbed in the stomach or in the large intestine of pigs (Moore and Tyler, 1955a, b; Partridge, 1978; Liu et al., 2000; Schröder and Breves, 2006; Metzler-Zebeli et al., 2010). Endogenous Ca may be lost over the total gastrointestinal tract (Moore and Tyler, 1955b; Hansard et al., 1961; Fernández, 1995), but there are no data to quantify the amounts of endogenous Ca that are lost from different segments of the gastro-intestinal tract. Therefore, the objective of this experiment was to test the hypothesis that the digestibility of Ca in $CaCO_3$ and *Lithothamnium calcareum* are not different regardless of the level of Ca in the diet, and that phytate affects the digestibility of Ca in these 2 ingredients to the same degree. The second objective was to determine if Ca absorption takes place only in the small intestine or if absorption also occurs in the stomach or in the large intestine. The third objective was to determine if measurable quantities of basal endogenous Ca are lost in the stomach, the small intestine, or in the large intestine.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment.

Diets, Animals, and Experimental Design

The 2 sources of Ca that were used in this experiment were Vistacal and calcium carbonate. Vistacal was produced by *Lithothamnium calcareum* algae and contained 32.7% Ca (as fed basis; Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). Calcium carbonate, which was mined in IA and contained 39% Ca, was procured from ILC Resources, Alden, IA (Table 4.1). A 50% solution of phytic acid (Sigma-Aldrich, St. Louis, MO) was also used.

A total of 9 diets were formulated (Tables 4.2 and 4.3). Eight diets were used in a 2 × 2 × 2 factorial design. There were 2 sources of Ca (calcium carbonate and Vistacal), 2 levels of phytic acid (0 and 1%), and 2 levels of dietary Ca (0.4 and 0.8%). Calcium carbonate or Vistacal were the sole source of Ca in these diets. A Ca-free diet based on sucrose, cornstarch, potato protein isolate, corn gluten meal, vitamins, and minerals was formulated and used to measure endogenous losses of Ca. Monosodium phosphate was the source of inorganic P. Chromic oxide was included in all diets at 0.4% as an indigestible marker.

Nine growing pigs (initial BW: 23.8 ± 1.3 kg) were surgically equipped with a T-cannula in the duodenum and another T-cannula in the distal ileum (McGinnis et al., 2007). After a 10 d recovery period, pigs were allotted to a 9 × 6 incomplete Latin square design with 9 diets and 6 periods.

Feeding and Sample Collections

Pigs were fed each experimental diet during six 10 d-periods and all pigs received each diet in 1 period. The daily allotments of feed were calculated as 3 times the daily energy

requirement for maintenance (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998) and divided into 2 equal meals that were fed at 0700 and 1900 h. Fecal samples were collected from the pigs on d 5 and 6. Ileal samples were collected consecutively for 8 h on d 7 and 8 starting at 0700 h. Duodenal samples were collected on d 9 from 0700 to 0900 h, from 1100 to 1300 h, and from 1500 to 1700 h. On d 10, duodenal samples were collected from 0900 to 1100 h, from 1300 to 1500 h, and from 1700 to 1900 h. Fecal, ileal, and duodenal samples were stored at -20°C immediately after collection.

Sample Analysis

Before chemical analysis, doudenal and ileal samples were thawed at room temperature and mixed within animal and diet, and 2 subsamples were collected. Duodenal and ileal samples were lyophilized and ground. Fecal samples were dried in a forced-air oven at 65ºC and then ground. Potato protein isolate, corn gluten meal, calcium carbonate, Vistacal, monosodium phosphate, diets, duodenal, ileal, and fecal samples were analyzed for Ca by an atomic absorption spectrophotometer procedure (Method 968.08; AOAC Int., 2007) after wet digestion sample preparation (Method 935.13; AOAC Int., 2007). The concentrations of P in potato protein isolate, corn gluten meal, monosodium phosphate, diets, duodenal, ileal, and fecal samples were analyzed using a colorimetric procedure (Method 931.01; AOAC Int., 2007). Potato protein isolate, corn gluten meal, calcium carbonate, Vistacal, monosodium phosphate, diets, duodenal, ileal, and fecal samples were also analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007) and these samples, except for duodenal, ileal, and fecal samples, were also analyzed for ash (Method 942.05; AOAC Int., 2007). Potato protein isolate, corn gluten meal, and diets were analyzed for phytase activity (Engelen et al.; 2001), and for phytic acid (Megazyme method; AB Vista, Ystrad Mynach, UK). These samples were also

analyzed for GE using an adiabatic bomb calorimeter (Model 6300, Parr Instruments, Moline, IL), for CP using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ), and for acid-hydrolyzed ether extract (**AEE**) using 3N HCl (Sanderson, 1986) followed by crude fat extraction with petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). These samples were also analyzed for ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). For diets, calcium carbonate, and Vistacal, 5 g of each sample was mixed with 15 mL of distilled water and filtered, and pH was measured in the solution with a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA). For duodenal and ileal samples, pH was measured in the collected samples. Diets, duodenal, ileal, and fecal samples were analyzed for chromium by Inductively Coupled Plasma Atomic Emission Spectrometry Method (method 990.08; AOAC Int., 2007) after nitric acid-perchloric acid wet ash sample preparation (method 968.08D; AOAC Int., 2007).

Calculations

Apparent duodenal digestibility (**ADD**) of Ca was calculated using the following equation (Stein et al., 2007):

$$ADD\ (\%) = [1 - (Ca_{digesta} / Ca_{diet}) \times (M_{diet}/M_{digesta})] \times 100, \qquad [1]$$

where ADD is the apparent duodenal digestibility (%) of Ca, $Ca_{digesta}$ and Ca_{diet} are the Ca concentration (g/kg DM) in duodenal samples and diets, respectively, and M_{diet} and $M_{digesta}$ are the marker concentrations (g/kg DM) in diet and duodenal samples, respectively.

The apparent ileal digestibility (**AID**) and the apparent total tract digestibility (**ATTD**) of Ca (%) were calculated as ADD except that Ca and marker concentrations in ileal or fecal samples were used rather than in duodenal samples.

Basal endogenous duodenal losses of Ca (**ECaL**) were calculated from pigs fed the Ca-free diet according to equation 2 (Stein et al., 2007):

$$\text{Duodenal ECaL (g/kg DMI)} = Ca_{digesta} \times (M_{diet} / M_{digesta}), \quad [2]$$

where ECaL is the basal duodenal loss of Ca (g/kg DMI). The basal ileal and the basal total tract ECaL were calculated as duodenal ECaL except that Ca and marker concentrations in ileal digesta or fecal samples were used rather than in duodenal samples.

The standardized duodenal digestibility (**SDD**) of Ca was calculated according to equation 3 (Stein et al., 2007):

$$\text{SDD (\%)} = \text{ADD} + [({}_{duodenal}\text{ECaL} / Ca_{diet}) \times 100], \quad [3]$$

where SDD is the standardized duodenal digestibility (%) of Ca. The standardized ileal digestibility (**SID**), and standardized total tract digestibility (**STTD**) of Ca (%) were calculated as the SDD of Ca with the exception that values for the AID or ATTD were used instead of ADD, and values for ileal or total tract ECaL were used rather than the duodenal ECaL.

Statistical Analyses

For Ca digestibility, data were initially analyzed as a 2 × 2 × 2 × 3 factorial using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included the fixed effects of Ca level, phytate level, Ca source, collection site, and all possible interactions, and the random effects of period and pig. However, because effect of phytate was not significant, phytate was removed from the final model and data were analyzed as a 2 × 2 × 3 factorial. Likewise, for P digestibility, data were analyzed as a 2 × 2 × 3 factorial using the MIXED procedure with Ca level, Ca source, and collection site, and all possible interactions as the fixed effects, and period and pig as the random effects. For digesta pH, data were analyzed as a 2 × 2 × 2 × 2 factorial using the MIXED procedure. The model included the fixed effects of Ca level, phytate level, Ca

source, collection site, and all possible interactions, and the random effects of period and pig. Means were calculated using the LSMEANS statement and if significant differences were observed, means were separated using the PDIFF option. For endogenous losses of Ca, data were analyzed using the GLM procedure, the model included the effect of collection site, and means were separated with the Tukey adjustment. The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to determine normality of residuals and this procedure was also used to identify outliers. Pig was the experimental unit for all analyses. An α-value of 0.05 was used to assess significance among treatments.

RESULTS

Pigs remained healthy and consumed their respective diets. However, 2 pigs had problems with the ileal cannula in the fifth period and were removed from the experiment and the extra pig was included in the experiment at this point.

Values for the analyzed concentration of Ca in diets were up to 0.08% greater than expected values and for analyzed P values in the diets were from 0.06% less to 0.05% greater than expected values (Table 4.3). However, it was assumed that results of the experiment were not affected by the differences between analyzed and expected values because the expected differences among diets were obtained. Analyzed values for phytic acid were up to 0.18% units greater than expected values for the diets that contained 0% phytic acid, but for diets containing 1% phytic acid, all analyzed values were close to expected values. The pH of diets that contained 0% phytic acid range from 5.38 to 6.23 and the pH of diets that contained 1% phytic acid range from 3.46 to 4.53, and the pH of Ca-free diet was 5.27.

There was no effect of phytate on ADD, AID, ATTD, SDD, SID, or STTD of Ca. If calcium carbonate was the source of Ca, the ADD, AID, and ATTD of Ca were not different and this was also the case for SDD, SID, and STTD of Ca (Tables 4.4 and 4.5). If Vistacal was the source of Ca, the AID and ATTD of Ca were not different, but were greater ($P < 0.05$) than the ADD of Ca (interaction, $P \leq 0.01$). Likewise, the SID and STTD of Ca were not different, but were greater ($P < 0.05$) than the SDD of Ca. For all sites of absorption, values for ADD, AID, and ATTD of Ca were greater ($P < 0.05$) if calcium carbonate was the source of Ca than if Vistacal was used, and this was also the case for SDD, SID, and STTD of Ca. If calcium carbonate was the source of Ca, the ADD, AID, and ATTD of Ca were not different between diets containing 0.4 and 0.8% Ca and this was also the case for the SDD, SID, and STTD of Ca. However, if Vistacal was the source of Ca, the ADD, AID, and ATTD of Ca were not different between diets containing 0.4 and 0.8% Ca, but the SDD, SID, and STTD of Ca were greater ($P < 0.05$) if diets contained 0.4% Ca than if diets contained 0.8% Ca (interaction, $P < 0.05$). For both levels of Ca, values for ADD, AID, and ATTD of Ca in calcium carbonate were greater ($P < 0.05$) than in Vistacal, and this was also the case for SDD, SID, and STTD of Ca. Duodenal endogenous losses of Ca were greater ($P < 0.05$) than ileal and total tract endogenous losses of Ca, but no differences were observed between ileal and total tract endogenous losses of Ca.

If no phytate was used and calcium carbonate was the source of Ca, the ADD, AID, and ATTD of P were not different between diets containing 0.4 and 0.8% Ca, and this was also the case for ADD, AID, and ATTD of P when 1% phytate was included in the diet (Tables 4.6 and 4.7). However, if no phytate was used and Vistacal was the source of Ca, the ADD, AID, and ATTD of P in Vistacal were greater ($P < 0.05$) in diets contained 0.4% Ca than in diets contained 0.8% Ca, and this was also the case for ADD, AID, and ATTD of P if 1% phytate was included

in the diet (interaction, $P < 0.05$). If no phytate was used and diets contained 0.4% Ca, the ADD, AID, and ATTD of P were not different between calcium carbonate and Vistacal, but if diets contained 0.8% Ca, the ADD, AID, and ATTD of P in calcium carbonate were greater ($P < 0.05$) than in Vistacal (interaction, $P < 0.05$). Likewise, if 1% phytate was included in diets containing 0.4% Ca, the ADD, AID, and ATTD of P were not different between calcium carbonate and Vistacal, but if diets contained 0.8% Ca, the ADD, AID, and ATTD of P in calcium carbonate were greater ($P < 0.05$) than in Vistacal (interaction, $P < 0.05$). If no phytate was used and diets contained 0.4 or 0.8% Ca, values for AID and ATTD of P were not different, but were greater ($P < 0.05$) than ADD of P. The ADD of P was not different between diets containing 0.4 and 0.8% Ca, but the AID and ATTD of Ca was greater ($P < 0.05$) if diets contained 0.4% Ca than if diets contained 0.8% Ca (interaction, $P < 0.05$). If 1% phytate was included in the diet, for both sources of Ca, the ATTD of P was greater ($P < 0.05$) than the AID and ADD of P, and the AID of P was greater ($P < 0.05$) than the ADD of P. Values for ADD and AID of P were not different between calcium carbonate and Vistacal, but the ATTD of P in calcium carbonate was greater ($P < 0.05$) than in Vistacal (interaction, $P < 0.05$).

If no phytate was used, ileal samples had greater ($P < 0.05$) pH than duodenal samples and this was also the case if 1% phytate was included in the diet (Table 4.8). The pH in duodenal samples decreased ($P < 0.01$) if 1% phytate was included in the diets, but the pH in ileal samples was not affected by level of dietary phytate (interaction, $P < 0.01$). Digesta pH decreased ($P < 0.05$) by adding 1% phytate to calcium carbonate diets, but digesta pH was not affected by adding 1% phytate to Vistacal diets (interaction, $P < 0.05$). Digesta pH was not different between the 2 sources of Ca if no phytate was used, but if 1% phytate was included in the diets, digesta pH was greater ($P < 0.05$) if Vistacal was the source of Ca than if calcium carbonate was used. If

phytate was not used, digesta pH was not different between diets contained 0.4 and 0.8% Ca, and this was also the case if 1% phytate was included in the diet. If diets contained 0.4% Ca, digesta pH was not affected by level of phytate, but if diets contained 0.8% Ca, digesta pH decreased ($P < 0.05$) if 1% phytate was added to the diet (interaction, $P < 0.05$).

DISCUSSION

The concentrations of Ca in calcium carbonate and in *Lithothamnium calcareum* that were analyzed in this experiment is in agreement with reported values (Sauvant et al., 2004; Melo and Moura, 2009; Stein et al., 2011; NRC, 2012). However, the concentration of P in the monosodium phosphate used in this experiment was slightly greater than previous values (Sauvant et al., 2004; NRC, 2012).

The relatively high duodenal endogenous loss of Ca is likely caused by Ca secreted in saliva (Tryon and Bibby, 1966), gastric juice (Trautmann and Kirchhof, 1937; Moore and Tyler, 1955b), pancreatic juice (Gamble and McIver, 1928; Partridge et al., 1982; Bronner, 1997; Fernández, 1995), and bile (Sullivan et al., 1981; Allen, 1982; Bronner, 1997). It is likely that a portion of these endogenous secretions of Ca was reabsorbed in the small intestine (Allen, 1982), which is the reason the ileal endogenous loss was less than the duodenal loss. The observation that there were no differences between ileal and total tract endogenous losses of Ca indicates that there are no endogenous losses of Ca in the large intestine. This observation concurs with previous data (Partridge, 1978).

It was surprising that Ca digestibility was not affected by inclusion of phytate to the diets, because phytate may bind Ca, which is believed to reduce the digestibility of Ca (Selle et al., 2009). Phytate in plant ingredients is often bound to magnesium and potassium ions and has

greater affinity for zinc and copper than for Ca, but because Ca is included in high amounts in mixed diets, Ca-phytate complexes may be formed (Selle et al., 2009). It is, however, possible that there are differences in the properties of phytate between natural phytate in plant ingredients and synthetic phytate, which was used in this experiment (Onyango et al., 2008; Santos, 2012). Solubility of free synthetic phytate is greater than magnesium-potassium phytate, which indicates that free synthetic phytate is more susceptible to be hydrolyzed than magnesium-potassium phytate (Onyango et al., 2008). Factors such as pH, temperature, and ionic strength also influence the binding between phytate and Ca (Graft, 1983).

The observation that the ATTD of Ca was not affected by the level of dietary Ca is in agreement with the observation that increasing dietary Ca levels from 55 to 173% of the requirement does not influence the ATTD of Ca (Stein et al., 2011). The fact that the STTD of Ca in Vistacal, but not in calcium carbonate, was negatively affected by increasing dietary Ca level may be explained by possible formations of Ca-P complexes (Clark, 1969; Brink et al., 1992; Stein et al., 2011). The greater solubility of Ca in Vistacal than of Ca in calcium carbonate may result in increased formation of Ca-P complexes in the intestinal tract of pigs than if calcium carbonate is used (Walk et al., 2012), which may result in reduced digestibility. Another reason for the reduced formation of Ca-P complexes if calcium carbonate is used may be that most Ca in calcium carbonate was absorbed before the duodenum, and only a small amount of Ca was available in the remaining portion of the small intestine to form Ca-P complexes. In contrast, absorption prior to the duodenal cannula of Ca from Vistacal was relatively minor so more Ca entered the distal small intestine, where complexes could be formed.

Although most Ca is absorbed in the small intestine (Moore and Tyler, 1955a; b; Partridge, 1978; Liu et al., 2000), absorption of Ca in the colon has also been observed (Liu et

al., 2000), and Ca absorption is affected by the type of diet that is fed (Partridge, 1978). In this experiment, Ca from calcium carbonate diets was mainly absorbed in the region before the duodenal cannula, which was placed approximately 10 cm behind the pancreatic duct. This observation indicates that Ca from calcium carbonate is absorbed in the stomach or the early part of the duodenum. However, most of the Ca from Vistacal was absorbed between the duodenal and the ileal cannula, indicating that Ca in Vistacal is released later in the small intestine. Calcium-binding proteins transport the Ca through the enterocyte in the small intestine (Bronner, 1998) but they have also been identified in the stomach of pigs (Raeymaekers et al., 1993), indicating that Ca may be absorbed before the duodenum. Secretion of gastric acids and enzymes contribute to the release of Ca from the diet to a soluble or ionic form, which is required for Ca to be absorbed, and greater absorption of Ca in the proximal small intestine than in jejunum is promoted by the greater concentration of Ca-binding proteins in the enterocytes in the duodenum than in the jejunum (Allen, 1982). Result of this experiment support this hypothesis, but also indicate that some variation among feed ingredients exists, which results in differences in the site where Ca is absorbed. However, the present results do not support the hypothesis that Ca is also absorbed in the large intestine because no differences between ileal and total tract digestibility were observed. This conclusion is in agreement with data from Bohlke et al. (2005) who reported that there is no absorption of Ca in the large intestine of pigs.

The reason Ca in calcium carbonate had greater digestibility than Ca in Vistacal may be that there are differences in solubility between the 2 ingredients, which may result in formation of more Ca-P complexes in the intestinal tract of pigs fed Vistacal compared with pigs fed calcium carbonate. The fact that the digestibility of P was also less if Vistacal was used, support this hypothesis. To our knowledge there are no other data for the digestibility of Ca in Vistacal

fed to pigs, but the digestibility of Ca in Vistacal fed to broilers is not less than in calcium carbonate (Walk et al., 2012). Differences in pH, transit time, and in enzyme properties between pigs and chickens (Crévieu-Gabriel et al., 1999) may explain the differences observed between pigs and broilers fed Vistacal.

One of the factors that may affect P digestibility is the dietary level of Ca, and increasing dietary Ca may reduce P digestibility (Stein et al., 2011). In this experiment, this was true only for Vistacal diets, but not for calcium carbonate diets. The reduction of P digestibility in Vistacal diets that were observed as Ca in the diets increased is likely a result of increased formation of Ca-P complexes in the small intestine, which reduced the availability of P for absorption (Clark, 1969; Stein et al., 2011). However, the reason P digestibility was not affected by Ca level in the calcium carbonate diets may be the low concentration of Ca in the small intestine, because most Ca was absorbed before the early part of duodenum. This hypothesis explains the fact that no differences were observed between calcium carbonate and Vistacal diets in the ADD of P, but the AID and ATTD of P were greater in calcium carbonate diets than in Vistacal diets if no phytate was added to the diets. In a recent experiment with broilers, it was also observed that the digestibility of P was less in birds fed Vistacal diets than in birds fed limestone diets (Walk et al., 2012).

It is believed that most P is absorbed in the small intestine and neither secretion nor absorption of P occurs in the large intestine (Crenshaw, 2001; Bohlke et al., 2005). Results from this experiment obtained for diets that contained no phytate support this hypothesis, but data obtained for diets containing 1% phytate indicate that P may be absorbed in the large intestine. We do not have an explanation for this observation.

It has been reported that the pH in the stomach, duodenum, jejunum, and ileum of a weaned pig is 3.2, 5.7, 5.9, and 6.9, respectively (Li et al, 2008) and the pH in the stomach, duodenum, jejunum, and ileum of a finishing pig is 4.5, 6.3, 6.4, and 6.6, respectively (Merchant et al., 2011). Although, the values obtained in this experiment for duodenal pH are less and the values for ileal digesta are greater than the reported values, the digesta pH may vary depending on the diet and the feed intake. Inclusion of phytic acid decreased the pH of the diet, which is likely a result of the added acids from phytate. The fact that the digesta pH was greater in pigs fed diets containing Vistacal than in pigs fed calcium carbonate may be a result of the reduced absorption of Ca and P from Vistacal compared with calcium carbonate.

In conclusion, the duodenal endogenous losses of Ca were greater than the ileal and total tract endogenous losses, but ileal and total tract endogenous losses of Ca were not different. The ADD, AID, ATTD, SDD, SID, STTD of Ca were not affected by the level of synthetic phytate in the diet. Likewise, the ADD, AID, and ATTD of Ca were not affected by Ca level. However, the SDD, SID, and STTD of Ca decreased if the dietary concentration of Ca from Vistacal increased, but this was not the case if calcium carbonate was the source of Ca in the diet. Calcium from calcium carbonate was mostly absorbed in the stomach and the upper duodenum, but some of the Ca from Vistacal was absorbed in the jejunum or ileum. Because there was no net absorption or secretion of Ca in the large intestine, total tract collections can be used for Ca digestibility studies with pigs. The digestibility of P was reduced by increasing Ca level if Vistacal was the source of Ca, but that was not the case if calcium carbonate was the source of Ca.

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Tables

Table 4.1. Composition of ingredients as-fed basis

	Ingredient				
Item	Potato protein isolate	Corn gluten meal	Calcium carbonate	Vistacal[1]	Monosodium phosphate
GE, kcal/kg	5,268	5,380	-	-	-
DM, %	91.19	91.46	99.99	98.36	98.99
CP, %	80.75	63.3	-	-	-
Ash, %	0.48	2.59	96.08	91.14	90.61
AEE,[2] %	0.50	4.60	-	-	-
ADF, %	3.60	6.93	-	-	-
NDF, %	1.12	5.57	-	-	-
Ca, %	0.03	0.09	39.11	32.70	0.08
P, %	0.12	0.54	-	-	29.69
Phytase,[3] FTU/kg	52	94	-	-	-
Phytic acid, %	0.33	1.60	-	-	-
Phytate-bound P,[4] %	0.09	0.45	-	-	-

Table 4.1. (Cont.)

Non-phytate P,[5] %	0.03	0.09	-	-	-
pH	-	-	8.14	7.88	-

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

[2]AEE = acid-hydrolyzed ether extract.

[3]FTU = phytase units.

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

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

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

	Diet								
	Ca from calcium carbonate, %				Ca from Vistacal,[1] %				
	0% phytic acid		1% phytic acid		0% phytic acid		1% phytic acid		
Ingredient, % Ca, %:	0.40	0.80	0.40	0.80	0.40	0.80	0.40	0.80	Ca-free
Corn gluten meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cornstarch	47.94	46.94	45.94	44.94	47.69	46.42	45.69	44.42	48.94
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Potato protein isolate	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Calcium carbonate	1.00	2.00	1.00	2.00	-	-	-	-	-
Vistacal	-	-	-	-	1.25	2.52	1.25	2.52	-
Monosodium phosphate	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Phytic acid[2]	-	-	2.00	2.00	-	-	2.00	2.00	-
L-Lys HCL	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
DL-Met	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09

Table 4.2. (Cont.)

L-Trp	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Solka floc[3]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin mineral premix[4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Vistacal is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). Vistacal contains 32.7% Ca (as fed).

[2]Phytic acid, 50 %, Sigma-Aldrich, St. Louis, MO.

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

[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and nicotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

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

	Diet								
	Ca from calcium carbonate, %				Ca from Vistacal,[1] %				
Item	0% phytic acid[2]		1% phytic acid		0% phytic acid		1% phytic acid		Ca-free
Ca level, %:	0.4	0.8	0.4	0.8	0.4	0.8	0.4	0.8	0
GE, kcal/kg	4,223	4,286	4,272	4,153	4,213	4,164	4,195	4,116	4,389
DM, %	92.00	92.46	91.43	91.59	92.43	92.50	89.97	89.78	91.82
CP,%	17.0	16.7	16.4	16.4	16.5	16.9	16.8	16.7	16.3
Ash, %	3.13	3.95	3.85	4.43	3.03	4.43	3.98	5.02	2.33
AEE,[3] %	3.69	3.85	3.61	3.83	3.76	3.87	3.69	3.50	3.58
Ca, %	0.46	0.87	0.47	0.85	0.46	0.83	0.48	0.85	0.08
P, %	0.39	0.42	0.59	0.64	0.40	0.37	0.65	0.66	0.37
ADF, %	3.36	3.44	3.19	3.36	3.11	3.60	3.31	3.84	3.14
NDF, %	3.16	3.25	2.89	2.83	3.39	2.90	2.91	3.09	3.29
Phytase, FTU[4]/kg	<50	<50	<50	<50	<50	<50	<50	<50	<50
Phytic acid, %	0.38	0.37	1.25	1.25	0.37	0.36	1.24	1.21	0.36

Table 4.3. (Cont.)

Phytate-bound P,[5] %	0.13	0.14	0.35	0.35	0.14	0.12	0.35	0.34	0.13
Non-phytate P,[6] %	0.19	0.22	0.24	0.31	0.23	0.24	0.28	0.29	0.27
pH	5.38	6.23	3.79	3.46	5.72	5.71	4.28	4.53	5.27

[1]Vistacal is a source of Ca produced by *Lithothamnium calcareum* (Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). Vistacal contains 31.4% Ca (as fed).

[2]Phytic acid, 50 %, Sigma-Aldrich, St. Louis, MO.

[3]AEE = acid-hydrolyzed ether extract.

[4]FTU = phytase units.

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

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

Table 4.4. Apparent duodenal, ileal, and total tract digestibility of Ca by pigs fed diets containing calcium carbonate or Vistacal included in quantities that provided 0.4 or 0.8% Ca[1,2]

	Ca from calcium carbonate			Ca from Vistacal				*P*-value		
Site	0.4%	0.8%	Source × site[3]	0.4%	0.8%	Source × site[3]	Pooled SEM	Ca source	Ca level	Site of absorption
Duodenal	37.78	46.61	abc	16.27	14.30	d				
Ileal	40.55	49.24	ab	39.97	30.00	c	4.89	<0.01	0.925	<0.01
Total tract	49.67	48.51	a	42.13	36.20	bc				
Ca level × source[4]	x	x	-	y	y	-				

[1]Effect of phytate was not significant and effect of phytate was, therefore, removed from the final model and data are presented only for Ca source, Ca level, and site of absorption.

[2]Interactions between Ca level and site of absorption and between Ca level, Ca source, and site of absorption were not significant.

[3]The interaction between Ca source and site of absorption was significant ($P \leq 0.01$). For calcium carbonate, no differences among absorption sites were observed regardless of the level of Ca in the diet. However, for Vistacal, apparent ileal and total tract digestibility of Ca were greater ($P < 0.05$) than apparent duodenal digestibility of Ca for both levels of Ca, but apparent ileal and total tract digestibility of Ca were not different. Apparent duodenal, ileal, and total tract digestibility of Ca were greater ($P < 0.05$) in calcium carbonate than in Vistacal. Means within a column or within a row lacking a common superscript letter differ ($P < 0.05$).

[4]The interaction between Ca level and Ca source was significant ($P < 0.05$). However, for both calcium carbonate and Vistacal, no differences between levels of Ca were observed regardless of site of absorption, but the apparent total tract digestibility of Ca from calcium carbonate was greater ($P < 0.05$) than the apparent total tract digestibility of Ca from Vistacal. Means within a row lacking a common superscript letter are different ($P < 0.05$).

Table 4.5. Standardized duodenal, ileal, and total tract digestibility of Ca by pigs fed diets containing calcium carbonate or Vistacal included in quantities that provided 0.4 or 0.8% Ca[1,2,3]

	Ca from calcium carbonate			Ca from Vistacal				*P*-value		
Site	0.4%	0.8%	Source × Site[4]	0.4%	0.8%	Source × Site[4]	Pooled SEM	Ca source	Ca level	Site of absorption
Duodenal	58.09	57.63	ab	36.26	25.38	d	4.90	<0.01	0.017	<0.01
Ileal	48.87	53.70	ab	48.11	34.49	c				
Total tract	62.81	55.63	a	55.09	43.37	bc				
Ca level × Source[5]	x	x	-	y	z	-				

[1]Effect of phytate was not significant and effect of phytate was, therefore, removed from the final model and data are presented only for Ca source, Ca level, and site of absorption.

[2]Standardized duodenal, ileal, and total tract digestibility were calculated by correcting the apparent duodenal, ileal, and total tract digestibility values for the duodenal (1.03 g/kg of DMI), ileal (0.42 g/kg of DMI), and total tract (0.67 g/kg of DMI) basal endogenous loss of Ca, respectively. Basal endogenous losses of Ca were determined using pigs fed the Ca-free diet.

[3]Interactions between Ca level and site of absorption and between Ca level, Ca source, and site of absorption were not significant.

[4]The interaction between Ca source and site of absorption was significant ($P \leq 0.01$). For calcium carbonate, no differences among absorption sites were observed regardless of the level of Ca in the diet. However, for Vistacal, standardized ileal and total tract digestibility of Ca were greater ($P < 0.05$) than standardized duodenal digestibility of Ca for both levels of Ca, but standardized ileal and total tract digestibility of Ca were not different. Standardized duodenal, ileal, and total tract digestibility were greater ($P < 0.05$) in calcium carbonate than in Vistacal.

[5]The interaction between Ca level and Ca source was significant ($P < 0.05$). For calcium carbonate, no differences between levels of Ca were observed regardless of the site of absorption, but for Vistacal, standardized digestibility of Ca was greater ($P < 0.05$) at all absorption sites if the diet contained 0.4% Ca than if diet contained 0.8% Ca. However, for both levels of Ca, standardized digestibility of Ca in calcium carbonate was greater ($P < 0.05$) than in Vistacal. Means within a row lacking a common superscript letter are different ($P < 0.05$).

Table 4.6. Apparent duodenal, ileal, and total tract digestibility of P by pigs fed calcium carbonate or Vistacal included in quantities that provided 0.4 or 0.8% Ca, and 0% phytate[1]

	Ca from calcium carbonate		Ca from Vistacal		Ca level × site[2]			*P*-value		
Site	0.4 %	0.8 %	0.4 %	0.8 %	0.4 %	0.8 %	Pooled SEM	Ca source	Ca level	Site
Duodenal	24.46	38.73	28.31	17.63	d	d	4.79	<0.01	<0.01	<0.01
Ileal	73.09	73.76	63.63	40.75	ab	c				
Total tract	75.21	74.43	69.26	47.50	a	bc				
Ca level × source[3]	ab	a	b	c	-	-				

[1]Interactions between Ca source and site of absorption and between Ca level, Ca source, and site of absorption were not significant.

[2]The interaction between Ca level and site of absorption was significant ($P < 0.05$). If diets contained 0.4 or 0.8% Ca, no differences were observed between apparent ileal and apparent total tract digestibility of P, but both of these values were greater ($P < 0.05$) than the apparent duodenal digestibility of P. For duodenal digestibility, no differences were observed between the 2 Ca levels. However, values for apparent ileal and apparent total tract digestibility of P were greater ($P < 0.05$) at 0.4% Ca than at 0.8% Ca. Means within a column and within a row lacking a common superscript letter differ ($P < 0.05$).

[3]The interaction between Ca level and Ca source was significant ($P < 0.01$). If calcium carbonate, was included in the diets no differences were observed between the 2 Ca levels. However, if Vistacal was the source of Ca, digestibility of P was greater ($P < 0.05$) at 0.4% Ca than at 0.8% Ca. If diets contained 0.4% Ca, no differences in the apparent digestibility of P were observed between the 2 sources of Ca, but if diets contained 0.8%, the apparent digestibility of P was greater ($P < 0.05$) in diets containing calcium carbonate than in diets containing Vistacal. Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 4.7. Apparent duodenal, ileal, and total tract digestibility of P by pigs fed calcium carbonate or Vistacal included in quantities that provide 0.4 or 0.8% Ca, and 1% phytate[1]

	Ca from calcium carbonate			Ca from Vistacal				*P*-value		
Site	0.4 %	0.8 %	Ca source × site[3]	0.4 %	0.8 %	Ca source × site[2]	Pooled SEM	Ca source	Ca level	Site
Duodenal	20.77	25.26	d	29.37	20.54	d				
Ileal	44.94	50.12	bc	49.15	33.00	c	5.23	0.017	0.088	<0.01
Total tract	64.43	64.51	a	57.61	45.49	b				
Ca level × source[3]	a	a	-	a	b	-				

[1]Interactions between Ca level and site of absorption and between Ca level, Ca source, and site of absorption were not significant.

[2]The interaction between Ca source and site of absorption was significant ($P < 0.05$). For both sources of Ca, apparent total tract digestibility of P was greater ($P < 0.05$) than apparent ileal and apparent duodenal digestibility of P, and apparent ileal digestibility of P was greater ($P < 0.05$) than apparent duodenal digestibility of P. There was no influence of Ca source on the apparent duodenal and the apparent ileal digestibility of P, but apparent total tract digestibility of P was greater ($P < 0.05$) if calcium carbonate was used than if Vistacal was included in the diet. Means within a column and within a row lacking a common superscript letter differ ($P < 0.05$).

[3]The interaction between Ca level and Ca source was significant ($P < 0.05$). For calcium carbonate, no differences in the apparent digestibility of P were observed between the 2 levels of Ca. However, if Vistacal was included in the diet, the apparent digestibility of P was greater ($P < 0.05$) at 0.4% Ca than at 0.8% Ca. If diets contained 0.4% Ca, the apparent digestibility of P was not influenced by source of Ca, but if diets contained 0.8% Ca, the apparent digestibility of P was greater ($P < 0.05$) if calcium carbonate was used than if Vistacal was included in the diets. Means within a row lacking a common superscript letter differ ($P < 0.05$).

Table 4.8. Duodenal and ileal digesta pH in pigs fed calcium carbonate or Vistacal, 0.4 or 0.8% Ca, and 0 or 1% phytate[1, 5]

	0% phytate					1% phytate					
	Calcium carbonate		Vistacal		Phytate	Calcium carbonate		Vistacal		Phytate	Pooled
Site Ca,%:	0.4%	0.8%	0.4%	0.8%	× site[2]	0.4%	0.8%	0.4%	0.8%	× site[2]	SEM
Duodenal	5.13	5.43	5.29	5.37	b	4.69	4.63	5.06	4.94	c	0.11
Ileal	7.32	7.24	7.08	7.32	a	7.43	7.20	7.43	7.37	a	
Phytate×Source[3]	a		a		-	b		a		-	
Phytate×Ca level[4]	ab	a	ab	a	-	bc	c	bc	c	-	

[1]The effects of phytate level and site of absorption were significant ($P < 0.01$), but the effects of Ca source and Ca level were not significant.

[2]The interaction between phytate level and site of absorption was significant ($P < 0.01$). Ileal samples had greater ($P < 0.05$) pH than duodenal samples regardless of the level of phytate in the diet. The pH in duodenal samples was greater ($P < 0.05$) if no phytate was used than if 1% phytate was included in the diets, but the pH of ileal samples was not influenced by the level of phytate.

[3]The interaction between phytate level and Ca source was significant ($P < 0.05$). If phytate was not used, pH was not affected by the source of Ca. However, if 1% phytate was added to the diet, the pH of digesta from pigs fed Vistacal was greater ($P < 0.05$) than if calcium carbonate was included in the diets. For diets containing calcium carbonate, digesta pH was greater ($P < 0.05$) if phytate was not used than if 1% phytate was included in the diet, but for diets containing Vistacal, no differences in digesta pH were observed between levels of dietary phytate.

[4]The interaction between phytate level and Ca level was also significant ($P < 0.05$). Regardless of the level of phytate in the diet, no differences in digesta pH were observed between diets contained 0.4% and 0.8% Ca. If diets contained 0.4% Ca, no differences in digesta pH were observed between dietary levels of phytate. However, if diets contained 0.8% Ca, digesta pH was greater ($P < 0.05$) if no phytate was used than if 1% phytate was included in the diet.

[5]All other possible interactions were not significant.

CHAPTER 5

CONCLUSIONS

It is concluded that endogenous Ca is lost from the gastrointestinal tract of pigs, therefore, values for apparent total tract digestibility (ATTD) of Ca are influenced by Ca level, but that is not the case for true total tract digestibility (TTTD) of Ca. Thus, TTTD values for Ca are expected to be additive in mixed diets. Endogenous Ca losses before the duodenum are greater than the endogenous Ca losses from jejunum, ileum, and large intestine, but there are no endogenous losses of Ca in the large intestine. Depending on Ca source, Ca may be absorbed before the duodenum or in the jejunum and ileum, but no absorption of Ca occurs in the large intestine. Therefore, total tract collections can be used for Ca digestibility studies with pigs.

It is also concluded that adding microbial phytase to the diets increases Ca and P digestibility, but microbial phytase does not influence the endogenous losses of Ca. Adding synthetic phytate to the diets does not affect the digestibility of Ca. Depending on the source of Ca, standardized duodenal, ileal, and total tract digestibility of Ca may be negatively affected by dietary Ca level, and this is also the case for apparent duodenal, ileal, and total tract digestibility of P.

Values for STTD or TTTD of Ca in feed ingredients need to be determined and if swine diets are formulated based on these values, a better utilization not only of Ca, but also of P, is expected. Factors such as Ca source, dietary Ca level, and inclusion of microbial phytase also need to be considered as diets for pigs are formulated because they influence Ca digestibility.