BASAL ENDOGENOUS LOSS OF CALCIUM AND PHOSPHORUS AND USE OF PHYTASE IN DIETS FOR PIGS

BY

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THESIS

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Abstract

Three experiments were conducted to test a series of hypotheses regarding basal endogenous loss (**BEL**) of Ca and P and the use of microbial phytase in diets for pigs. In experiment 1, the hypothesis was that BEL of P from pigs fed a diet containing spray dried plasma, casein, or potato protein concentrate is not different from that of pigs fed a diet containing gelatin. Forty growing pigs (body weight: 19.34 kg; SD = 0.80) were allotted to four low-P diets using a completely randomized block design with two blocks of 20 pigs and five pigs per diet in each block. The diets were based on cornstarch and sucrose and were formulated to contain 20% gelatin, 20% spray dried plasma, 18.5% casein, or 20% potato protein concentrate as the only source of P in the diets. Results indicated that the BEL of P was greater ($P < 0.001$) in pigs fed diets containing spray dried plasma or potato protein concentrate compared with pigs fed diets containing gelatin or casein, but no differences in BEL of P were observed between pigs fed diets containing casein or gelatin. It was concluded that casein may be used as an alternative to gelatin to estimate BEL of P. In experiment 2, the hypothesis was that increasing dietary phytase reduces BEL of Ca and increases P balance in pigs. Seventy growing pigs (body weight: 17.66 kg; SD = 1.69) were allotted to seven Ca-free diets using a completely randomized block design with two blocks and five pigs per diet in each block. Experimental diets based on corn, potato protein concentrate, and full-fat rice bran were formulated. A positive control diet was formulated to contain P at the requirement for standardized total tract digestible (**STTD**) P and six negative control diets were formulated by reducing the provision of digestible P by 0.15% and adding 0, 250, 500, 1,000, 2,000, or 4,000 phytase units/kg of diet (**FYT**). Results indicated that BEL of Ca was not affected by dietary P, but exponentially decreased ($P = 0.030$) as phytase concentration increased in diets. The STTD of P exponentially increased ($P < 0.001$) as phytase

level increased in diets, but because of the lack of Ca, P retention linearly decreased ($P < 0.001$) as phytase increased in diets. It was concluded that BEL of Ca decreased as dietary phytase increased demonstrating that endogenous Ca can be bound by phytate in the intestinal tract of pigs, but STTD of P increased as phytase level increased in the diets. In experiment 3, the hypothesis was that there are differences in the apparent total tract digestibility (**ATTD**) of Ca and in the response to microbial phytase among sources of Ca carbonate obtained from different parts of the world. Three hundred and twenty growing pigs (body weight: 17.47 kg; SD = 1.28) were allotted to 40 diets using a completely randomized block design with eight blocks of 40 pigs for a total of eight replicate pigs per diet. All experimental diets were based on corn and potato protein concentrate. Twenty sources of Ca carbonate were obtained from different regions of the world (i.e., Europe, United States, Asia, and South Africa), and each source was used in two diets, one diet without microbial phytase and one diet that contained 1,000 FYT. Results indicated that there were no interactions between source of Ca carbonate and phytase, or region and phytase. Differences in ATTD and STTD of Ca were observed among pigs fed diets containing different sources of Ca carbonate ($P < 0.001$) and pigs fed diets containing 1,000 FYT had greater ($P < 0.001$) ATTD and STTD of Ca compared with pigs fed diets containing no phytase. There was a tendency ($P = 0.050$) for pigs fed diets containing different sources of Ca carbonate to have different ATTD of P, but pigs fed diets containing 1,000 FYT had greater ($P < 0.001$) ATTD of P compared with pigs fed diets containing no phytase. The ATTD and STTD of Ca in Ca carbonate from the United States was less ($P < 0.001$) than in Ca carbonate from Europe, Asia, or South Africa. In conclusion, differences in ATTD and STTD of Ca were observed among Ca carbonate sources obtained from different regions of the word, and inclusion of microbial phytase increased the ATTD and STTD of Ca in the sources of Ca carbonate.

Key words: basal endogenous loss, calcium, digestibility, phosphorus, phytase, pigs

To my family and Aaron, thank you for always believing in me.

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Chapter 1: Introduction

Phosphorus and Ca are essential nutrients that must be present in adequate amounts for bone tissue synthesis and for many physiological functions (Crenshaw, 2001). Phosphorus is one of the most expensive nutrients in swine diets and has been the focus of much research because phosphate used to supplement P in diets is a non-renewable resource and P excreted in feces may contribute to environmental pollution (Lautrou et al., 2021). Phosphorus absorption is reduced by phytate and excess Ca in diets (Selle and Ravindran, 2008; Selle et al., 2009), which results in reduced P digestibility and increased P excretion in feces, but inclusion of phytase in diets helps ameliorate some of these negative effects (González-Vega et al., 2013). Inclusion of phytase in diets also increases Ca digestibility (Traylor et al., 2001; Almeida and Stein, 2010; González-Vega et al., 2015), which may lead to an increase in the Ca to P ratio if the Ca released by phytase is not taken into account when formulating diets. Therefore, determining digestibility of dietary Ca and P in feed ingredients and their response to phytase is necessary to accurately formulate diets to meet animal requirements for Ca and P.

It is recommended that diets are formulated using standardized total tract digestibility (STTD) values for Ca and P instead of values for apparent total tract digestibility (ATTD) because STTD values are additive in mixed diets (NRC, 2012). To determine STTD, it is necessary to correct ATTD values for the basal endogenous loss (BEL) of Ca and P (Stein et al., 2007). The BEL of Ca and P can be determined by feeding a diet that is deficient in the respective mineral (Lautrou et al., 2021). Typically, gelatin is used in diets formulated to determine BEL of P because it contains essentially no P and is a good source of protein (Petersen and Stein, 2006). However, other protein sources have been used as alternatives to gelatin in diets used to determine BEL of P (Bünzen et al., 2012; Lopez et al., 2022), but there is a lack of

data to demonstrate if BEL of P obtained from pigs fed diets using these alternatives is the same as that of pigs fed a gelatin diet.

Phytate in plant-based feed ingredients limits the amount of P available for absorption and may form indigestible complexes with dietary and endogenous Ca, resulting in decreased digestibility of P and Ca (Selle and Ravindran, 2008; Selle et al., 2009). Including phytase in pig diets increases the digestibility of P by hydrolyzing phytate molecules and liberating phytate-bound P (Akinmusire and Adeola, 2009; Almeida and Stein, 2010; Poulsen et al., 2010), and results of recent experiments demonstrated a decrease in BEL of Ca when phytase was supplemented to diets for sows (Lee et al., 2019b, a). However, there is no information about how increasing levels of phytase effects BEL of Ca in growing pigs.

Plant-based feed ingredients have low concentrations of Ca and, therefore, Ca must be supplemented by feed mineral sources, such as limestone and Ca carbonate, to meet dietary requirements for pigs. Phytase increases the digestibility of Ca in Ca carbonate and differences in ATTD and STTD of Ca in Ca carbonate produced in the U.S. have been reported (González-Vega et al., 2015; Lee et al., 2019b). These differences may be due to variability in raw materials as well as differences is processing methods. Calcium is commonly oversupplied in diets (Walk, 2016; Lagos et al., 2023) and excess Ca negatively affects phytase activity (Qian et al., 1996; González-Vega and Stein, 2014) and reduces P digestibility, which may result in reduced growth performance in pigs (Stein et al., 2011; González-Vega et al., 2016). Thus, it is important to determine the digestibility of Ca in Ca carbonate so that diets may be formulated using values for digestible Ca rather than total Ca. However, possible differences in digestibility of Ca among different sources of Ca carbonate and their responses to microbial phytase need to be determined. Therefore, the objective of the work included in this thesis was to determine if protein

alternatives to gelatin can be used in diets formulated to determine the BEL of P, to determine the effects of increasing levels of dietary phytase on BEL of Ca, and to determine if there are differences in digestibility of Ca and response to microbial phytase among Ca carbonate obtained from different regions around the world.

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CHAPTER 2: Digestibility of calcium and phosphorus, phytate, and phytase: Literature review

Introduction

Calcium and P are essential minerals that are required to be included in diets for pigs at adequate levels for bone tissue synthesis as well as many other physiological functions. Concentrations of Ca and digestible P in plant-based feed ingredients are often not sufficient to meet the requirements for Ca and P by pigs and, therefore, Ca and P is supplemented by feed mineral ingredients. Plant-based ingredients contain a considerable amount P in the form of phytate-P, but because pigs lack sufficient amounts of mucosal phytase, the majority of this P is inaccessible to pigs (She et al., 2017). Phosphorus that is not absorbed by the pig will be excreted in the feces, which may pose a threat to the environment as it may lead to eutrophication of water sources (Yang et al., 2008). Therefore, microbial phytases are included in swine diets to increase P digestibility, thereby decreasing the need for mineral phosphates and reducing the amount of P excreted into feces (Selle and Ravindran, 2008).

Most of the Ca supplemented in swine diets originates from limestone. Unlike P, Ca is inexpensive and does not pose an environmental threat (Lautrou et al., 2021) and because of this, Ca is commonly oversupplied in swine diets (Walk, 2016). Excess Ca reduces the digestibility of P due to formation of indigestible Ca-P complexes that precipitate in the intestines of the pig (Stein et al., 2011). Phytase increases the digestibility of Ca by hydrolyzing the phytate molecule and preventing formation of indigestible Ca-phytate complexes (Selle et al., 2009), but the efficacy of phytase can be negatively affected by excess dietary Ca (Qian et al., 1996; González-

Vega and Stein, 2014). Therefore, this review focuses on the digestibility of Ca and P and the use of phytase in diets for pigs.

Sources of dietary Ca and P

Calcium and P may be provided in swine diets in the form of plant-based ingredients, animal-based ingredients, and mineral sources where mineral sources usually contribute the greatest amount of Ca and P to diets. Concentrations of Ca and digestible P in plant-based ingredients are often not sufficient to meet the requirement for pigs. Calcium and P provided by cereal grains and cereal grain co-products range from 0.02 to 0.22% and 0.26 to 1.27%, respectively, whereas Ca and P provided by oilseed meals range from 0.33 to 0.69% and 0.71 to 1.08%, respectively (NRC, 2012). Although plant-based feed ingredients contain a significant amount of P, the majority of P is bound to phytate and is, therefore, poorly digested by pigs. In cereal grains and oilseeds, the amount of phytate-P ranges from 0.18 to 0.22% and 0.38 to 0.84%, respectively (NRC, 2012).

Animal-based feed ingredients, including milk, blood meal, fish meal, meat and bone meal, and poultry byproducts, provide 0.02 to 10.94% Ca and 0.21 to 5.26% P (NRC, 2012). Phosphorus in ingredients of animal origin are not bound to phytate, and therefore, the P in these ingredients is highly available to pigs (Kiarie and Nyachoti, 2010). Although P from animal-based ingredients is more available than P from plant-based ingredients, plant ingredients are less expensive, and therefore, are typically the main ingredients in pig diets. In addition to the Ca and P provided by plant and animal feed ingredients, mineral sources of Ca and P are also included to meet dietary requirements for pigs.

Phosphorus is one of the most expensive nutrients in pig diets and is typically provided in the form of mineral phosphates. Monocalcium phosphate (MCP) and dicalcium phosphate (DCP) are the most common sources of mineral P. Both feed phosphates, DCP ($CaHPO_4$) and MCP [$Ca(H_2PO_4)_2$], are produced using the same process from defluroinated phosphoric acid and calcium carbonate (Leikam and Achron, 2005; Lee et al., 2023). Based on their molecular weights, DCP and MCP should contain 29.5 and 17.1% Ca and 22.8 and 26.5% P, respectively. However, actual concentrations of Ca and P are typically less than calculated values due to contaminants that may be present in these ingredients (Baker, 1989; Lima et al., 1995) and average analyzed values are around 24.8 and 16.9% Ca and 18.8 and 21.5% P in DCP and MCP, respectively (NRC, 2012). The addition of microbial phytase to pig diets does not affect the digestibility of Ca or P in MCP and DCP (Lee et al., 2019b; Lopez et al., 2022).

Limestone is the main mineral Ca source used to supplement Ca to diets. Although MCP and DCP contain a significant amount of Ca, they are mainly utilized as sources of P. Limestone predominantly consists of calcium carbonate ($CaCO_3$) and, based on molecular weight, should contain 40.0% Ca and no P; however, due to contaminations with other minerals, analyzed concentrations of Ca and P in calcium carbonate have an average value of 38.5 and 0.02%, respectively (NRC, 2012). Differences in raw materials and production practices among suppliers of calcium carbonate can affect the digestibility of Ca from this ingredient (Lee et al., 2019b). Particle size of calcium carbonate does not affect the digestibility of Ca in pigs (Merriman and Stein, 2016), but the inclusion of phytase increases the digestibility of Ca from calcium carbonate (González-Vega et al., 2015b; Lee et al., 2019b).

Digestibility of Ca and P

Digestibility is the measure of disappearance of a nutrient from the intestines and can be determined by calculating the difference between the amount of nutrient ingested and the amount of nutrient excreted, either in the feces when determining apparent total tract digestibility (ATTD) or in ileal digesta when determining apparent ileal digestibility (AID; Stein, 2017). Apparent ileal digestibility is often used when the digestibility of nutrients is affected by microbial fermentation in the hindgut; however, no significant differences have been observed between AID and ATTD values for Ca and P because no net absorption or secretions of Ca and P occurs in the large intestine (Bohlke et al., 2005; Dilger and Adeola, 2006). Therefore, determining ATTD values of Ca and P is the preferred method because it is easier and more cost effective (Stein, 2017).

Values for ATTD are determined by subtracting the amount of Ca and P excreted in the feces from the intake of Ca and P. Therefore, ATTD of Ca and P can be determined using the Eq. [1] (Almeida and Stein, 2010):

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

where intake and output of Ca and P are expressed in grams per day.

Dietary Ca does not affect the ATTD of Ca when provided between 50 and 150% of the requirement (Stein et al., 2011; González-Vega et al., 2014). Concentrations of dietary P do not affect the ATTD of Ca in diets (Stein et al., 2006; Stein et al., 2008; González-Vega et al., 2016) but increasing concentrations of Ca reduces the ATTD of P (Stein et al., 2011; González-Vega et al., 2013; Velayudhan et al., 2019; Lee et al., 2020). This is due to formation of indigestible Ca-P

complexes that develop and precipitate in the intestines of the pig (Stein et al., 2011). When calculating ATTD, it is assumed that the Ca and P in the feces is from unabsorbed Ca and P that were provided by the diet; however, this calculation does not account for endogenous losses. Increases of ATTD of Ca and P are observed when dietary concentrations increase because endogenous Ca and P, as a percent of total Ca and P excreted, decreases proportionately as Ca or P intake increases (González-Vega et al., 2013; Alves et al., 2016). Therefore, values for ATTD may be underestimated in diets that have low concentrations of the nutrient being tested and, as a consequence, ATTD values are not always additive in mixed diets (Fan and Sauer, 2002; Almeida and Stein, 2010; Zhang and Adeola, 2017) .

Endogenous Ca and P can originate from secretions of the salivary glands, pancreas, gall bladder, and stomach as well as from sloughed-off enterocytes, and mucin (Fan et al., 2001; Vitti and Da Silva Filho, 2010). Endogenous losses can be categorized as either specific endogenous losses, which are losses that are affected by the diet, or basal endogenous losses (BEL), which represent the minimum loss of a nutrient determined by dry matter intake (DMI; Stein et al., 2007).

The BEL of Ca and P can be determined by feeding a diet that is deficient in the respective mineral (Lautrou et al., 2021) and calculated using Eq [2] (adapted from Almeida and Stein, 2010):

$$\text{Basal endogenous loss} = \frac{\text{output of Ca/P}}{\text{DMI}} \times 100, \quad [2]$$

where BEL is expressed in milligrams per kilogram DMI, DMI is expressed as kilogram DMI per day, and fecal output is expressed in grams per day. The value for BEL of P is estimated to be 190 mg/kg DMI and remains relatively constant among experiments regardless of the body

weight (NRC, 2012). This value is consistent with reported values ranging from 139 to 252 mg/kg DMI when a P-free, cornstarch and gelatin-based diet was used (Petersen and Stein, 2006; Almeida and Stein, 2010; Son et al., 2013). Values for BEL of Ca appear to be more variable ranging from 123 to 220 mg/kg DMI if a Ca-free cornstarch diet is used (González-Vega et al., 2015b, a) and 329 to 659 mg/kg DMI when diets are corn-based (Merriman and Stein, 2016; Lee et al., 2019b; Sung et al., 2020). The greater BEL of Ca observed when a corn-based diet is utilized may be due to fiber and anti-nutritional factors, such as phytate, that are present in corn but not in cornstarch (NRC, 2012).

By correcting ATTD values for total BEL, standardized total tract digestibility (STTD) can be calculated for Ca and P using Eq [3] (adapted from Almeida and Stein, 2010):

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

where intake, output, and BEL are expressed in gram per day. Because STTD is not affected by the level of nutrient in the diet, values for STTD are believed to be additive in mixed diets (NRC, 2012; She et al., 2018).

Total endogenous loss (TEL) represents both specific endogenous loss and BEL from the diet (She et al., 2017) and may be determined using the regression method that regresses digested nutrients on different levels of ingested nutrients (Fan et al., 2001). The y-intercept of the regression represents the endogenous loss, whereas the slope represents the value of true total tract digestibility (TTTD). True total tract digestibility can also be determined by correcting ATTD for total endogenous loss using Eq. [4] (Petersen and Stein, 2006):

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

where intake, output, and TEL are expressed in gram per day. Values of TEL for Ca and P range from 160 to 316 mg/kg of DMI (González-Vega et al., 2013; Zhang and Adeola, 2017) and 101 to 670 mg/kg of DMI (Fan et al., 2001; Shen et al., 2002; Ajakaiye et al., 2003; Akinmusire and Adeola, 2009), respectively. Like STTD, TTTD is not affected by the level of nutrient in the diet and, therefore, values for TTTD are believed to be additive in mixed diets (Fang et al., 2007; Zhang and Adeola, 2017).

Phytate

Myo-inositol hexaphosphate (IP6), more commonly known as phytate, is a salt form of phytic acid consisting of an inositol ring and 6 phosphates that are connected to the ring via ester bonds (Selle and Ravindran, 2008). Phytate-P accounts for 50 to 80% of total P in plants and serves as P storage that the plant can utilize during different stages of growth (Viveros et al., 2000; NRC, 2012). A molecule of phytate can carry up to 12 negative charges (Humer et al., 2015) and, because of its strong negative charge at intestinal pH, phytate may chelate positively charged cations such as Ca, Fe, Zn, Mg, K, and Mn, resulting in indigestible mineral-phytate complexes precipitating in the small intestine (Selle et al., 2009). Phytate may also form insoluble complexes with proteins and starch, decreasing the digestibility and utilization of these nutrients (Selle and Ravindran, 2008). In pig diets, the main ingredients are typically plant-based and phytate-P in grains and oilseeds is not fully available for absorption (Selle and Ravindran, 2008). Therefore, a considerable amount of P is excreted into the feces.

Phytate-P is a potential source of P for pigs, but because pigs do not produce a sufficient amount of phytase to hydrolyze all the dietary phytate (Jongbloed et al., 1992), phytate has a negative effect on the digestibility of P (Almaguer et al., 2014; Misiura et al., 2018). Phytate may

bind up to 5 Ca cations, forming indigestible Ca-phytate complexes, and therefore, reduce the digestibility of Ca as well as the digestibility of P (Selle et al., 2009). Although phytate has a lower affinity for Ca cations compared with Zn and Fe, because of the abundance of Ca provided in swine diets, Ca is the cation most likely to form complexes with phytate (Angel et al., 2002; Humer et al., 2015). Calcium-phytate complexes may form in the intestines from Ca provided by plant-based ingredients, mineral supplements, or endogenous Ca secretions (Selle et al., 2009; González-Vega et al., 2015b; Lee et al., 2019a). High concentrations of Ca provided by limestone tend to increase gut pH, which may result in a decrease in exogenous phytase activity and increased precipitation of indigestible Ca-phytate complexes (Selle et al., 2009). Extra dietary Ca may also compete for active sites on phytase, thereby reducing the efficacy of the enzyme (Qian et al., 1996; González-Vega and Stein, 2014). However, Ca provided by DCP or MCP has less binding capacity for the phosphates on phytate because the Ca in these ingredients is already bound to phosphate from phosphoric acid (Walk, 2016; Lee et al., 2019b).

Phytase

For phytate-P to be utilized by pigs, phytase (myo-inositol hexaphosphate phosphohydrolase) must hydrolyze IP6 to release the phytate-P (Selle and Ravindran, 2008). Phytate is hydrolyzed through a series of dephosphorylation reactions that occur in a decremental manner, producing lower inositol phosphate (IP) esters, IP5 to IP1, and ultimately, mineral phosphate and myo-inositol (Selle and Ravindran, 2008; Humer et al., 2015). The complete hydrolysis of phytate will result in the release of 282 g P per kg of phytate (Selle and Ravindran, 2008). Phytase activity is expressed in phytase units (FTU) where one FTU represents the amount of enzyme activity that releases 1 μmol mineral orthophosphate from hydrolysis of

sodium phytate at pH 5.5 at 37 °C (Kornegay, 2001; Engelen et al., 2020). Phytase efficiency can be affected by the stability of pH, proteolytic stability, and temperature stability (Humer et al., 2015). Phytase may originate from feed ingredients, intestinal mucosa, resident gut microbes, or commercially available microbial phytases that are provided as a feed additive (Ravindran, 1995; Selle and Ravindran, 2008).

Corn and oilseeds contain low amounts of intrinsic phytase whereas ingredients such as rye, wheat, triticale, and barley have greater amounts of intrinsic phytase, which contributes to the breakdown of phytate in the gastrointestinal tracts of the pig (Viveros et al., 2000; Rodehutscord et al., 2016). The intrinsic phytase may also increase P digestibility in other feed ingredients (Zimmermann et al., 2003; Archs Toledo et al., 2020). However, phytase in cereal grains is not sufficient to hydrolyze all the dietary phytate (McGhee and Stein, 2019) and is 40% less efficient than microbial phytase (Zimmermann et al., 2002). Intrinsic phytase in plant-based feed ingredients can be affected by the low pH in the upper gastrointestinal tract and by high heat during feed manufacturing and processing, which may result in reduced efficacy of the enzyme (Angel et al, 2002; Rodehutschord and Rosenfelder, 2016).

Pigs have small amounts of phytase in the gastrointestinal tract originating from gut microbiota and intestinal mucosa. The hydrolysis of phytate by endogenous microbial phytase mainly occurs in the hindgut and is considered physiologically irrelevant because no net absorption of P occurs in the large intestine and is, therefore, unavailable for use by pigs (Selle and Ravindran, 2008). Mucosal phytase activity is greatest in the jejunum for IP3, but declines as phosphorylation of the phytate molecule increases, indicating that mucosal phytase may complement exogenous phytase by dephosphorylating lower IP esters (Hu et al., 1996; Selle and Ravindran, 2008). Because of the limited amount of phytase in the mucosa and the location of

the microflora populations, their phytase activity is considered negligible (Schlemmer et al., 2001) and exogenous microbial phytase needs to be supplemented to achieve a measurable release of P from phytate.

Most commercial microbial phytases are obtained from bacteria (Escherichia coli) and fungi (Aspergillus niger and Peniophora lycii; Lautrou et al., 2021). An ideal phytase will remain stable in the gastrointestinal tract, remain active through feed processing and storage, and have low cost (Greiner and Konietzny, 2010). Phytases have many different properties that can affect their efficacy, including pH activity profiles, susceptibility to proteolytic activity, specific activity, and the initiation site of dephosphorylation (Greiner and Konietzny, 2010). The activity of microbial phytase is optimized at pH 5.0 for acid phytases and pH 8.0 for alkaline phytases. The low pH (pH 2.0 to 5.0) in the stomach increases the activity of microbial phytase, whereas the neutral pH in the small intestine (pH 6.5 to 7.5) may result in diminished enzymatic activity (Greiner and Konietzny, 2010); therefore, phytase activity is greatest in the stomach and the upper portion of the small intestine (Jongbloed et al., 1992). Indeed, the main site for hydrolysis of phytate is the stomach (Schlemmer et al., 2001; Blaabjerg and Poulsen, 2010; Mesina et al., 2018). Hydrolysis of phytate in the stomach is ideal because it is upstream of the absorption site of Ca and P (Lautrou et al., 2021) and the low pH may prevent precipitation of Ca-phytate complexes (Selle et al., 2009). Inclusion of exogenous microbial phytase to pig diets may not lead to complete hydrolysis of IP6, but instead may lead to generation of lower IP esters that may be hydrolyzed by mucosal phytase in the small intestine of the pig (Hu et al., 1996) which increases the amount of P released.

For over 30 years, microbial phytases have been available for use in commercial pig diets to increase the availability and utilization of phytate-bound nutrients and reduce the amount of P

excreted in feces (Lei et al., 2013). In a standard commercial swine diet, up to 60% of the phytate (2.8 g of phytate-P/kg) may be hydrolyzed by phytase, resulting in increases of digestible P up to 0.17 percentage units depending on the source and concentration of phytase used, dietary mineral and vitamin D concentrations, and the age of the animals (Adeola and Cowieson, 2011). Phytase is typically included in commercial diets at a concentration between 500 and 1,000 FTU per kg of diet. The addition of 500 FTU to corn and soybean meal diets resulted in approximately 50% of phytate-P being released (Dersjant-Li et al., 2015) and increasing the concentration of phytase to 1,000 FTU per kg of diet degrades approximately 60% of phytate (Létourneau-Montminy et al., 2011).

Including microbial phytase in diets has a positive effect on the digestibility of Ca (Traylor et al., 2001; Almeida and Stein, 2010; Poulsen et al., 2010; González-Vega et al., 2015b). In diets containing up to 4,000 FTU, Ca digestibility was maximized at an inclusion of 1,000 FTU indicating that high levels, or super-dosing, of phytase may not be needed to maximize digestibility of Ca (Almeida et al., 2013). However, in experiments with newer phytases, linear increases in the digestibility of Ca and P were observed even when up to 4,000 FTU of phytase was used (Espinosa et al., 2022; Lagos et al., 2022). It therefore appears that some of the newer phytases are more efficient than older phytases and release of up to 80% of the phytate bound P and Ca appear to be possible. Microbial phytases increase the digestibility of calcium carbonate, but have no effect on the digestibility of MCP and DCP (Lee et al., 2019b). This is likely due to Ca in DCP and MCP being bound to phosphates in the phosphoric acid, resulting in Ca being less available to bind to phytate in the intestines (Walk, 2016; Lee et al., 2019b). The inclusion of 500 FTU microbial phytase decreased basal endogenous loss of Ca in growing pigs and sows (Lee et al., 2019b, a). Reducing dietary Ca in pig diets supplemented with

phytase should be considered because phytase may widen the Ca to P ratio in pig diets when endogenous Ca and Ca released from phytate are not accounted for in diet formulations (Walk, 2016).

Conclusion

Calcium and P are important minerals needed for bone growth and several physiological functions. Due to the low concentration of Ca and digestible P in plant-based ingredients, these minerals must be supplemented in swine diets to meet the requirements for pigs. Phytase is mainly used in diets to increase the digestibility of P in plant-based ingredients, thereby reducing the amount of P that needs to be supplemented in the diet. As the requirement for feed phosphates such as MCP and DCP is reduced by the addition of phytase, more Ca may need to be supplemented by limestone orcalcium carbonate. Phytase increases the digestibility of Ca from calcium carbonate and reduces the BEL of Ca in pigs, but differences in the digestibility of Ca among sources of calcium carbonate have been reported. An oversupply of dietary Ca can result in reduced P digestibility and may negatively affect exogenous phytase activity. Therefore, more research is needed to determine the digestibility of Ca in different sources of calcium carbonate as well as the effect of phytase on dietary and endogenous Ca.

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CHAPTER 3: Effects of different protein sources in low-phosphorus diets on the basal endogenous loss of phosphorus by growing pigs

Abstract

The objective of this experiment was to test the hypothesis that the basal endogenous loss (**BEL**) of P from pigs fed a diet containing spray dried plasma, casein, or potato protein concentrate is not different from that of pigs fed a diet containing gelatin. Forty pigs (body weight: 19.34 kg; SD = 0.80) were housed individually in metabolism crates and allotted to four low-P diets using a complete randomized block design with two blocks of 20 pigs and five pigs per diet in each block. The diets were based on cornstarch and sucrose and contained 20% gelatin, 20% spray dried plasma, 18.5% casein, or 20% potato protein concentrate. With the exception of Ca and P, diets were formulated to meet requirements. Feces and urine samples were collected separately for 4 d following a 5 d adaptation period. Feces samples were dried, ground, and feces and urine samples were analyzed for P. Data were analyzed using a model that included diet as fixed effect and group as random effect. Results indicated that feed intake and fecal excretion of dry matter were greater ($P < 0.05$) in pigs fed diets containing spray dried plasma, casein, and potato protein concentrate compared with pigs fed the gelatin diet, with pigs fed potato protein concentrate having the greatest ($P < 0.05$) excretion. The apparent total tract digestibility (**ATTD**) of dry matter was least ($P < 0.001$) in pigs fed the diet containing potato protein concentrate, but there were no differences among gelatin, spray dried plasma, and casein diets. The ATTD of P was greater ($P < 0.001$) in pigs fed diets containing spray dried plasma and casein compared with pigs fed gelatin and potato protein concentrate diets. The BEL of P was not different between the gelatin and casein diets, but pigs fed diets containing spray dried plasma or potato protein

concentrate had greater ($P < 0.001$) BEL of P compared with pigs fed gelatin or casein diets. In conclusion, the BEL of P was greater if calculated from diets containing spray dried plasma or potato protein concentrate compared with gelatin. However, casein may be an alternative to gelatin to estimate the BEL of P because casein provides a greater amount of P compared with gelatin which compensates for the deficient level of P in gelatin, but the endogenous loss of P determined from pigs fed a casein-based diet is not different from that of pigs fed a gelatin-based diet.

Key words: digestibility, endogenous loss, P-free diet, pig, phosphorus

Abbreviations: ATTD, apparent total tract digestibility; BEL, basal endogenous loss; DM, dry matter; DMI, dry matter intake; STTD, standardized total tract digestibility

Introduction

Phosphorus is one of the most expensive nutrients used in swine diets. Formulating diets based on values for standardized total tract digestibility (**STTD**) of P instead of values for apparent total tract digestibility (**ATTD**) may reduce the cost of diets because STTD values, unlike ATTD values, are additive in mixed diets, which may prevent an oversupply of P (Almeida and Stein, 2010). Values for STTD of P in plant ingredients, animal proteins, and feed phosphates have been determined (NRC, 2012; Stein et al., 2016) by correcting ATTD of P for the endogenous P loss. The basal endogenous loss (**BEL**) of P can be estimated using a P-free diet and values are relatively constant for growing-finishing pigs regardless of the body weight and dietary Ca concentration (Baker et al., 2013; Son and Kim, 2015; Kim et al., 2017). There are, however,

differences in endogenous loss of P between growing pigs and gestating sows (Son and Kim, 2015; Bikker et al., 2017; Lee et al., 2018).

Gelatin has been widely used in P-free diets because it does not contain any P and has high levels of digestible amino acids (Petersen et al., 2005; Petersen and Stein, 2006). However, most gelatin products are in a powder form that may make diets dusty and sticky, which can make it difficult to work with and may result in reduced palatability of diets. In addition, feeding pigs a diet containing no P may result in physiological issues in pigs (Fan et al., 2001; She et al., 2017). Therefore, spray dried plasma, casein, or potato protein concentrate are possible protein alternatives to gelatin because P in spray dried plasma and casein is close to 100% digestible and potato protein concentrate has very low concentrations of P (NRC, 2012). Indeed, blood plasma is sometimes used to determine the BEL of P (Bünzen et al., 2012) because it is assumed that the P contributed by plasma is 100% absorbed (Almeida and Stein 2011). However, to our knowledge, it has never been confirmed that the BEL of P is the same if estimated from diets containing gelatin, spray dried plasma, casein, or potato protein concentrate. Therefore, the objective of this experiment was to test the hypothesis that the BEL of P from pigs fed a diet containing spray dried plasma, casein, or potato protein concentrate are not different from that of pigs fed a diet containing gelatin.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Diets, animals, and experimental design

Four semi-purified, low-P diets were formulated based on cornstarch and sucrose (Table 3.1). These diets also contained 20% gelatin, 20% spray dried plasma, 18.5% casein, or 20% potato protein concentrate which were the only source of P provided in the diets. Crystalline amino acids, vitamins, and minerals with the exception of Ca and P were included in the diets to meet the current requirements for 11 to 25 kg pigs (NRC, 2012).

Forty pigs (body weight: 19.34 kg; SD = 0.80) were allotted to the four diets using a complete randomized block design with two blocks of 20 pigs for a total of 10 replicate pigs per diet. Pigs were individually housed in metabolism crates that were equipped with a nipple waterer, a feeder, and fully slated floors. A mesh screen and pan were installed under the slatted floor during the collection period allowing for total, but separate, feces and urine collection.

Feeding and sample collection

Daily feed allotments were divided into two equal meals that were provided at 0800 and 1600 h and pigs were provided feed at 2.5 times the daily maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy per kg body weight$^{0.60}$; NRC, 2012). Experimental diets were fed for 12 d, with the initial 5 d considered the adaptation period. Samples of the diets and main ingredients were collected at the time of mixing. Feces were collected for 4 d following the adaptation period using the marker-to-marker procedure (Adeola, 2001). Fecal collection began when the first marker (i.e., chromium oxide) fed in the morning of d 6 appeared in the feces and ceased once the second marker (i.e., ferric oxide), which was fed in the morning of d 10, appeared in the feces. Urine was collected from d 6 to d 10. Feed consumption was recorded daily, and orts were collected to determine feed intake from d 6 to d 10. Pigs had free access to water throughout the experiment.

Fecal collection occurred twice daily, and samples were stored at −20 °C immediately following collection. Buckets containing a preservative of 50 mL of 6*N* HCl were placed under each crate for urine collection. Urine buckets were weighed and emptied once per day and 10% of the collected urine was stored at −20 °C. At the conclusion of the experiment, urine samples were thawed at room temperature and subsamples were collected for analysis.

Chemical analysis

Fecal samples were thawed, dried in a 50 °C forced air drying oven, and finely ground using a 500G swing type grain mill (RRH, Zhejiang, China) prior to analysis. The dry matter (**DM**) in diets and ingredients was determined in duplicate by oven drying at 135 °C for 2 h (method 930.15; AOAC Int., 2019). Diets were also analyzed in duplicate for ash at 600 °C for 2 h (method 942.05; AOAC Int., 2019). Diet and ingredient samples were analyzed for Ca and P and feces and urine samples were analyzed for P (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). Diets were also analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL) and diets and ingredients were analyzed for N using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI). Crude protein was calculated as N × 6.25.

Calculations

The BEL of P was calculated using the fecal flow of P and feed intake of pigs and was expressed as mg/kg of DM intake (**DMI**) using the following equation (adapted from Almeida and Stein, 2010):

$$\text{Basal endogenous loss} = \frac{\text{fecal P output}}{\text{DMI}} \times 100,$$

where BEL is in mg/kg of DMI, DMI is in kg DM per day, and fecal output is expressed in grams per day.

The ATTD of P in each experimental diet was calculated using the following equation (Almeida and Stein, 2010):

$$\text{ATTD (\%)} = \frac{\text{P intake - P output}}{\text{P intake}} \times 100,$$

where both P intake and fecal P output are expressed in grams per day.

Retention of P was calculated using the following equation (Fernández, 1995):

$$\text{Retention (\%)} = \frac{\text{P intake - (fecal P output + urine P output)}}{\text{P intake}} \times 100,$$

where intake, fecal output, and urine output are expressed in grams per day.

Statistical analysis

Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures (SAS Inst. Inc., Cary, NC, USA) and outliers were identified using Internally Studentized Residuals (Tukey, 1977). Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC, USA). The model included diet as fixed effect and group as random effect. Pig was the experimental unit for all analyses and results were considered significant at *P*

≤ 0.05 and a trend at $P \leq 0.10$. Treatment mean values were calculated using the LSMeans statement in SAS and, if significant, were separated using PDIFF option with Tukey's adjustment. An alpha value of 0.05 was used to assess significance among means.

Results

Feed intake and fecal excretion were greater ($P < 0.05$) for pigs fed diets containing spray dried plasma, casein, or potato protein concentrate compared with pigs fed the gelatin diet, but pigs fed the diet containing potato protein concentrate had the greatest ($P < 0.05$) fecal excretion compared with pigs fed the other protein sources (Table 3.4). The ATTD of DM was lower ($P < 0.05$) for the diet containing potato protein concentrate compared with diets containing gelatin, spray dried plasma, or casein. Urine excretion of P from pigs fed the gelatin diet was not different from that of pigs fed the other diets, but pigs fed the spray dried plasma diet had greater ($P < 0.05$) urine excretion compared with pigs fed diets containing casein or potato protein concentrate.

Phosphorus intake was different ($P < 0.05$) between all diets, with pigs fed the diet containing gelatin having the lowest and pigs fed the diet containing spray dried plasma having the greatest P intake. There was no difference in concentration of fecal P (%) from pigs fed the gelatin diet and pigs fed the casein diet, but pigs fed the gelatin diet had less ($P < 0.05$) fecal P output (g/d) compared with pigs fed diets containing the other protein sources. Absorbed P was greatest ($P < 0.05$) by pigs fed the diet containing spray dried plasma, but no differences in absorbed P were observed between pigs fed the gelatin and casein diets. Pigs fed diets containing spray dried plasma or casein had greater ($P < 0.05$) ATTD of P compared with pigs fed diets containing gelatin and potato protein concentrate. The BEL of P was not different between the

gelatin and casein diets, but pigs fed diets containing spray dried plasma or potato protein concentrate had greater ($P < 0.05$) BEL of P than pigs fed the other diets. Phosphorus excreted in urine, expressed as % or g/d, was not different among diets containing gelatin, casein, or potato protein concentrate, but pigs fed diets containing spray dried plasma had greater ($P < 0.05$) urine P excretion compared with pigs fed the other protein sources. Retention of P was lowest ($P < 0.05$) in pigs fed diets containing gelatin or potato protein concentrate and was greatest ($P < 0.05$) in pigs fed spray dried plasma diet.

Discussion

Concentrations of DM and crude protein in gelatin, spray dried plasma, casein, and potato protein concentrate and analyzed concentrations of Ca and P in spray dried plasma and casein were in agreement with expected values (NRC, 2012). Calcium and P concentrations in potato protein concentrate were consistent with reported results (Nelson et al., 2022).

Differences observed in P intake among the diets were expected due to the different amounts of P in each diet. The observation that pigs fed the gelatin diet had the lowest feed intake is in agreement with previous observations (Stein et al., 2006; Alves et al., 2016) which may be due to the reduced palatability caused by the gelatin. Differences observed in P intake, fecal P (expressed as % and g/d), and absorbed P were expected due to the differences in P provided by the diets. However, although no differences in feed intake were observed among pigs fed spray dried plasma, casein, or potato protein concentrate diets, pigs fed the diet containing potato protein concentrate had greater fecal P output (g/d). This is likely due to increased fecal excretion observed by pigs fed the potato protein concentrate diet, which could be the result of fiber and phytate in the potato protein concentrate (NRC, 2012; Nelson et al.,

2022). The observation that P concentration in the urine, expressed as % or g/d, was greatest in pigs fed spray dried plasma diet was expected due to greater dietary concentration of P.

The observation that apparent total tract digestibility of P was negative in pigs fed diets containing gelatin and potato protein concentrate is a result of the low concentration of P in these diets and pigs were, therefore, excreting more P than they were ingesting from the diets. In contrast, the greater ATTD of P in the spray dried plasma and casein diets was a result of the greater concentration of P in these diets and reflects that endogenous P, as a percent of the total P excreted, decreases proportionately as P intake increases (Alves et al., 2016). Values for ATTD of P in pigs fed spray dried plasma and casein diets are consistent with reported values (NRC, 2012).

The BEL of P observed in pigs fed the gelatin diet (176 mg/kg of DMI) is close to the 190 mg/kg of DMI, which the NRC (2012) designated as a representative value for BEL of P. Observed BEL of P from pigs fed the diet containing spray dried plasma (338 mg/kg of DMI) is consistent with the value of 370 mg/kg of DMI that was recently observed by Bailey et al. (2023), where spray dried plasma was the only source of P in the diet. In Brazil, blood plasma is sometimes utilized as a protein source in low-P diets to determine the BEL of P (Bünzen et al., 2012). Due to the high digestibility of P in spray dried plasma, it is assumed that P provided by spray dried plasma is 100% absorbed, and therefore, any P present in the feces is of endogenous origin (Almeida and Stein, 2011). However, BEL of P measured from the diet containing spray dried plasma was greater than that measured from pigs fed the gelatin diet, indicating that not all P provided by spray dried plasma was absorbed by the pig. Therefore, spray dried plasma may result in an overestimation of BEL of P. The BEL of P observed in pigs fed potato protein concentrate (374 mg/kg of DMI) is slightly greater than results observed by Lopez et al. (2022)

who reported a value of 308.5 of mg/kg of DMI when utilizing potato protein concentrate as the only source of P in the diet. The BEL of P observed from pigs fed the potato protein concentrate diet was greater than that observed in pigs fed the gelatin diet, indicating that the P provided from potato protein concentrate is not 100% absorbed by the pig. Therefore, potato protein concentrate may also result in an overestimation of BEL of P. The observed BEL of P in pigs fed the casein diet (234 mg/kg DMI) is close to the range (110 to 226 mg/kg DMI) reported by Pettey et al. (2006) and because this value was not different from the value obtained for the gelatin diet it is concluded that casein may be used as an alternative to gelatin in P-free diets used to estimate the BEL of P.

In conclusion, BEL of P was greater if calculated from pigs fed diets containing spray dried plasma or potato protein concentrate compared with gelatin. However, no difference between the casein and gelatin diet was observed and casein may be used as an alternative to gelatin to estimate the BEL of P. Casein provides a greater amount of P compared with gelatin, which compensates for the deficient level of P in gelatin, but does not affect values for the BEL of P.

Tables

Table 3.1. Ingredient composition of experimental diets

Item,%	Protein Source			
	Gelatin	Spray dried plasma	Casein	Potato protein concentrate
Corn, starch	46.81	49.38	51.36	49.50
Sucrose	20.00	20.00	20.00	20.00
Gelatin	20.00	-	-	-
Spray dried plasma	-	20.00	-	-
Casein	-	-	18.50	-
Potato protein concentrate	-	-	-	20.00
Soybean oil	4.00	4.00	4.00	4.00
Cellulose	4.00	4.00	4.00	4.00
$_{L}$-Arg	-	-	-	-
$_{L}$-His	0.30	-	-	-
$_{L}$-Ile	0.39	0.18	-	-
$_{L}$-Leu	0.75	-	-	-
$_{L}$-Lys·HCL	0.66	0.04	-	0.20
$_{DL}$-Met	0.20	0.24	-	0.05
$_{L}$-Thr	0.38	0.02	0.08	-
$_{L}$-Trp	0.18	-	-	0.05
$_{L}$-Val	0.38	-	-	-
Limestone, ground	0.55	0.74	0.66	0.80
Potassium carbonate	0.40	0.40	0.40	0.40

Table 3.1. (cont.)

Magnesium oxide	0.10	0.10	0.10	0.10
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin-mineral premix	0.50	0.50	0.50	0.50

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D3 as cholecalciferol, 1,660 IU; vitamin E as DL-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 3.2. Analyzed nutrient composition of experimental diets (as-fed basis)

Item, %	Protein source			
	Gelatin	Spray dried plasma	Casein	Potato protein concentrate
Dry matter, %	91.62	91.76	92.01	92.95
Crude protein, %	21.12	17.91	16.30	15.87
Gross Energy	4,107	4,045	4,113	4,212
Ash, %	1.44	3.36	1.76	2.07
Ca, %	0.30	0.37	0.28	0.38
P, %	0.01	0.31	0.14	0.03
Calculated				
Ca, %	0.21	0.30	0.26	0.31
P, %	0.01	0.32	0.13	0.02

Table 3.3. Analyzed nutrient composition of ingredients (as-fed basis)

Item, %	Protein source			
	Gelatin[1]	Spray dried plasma[2]	Casein[3]	Potato protein concentrate[4]
Dry matter, %	87.49	87.70	88.71	91.84
Crude protein, %	101.04	75.96	87.83	82.31
Ca, %	0.04	0.12	0.03	0.02
P, %	0.05	1.62	0.71	0.10

[1]Gelatin obtained from Gelita USA Inc. (Sioux City, IA, USA).

[2]Spray dried plasma was obtained from APC Inc. (Ankeny, IA, USA).

[3]Casein was obtained from NZMP (Auckland, New Zealand).

[4]Potato protein concentrate was obtained from Royal Avebe (Veendam, Netherlands).

Table 3.4. Basal endogenous loss and retention of P by pigs fed low-P diets[1,2]

Item, %	Protein source					
	Gelatin	Spray dried plasma	Casein	Potato Protein Concentrate	SEM	*P*-value
Feed intake, kg/d	0.59[b]	0.72[a]	0.73[a]	0.73[a]	0.02	< 0.001
Fecal excretion, g DM/d[1]	21.13[c]	31.12[b]	29.07[b]	43.16[a]	0.002	< 0.001
ATTD of DM[1], %	96.09[a]	95.29[a]	95.65[a]	93.60[b]	0.31	< 0.001
Urine excretion, kg/d	3.93[ab]	4.93[a]	2.93[b]	2.59[b]	0.57	0.004
P						
P intake, g/d	0.09[d]	2.23[a]	1.12[b]	0.23[c]	0.02	< 0.001
P in feces, %	0.45[c]	0.71[a]	0.53[bc]	0.57[b]	0.04	< 0.001
Fecal P output, g/d	0.10[c]	0.22[a]	0.16[b]	0.25[a]	0.01	< 0.001
Absorbed P, g/d	-0.01[c]	2.01[a]	0.96[b]	-0.03[c]	0.02	< 0.001
ATTD of P	-6.76[b]	92.03[a]	84.94[a]	-12.10[b]	5.50	< 0.001
BEL of P, mg/kg DMI[1]	176[b]	338[a]	234[b]	374[a]	16.8	< 0.001
P in urine, %	0.0003[b]	0.0144[a]	0.0005[b]	0.0005[b]	0.001	< 0.001
Urine P output, g/d	0.01[b]	0.68[a]	0.01[b]	0.01[b]	0.04	< 0.001

Table 3.4. (cont.)

P retention, g/d	-0.02[c]	1.33[a]	0.96[b]	-0.04[c]	0.04	< 0.001

[a,b,c,d]Means within a row without a common superscript letter are different ($P < 0.05$).

[1]DM = dry matter; ATTD = apparent total tract digestibility; BEL = basal endogenous loss; DMI = dry matter intake.

[2]Least squares means represent 10 observations per diet.

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CHAPTER 4: Microbial phytase reduces basal endogenous loss of calcium in pigs fed diets containing phytate phosphorus at commercial levels

Abstract

The objective of this experiment was to test the hypothesis that increasing dietary phytase reduces basal endogenous loss of Ca and increases P balance in pigs. Seventy barrows (body weight: 17.66 kg; SD = 1.69 kg) were allotted to seven Ca-free diets using a randomized complete block design with two blocks and five pigs per diet in each block. All diets were based on corn, potato protein concentrate, and full-fat rice bran. A positive control (**PC**) diet was formulated to contain P at the requirement for standardized total tract digestible (**STTD**) P by 11 to 25 kg pigs. Six negative control (**NC**) diets were formulated by reducing the provision of digestible P by 0.15% and adding 0, 250, 500, 1,000, 2,000, or 4,000 phytase units/kg diet. Pigs were housed individually in metabolism crates that allowed for total, but separate, collection of urine and feces. Daily feed allowance was 3.0 times the maintenance requirement for metabolizable energy and was divided into two equal meals. Diets were fed for 12 d with the first 5 d considered the adaptation period. Urine collections started on d 6 in the morning and ceased on d 10 in the morning. Fecal markers were also included in the morning meals fed on d 6 and d 10 and feces were collected according to the marker-to-marker procedure (Adeola, 2001). Results indicated that the apparent total tract digestibility of dry matter was not affected by dietary P or phytase levels. The basal endogenous loss of Ca was not affected by dietary P, but exponentially decreased ($P = 0.030$) as phytase level increased in the diets. Phosphorus retention (g/d) and standardized total tract digestibility of phosphorus were greater ($P < 0.05$) in pigs fed

the PC diet compared with pigs fed the NC diet with no phytase. The STTD of P exponentially ($P < 0.001$) increased as phytase level increased in the diets, but because of the lack of Ca, retention of P (% of absorbed) linearly decreased ($P = 0.006$) as phytase increased. In conclusion, basal endogenous loss of Ca decreased as dietary phytase increased demonstrating that endogenous Ca can be bound to phytate in the intestinal tract of pigs. However, STTD of P increased as phytase level in the diets increased.

Key words: calcium, digestibility, endogenous loss, phosphorus, phytase, phytate

Abbreviations: ATTD, apparent total tract digestibility; DM, dry matter; DMI, dry matter intake; FTU, phytase unit; NC, negative control; PC, positive control; STTD; standardized total tract digestibility

Introduction

Most P in plant-based feed ingredients is bound to phytate, which limits the amount of P that is available for absorption (Selle and Ravindran, 2008), but microbial phytase in pig diets increases the digestibility of P (Poulsen et al., 2010; Rojas and Stein, 2012). Digestibility of P may be negatively affected by excess dietary Ca (Stein et al., 2011; Lee et al., 2020), but to a lesser extent if phytase is included in the diet than if no phytase is used (González-Vega et al., 2013). A molecule of phytate can chelate Ca cations resulting in the formation of insoluble Ca-phytate complexes that reduce digestibility of both Ca and P (Selle et al., 2009). Although inclusion of 1,500 units/kg of an E-coli microbial phytase to diets based on canola meal did not influence the total endogenous loss of Ca in growing pigs (González-Vega et al., 2013), addition of 500 units of an E-coli microbial phytase reduced the basal endogenous loss of Ca and increased

digestibility of Ca and P (Lee et al., 2019a; 2019b). The basal endogenous loss of Ca is determined using a Ca-free diet whereas the total endogenous loss often is determined using the regression procedure (NRC, 2012). The decrease in basal endogenous loss of Ca may be due to a reduction in the amount of phytate (i.e., inositol hexakisphosphate) that can form Ca-phytate complexes if phytase is added to the diet, resulting in an increase in absorption of endogenous Ca and a reduced amount of endogenous Ca being excreted in feces (Lee et al., 2019a). If indeed the reduced endogenous loss of Ca is a result of degradation of phytate, it is expected that increased doses of dietary phytase will reduce endogenous losses of Ca, but this hypothesis has not been experimentally verified. Therefore, an experiment was conducted to test the hypothesis that increasing dietary phytase reduces basal endogenous loss of Ca and increases P digestibility in growing pigs.

Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. The pigs used in the experiment were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN).

Diets, animals, and experimental design

Seven Ca-free diets were formulated (Tables 4.1 and 4.2). The positive control (**PC**) diet contained P at the requirement for standardized total tract digestible (**STTD**) P for 11 to 25 kg pigs (i.e., 0.33% STTD P; NRC, 2012). A negative control (**NC**) diet was formulated by reducing the provision of STTD P by 0.15 percentage units, which was the assumed release of STTD P obtained by inclusion of 1,000 phytase units (**FTU**)/kg. The NC diet, therefore,

contained 0.18% STTD P. Five additional diets were formulated by adding 250, 500, 1,000, 2,000, or 4,000 FTU/kg of a novel consensus bacterial 6-phytase variant (Danisco Animal Nutrition & Health – IFF, Oegstgeest, The Netherlands) to the NC diet. All diets were based on corn, potato protein concentrate, and full-fat rice bran (Table 4.3). Vitamins and minerals, with the exception of Ca and P, were included in all diets to meet or exceed current requirement estimates (NRC, 2012).

Seventy barrows (initial body weight: 17.66 kg; SD = 1.69 kg) were allotted to the seven diets using a randomized complete block design with two blocks of 35 pigs. Weaning group was the block and there were five replicate pigs per diet in each block for a total of 10 replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a feeder, a nipple drinker, and a fully slatted floor. A screen floor and a urine tray were placed under the slatted floor to allow for total, but separate, collection of urine and feces.

Feeding and sample collection

Pigs were provided feed at 3.0 times the daily maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy per kg body weight$^{0.60}$; NRC, 2012). Daily feed allotments were divided into two equal meals provided at 0800 and 1600 h. Pigs were fed experimental diets for 12 d, with the initial 5 d considered the adaptation period. Feces were collected for 4 d following the adaptation period using the marker-to-marker procedure (Adeola, 2001). Indigo carmine was fed in the morning of d 6 and fecal collection began when the marker appeared in the feces. Fecal collection ceased when the second marker, ferric oxide, which was fed in the morning of d 10, appeared in the feces. Urine was collected from d 6 to d 10. Feed consumption was recorded daily and orts were collected to determine feed intake from d 6 to d 10. Pigs had free access to water throughout the experiment.

Fecal collection occurred twice daily and samples were stored at −20 °C immediately after collection. Urine was collected in buckets containing a preservative of 50 mL of 6*N* HCl that were placed under each metabolism crate. The buckets were weighed and emptied once per day and 10% of the collected urine was stored at −20 °C.

Chemical analysis

Samples of the main ingredients and diets were collected at the time of diet mixing for chemical analysis. Ingredients were analyzed for phytic acid before diets were formulated (Ellis et al., 1977), and diets were analyzed for phytase activity (method 2000.12; AOAC Int., 2019) before feeding was initiated. Fecal samples were thawed and then dried in a 65°C forced air drying oven and ground using a 500G stainless steel mill grinder (RRH, Zhejiang, China). Urine samples were thawed at room temperature and subsamples were collected and filtered for analysis. Diet, ingredient, and dried fecal samples were analyzed in duplicate for dry matter (**DM**) by oven drying at 135 °C for 2 h (method 930.15; AOAC Int., 2019). These samples were also analyzed for ash at 600 °C for 2 h (Method 942.05; AOAC Int., 2019), and for N using the combustion procedure (Method 990.03; AOAC Int., 2019). Crude protein was calculated as N × 6.25. Fecal, urine, diet, and ingredient samples were analyzed for Ca and P (Method 985.01 A, B, and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA) after wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000).

Calculations

Phytate in the diets was calculated by multiplying the analyzed phytate in corn, potato protein concentrate, and rice bran by the inclusion rate of each ingredient in the diet and by adding the

values. Phytate-P was calculated by multiplying phytate by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated as the difference between phytate-P and total P.

The basal endogenous loss of Ca was calculated using the fecal flow of Ca and feed intake of pigs and was expressed as mg/kg DM intake (**DMI**) using the following equation (adapted from Almeida and Stein, 2010):

$$\text{Basal endogenous loss} = \frac{\text{fecal Ca output}}{\text{DMI}} \times 100,$$

where basal endogenous loss is in mg/kg DMI, DMI is in kg DM per day, and fecal output of Ca is in gram per day.

The apparent total tract digestibility (**ATTD)** of P in each experimental diet was calculated according to the following equation (Almeida and Stein, 2010):

$$\text{ATTD (\%)} = \frac{\text{P intake - fecal P output}}{\text{P intake}} \times 100,$$

where both P intake and fecal P output are expressed in grams per day.

The STTD (%) of P in each experimental diet was calculated by correcting the ATTD of P for the average basal endogenous loss of P (i.e., 190 mg/kg DMI; NRC, 2012).

Retention of P (%) was calculated using the following equation (Fernández, 1995):

$$\text{Retention (\%)} = \frac{\text{P intake - (fecal P output + urine P output)}}{\text{P intake}} \times 100,$$

where P intake, fecal P output, and urine P output are expressed in grams per day.

Statistical analysis

Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures (SAS Inst. Inc., Cary, NC, USA) and outliers were identified using Internally Studentized Residuals (Tukey, 1977). Identified outliers were excluded from the final statistical analysis; if the number of observations was not identical among the dietary treatments, an average SEM was used. Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC, USA). The model included diet as fixed effect and block as random effect. Mean values were calculated using the LSMeans statement. Contrast statements were used to analyze PC vs. NC diets and linear effects of increasing phytase in NC diets. Using JMP software in SAS, exponential curve fitting was also analyzed with level of phytase in NC diets as the X variable and response criteria as the Y variable. Pig was the experimental unit for all analyses and results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

Results

The basal endogenous loss of Ca by one pig fed the PC diet and one pig fed the diet containing 2,000 FTU/kg were identified as outliers and removed. Therefore, these diets had 9 observations for the endogenous loss of Ca. No other outliers were identified and there were, therefore, 10 observations for all other criteria. Feed intake, weights of fecal and urine excretion, and the ATTD of DM were not affected by experimental diets (Table 4.4). Calcium excretion in feces expressed as percent of feces and as g/d was not different between the PC and NC diets, but linearly ($P < 0.05$) decreased as phytase increased in the diets. Basal endogenous loss of Ca was not different between the PC and NC diet. However, as dietary phytase increased, basal endogenous loss of Ca exponentially ($P = 0.030$) decreased with the basal endogenous loss of Ca

in the diet containing 4,000 FTU/kg being the least among all diets. Calcium excretion in urine expressed in mg/kg and g/d was not affected by increasing phytase in the NC diets, but Ca in urine tended ($P = 0.06$) to be less from pigs fed the NC diet compared with pigs fed the PC diet.

Phosphorus intake, concentration of P in feces, absorbed P, the ATTD of P, and the STTD of P were greater ($P < 0.05$) for pigs fed the PC diet compared with pigs fed the NC diet (Table 4.5). Concentration of P in the urine and P retention (g/d) were greater ($P < 0.001$) for pigs fed the PC diet compared with those fed the NC diet. However, P retention calculated as percent of intake and absorbed P was not different between the 2 diets.

As phytase level increased in the NC diets, P excretion in feces, expressed as percent of feces or as g/d, exponentially ($P < 0.001$) decreased, which resulted in an exponential increase ($P < 0.001$) in the ATTD and STTD of P. Phosphorus excretion in urine (g/d) exponentially ($P = 0.009$) increased as phytase in the NC diet increased. Retention of P (percent of intake) was not affected by dietary phytase, but retention of P calculated as percent of absorbed P linearly ($P = 0.006$) decreased as dietary phytase increased.

Discussion

The basal endogenous loss of Ca is a loss of Ca that is diet-independent whereas the specific endogenous loss is diet-dependent as has been described for amino acids and P (Stein et al., 2007; NRC, 2012). Total endogenous loss of Ca, thus, includes both the basal and specific endogenous losses. The basal endogenous loss of Ca by pigs fed cornstarch-based Ca-free diets is less compared with pigs fed a corn-based diet (González-Vega et al., 2015), but because corn-based diets have concentrations of fiber and phytate resembling commercial diets, corn based diets usually are used to determine the basal endogenous loss of Ca (Lee et al., 2019a; 2019b).

The very low levels of Ca (0.02 to 0.04%) in the diets used in the experiment was assumed not to impact calculated endogenous losses because only approximately 0.25% of diet Ca will be excreted in the feces.

Analyzed concentrations of P and Ca in corn, potato protein concentrate, and full-fat rice bran were consistent with previous data (Casas and Stein, 2015; Lee et al., 2019a). The basal endogenous loss of Ca in pigs fed the PC and NC diets was within the range of values observed in growing pigs fed corn-based, Ca-free diets (i.e., 329 to 659 mg per kg DMI; Merriman and Stein, 2016; Lee et al., 2019b; Sung et al., 2020). The observation that the basal endogenous loss of Ca was reduced by inclusion of microbial phytase in the diets was consistent with results from both gestating sows and growing pigs (Lee et al., 2019a; 2019b). The negatively charged phytate molecule has the ability to chelate endogenous Ca cations resulting in formations of insoluble Ca-phytate complexes (Selle et al., 2009). Therefore, it is likely that the observed reduction in basal endogenous loss of Ca that was caused by dietary phytase is due to the decreased concentration of phytate molecules that may form non-digestible complexes with Ca when phytase was added to experimental diets, resulting in less endogenous Ca being excreted (Lee et al., 2019a).

The STTD of P in the PC diet was greater than the calculated STTD of P (NRC, 2012), which may be due to the lack of dietary Ca in the PC diet. Increasing dietary Ca reduces the digestibility of P in gestating sows and growing pigs (Stein et al., 2011; Lee et al., 2020) and the ATTD of P in a Ca-free diet is greater than in diets containing calcium carbonate as a Ca source (Lee et al., 2019a). An increase in dietary Ca may result in increased formations of Ca-P complexes that prevent P from being absorbed, resulting in decreased P digestibility (Stein et al.,

2011). It is, therefore, likely that the greater than expected STTD of P is a result of the lack of Ca in the PC diet.

The observation that STTD of P increased and P excretion in feces decreased as dietary phytase increased was expected due to the liberation of phytate-bound P by microbial phytase and is consistent with previous data (Casas and Stein, 2015; Blavi et al., 2017; She et al., 2017). Likewise, the exponential increase in the ATTD of P that was observed as microbial phytase was included in the diet is consistent with data demonstrated that using from 500 to 4,000 FTU of the same consensus bacterial 6-phytase variant as used in the present experiment resulted in an increased ATTD of P regardless of the phytate concentration in the diet (Espinosa et al., 2021). The majority of P stored in plant-based ingredients is bound to phytate, limiting the amount of P available for utilization by pigs (Selle and Ravindran, 2008). Phytase hydrolyzes the bonds between P and phytate, releasing some of the phytate-bound P, which increases digestibility of P (Poulsen et al., 2010). Although pigs have some mucosal phytase activity, it is not enough to effectively release phytate-bound P in plant based feed ingredients (Selle and Ravindran, 2008). As expected, phytase released some of the P from phytate and the quantities of released P (between 0.04 and 0.10%, depending on the level of phytase in the diet) was within the range of values previously reported (Rojas and Stein, 2012; She et al., 2017). However, the released P in this experiment was less than sometimes observed because the ATTD and STTD of P in the control diets were greater than usual as discussed above, and there was, therefore, not as much opportunity to increase digestibility as in more practical diets.

Calcium and P must both be available in sufficient quantities for bone tissue synthesis to occur (Crenshaw, 2001). Because no dietary Ca was included in the experimental diets, absorbed P was not used to synthesize bone tissue, which resulted in an increase in P excretion in the urine

as well as a decrease in P retention, calculated as a percentage of absorption, as dietary phytase levels increased. This observation demonstrates that the extra P that was absorbed as phytase was included in the diet had to be excreted in the urine due to the lack of Ca for bone tissue synthesis. Stein et al. (2006) reported a decrease in Ca retention in pigs fed a P-free diet compared with pigs fed diets containing field peas without or with phytase. Thus, an insufficient amount of Ca or P in diets will result in decreased retention of P or Ca, respectively.

The observation that phytase reduced endogenous losses of Ca and at the same time increased digestibility of P, indicates that less Ca is needed in diets containing microbial phytase because a greater proportion of both dietary calcium and reabsorbed endogenous Ca can be used for bone tissue synthesis. Because the response to increasing doses of microbial phytase on the endogenous loss of Ca was linear and exponential, it will be necessary to use different values for the reduction in the endogenous loss of Ca as the phytase dose is increased to capture the true value of phytase. Although the reductions in endogenous loss of Ca caused by phytase may seem small, it is important that these values are accounted for in diet formulations and that Ca inclusion is reduced accordingly because even a small excess of Ca will reduce growth performance of growing pigs (González-Vega et al., 2016a; 2016b; Merriman et al., 2017; Lagos et al., 2019a; 2019b). To quantify the effect of phytase on Ca digestibility it will, therefore, be necessary to use values for STTD Ca rather than ATTD values in diet formulation because values for STTD of Ca are additive in mixed diets (Stein et al., 2016). In addition, it is important to reduce the provision of dietary Ca as the dose of phytase is increased to avoid the negative effects of oversupplying Ca in the diets. However, by reducing Ca in diets containing microbial phytase, fecal excretion of P due to formation of Ca-P complexes will be reduced (Stein et al., 2011), and the provision of P can, therefore, also be reduced. Future research should be directed

at quantifying the effects of increasing dietary provisions of microbial phytase on the STTD of both Ca and P.

In conclusion, ATTD and STTD of P increased by increasing phytase doses in low-P Ca-free diets, but dietary concentration of P did not affect basal endogenous loss of Ca in pigs fed Ca-free diets. However, increasing dietary phytase reduced the basal endogenous loss of Ca. This indicates that phytate and endogenous Ca form insoluble complexes in the gastrointestinal tract of pigs, but if phytase is included in the diet, phytate is hydrolyzed by phytase before it forms an insoluble complex with Ca, which results in the reduced endogenous loss of Ca. Consideration should be given to the effect of dietary phytase on basal endogenous loss of Ca when formulating diets for pigs because the increased absorption of Ca caused by phytase indicates that dietary Ca can be reduced if phytase is added to the diet, which may increase P digestibility.

Tables

Table 4.1. Ingredient composition of experimental diets

Item,%	Positive control	Negative control
Ground yellow corn	71.88	72.04
Full-fat rice bran	6.00	6.00
Potato protein concentrate	18.20	18.20
Soybean oil	2.00	2.00
Monosodium phosphate	1.02	0.36
Sodium chloride	0.40	0.40
Vitamin mineral premix[2]	0.50	0.50
Corn-phytase premix	-	0.50

[1]There were 6 negative control diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of phytase/kg diet, but the positive control diet did not contain phytase.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D3 as cholecalciferol, 1,660 IU; vitamin E as DL-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 4.2. Analyzed nutrient composition of experimental diets (as-fed basis)[1]

Item		NC (phytase, FTU/kg)[2]					
	PC[1]	0	250	500	1,000	2,000	4,000
Dry matter, %	90.84	90.62	90.56	90.58	90.83	90.85	90.72
Crude protein, %	19.28	19.83	19.67	19.76	19.71	20.96	20.40
Ash, %	8.83	8.97	7.67	7.51	8.82	7.69	7.21
Ca, %	0.03	0.03	0.03	0.03	0.03	0.05	0.04
P, %	0.66	0.46	0.45	0.46	0.48	0.49	0.45
Phytate[3], %	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Phytate P[4], %	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Non-phytate P[5], %	0.39	0.19	0.18	0.19	0.21	0.22	0.18
Phytase activity, FTU/kg	< 70	< 70	260	560	1,200	1,800	3,900

[1]All analyzed data are the average of two duplicate analyses.

[2]NC = negative control; PC = positive control; FTU = phytase unit.

[3]Phytate was calculated by multiplying the analyzed phytate in the ingredients by the inclusion rate of the ingredients in the diet.

[4]Phytate-P was calculated by multiplying analyzed phytate by 0.282 (Tran and Sauvant, 2004).

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

Table 4.3. Analyzed nutrient composition of ingredients (as-fed basis)[1]

Item	Ground yellow corn	Potato protein concentrate	Full-fat rice bran
Dry matter, %	89.13	93.48	96.96
Crude protein, %	6.46	81.18	15.27
Ash, %	2.08	1.48	10.92
Ca, %	0.02	0.02	0.06
P, %	0.30	0.10	2.10
Phytate, %	0.78	0.23	5.98
Phytate P[2], %	0.22	0.06	1.68
Non-phytate P[3], %	0.08	0.04	0.42

[1] All analyzed data are the average of two duplicate analyses.

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

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

Table 4.4. Basal endogenous loss of Ca and excretion of Ca in urine by pigs fed Ca-free diets[1,2]

Item, %	NC (FTU/kg)[1]								Contrast *P*-value			Exponential *P*-value
	PC[1]	0	250	500	1,000	2,000	4,000	SEM	PC vs. NC	Linear	SE[3]	
Feed intake, kg/d	0.90	0.91	0.89	0.89	0.92	0.87	0.90	0.02	0.763	0.712	0.02	0.987
Fecal excretion, g DM/d[1]	58.15	60.41	61.80	65.72	70.78	60.47	64.09	5.86	0.692	0.922	0.01	0.659
ATTD of DM[1], %	92.92	92.71	92.27	91.88	91.52	92.35	92.14	0.76	0.758	0.832	0.01	0.629
Urine excretion, kg/d	2.89	4.05	3.98	4.24	4.11	4.04	4.95	0.83	0.287	0.363	0.01	0.685
Ca excretion												
Ca in feces, %	0.75	0.67	0.35	0.29	0.24	0.40	0.22	0.11	0.382	0.010	0.004	0.133
Fecal Ca output, g/d	0.48	0.42	0.23	0.20	0.17	0.25	0.15	0.06	0.427	0.023	0.01	0.187
Basal endogenous loss of Ca, mg/kg DMI[1]	512	501	282	246	211	239	186	63	0.885	0.002	0.002	0.030
Ca in urine, mg/kg	65	23	45	25	34	45	18	18	0.060	0.601	0.002	0.761
Urine Ca output, g/d	0.22	0.09	0.23	0.09	0.15	0.17	0.14	0.09	0.232	0.901	0.002	0.801

[1]NC = negative control; PC = positive control; DM = dry matter; ATTD = apparent total tract digestibility; FTU = phytase unit; DMI = dry matter intake.

[2]Least squares means represent 10 observations with the exception that there were 9 observations for basal endogenous loss of Ca by pigs fed the PC diet and pigs fed the NC diet containing 2,000 FTU/kg.

[3]Standard error for the exponential model.

Table 4.5. Phosphorus balance in growing pigs fed Ca-free diets[1,2]

Item, %	NC (FTU/kg diet)[1]								Contrast *P*-value			Exponential *P*-value
	PC[1]	0	250	500	1,000	2,000	4,000	SEM	PC vs. NC	Linear	SE[3]	
P intake, g/d	5.91	4.23	4.13	4.15	4.28	4.04	4.17	0.12	< 0.001	0.735	0.02	1.000
P in feces, %	2.30	2.15	1.55	1.22	1.02	1.00	0.74	0.04	0.009	< 0.001	0.0002	< 0.001
Fecal P output, g/d	1.42	1.38	1.01	0.85	0.75	0.64	0.50	0.08	0.685	< 0.001	0.0004	< 0.001
Absorbed P, g/d	4.48	2.85	3.12	3.30	3.52	3.40	3.68	0.14	< 0.001	< 0.001	0.0007	0.024
ATTD of P[1], %	75.96	67.49	75.48	79.63	82.28	84.12	87.97	1.95	< 0.001	< 0.001	0.0004	< 0.001
Digestible P in diet[4], %	0.50	0.31	0.35	0.37	0.38	0.39	0.41	0.01	< 0.001	< 0.001	0.0004	< 0.001
STTD of P[1,5], %	78.60	71.20	79.19	83.33	86.00	87.83	91.73	1.95	0.001	< 0.001	0.0004	< 0.001
Digestible P in diet[4], %	0.52	0.33	0.37	0.39	0.40	0.41	0.43	0.01	< 0.001	< 0.001	0.0004	< 0.001
P in urine, %	0.06	0.03	0.04	0.05	0.05	0.06	0.04	0.01	0.014	0.379	0.004	0.363
Urine P output, g/d	1.40	0.81	1.36	1.33	1.63	1.68	1.66	0.19	< 0.001	< 0.001	0.001	0.009
P retention, g/d	3.08	2.04	1.76	1.96	1.89	1.72	2.01	0.29	< 0.001	0.845	-	Not good fit
P retention, % of intake	52.25	48.22	42.53	47.31	44.05	42.47	47.71	6.00	0.338	0.808	-	Not good fit
P retention, % of absorbed	68.45	71.20	56.26	59.10	53.28	50.26	53.95	6.45	0.552	0.006	0.003	0.108

[1]ATTD = apparent total tract digestibility; STTD = standardized total tract digestibility; FTU = phytase unit; PC = positive control;

Table 4.5. (cont.)

NC = negative control[2]Least squares means represent 10 observations.

[3]Standard error for the exponential model.

[4]Digestible P was calculated by multiplying concentration of P in each diet by the ATTD or STTD of P.

[5]Calculated by correcting the ATTD of P for the average basal endogenous loss of P (i.e., 190 mg/kg DMI; NRC, 2012).

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CHAPTER 5: Effects of source of calcium carbonate and microbial phytase on apparent total tract digestibility of calcium

Abstract

The objective of this experiment was to test the hypothesis that there are differences in the apparent total tract digestibility (**ATTD**) of Ca and in the response to microbial phytase among sources of Ca carbonate obtained from different regions of the world. Three hundred and twenty barrows (body weight: 17.47 kg; SD = 1.28) were allotted to 40 diets using a completely randomized block design with eight blocks of 40 pigs for a total of eight replicate pigs per diet. All diets were based on corn and potato protein concentrate. Twenty sources of Ca carbonate were obtained from different regions of the world, including the United States, Europe, Asia, and South Africa. Each source of Ca carbonate was used in two diets, one diet without microbial phytase and one diet that contained 1,000 phytase units/kg of diet (**FYT**). Pigs were housed individually in metabolism crates and were fed experimental diets for 12 d, with the initial 5 d being the adaptation period. Daily feed allotments were divided into two equal meals and pigs were provided feed at 3.0 times the maintenance requirement for metabolizable energy. Feces were collected for 4 d following the adaptation period, and at the conclusion of the experiment, fecal samples were dried, ground, and analyzed for Ca and P. Results indicated that there were no interactions between source of Ca carbonate and phytase. Differences in ATTD and standardized total tract digestibility (**STTD**) of Ca were observed among pigs fed diets containing different sources of Ca carbonate ($P < 0.001$). Pigs fed diets containing 1,000 FYT had greater ($P < 0.001$) ATTD and STTD of Ca compared with pigs fed diets containing no phytase. There was a tendency ($P = 0.050$) for pigs fed diets containing different sources of Ca

carbonate to have different ATTD of P, but pigs fed diets containing 1,000 FYT had greater ($P < 0.001$) ATTD of P compared with pigs fed diets without phytase. No interactions were observed between region and phytase. The ATTD and STTD of Ca in Ca carbonate from the United States was less ($P < 0.001$) than in Ca carbonate from Europe, Asia, or South Africa. In conclusion, differences in ATTD and STTD of Ca were observed among Ca carbonate obtained from four regions of the world, and inclusion of microbial phytase increased the ATTD and STTD of Ca in Ca carbonate regardless of the region where the Ca carbonate was produced.

Key words: Calcium, calcium carbonate, digestibility, phosphorus, phytase, pig

Abbreviations: ATTD, apparent total tract digestibility; FYT, phytase unit; STTD, standardized total tract digestibility.

Introduction

Only a small amount of the Ca required by pigs is provided by plant-based ingredients and supplementation of Ca from inorganic sources, such as calcium carbonate, is usually required to meet the requirement by pigs (González-Vega et al., 2015). The apparent total tract digestibility (**ATTD**) of Ca in calcium carbonate is not affected by dietary Ca concentration (Stein et al., 2011), but addition of microbial phytase to diets supplemented with calcium carbonate resulted in an increase in the ATTD of Ca (González-Vega et al., 2015; Lee et al., 2019). Excess dietary Ca can negatively affect growth performance of pigs by reducing absorption and digestibility of P (Stein et al., 2011; González-Vega et al., 2016) and it is, therefore, important that the digestibility of Ca in calcium carbonate is known to formulate diets based on values for digestible Ca rather than total Ca.

The concentration of Ca in different sources of calcium carbonate may vary due to differences in raw materials and processing methods that suppliers utilize. Differences in ATTD and standardized total tract digestibility (**STTD**) of Ca in calcium carbonate produced in the U.S. have been observed (Lee et al., 2019), but it is unknown if there are differences in the ATTD of Ca in calcium carbonate sources produced outside the U.S. Therefore, the objective of this experiment was to test the hypothesis that there are differences in the ATTD of Ca and in the response to microbial phytase among sources of calcium carbonate obtained from different parts of the world.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before animal work was initiated. Pigs used in this experiment were offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Diets, animals, and experimental design

A total of 40 diets were formulated based on corn and potato protein concentrate (Table 5.1). Twenty sources of calcium carbonate were obtained from different regions of the world, including the United States, Europe, Asia, and South Africa. Each source of calcium carbonate was used in two diets, one diet without microbial phytase and one diet that contained 1,000 phytase units/kg of diet (**FYT**; HiPhos, DSM, Kaiseraugst, Switzerland). Crystalline amino acids, vitamins, and minerals were included in all diets to meet requirements for 11 to 25 kg pigs (NRC, 2012).

Three hundred and twenty barrows (body weight: 17.47 kg; SD = 1.28) were allotted to

the 40 diets using a completely randomized block design with eight blocks of 40 pigs for a total of eight replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a fully slatted tri-bar floor, a nipple waterer, and a feeder. A mesh screen and a pan were installed under the slatted floor during the collection period to allow for separate collection of feces and orts.

Feeding and sample collection

Daily feed allotments were divided into two equal meals that were provided at 0800 and 1600 h, and pigs were provided feed in the amount of 3.0 times the maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy per kg of body weight$^{0.60}$; NRC, 2012). Pigs had free access to water throughout the experiment. Experimental diets were fed for 12 d, with the initial 5 d being the adaptation period to the diets followed by 4 d of collection using the marker-to-marker procedure (Adeola, 2001). Fecal collection commenced when the first marker (i.e., indigo carmine), which was supplemented in the morning of d 6, appeared in the feces, and ceased when the second marker (i.e., ferric oxide), which was supplemented in the morning of d 10, appeared in the feces (Adeola, 2001). Feces were stored at -20 °C immediately following collection.

Chemical Analysis

At the conclusion of the experiment, fecal samples were thawed, dried at 65 °C in a forced air oven, and finely ground using a 500G swing type grain mill (RRH, Zhejiang, China) prior to analyses. Main ingredients, diets, and fecal samples were analyzed in duplicate for dry matter (method 930.15; AOAC Int., 2019), and ash (method 942.05; AOAC Int., 2019). Diets, main ingredients, and feces were also analyzed for Ca and P (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectroscopy (ICP-OES; Avio 200,

PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). Diets were analyzed for phytase activity (method 2000.12; AOAC Int., 2019) and corn and potato protein concentrate were analyzed for phytic acid (Ellis et al., 1977).

Calculations

Phytate in the diets was calculated by multiplying the analyzed phytate in corn and potato protein concentrate by the inclusion rate of each ingredient in the diet and by adding the values. Phytate-P was calculated by multiplying phytate by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated as the difference between phytate-P and total P.

The ATTD of Ca and P in each experimental diet was calculated according to the following equation (Almeida and Stein, 2010):

$$\text{ATTD (\%)} = \frac{\text{intake - output}}{\text{intake}} \times 100,$$

where both intake and output are expressed in grams per day. The standardized total tract digestibility (**STTD**) of Ca in each experimental diet was determined by correcting the ATTD of Ca for an average basal endogenous loss of Ca (i.e., 433 mg/kg dry matter intake) obtained by Lee and Stein (2023). Digestible Ca in source was calculated by multiplying the concentration of Ca in source by the STTD of Ca and diving by 100.

Statistical Analysis

Normality and homogeneity of variances were verified (SAS Inst. Inc., Cary, NC, USA) and outliers were identified as values that deviated from the first or third quartiles by more than 3 times the interquartile range using Internally Studentized Residuals (Tukey, 1977). Pig was the

experimental unit for all analyses. Seven outliers were identified and removed from the final statistical analysis and an average SEM was used if the number of observations was not identical among dietary treatments. Data were analyzed using the PROC GLM in SAS (SAS Institute Inc., Cary, NC, USA). The initial statistical model included Ca carbonate source, phytase, and the Ca-source × phytase interaction as fixed effects; however, no interactions between Ca source and phytase were observed. Therefore, the final statistical model included only Ca source and phytase as fixed effects. A second analysis was performed to compare digestibility of Ca carbonate obtained from different regions of the world (i.e., Europe, Asia, United States, and South Africa). In this model region, phytase, and the region × phytase interaction were fixed effects. However, no interactions between region and phytase were observed and therefore, the final statistical model included only region and phytase as fixed effects. Means were calculated, and least significant differences were used to separate the means. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

There were no interactions between Ca carbonate source and phytase on intake, output, or digestibility of Ca, and therefore, main effects of Ca source and phytase are presented (Table 5.4). Feed intake, fecal excretion, and ATTD of dry matter were not different among pigs fed diets containing different sources of Ca carbonate. There were differences in Ca intake among pigs fed different Ca carbonate sources ($P = 0.007$) and values ranged from 5.20 to 6.46 g/d. Differences were also observed in Ca concentration in the feces ($P < 0.001$) and fecal Ca output ($P = 0.002$) among pigs fed experimental diets. Values for Ca in feces, measured as % or g/d, ranged from 1.33 to 2.41% and 1.14 to 1.98 g/d, respectively. There were also differences in

ATTD and STTD of Ca among pigs fed diets containing different sources of Ca carbonate ($P < 0.001$). Apparent total tract digestibility and STTD of Ca ranged from 65.91 to 79.88% and 71.48 to 85.29%, respectively. Differences in the concentration of digestible Ca among the 20 sources of Ca carbonate were also observed ($P < 0.001$) and values ranged from 26.14 to 34.58%.

No effects of phytase were observed for feed intake, fecal excretion, ATTD of dry matter, or Ca intake. Pigs fed diets containing 1,000 FYT/kg had less ($P < 0.001$) Ca in feces, expressed as % or g/d, compared with pigs fed diets without phytase. Apparent total tract digestibility and STTD of Ca were greater ($P < 0.001$) in pigs fed diets containing 1,000 FYT/kg compared with pigs fed diets without phytase. The concentration of digestible Ca in Ca carbonate increased ($P < 0.001$) when 1,000 FYT/kg was included in the diet compared with diets without phytase.

There were no interactions between Ca source and phytase on intake, output, or ATTD of P, and therefore, main effects of Ca source and phytase are presented (Table 5.5). No differences were observed in P intake, but differences were observed in P concentration in the feces ($P < 0.01$) and fecal P output ($P < 0.05$) among pigs fed experimental diets. Values for P in feces, expressed, as % or g/d, ranged from 1.26 to 1.48% and 1.01 to 1.35 g/d, respectively. There was a tendency ($P = 0.05$) for pigs fed diets containing different sources of Ca carbonate to have different ATTD of P. Values for ATTD of P ranged from 68.16 to 75.66%. No differences were observed in feed intake between pigs fed diets with 1,000 FYT/kg and pigs fed no phytase, but pigs fed diets containing 1,000 FYT/kg had less ($P < 0.001$) P in feces, expressed as % or g/d, compared with pigs fed diets without phytase. Pigs fed diets containing 1,000 FYT/kg also had greater ($P < 0.001$) ATTD of P compared with pigs fed diets without phytase.

There were no interactions between region and phytase for intake, output, or digestibility of Ca, and therefore, main effects of region and phytase are reported (Table 5.6). Calcium intake

was not different among pigs fed diets containing Ca carbonate sourced from Europe, Asia, United States, or South Africa. However, pigs fed diets containing Ca carbonate from the United States had greater ($P < 0.001$) concentration of Ca in feces, expressed as % or g/d, than pigs fed Ca carbonate from other regions. As a consequence, the ATTD and STTD of Ca in Ca carbonate from the United States was less ($P < 0.001$) than in Ca carbonate from other regions of the world, but no differences among Europe, Asia, and South Africa were observed. Calcium carbonate from Europe containing more ($P < 0.05$) digestible Ca than Ca carbonate from the other three regions in the world, and Ca carbonate from Asia contained more ($P < 0.05$) digestible Ca than Ca carbonate from South Africa or United States. There was no effect of phytase on Ca intake, but pigs fed diets containing 1,000 FYT/kg had less ($P < 0.001$) Ca excreted in feces, expressed as % or g/d, compared with pigs fed diets containing no phytase and consequently, pigs fed diets containing 1,000 FYT/kg had greater ($P < 0.001$) ATTD and STTD of Ca compared with pigs fed diets with no phytase. Concentration of digestible Ca in Ca carbonate sources increased ($P < 0.001$) when 1,000 FYT/kg was included in diets compared with diets containing no phytase.

Discussion

Analyzed concentrations of Ca and P in corn and potato protein concentrate were consistent with reported values (NRC, 2012; Nelson et al., 2022). Based on the molecular weight, Ca carbonate should contain 40% Ca; however, due to impurities in raw materials, the analyzed concentration of Ca in Ca carbonate is typically lower and has an average value of 38.5% (NRC, 2012). Calcium concentrations in the 20 Ca carbonate sources used in this experiment ranged from 32.1

to 40.9%, demonstrating that there is considerable variation in Ca concentration among Ca carbonate obtained from four different regions around the world.

Calculated concentrations of Ca in diets ranged from 0.59 to 0.75%, whereas analyzed concentrations of Ca in diets ranged 0.52 to 0.74% depending on the source of Ca carbonate being used. Variability in analyzed values for dietary Ca have been reported (Wu et al., 2018); however, calculated dietary Ca concentrations were used in all calculations, and therefore, any analytical discrepancies in diets did not impact calculated values for digestibility of Ca.

The observation that ATTD and STTD of Ca were different among the 20 sources of Ca carbonate is consistent with previous results where differences in Ca digestibility were observed among four Ca carbonate sources obtained from the United States (Lee et al., 2019). The observation that Ca carbonate sources from the U.S. had lower ATTD and STTD of Ca compared with Ca carbonate from Asia, Europe, and South Africa, indicates that differences exist in commercial sources of Ca carbonate obtained from four continents. These differences may be the result of differences in raw materials and processing practices used by producers of Ca carbonate. Values for ATTD and STTD of Ca among Ca carbonate sources were in agreement with previous data (González-Vega et al., 2015; Merriman and Stein, 2016; Lee et al., 2019). The observation that inclusion of 1,000 FYT in diets reduced Ca excretion in feces, and resulted in increased ATTD and STTD of Ca concurs with previous studies where increases in ATTD and STTD of Ca from Ca carbonate were observed when 500 FYT were added to diets (González-Vega et al., 2015; Lee et al., 2019). Phytate has the capacity to chelate up to five Ca cations resulting in the formation of indigestible Ca-phytate complexes (Selle et al., 2009). Observations from this experiment further demonstrate that Ca in Ca carbonate may be chelated

by phytate, but addition of phytase to diets reduces chelation and increases the digestibility of Ca in Ca carbonate.

The observation that inclusion of microbial phytase increased ATTD of P has been documented in previous studies (Poulsen et al., 2010; Almeida and Stein, 2012; She et al., 2017). Phytase hydrolyzes the phytate molecule, thereby liberating phytate-bound P and increasing P absorption, resulting in less P excreted in the feces (Selle and Ravindran, 2008). Differences observed in P excretion among diets containing different sources of Ca carbonate may be due to the differences observed in Ca concentration of diets, which ranged from 0.59 to 0.75%. Increases in Ca to P ratio negatively affect P digestibility by the formation of indigestible Ca-P and Ca-phytate complexes that precipitate in the small intestine and result in greater excretion of P in the feces (Selle et al., 2009).

Calcium is relatively inexpensive to supplement in diets and, as a result, is often oversupplied in commercial U.S. and European pig diets (Walk, 2016; Lagos et al., 2023). Calcium carbonate is used most often to supplement Ca in diets, but some Ca may also be provided by mineral feed phosphate sources such as monocalcium phosphate (**MCP**) and dicalcium phosphate (**DCP**). Unlike Ca carbonate, microbial phytases have no effect on digestibility of Ca in MCP or DCP (González-Vega et al., 2015; Lee et al., 2019). Calcium in MCP and DCP is bound to phosphates in phosphoric acid, resulting in Ca cations being less available to bind to phytate in the intestines (Walk, 2016), and thus, when phytase is included in the diet, there is no observed increase in Ca digestibility in these sources. With the possibility of up to 80% of phytate-bound P being released with newer phytases (Espinosa et al., 2022; Lagos et al., 2022), the need to supplement P from MCP and DCP is decreasing, and consequently, more Ca may need to be supplemented by Ca carbonate.

In conclusion, calcium concentration varied considerably among sources of Ca carbonate used in this experiment. Differences in ATTD and STTD of Ca were observed among sources of Ca carbonate obtained from four regions of the world and inclusion of microbial phytase increased ATTD and STTD of Ca in the sources of Ca carbonate. Therefore, consideration should be given to differences in analyzed concentrations of Ca, values for STTD of Ca, and response to microbial phytase among different sources of Ca carbonate to ensure Ca is not being oversupplied when formulating diets.

Tables

Table 5.1. Ingredient composition of diets (as-is basis)

Item, %	Diet[1]
Ingredient composition, as-fed basis	
Corn	76.87
Potato protein concentrate	18.00
Calcium carbonate	1.75
Soybean oil	1.20
$_{L}$-Lys·HCL	0.14
$_{DL}$-Met	0.03
$_{L}$-Trp	0.01
Monosodium phosphate	1.09
Salt	0.40
Vitamin-mineral premix[2]	0.50
Phytase	0.01
Calculated nutrient composition[3]	
Metabolizable energy, kcal/kg	3,350
Crude protein, %	20.86
Ca, %	0.70
Total P, %	0.50
Standardized total tract digestible P, %	0.33

[1]Twenty sources of Ca carbonate were included in one diet without microbial phytase and in one diet with microbial phytase (1,000 phytase units/kg of diet).

Table 5.1. (cont.)

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622IU; vitamin D3 as cholecalciferol, 1,660IU; vitamin E as DL-alpha-tocopheryl acetate, 66IU; vitamin K as menadione nicotinamide bisulfate, 1.40mg; thiamin asthiamine mononitrate,1.08mg; riboflavin, 6.49mg; pyridoxine as pyridoxine hydrochloride, 0.98mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2mg; niacin, 43.4mg; folic acid, 1.56mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

[3]Calculated from NRC (2012).

Table 5.2. Analyzed nutrient composition of feed ingredients (as-is basis)[1]

Item	Dry matter, %	Ash, %	Ca, %	P, %	Phytate, %	Phytate P[3], %	Non-phytate P[3], %
Corn	85.9	1.19	0.03	0.23	0.64	0.18	0.05
Potato protein concentrate	91.0	0.37	0.02	0.05	0.17	0.05	0.00
Ca carbonate							
A	100.0	99.4	37.3	-	-	-	-
B	100.0	99.9	39.0	-	-	-	-
C	100.0	99.6	39.0	-	-	-	-
D	100.0	99.5	38.0	-	-	-	-
E	100.0	99.3	37.7	-	-	-	-
F	100.0	99.4	39.7	-	-	-	-
G	100.0	99.6	37.3	-	-	-	-
H	100.0	99.2	35.9	-	-	-	-
I	100.0	98.4	36.5	-	-	-	-
J	100.0	99.7	39.3	-	-	-	-
K	100.0	98.9	37.5	-	-	-	-
L	99.9	97.5	34.8	-	-	-	-
M	100.0	99.4	40.9	-	-	-	-
N	99.9	99.5	39.2	-	-	-	-
O	99.9	99.3	36.8	-	-	-	-
P	99.7	99.0	37.7	-	-	-	-
Q	100.0	99.7	37.6	-	-	-	-

Table 5.2. (cont.)

R	99.9	97.6	32.1	-	-	-	-
S	98.7	95.4	36.5	-	-	-	-
T	99.9	92.3	36.6	-	-	-	-

[1]All analyzed data are the average of two duplicate analyses.

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

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

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

Item	Dry matter, %	Ash, %	Ca, %	P, %	Phytase, FYT/kg	Phytate[3], %	Phytate P[4], %	Non-phytate P[5], %
Ca carbonate (0 FYT/kg)								
A	87.93	3.60	0.69	0.54	< 70	0.52	0.15	0.39
B	87.97	3.62	0.73	0.51	< 70	0.52	0.15	0.36
C	87.97	3.66	0.69	0.55	< 70	0.52	0.15	0.40
D	87.86	3.72	0.68	0.48	< 70	0.52	0.15	0.33
E	87.92	3.58	0.70	0.54	< 70	0.52	0.15	0.39
F	88.02	3.51	0.64	0.49	< 70	0.52	0.15	0.34
G	88.20	3.40	0.72	0.49	< 70	0.52	0.15	0.34
H	88.00	3.57	0.63	0.55	< 70	0.52	0.15	0.40
I	87.94	3.53	0.68	0.49	< 70	0.52	0.15	0.34
J	87.96	3.72	0.71	0.50	< 70	0.52	0.15	0.35
K	87.59	3.37	0.69	0.47	< 70	0.52	0.15	0.32
L	87.72	3.59	0.65	0.52	< 70	0.52	0.15	0.37
M	87.56	3.62	0.71	0.53	< 70	0.52	0.15	0.38
N	87.17	3.65	0.69	0.56	< 70	0.52	0.15	0.41
O	87.36	3.96	0.64	0.51	< 70	0.52	0.15	0.36
P	87.37	4.05	0.69	0.51	< 70	0.52	0.15	0.36
Q	87.33	3.47	0.65	0.48	< 70	0.52	0.15	0.33
R	87.42	3.33	0.54	0.48	< 70	0.52	0.15	0.33
S	87.36	3.92	0.67	0.52	< 70	0.52	0.15	0.37
T	87.29	3.17	0.68	0.47	< 70	0.52	0.15	0.32

Table 5.3. (cont.)

Ca carbonate (1,000 FYT/kg)								
A	86.95	4.08	0.64	0.49	995	0.52	0.15	0.34
B	87.09	4.26	0.71	0.50	1,550	0.52	0.15	0.35
C	87.16	4.23	0.74	0.54	1,150	0.52	0.15	0.39
D	87.31	3.43	0.64	0.55	1,135	0.52	0.15	0.40
E	87.33	4.04	0.72	0.52	895	0.52	0.15	0.37
F	87.30	4.04	0.73	0.54	1,200	0.52	0.15	0.39
G	87.20	4.26	0.71	0.53	1,350	0.52	0.15	0.38
H	87.23	3.43	0.66	0.47	1,250	0.52	0.15	0.32
I	88.17	3.89	0.68	0.52	1,400	0.52	0.15	0.37
J	87.12	3.82	0.71	0.54	1,450	0.52	0.15	0.39
K	86.85	4.34	0.69	0.49	1,075	0.52	0.15	0.34
L	87.30	4.05	0.60	0.54	1,200	0.52	0.15	0.39
M	86.80	3.42	0.70	0.52	1,230	0.52	0.15	0.37
N	86.95	3.36	0.72	0.49	1,200	0.52	0.15	0.34
O	87.21	4.07	0.71	0.50	1,400	0.52	0.15	0.35
P	87.16	3.63	0.65	0.48	1,365	0.52	0.15	0.33
Q	87.24	3.39	0.64	0.51	1,200	0.52	0.15	0.36
R	87.23	3.48	0.52	0.48	1,400	0.52	0.15	0.33
S	87.21	4.35	0.66	0.48	1,300	0.52	0.15	0.33
T	87.13	3.40	0.69	0.47	1,245	0.52	0.15	0.32

[1]All analyzed data are the average of two duplicate analyses.

[2]FYT = phytase unit.

Table 5.3. (cont.)

[3]Phytate was calculated by multiplying the analyzed phytate in the ingredients by the inclusion rate of the ingredients in the diet.

[4]Phytate-P was calculated by multiplying analyzed phytate by 0.282 (Tran and Sauvant, 2004).

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

Table 5.4. Main effects of Ca source and phytase on apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca in diets containing 20 different sources of calcium carbonate[1]

Item	Feed intake, kg/d	Dried fecal excretion, g/d	ATTD of DM, %	Ca intake, g/d	Ca in feces, %	Fecal Ca output, g/d	ATTD of Ca, %	STTD of Ca, %	Digestible Ca in source[4], %
Ca carbonate[2]									
A	0.82	81.92	89.07	5.62	1.40	1.14	79.44	84.99	31.70
B	0.85	89.33	88.59	6.09	1.66	1.50	75.43	80.75	31.49
C	0.87	88.10	88.94	6.22	1.42	1.24	79.88	85.20	33.23
D	0.84	87.50	88.34	5.81	1.33	1.18	79.83	85.29	32.41
E	0.86	86.33	88.95	5.90	1.53	1.33	77.58	83.09	31.32
F	0.89	88.33	89.18	6.46	1.66	1.47	77.29	82.52	32.76
G	0.86	83.45	89.33	5.87	2.41	1.98	65.91	71.48	26.66
H	0.89	91.44	88.80	5.88	1.49	1.35	76.92	82.68	29.68
I	0.83	90.14	87.94	5.53	1.95	1.78	67.95	73.66	26.88
J	0.84	86.15	88.73	6.03	2.02	1.75	71.06	76.33	30.00
K	0.84	82.08	89.20	5.75	1.85	1.49	74.04	79.54	29.83

Table 5.4. (cont.)

L	0.85	85.95	88.84	5.41	1.55	1.33	75.54	81.47	28.35
M	0.86	81.68	89.44	6.38	1.60	1.32	79.49	84.55	34.58
N	0.84	87.27	88.57	5.99	1.64	1.39	76.74	82.00	32.14
O	0.78	86.45	87.61	5.29	1.63	1.41	72.95	78.55	28.91
P	0.85	85.98	88.87	5.88	1.58	1.37	76.83	82.30	31.03
Q	0.84	92.00	88.01	5.81	1.50	1.39	76.24	81.73	30.73
R	0.88	93.95	88.31	5.20	1.40	1.29	75.04	81.43	26.14
S	0.85	86.75	88.80	5.71	1.45	1.26	77.90	83.56	30.50
T	0.89	93.07	88.41	5.94	1.80	1.71	71.08	76.72	28.08
SEM	0.03	4.53	0.47	0.23	0.11	0.12	1.81	1.81	0.77
LSD	0.09	12.6	1.30	0.62	0.26	0.33	4.71	4.71	1.76
P-value	0.890	0.912	0.391	0.007	< 0.001	0.002	< 0.001	< 0.001	< 0.001
Phytase[3], FYT/kg									
0	0.85	87.41	88.69	5.79	2.01	1.75	69.74	75.30	28.21
1,000	0.86	87.40	88.70	5.89	1.29	1.12	80.86	86.38	32.38

Table 5.4. (cont.)

SEM	0.01	1.43	0.15	0.07	0.03	0.04	0.57	0.57	0.24
LSD	0.03	4.00	0.41	0.20	0.08	0.10	1.49	1.49	0.56
P-value	0.391	1.00	0.996	0.402	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1]DM = dry matter; ATTD = Apparent total tract digestibility; STTD = Standardized total tract digestibility; FYT = phytase unit.

[2]Each mean represents 16 observations per Ca carbonate source with the exception that there were 15 observations for diets containing Ca carbonate sources C, J, L, M, O, S, and T.

[3]Each mean represents 155 observations for diets containing no phytase and 158 observations for diets containing 1,000 FYT/kg.

[4]STTD of Ca was calculated by correcting the ATTD of Ca for an average basal endogenous loss of Ca (i.e., 433 mg/kg dry matter intake) obtained from 7 experiments (Lee and Stein, 2023).

[5]Digestible Ca in source was calculated by multiplying the concentration of Ca in source by the STTD of Ca and dividing by 100.

T**able 5.5.** Main effects of Ca source and phytase on apparent total tract digestibility (ATTD) of P in diets containing 20 different sources of Ca carbonate[1]

Item	P intake, g/d	P in feces, %	Fecal P output, g/d	ATTD of P, %
Ca carbonate[2]				
A	4.07	1.29	1.04	73.78
B	4.23	1.48	1.35	68.16
C	4.31	1.27	1.12	73.85
D	4.14	1.45	1.27	68.80
E	4.23	1.41	1.23	71.13
F	4.40	1.43	1.27	71.25
G	4.25	1.26	1.01	75.66
H	4.42	1.45	1.34	69.56
I	4.09	1.28	1.11	71.75
J	4.16	1.29	1.11	72.94
K	4.14	1.48	1.17	71.13
L	4.18	1.33	1.16	72.33
M	4.23	1.35	1.09	74.05
N	4.13	1.43	1.20	70.62
O	3.88	1.36	1.17	68.44
P	4.21	1.39	1.17	71.99
Q	4.18	1.30	1.17	71.83
R	4.35	1.32	1.21	71.79
S	4.23	1.30	1.13	73.14
T	4.38	1.26	1.18	73.07
SEM	0.16	0.05	0.07	1.57

Table 5.5. (cont.)

LSD	0.45	0.14	0.19	4.29
P-value	0.890	0.009	0.029	0.050
Phytase[3], FYT/kg				
0	4.18	1.72	1.49	64.09
1,000	4.24	1.00	0.87	79.25
SEM	0.05	0.02	0.02	0.50
LSD	0.14	0.05	0.06	1.36
P-value	0.391	< 0.001	< 0.001	< 0.001

[1]ATTD = Apparent total tract digestibility; FYT = phytase unit.

[2]Each mean represents 16 observations per Ca carbonate source with the exception that there were 15 observations for diets containing Ca carbonate source C, J, L, M, O, S, and T.

[3]Each mean represents 155 observations for diets containing no phytase and 158 observations for diets containing 1,000 FYT/kg.

Table 5.6. Main effects of region and phytase on apparent total tract digestibility (**ATTD**) and standardized total tract digestibility (**STTD**) of Ca in diets containing 20 different sources of Ca carbonate[1]

Item	Regions[2]							Phytase[3], FYT/kg				
	Europe	Asia	United States	South Africa	SEM	LSD	*P*-value	0	1,000	SEM	LSD	*P*- value
Ca												
Ca intake, g/d	5.92	5.89	5.81	5.57	0.11	0.30	0.153	5.79	5.89	0.04	0.20	0.304
Ca in feces, %	1.56^{cb}	1.66^{b}	1.90^{a}	1.45^{c}	0.05	0.14	< 0.001	2.01	1.29	0.02	0.09	< 0.001
Fecal Ca output, g/d	1.34^{b}	1.44^{b}	1.68^{a}	1.32^{b}	0.06	0.16	< 0.001	1.75	1.12	0.02	0.11	< 0.001
ATTD of Ca, %	77.25^{a}	75.51^{a}	71.03^{b}	76.36^{a}	0.84	2.38	< 0.001	69.74	80.86	0.31	1.59	< 0.001
STTD[4] of Ca, %	82.62^{a}	81.08^{a}	76.56^{b}	82.21^{a}	0.84	2.37	< 0.001	75.30	86.38	0.31	1.59	< 0.001
Digestible Ca in source[5], %	31.86^{a}	30.22^{b}	28.69^{c}	29.09^{c}	0.36	1.10	< 0.001	28.21	32.38	0.13	0.68	< 0.001

[1]ATTD = apparent total tract digestibility; STTD = standardized total tract digestibility; FYT = phytase unit.

[2]Each mean represents 109, 95, 62, and 47 observations for Europe, Asia, United States, and South Africa, respectively.

[3]Each mean represents 155 and 158 observations for diets containing no phytase and 1,000 FYT/kg, respectively.

[4]STTD of Ca was calculated by correcting the ATTD of Ca for an average basal endogenous loss of Ca (i.e., 433 mg/kg dry matter intake) obtained from 7 experiments (Lee and Stein, 2023).

[5]Digestible Ca was calculated by multiplying the concentration of Ca in each source by the STTD of Ca and dividing by 100.

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CHAPTER 6: Conclusions

Results for experiment 1 indicated that not all P provided by spray dried plasma and potato protein concentrate is absorbed by the pigs, which may result in an overestimation of basal endogenous loss (**BEL**) of P if these ingredients are used in diets to estimate BEL of P. However, no differences in BEL of P were observed between pigs fed the gelatin and casein diets, and therefore, casein may be used as an alternative to gelatin to estimate BEL of P. Casein provides a greater amount of P compared with gelatin, but this does not affect values for BEL of P.

Results from experiment 2 indicated that apparent total tract digestibility (**ATTD**) and standardized total tract digestibility (**STTD**) of P increased by increasing phytase concentration. The BEL of Ca was not affected by dietary P concentration, but increasing dietary phytase reduced BEL of Ca. This demonstrates that phytate may chelate endogenous Ca, resulting in the formation of indigestible Ca-phytate complexes. However, by including phytase in the diet, phytate is hydrolyzed before it can form these complexes with endogenous Ca, thereby increasing the absorption of endogenous Ca. Therefore, less dietary Ca may need to be included in diets when phytase is used.

In experiment 3 it was observed that the concentration of Ca varied considerably among the sources of Ca carbonate used. Results also indicated that there were differences in ATTD and STTD of Ca among sources of Ca carbonate from different regions of the world and that Ca carbonate sources form the United States had the lowest ATTD and STTD of Ca compared with Ca carbonate from the other three regions. However, inclusion of microbial phytase increased the digestibility of ATTD of Ca and P and increased the STTD of Ca.

Overall, it is important to consider Ca concentrations and STTD of Ca in feed ingredients, as well as effects of phytase on the digestibility of Ca to ensure Ca is not being oversupplied in diets.