DIGESTIBILITY OF CALCIUM AND PHOSPHORUS AND EFFECTS OF MICROBIAL PHYTASE IN DIETS FED TO GROWING PIGS OR GESTATING SOWS

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DISSERTATION

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ABSTRACT

Five experiments were conducted to determine Ca digestibility in feed ingredients and to demonstrate the interactions among Ca, P, and phytase in diets fed to gestation sows or growing pigs. In Exp. 1, two experiments were conducted to test the hypothesis that standardized total tract digestibility (**STTD**) of Ca and the response to microbial phytase is constant among different sources of calcium carbonate and that the STTD of Ca is constant among different sources of dicalcium phosphate (**DCP**) when fed to growing pigs. Results indicated that there were no interactions between phytase and source of calcium carbonate. Values for STTD of Ca in calcium carbonate were greater ($P < 0.001$) for diets containing microbial phytase compared with diets without exogenous phytase, but values for STTD of Ca differed ($P = 0.006$) among the 4 sources of calcium carbonate. Values for STTD of Ca in DCP appears to be constant regardless of origin. In Exp. 2, the objective was to determine correlations between individual bones in the body and total bone ash to identify the bone that is most representative of total body bone ash in growing pigs. Pigs were fed diets containing 60 or 100% of the requirement for STTD Ca and STTD P. Results indicated that growth performance of pigs and bone ash were negatively affected by dietary Ca and P below the requirement. Metacarpals, metatarsals, and tibia were more representative of total body bone ash compared with femur, fibula, and ribs. Experiment 3 was conducted to test the hypothesis that there are no differences between gestating sows and growing pigs for STTD and retention of Ca and P. Two diets containing normal- or high-phytate were fed to growing pigs and gestating sows. Phytate level did not affect the STTD of Ca or Ca retention by gestating sows whereas the STTD of Ca and Ca and P retentions were greater if growing pigs were fed the normal-phytate diet than if they were fed the high-phytate diet (physiological state × phytate level interaction, $P < 0.001$). The STTD of P was greater for the

normal-phytate diet than for the high-phytate diet, but the difference was greater for growing pigs than for gestating sows (physiological state × phytate level interaction; $P = 0.002$). Regardless of phytate level, gestating sows had reduced digestibility and retention of Ca and P compared with growing pigs. Experiment 4 was conducted to test the hypothesis that the STTD of Ca and the response to microbial phytase on STTD of Ca, apparent total tract digestibility (**ATTD**), and retention of Ca and P does not change during gestation. Throughout gestation, sows were fed 4 diets that were Ca-free diet or a corn-based diet in which Ca carbonate was the sole source of Ca without or with microbial phytase. Results indicated that there were no interactions between period of gestation and dietary phytase. Supplementation of microbial phytase did not affect STTD of Ca, Ca retention, ATTD of P, or P retention in sows fed the calcium carbonate-containing diet. The ATTD of Ca, the ATTD of P, and the retention of Ca were least ($P < 0.05$) in mid-gestation, followed by early- and late-gestation, respectively, and the STTD of Ca in mid-gestation was also reduced ($P < 0.05$) compared with sows in early- or late-gestation. Phosphorus retention was greater ($P < 0.05$) in late-gestation than in the earlier periods. In Exp. 5, the objective was to test the hypothesis that the Ca level in diets fed to late gestating sows affect the ATTD and retention of Ca and P, blood Ca and P, serum concentrations of hormones, and blood biomarker for bone synthesis and resorption. Sows in late-gestation were fed one of 4 experimental diets containing 25, 50, 75, or 100% of the requirement for Ca with a constant level of P. Results indicated that values for the ATTD of Ca increased quadratically ($P = 0.039$) as Ca in diets increased. Calcium retention increased quadratically ($P < 0.05$) as Ca intake increased. The ATTD of P linearly decreased ($P < 0.001$), but P retention increased as dietary Ca increased. Serum concentrations of Ca and P and estrogen, calcitonin, and parathyroid hormone were not affected by Ca concentrations in diets. The ratio between serum osteocalcin

and carboxyterminal cross-linked telopeptide of type I collagen tended to increase ($P = 0.055$) as dietary Ca increased, which indicated that there was more bone formation than resorption in sows as dietary Ca increased. In conclusion, the STTD of Ca in calcium carbonate differed among different suppliers, but the STTD of Ca in DCP did not vary depending on different suppliers. Tibia, metacarpals, and metatarsals were the best indicators to predict total body bone ash. Gestating sows had much lower values for STTD of Ca and P than growing pigs and effects of microbial phytase on digestibility of Ca and P were much less predictable in gestating sows than in growing pigs. A wide Ca:P ratio decreased ATTD of P, but increased ATTD of Ca and retention of Ca and P in sows in late-gestation. Additional research is needed to determine the STTD of Ca and P in feed ingredients fed to sows and to elucidate interactions among dietary Ca and P, phytase, phytate, and biomarkers in both sows and growing pigs.

Key words: calcium, phosphorus, microbial phytase, phytate, pig, sows

To My Beloved Family (Beom Seok Lee, In Sook Han, and Young Ki Lee)

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CHAPTER 1: INTRODUCTION

The concentration of Ca in most plant feed ingredients is low compared with the requirement for pigs. Therefore, Ca is mostly provided by calcium carbonate or inorganic phosphate. Although Ca is an inexpensive nutrient compared with energy or AA, excess dietary Ca may result in reduced P digestibility, feed intake, and growth performance (Stein et al., 2011; González-Vega et al., 2016; Merriman et al., 2017). Excretion of P may also increase if dietary Ca is provided above the requirement, which may increase environmental pollution (Knowlton et al., 2004). Therefore, determination of digestibility of Ca and P in dietary sources of Ca is needed to reduce Ca and P excretion by preventing over- or under-formulation of Ca and P. Whereas the digestibility of P in most feed ingredients has been reported (NRC, 2012), there is a limited number of experiments in which the digestibility of Ca in feed ingredients used in diets for pigs was determined.

Absorption of Ca has been estimated using the total tract digestibility procedure (González-Vega et al., 2014; Zhang et al., 2016), and the concept of standardized total tract digestibility (**STTD**) of Ca has been introduced because the STTD values are calculated by excluding endogenous Ca (Stein et al., 2016). Once the values for STTD of Ca are evaluated in feed ingredients, diets for pigs can be formulated based on digestible Ca similar to the way values for digestible P in feed ingredients are used in diet formulation. Digestibility of Ca and P may be affected by a number of dietary factors including phytate, exogenous phytase, Ca to P ratio, and the concentration of other minerals. Interactions between dietary Ca and P have been studied for many years, but effects of dietary level of Ca and P on digestibility of Ca and P, growth performance, and bone ash need to be elucidated.

Besides dietary factors, values for apparent total tract digestibility (**ATTD**) of Ca and P

are also affected by the physiological status of pigs because growing pigs have greater ATTD of Ca and P compared with gestating and lactating sows (Kemme et al., 1997). It is also possible that different requirements for Ca and P during different periods of gestation affect the Ca and P balance by sows. However, most values for STTD of Ca and P were determined in ingredients fed to growing pigs and these values are subsequently applied to all categories of pigs, including sows in different gestation periods.

Blood biomarkers including carboxyterminal cross-linked telopeptide of type I collagen, osteocalcin, and bone-specific alkaline phosphatase have been used to predict bone turnover in humans, beef breeder cows, and growing pigs as indicators of Ca and P adequacy in the body (Vasikaran et al., 2011; Anderson et al., 2017; Sørensen et al., 2018). Retained Ca and P in the body and bone turnover may be estimated from serum concentrations of biomarkers, but this relationship has not been demonstrated in sows, and it is not known if blood biomarkers can be used to estimate Ca and P status of gestating sows.

Therefore, the objectives of this dissertation are to determine the STTD of Ca in different sources of calcium carbonate and dicalcium phosphate, to determine correlations between individual bones in the body and total bone ash to identify the bone that is most representative of total body bone ash in growing pig, to compare the STTD of Ca and P and retention of Ca and P in diets fed to sows in mid-gestation and growing pigs, to compare the STTD of Ca in calcium carbonate and Ca and P balance in diets fed to sows in different gestation periods, and to demonstrate the effects of dietary levels of Ca on Ca and P balance and blood biomarkers in late-gestating sows.

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CHAPTER 2: UTILIZATION OF CALCIUM AND PHOSPHORUS IN FEED INGREDIENTS BY SOWS AND GROWING PIGS: LITERATURE REVIEW

INTRODUCTION

Data for relative bioavailability of P have been used to determine the available P in inorganic P supplements as well as in feed ingredients of plant or animal origin and values have usually been determined relative to the availability of P in monosodium phosphate or monocalcium phosphate (NRC, 1998). Most values for bioavailability of P in feed phosphates are between 85 and 100% (NRC, 1998). Likewise, the relative bioavailability of Ca in most Ca supplements that are compared to limestone are between 90 and 100% (Ross et al., 1984; Kuznetsov et al., 1987), but there are very few data for the bioavailability of Ca in inorganic supplements of Ca (NRC, 2012). Values for relative bioavailability of P are variable among feed phosphates that have been used as standards (Petersen et al., 2011), and the relative bioavailability of P is always greater than digestibility values because the digestibility of P in the standard is less than 100% (Baker et al., 2013). Therefore, use of values for the digestibility of Ca and P has been suggested as a more accurate way of evaluating feed ingredients (Fan et al., 2001; Petersen and Stein, 2006; NRC, 2012; Baker et al., 2013; González-Vega et al., 2014).

DIGESTIBILITY AND RETENTION OF CALCIUM AND PHOSPHORUS BY GROWING PIGS

Digestibility

The digestibility of a nutrient represents the amount of that nutrient that disappears from the intestinal tract and it is generally assumed that this amount is also available for metabolism

after absorption (Stein, 2017). Total tract digestibility is used to determine the digestibility of Ca and P because there is limited net absorption or secretion of Ca and P into the large intestine (Fan and Sauer, 2002; Bohlke et al., 2005; González-Vega et al., 2014; Zhang et al., 2016; Stein, 2017). Apparent total tract digestibility (**ATTD**) values (%) can be calculated using Eq. [2.1] (Almeida and Stein, 2010):

$$\text{ATTD} = \frac{\text{intake - output}}{\text{intake}} \times 100, \qquad [2.1]$$

where intake and output of nutrients in feces are expressed as gram per day.

Values for ATTD are usually influenced by dietary nutrient levels because not only dietary nutrients that have not been digested and absorbed, but also nutrients of endogenous origin are excreted in the fecal output, which may result in an underestimation of ATTD values if pigs are fed a diet that is low in the nutrient that the ATTD is determined for (Fan et al., 2001; Zhai and Adeola, 2012). Endogenous losses of Ca and P include Ca and P from saliva juice, gastric juice, epithelial intestinal cells, biliary juice, pancreatic juice, and intestinal enzymes and mucin (Fan et al., 2001; González-Vega et al., 2013; Létourneau-Montminy et al., 2015). Endogenous losses of nutrients from pigs consist of basal endogenous losses and diet specific endogenous losses. Basal endogenous losses are considered an inevitable loss from the body that is related to dry matter intake (**DMI**), whereas the diet specific endogenous losses are losses that are influenced by dietary components (Stein et al., 2007). Values for ATTD can be corrected for either basal endogenous loss or total endogenous loss to calculate standardized total tract digestibility (**STTD**) or true total tract digestibility (**TTTD**), respectively. Because the STTD or TTTD values are not affected by the level of nutrients in the diet, the STTD and TTTD of Ca and P are believed to be additive in mixed diets (Table 2.1; Fan and Sauer, 2002; Fang et al., 2007; Kwon, 2016; Zhang and Adeola, 2017; She et al., 2018). The additivity of values for ATTD

depends on the concentration of the nutrients in the feed ingredients that are included in the mixed diet (Fan and Sauer, 2002; Fang et al., 2007; She et al., 2018).

Basal endogenous losses of Ca and P have been determined by feeding a Ca-free or P-free diet (Petersen and Stein, 2006; González-Vega et al., 2015a) and are calculated using Eq. [2.2] (adapted from Almeida and Stein, 2010):

$$\text{Basal endogenous loss} = \frac{\text{output of Ca or P}}{\text{DMI}} \times 1{,}000\,, \qquad [2.2]$$

where basal endogenous loss is expressed in milligram per kilogram of DMI, DMI in kilogram of DMI per day and the fecal output in gram per day. Once the basal endogenous loss has been determined, daily basal endogenous loss from pigs fed diets containing feed ingredients of interest is calculated as indicated below using Eq. [2.3]:

$$\text{Daily basal endogenous loss} = \text{basal endogenous loss} \div 1{,}000 \times \text{DMI}, \qquad [2.3]$$

where daily basal endogenous loss is in gram per day, basal endogenous loss is in milligram per kilogram of DMI, and DMI of each pig is in kilogram of DMI per day. The STTD values (%) can be calculated from the following Eq. [2.4] (adapted from Almeida and Stein, 2010):

$$\text{STTD} = \frac{\text{intake - (output - daily basal endogenous loss)}}{\text{intake}} \times 100\,, \qquad [2.4]$$

where intake, output, and daily basal endogenous loss are in gram per day.

Values for the basal endogenous loss of P that are estimated using a P-free diet are relatively constant regardless of BW and an average of a number of experiments indicated that a value of 190 mg/kg of DMI is representative of the basal endogenous loss of P (NRC, 2012). However, the basal endogenous loss of Ca that is estimated from pigs fed corn-based or cornstarch-based diets appears to be more variable and values ranging from 123 to 550 mg/kg of DMI have been reported (Table 2.2). Relatively lower values for the basal endogenous loss of Ca

and the ATTD of Ca have been observed in pigs fed cornstarch-based diets compared with pigs fed corn-based diets (González-Vega et al., 2015a). The increase in values for the basal endogenous loss of Ca from pigs fed corn-based diets may be explained by the presence of fiber in corn, which may prevent precipitation of Ca in the intestine. In addition, fermentation of fiber results in reduced pH in the small intestine, which may also result in increased absorption of Ca (González-Vega et al., 2015a). However, more studies are needed to elucidate the factors affecting the basal endogenous loss of Ca.

The total endogenous losses of Ca and P have been determined using a regression procedure that regresses digested Ca or P against different levels of intake of that nutrient (Dilger and Adeola, 2006; González-Vega et al., 2013; Zhang and Adeola, 2017). The negative y-intercept of the regression equation represents the endogenous loss and the slope represents the value for TTTD. The TTTD values (%) can also be calculated using Eq. [2.5] (Petersen and Stein, 2006):

$$\text{TTTD} = \frac{\text{intake - (output - daily total endogenous loss)}}{\text{intake}} \times 100, \qquad [2.5]$$

where intake, output, and daily total endogenous loss are in gram per day. The daily total endogenous loss is calculated in the same way as described above for the daily basal endogenous loss. The total endogenous losses of Ca ranged from 160 to 314 mg/kg DMI depending on the feed ingredients that were used in the diet and on the use of phytase.

Retention

Retention of a nutrient represents the amount of that nutrient that is absorbed and metabolized in the body and can be measured by quantifying the amount of nutrients that is excreted in fecal and urine outputs. Although the urinary Ca and P excretions give small contributions to the total excretion, values for retention of Ca and P may indicate the

relationships between the ratio among digested Ca and digested P, dietary Ca and P, and requirements for Ca and P (Jongbloed, 1987; Poulsen et al., 2010; Symeou et al., 2014; González-Vega et al., 2016b). Calcium and P need to be available in the body at the same time because both minerals are needed for bone tissue synthesis.

Retention values (%) can be calculated from Eq. [2.6] (adapted from Fernández, 1995):

$$\text{Retention} = \frac{\text{intake - (fecal output - urine output)}}{\text{intake}} \times 100\,, \qquad [2.6]$$

where intake and fecal and urine outputs are in gram per day.

SOURCES OF CALCIUM

Most dietary Ca is supplied by mineral supplements, but ingredients of animal origin or plant origin may also provide dietary Ca. Mineral supplements mostly include Ca carbonate and calcium phosphates and concentrations of Ca in Ca supplements range from 15 to 40% (NRC, 2012). Limestone, dicalcium phosphate (**DCP**), and monocalcium phosphate (**MCP**) are the 3 most commonly used Ca sources in animal diets. Calcium carbonate is a major component of ground limestone and contains 40.0% Ca (Table 2.3). Theoretically, based on the total molecular mass, DCP ($CaHPO_4$) and MCP [$Ca(H_2PO_4)_2$] should contain 29.46 and 17.12% Ca, respectively, and 22.77 and 26.47% P, respectively. However, most commercial DCP and MCP contain less Ca and P compared with expected values, which is a result of impurities in DCP and MCP (Baker, 1989; Table 2.4). The reason for the impurities is that the calcium phosphates are produced by reacting phosphoric acid (H_3PO_4) with limestone and impurities from either H_3PO_4 or limestone may result in the impurities in DCP and MCP. Therefore, most DCP and MCP in North America that are used in animal diets contain approximately 24.8 and 16.9% Ca, respectively, and 18.5 and 21.0% P, respectively (Baker, 1989).

Cereal grains and co-products of cereal grains and oilseed meals can also provide dietary Ca, although most of the plant ingredients are low in Ca (range = 0.02 to 1.17%; NRC, 2012). Animal origin feed ingredients including milk products and animal byproducts contain between 0.20 and 8.28% Ca (NRC, 2012).

The ATTD, STTD, and TTTD of Ca in feed ingredients have been determined in recent years and summarized values are presented in Table 2.5. The digestibility of Ca is greatest in animal feed ingredients, followed by mineral supplements and plant feed ingredients, respectively. Calcium digestibility in Ca carbonate or ingredients of animal origin is increased by use of phytase if the diets are formulated based on corn (González-Vega et al., 2015b; Merriman et al., 2016b, Univ. Illinois, 2018). It appears that digestibility of Ca in DCP and MCP is not affected by use of phytase (González-Vega et al., 2015b). The reason for this deviation may be that Ca from DCP or MCP is bound to phosphate, which results in Ca from DCP or MCP being less likely to bind to phytate in diets (Walk, 2016).

DIGESTIBILITY AND RETENTION OF CALCIUM AND PHOSPHORUS BY SOWS

Digestibility of Ca and P in Diets Fed to Gestating Sows and Lactating Sows

Values for ATTD of Ca in diets fed to gestating sows ranged from -5.9 to 63.1% and ATTD of P ranged from 12.2 to 48.7% depending on the use or not of exogenous phytase and on dietary sources of Ca and P (Table 2.6). Dietary Ca ranged from 0.40 to 1.16% and dietary P ranged from 0.36 to 1.09%, but it is not clear if there are correlations among dietary Ca and P levels, phytate level, and the ATTD of Ca and P in diets fed to gestating sows. However, it appears that responses to microbial phytase on the ATTD of Ca and P in diets fed to gestating sows is inconsistent and less predictable than in diets fed to growing pigs.

Values for ATTD of Ca in diets fed to lactating sows ranged from 19.5 to 63.0% and ATTD of P ranged from 19.2 to 72.0% for ATTD of P (Table 2.7). Generally, it appears that values for lactating sows are greater than values for gestating sows. As is the case for growing pigs, previous studies have demonstrated that values for ATTD of Ca and P are increased by inclusion of phytase in the diet for lactating sows and the digestibility of P increases if inorganic sources of P are used.

Sows in their first parity may need more Ca and P compared with multiparous sows because it is possible that they are still growing. Therefore, parity of sows may affect the digestibility of Ca and P because first parity sows need Ca and P not only for maintenance, fetus development, or recovery, but also for growth (Bikker and Blok, 2017). Few studies have demonstrated the effects of parity on the digestibility of Ca and P in gestating and lactating sows. Values for ATTD of P were greater if gestating sows were in their fourth parity compared with their third parity, but the ATTD of Ca was not affected by parity (Hanczakowska et al., 2009). During lactation, the ATTD of Ca and P was not different between parity 1 and parity 8 sows (Kemme et al., 1997b; Hanczakowska et al., 2009).

Comparison of Ca and P Digestibility in Sows and Growing Pigs

The STTD of Ca and P in most feed ingredients have been determined in recent years, but most values were determined in ingredients fed to growing pigs and these values are subsequently applied to all categories of pigs, including sows. It has been demonstrated that digestibility of energy and some nutrients are affected by the physiological state of the animal and sows usually have greater digestibility values than growing pigs (Le Goff and Noblet, 2001; Casas and Stein, 2017). Absorption and retention of Ca and P also increase during pregnancy in humans and rats compared with non-pregnant periods because of an increased need for maternal

body and fetus Ca and P (Institute of Medicine, 1990; Pérez et al., 2008; Kovacs, 2016). However, pig data have shown that ATTD of Ca and P in growing pigs are greater than in gestating and lactating sows when growing pigs were fed the same diet as the sows (Kemme et al., 1997a; Lee et al., 2018). Although the ATTD of Ca is not affected by dietary Ca concentration if a diet contains between 55 and 173% of the requirement for Ca (Stein et al., 2011), it is possible that a combination of different requirements for Ca and P and different ratios between Ca and P may result in differences in the digestibility of Ca and P.

Differences in feed intake between gestating sows and growing pigs may affect the ATTD values, but it is not likely that they are the main reason for the difference because feed intake of sows does not affect digestibility of Ca and P (Lee et al., 2018). Greater amount of endogenous losses from sows than from growing pigs may also affect ATTD of Ca and P, but there are no data comparing endogenous losses of Ca and P by sows and growing pigs.

It is possible that digestibility of Ca and P in sows is affected by the physiological stage of sows. The blood level of estrogen is related to Ca metabolism in the body (Heaney, 1990; Ross et al., 2011; Harmon et al., 2016), and estrogen increases in the blood during late gestation and during post-parturition to support development of mammary glands (Kensinger et al., 1982). Serum parathyroid hormone (**PTH**) and calcitriol that upregulate the para-cellular absorption of Ca also increase in pregnant women throughout pregnancy and further increases after parturition (Ardawi et al., 1997). Sows in mid- or late- gestation have reduced ATTD of Ca and P compared with lactating sows (Kemme et al., 1997a; Jongbloed et al., 2004; Männer and Simon, 2006; Nyachoti et al., 2006). Furthermore, the ATTD of Ca and P is reduced in mid-gestation compared with late-gestation (Kemme et al., 1997a; Jongbloed et al., 2004; Nyachoti et al., 2006; Jongbloed et al., 2013). Therefore, it is possible that an increase in estrogen results in an increase

in the digestibility of Ca and P by sows in late-gestation or lactation. However, research is needed to investigate if day of gestation and lactation influences basal endogenous losses and STTD of Ca and P in sows.

Calcium and P Retention by Sows

Considerably lower amounts of Ca and P relative to intake were retained by sows in early-, mid-, or late-gestation (Everts et al., 1998; Darriet et al., 2017). It is possible that the Ca and P needs in the body change depending on the stage of gestation. Very little Ca and P is needed for fetus development by sows in early- to mid-gestation compared with sows in late-gestation (Bikker and Blok, 2017). Furthermore, sows may not need nutrients for growth and may not need a high amount of Ca and P for maintenance because they have accumulated a large amount of Ca and P in the body over a long period of time. However, more research is needed to demonstrate if there is a relationship between Ca and P retention and dietary components or the reproductive status of the sow.

First parity sows are expected to retain more dietary Ca and P in the body compared with multiparous sows because of maternal growth, but very limited data are available to quantitate these effects. Based on computer models, it was proposed that more Ca and P relative to body size need to be retained in first parity sows compared with second or third parity sows (Everts et al., 1998), and Bikker and Blok (2017) suggested different Ca and P requirements for gestating and lactating sows in different parities.

FACTORS AFFECTING DIGESTIBILITY AND RETENTION OF CALCIUM IN PIGS

The digestibility or retention of Ca by growing pigs may be influenced by dietary phytate, exogenous phytase, Zn supplementation, sodium chloride, diet composition,

supplemental fat, or organic acids (Jongbloed et al., 2000; González-Vega et al., 2015a; Merriman, 2016; Merriman et al., 2016a; Blavi et al., 2017). Metabolism of Ca and P in the body is regulated by hormones including PTH and calcitonin, and estrogen (Pérez et al., 2008).

Regulation in the Body

Calcium in soluble or ionic forms is absorbed by both passive diffusion (paracellular) and active transport (transcellular) and the primary route for Ca absorption depends on dietary Ca levels (Bronner, 2003). Absorption of Ca by active transport in the small intestine is regulated by calcitriol that is the active form of vitamin D (1,25-dihydroxycholecalciferol) and the hormones including calcitonin and PTH (Crenshaw, 2001). If blood Ca concentration, i.e., ionic Ca++, is low, PTH is released from the parathyroid glands, which stimulates production of calcitriol in the kidney, and the calcitriol then binds to the vitamin D receptor in the intestinal tract to increase Ca absorption from the small intestine. The efflux of Ca from bones, and the reabsorption of Ca in the kidney are also increased by PTH, and combined, these effects result in a greater blood Ca concentration (Crenshaw, 2001; Molina, 2013; Blaine et al., 2015). Calcitonin, however, is released from the thyroid glands in response to high blood Ca level and the overall effect is to decrease blood Ca level by reducing active transport of Ca from the intestine, inhibiting reabsorption of Ca from the kidney, and storing Ca in the skeleton (Crenshaw, 2001; Molina, 2013). However, recent data indicate that the overall effect of calcitonin on regulation of intestinal absorption of Ca is limited (Stein et al., 2011).

Passive diffusion between cells is the primary route of Ca absorption if pigs are fed a diet containing Ca at or above the requirement, but active transport across the cell is the primary route if dietary Ca is below the requirement (González-Vega et al., 2016a; Lagos, 2018). It appears that the total absorption of Ca is not regulated by the concentration of Ca in the diet, but

retention of Ca is regulated by mechanisms of excretion and reabsorption of Ca by the kidney. Therefore, growing pigs tend to absorb a large amount of dietary Ca if they are fed above the requirement and subsequently excrete the excess Ca in the urine (Stein et al., 2011).

Bone Turnover Markers

Bone turnover is a continuous process that is remodeling bone by forming and removing bone tissue (also referred to as resorption) and this process is associated with osteocytes, osteoblasts, and osteoclasts for bone maintenance, formation, and resorption, respectively (Seibel, 2005). Therefore, several bone turnover markers that are associated with bone metabolism have been used to diagnose bone-related medical conditions in humans (Seibel, 2005; Vasikaran et al., 2011). Osteoblasts are bone-forming cells that synthesize cross-linked collagens and matrix proteins including osteocalcin and osteopontin, which produce alkaline phosphatase to mineralize the bone (Robey et al., 1993; Bassi et al., 2011; Niedźwiedzki and Filipowska, 2015). Therefore, the activity of osteoblasts can be measured by analyzing blood or urine for osteocalcin or bone-specific alkaline phosphatase (**BAP**), or procollagen peptides (Seibel, 2005; Vasikaran et al., 2011). Osteoclasts are cells that digest old bone by secreting acids and a collagenase (Bord et al., 1996). As bone undergoes resorption, there are several byproducts and activity of osteoblasts can also be estimated by analyzing blood or urine for inorganic P, pyridinoline, hydroxyproline, hydroxylysine-glycosides, or collagen cross-linked telopeptide (Seibel, 2005; Vasikaran et al., 2011).

Several experiments have been conducted with pigs to demonstrate relationships between bone turnover markers and dietary Ca, P, and vitamin D (Larsen et al., 2000; Weber et al., 2014; Sørensen et al., 2018) or parity of gestating sows (Weber et al., 2014; Schmidt et al., 2018).

In growing pigs, higher concentration of Ca in diets relative to requirement estimates

decreased plasma carboxyterminal cross-linked telopeptide of type I collagen (**CTX-I**), which is one of the collagen cross-linked telopeptides that is derived from bone resorption, but alkaline phosphatase and hydroxyproline levels were not affected by dietary Ca (Larsen et al., 2000). Osteocalcin level increased if concentration of P increased in diets and because of lower osteoblast activity, serum BAP decreased as pigs became older (Sørensen et al., 2018). Serum CTX-I decreased with increasing P concentrations in diets, which increased the osteocalcin to CTX-I ratio (Sørensen et al., 2018). Serum BAP (Liesegang et al., 2005; Verheyen et al., 2007; Lauridsen et al., 2010) and osteocalcin (Lauridsen et al., 2010; Weber et al., 2014; Schmidt et al., 2018) concentrations were greater by sows in early-gestation compared with sows in later gestation or lactation, which may be a result of an increase in the requirement for Ca and P in late gestation and lactation compared with early gestation. Gilts or sows in low parities had greater parameters for bone resorption and formation compared with sows in greater parities because they were growing (Weber et al., 2014; Schmidt et al., 2018). It appears that biomarkers may be used to predict bone metabolism in both growing pigs and sows and that there are certain correlations between biomarkers and dietary Ca and P.

Calcium and P Interactions

Excess dietary Ca reduces P digestibility and growth performance of pigs (Stein et al., 2011; González-Vega et al., 2016b; Merriman et al., 2017; Wu et al., 2017; Lagos, 2018). Calcium may be oversupplied to swine diets because limestone is less expensive than other feed ingredients and limestone is sometimes used as a carrier in vitamin and mineral premixes and nutritional additives or as a flow agent in feed mills. This may lead to greater Ca concentrations in diets compared with expected values (Walk, 2016; Wu et al., 2018). Dietary P level is not likely affecting Ca digestibility, but retention of Ca is reduced if digested P is not adequate

(Létourneau-Montminy et al., 2014; González-Vega et al., 2016a) because Ca and P can be retained in the body only if both Ca and P are available at the same time (Crenshaw, 2001). Unlike P digestibility, Ca digestibility is unlikely to be affected by Ca or P concentrations in diets if Ca and P are from inorganic sources (Stein et al., 2006; González-Vega et al., 2016a). Calcium absorption is negatively affected by phytate-P if exogenous phytase is not supplemented in diets (Misiura et al., 2018), which likely is a result of dietary Ca being bound to phytate, resulting in formulation of a Ca-phytate complex, which reduces Ca digestibility. However, Ca from Ca carbonate or animal or plant sources more actively binds to phytate compared with Ca from DCP or MCP (Walk, 2016).

Phytate and Exogenous Phytase

Phytic acid or phytate that consists of an inositol ring and 6 phosphates that are attached to the inositol ring by ester bonds is a primary form for storage of P in grains and oilseeds. As a consequence, most plant feed ingredients that are commonly used in swine diets have high concentrations of phytate-bound P relative to total P (Table 2.8). Pigs have low utilization of phytate-bound P because phytase is not produced in sufficient quantities by the body to hydrolyze the ester bonds in phytate and thus release the P. Phytate may also be bound to positively charged cations including Ca^{++} because of the negatively charged reactive sites on the phytate molecule, which results in chelated mineral-phytate compounds that may precipitate in the intestinal tract (Nelson and Kirby, 1987; González-Vega et al., 2015b). Calcium from plant ingredients as well as Ca from Ca carbonate tend to bind to phytate to form the Ca-phytate complex (Selle et al., 2009; González-Vega et al., 2015b).

Dietary phytate has a negative correlation with the digestibility of Ca and P by growing pigs (Almaguer et al., 2014; Misiura et al., 2018), and use of microbial phytase in diets for pigs

has increased Ca digestibility (Almeida et al., 2013; Rodríguez et al., 2013; González-Vega et al., 2015b). The quantity of phytase that is added to swine diets is usually between 250 and 1,000 phytase units/kg of diet although up to 2,500 units may sometimes be used (Walk, 2016). Efficacy of dietary exogenous phytase depends on its substrate, phytate, in the diets (Selle et al., 2009; Adeola and Cowieson, 2011), and the efficacy of phytase is reduced by a wide Ca to P ratio (Lei et al., 1994; Qian et al., 1996; Brady et al., 2002). Supplementary Zn may also reduce the efficacy of phytase, but effects of microbial phytase on ATTD and STTD of Ca was not affected by ZnO in growing pigs (Blavi et al., 2017).

Physiological Status and Exogenous Phytase

Efficacy of phytase may be influenced by the physiological status of pigs and it may be greater in lactating sows compared with growing-finishing pigs, late-gestating sows, weanling pigs, and mid-gestating sows (Kemme et al., 1997a; Sulabo, 2004).

During gestation, it is possible that reduced feed intake and the longer gastrointestinal tract in sows compared with growing pigs affect the retention time and, therefore, the efficacy of phytase to release P and Ca may be low. The activity of phytase is maximized if pH is between 2 and 5, depending on the phytase used (Tomschy et al., 2002; Kim et al., 2006; Selle and Ravindran, 2008). Therefore, the main site of activity of exogenous phytase is the stomach and the upper part of the small intestine (Jongbloed et al., 1992). Gestating sows may have lower pH compared with growing pigs because of lower feed allowance and longer intervals between meals, which theoretically should result in greater activity of phytase (Wang et al., 2003; Blaabjerg et al., 2011). However, the response to phytase appears to be less if phytase is used in diets for gestating sows compared with growing pigs (Kemme et al., 1997a), and lactating sows have a greater response to exogenous phytase than gestating sows despite the much greater feed

intake by lactating sows (Kemme et al., 1997a; Nyachoti et al., 2006; Jang et al., 2014). It, therefore, appears that there are factors determining the response to phytase that have not yet been elucidated.

Other Factors Influencing Ca Digestibility

Absorption of Ca or P may be decreased if insoluble fiber or phytate is included in the diet, which results in less transit time (Nortey et al., 2007; Hill et al., 2008). Likewise, the ATTD of Ca in Ca carbonate decreased if inclusion rate of cellulose increased from 4% to 12% in the diet (Son and Kim, 2015). Thus, it appears that insoluble fiber, which is largely unfermentable, reduces ATTD of Ca. However, Ca and P absorption may increase if fermentable dietary fiber is used. Calcium, Na, K, and Mg absorption in the cecum increased in rats fed a high fiber diet compared with a fiber-free diet and this was explained by an increase in mineral absorption via diffusion because of the lower intestinal pH, which was a result of fiber fermentation and synthesis of volatile fatty acids (Demigné and Rémésy, 1985). Cecal pH decreased with increased volatile fatty acids, and absorption of Ca, P, and Mg increased if inulin was included in diets fed to rats (Levrat et al., 1991). Likewise, use of corn and corn germ meal rather than cornstarch increased ATTD of Ca and P in pigs (González-Vega et al., 2015a), which may also be a result of reduced pH in the intestinal tract of pigs. Supplementation of organic acids increased the ATTD of Ca and P in growing pigs (Radcliffe et al., 1998; Kemme et al., 1999; Jongbloed et al., 2000), and lactation sows (Liu et al., 2014), which further indicated that a reduced intestinal pH tended to reduce chelation of minerals, which resulted in greater solubility and absorption (Ravindran and Kornegay, 1993; Kil et al., 2011). Particle size of Ca carbonate may affect the digestibility of Ca because reduced particle size increases in vitro solubility (Zhang and Coon, 1997). In poultry, finer particle size of Ca carbonate reduces the transit time

and, therefore, reduces the solubility in the gizzard compared with a coarser particle size (Rao and Roland, 1990; Zhang and Coon, 1997). Transit time and pH in the gastrointestinal tract of poultry and the presence of a gizzard are factors that influence digestibility of nutrients (Svihus, 2014; Li et al., 2015) and poultry data are, therefore, not always representative of pigs. The particle size of Ca carbonate did not influence the relative bioavailability of Ca, the digestibility and retention of Ca, or growth performance in growing pigs (Ross et al., 1984; Merriman and Stein, 2016). The reason for the difference between broiler chickens and pigs may be that pigs can digest and absorb Ca along the gastrointestinal tract until the end of the ileum, whereas poultry have a relatively shorter small intestine and much greater digesta viscosity compared with pigs. Fatty acids may also form complexes between Ca and fat in the gastrointestinal tract, which may reduce the digestibility Ca and specifically saturated fatty acids increase the formation of complexes (Mattson et al., 1979). However, values for ATTD of Ca were not affected if choice white grease or plant oils were used (Steiner et al., 2006; González-Vega et al., 2015a; Merriman et al., 2016a). In contrast, the ATTD of Ca may be increased by addition of tallow or plant oils to diets for pigs (Merriman et al., 2016a), which may be a result of reduced passage rate of digesta in the small intestine of pigs fed diets containing increased concentrations of fat.

CONCLUSIONS

In recent years, values for STTD of Ca and P in feed ingredients fed to growing pigs have been determined because STTD values are believed to be additive in a complete diet. Therefore, use of STTD may result in the most accurate diet formulations. There is, however, a lack of information if the STTD of Ca is different among different suppliers of inorganic sources without or with exogenous phytase fed to growing pigs. It is also not known if values for the STTD of Ca and P can be applied to diet formation for sows in different status.

Calcium and P utilization for bone turnover in the body may be estimated by using blood biomarkers, but there is a lack of information if the blood biomarkers also can be used for gestating sows. There is a need for further research to determine Ca digestibility in feed ingredients and to demonstrate the interactions among dietary Ca and P, phytase, and blood biomarkers in diets fed to gestating sows and growing pigs.

TABLES

Table 2.1. Additivity of values for apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and true total tract digestibility (TTTD) of Ca and P in mixed diets fed to growing pigs

Feed ingredients in diets	ATTD				STTD				TTTD			
	Measured	Predicted	Diff.	SE	Measured	Predicted	Diff.	SE	Measured	Predicted	Diff.	SE
Calcium												
Limestone, dicalcium phosphate[1]	69.3	68.1	1.3[b]	-	-	-	-	-	73.7	72.7	1.1	-
Phosphorus												
Barley, canola meal[2]	22.8	23.8	-1.0	-	-	-	-	-	-	-	-	-
Wheat, pea[2]	45.1	38.1	7.0[a]	-	-	-	-	-	-	-	-	-
Soybean meal, oat, rough rice, broken rice, corn[3]	15.5	27.3	-11.8[a]	5.3	-	-	-	-	40.4	42.0	-1.6	3.9
Soybean meal, buckwheat, pea,faba bean, sorghum[3]	21.2	29.9	-8.7[a]	4.9	-	-	-	-	42.3	41.0	1.3	3.0
Wheat, soybean meal[4]	45.1	41.3	3.8[a]	1.6	49.7	47.8	1.9	1.6	-	-	-	-
Corn, soybean meal[5]	40.9	42.8	1.9	3.0	44.7	49.0	-4.3	3.0	-	-	-	-
Corn, soybean meal, canola meal[5]	41.0	37.0	-4.0[a]	1.1	44.1	42.9	1.3	1.1	-	-	-	-

[a]Measured and predicted values differ, $P < 0.05$.

[b]Measured and predicted tend to be different, $0.05 < P < 0.10$.

[1]Zhang and Adeola, 2017.

Table 2.1. (Cont.)

[2]Fan and Sauer, 2002.

[3]Fang et al., 2007

[4]Kwon, 2016.

[5]She et al., 2018.

Table 2.2. Estimates of basal endogenous loss and total endogenous loss of Ca by growing pigs

Reference	Initial BW, kg	Endogenous loss of Ca, mg/kg DMI	Main ingredients
Ca-free diet feeding method (basal endogenous loss)			
González-Vega et al. (2015a)	19.2	220	Cornstarch
	19.4	396	Corn
González-Vega et al. (2015b)	17.7	123	Cornstarch
Merriman and Stein (2016)	15.4	329	Corn
Merriman (2016)	14.9	550	Corn
Blavi et al. (2017)	15.4	430	Corn
Santana et al. (2017)	20.5	140	Corn, soybean meal (1.25% soybean meal)
Regression method (total endogenous loss)			
Zhang and Adeola (2017)	20.0	207	Corn, corn gluten meal, limestone
	20.0	316	Corn, corn gluten meal, dicalcium phosphate
	20.0	264	Corn, corn gluten meal, limestone, dicalcium phosphate
González-Vega et al. (2013)	16.7	160	Cornstarch, canola meal
	16.7	189 (with phytase)	Cornstarch, canola meal

Table 2.3. Total molecular weight of Ca carbonate, dicalcium phosphate (DCP), monocalcium phosphate (MCP) and percentage composition of chemically pure inorganic supplements

	Total molecular mass (g/mol)	Percentage (%)
Calcium carbonate [$CaCO_3$]		
Ca × 1	40.08	40.04
C × 1	12.01	12.00
O × 3	48.00	47.96
Total	100.09	100.00
DCP [$CaHPO_4$]		
Ca × 1	40.08	29.46
H × 1	1.01	0.74
P × 1	30.97	22.77
O × 4	64.00	47.04
Total	136.06	100.00
MCP [$Ca(H_2PO_4)_2$]		
Ca × 1	40.08	17.12
H × 4	4.03	1.72
P × 2	61.95	26.47
O × 8	128.00	54.69
Total	234.05	100.00

Table 2.4. Impurities in commercial dicalcium phosphate (DCP) and monocalcium phosphate (MCP)[1,2]

Component	Chemical formula	Limestone	DCP (18.5% P)	MCP (21.0% P)
Calcium carbonate	$CaCO_3$	91.8	6.74	6.00
DCP and MCP				
Monocalcium phosphate	$Ca(H_2PO_4)_2 \cdot H_2O$	-	14.19	60.98
Dicalcium phosphate	$CaHPO_4$	-	26.42	12.54
Hydrated dicalcium phosphate	$CaHPO_4 \cdot H_2O$	-	34.65	-
Others				
Silica	SiO_2	3.5	0.15	0.13
Calcium fluoride	CaF_2	-	0.32	0.44
Sodium phosphate	$NaH_2PO_4 \cdot 2H_2O$	-	0.54	0.61
Phosphoric acid	H_3PO_4	-	0.80	1.00
Water	H_2O	-	0.80	1.00
Aluminum phosphate	$AlPO_4$	-	2.21	2.48
Alumina	Al_2O_3	2.5	-	-
Ferrous phosphate	$FePO_4 \cdot 2H_2O$	-	2.65	2.98
Calcium sulfate	$CaSO_4 \cdot H_2O$	-	3.51	3.95
Magnesium oxide	$CaMg(CO_3)_2$	2.2	-	-
Magnesium phosphate, dibasic	$Mg(H_2PO_4)_2 \cdot 4H_2O$	-	7.02	7.89
Total		100.00	100.00	100.00
Nutrient composition[3]				
Calcium	Ca	38.5	24.8	16.9

Table 2.4. (Cont.)

Phosphorus	P	-	18.8	21.5

[1]Values for limestone are estimated from Spiropoulos (1985) and unpublished data from the University of Illinois (2017).

[2]Values for DCP and MCP are adapted from Baker (1989).

[3]Values are from NRC (2012).

Table 2.5. Apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and true total tract digestibility (TTTD) of Ca in feed ingredients without and with phytase added to the diet fed to growing pigs

Item, %	ATTD of Ca		STTD of Ca		TTTD of Ca	
Supplementation of phytase[1]	-	+	-	+	-	+
Mineral supplements						
Monocalcium phosphate[2]	83	83	86	86	-	-
Dicalcium phosphate[2, 3]	73	76	77	79	76	-
Calcium carbonate[2, 3, 4, 5, 6, 7, 8, 9]	68	74	71	77	70	-
Calcium carbonate without fat source[6]	52	-	-	-	-	-
Lithothamnium calcareum[2]	63	66	65	69	-	-
Plant feed ingredients						
Canola meal[7, 8, 10]	41	-	45	70	47	70
Soybean meal[7, 8, 11]	53	-	78	-	-	-
Sugar beet co-product[2]	66	63	68	65	-	-
Sunflower meal[8]	22	-	-	-	-	-
Animal feed ingredients						
Meat and bone meal[12]	75	-	77	82	-	-
Meat meal[12]	75	-	77	86	-	-
Fish meal[13]	62	71	65	73	-	-
Poultry meal[12]	85	74	82	76	-	-
Poultry by product meal[12]	81	84	88	87	-	-
Skim milk powder[7]	95	-	97	-	-	-

Table 2.5. (Cont.)

Whey powder[7]	97	-	99	-	-	-
Whey permeate[7]	61	-	63	-	-	-

[1]Phytase level varies from 500 to 1,500 phytase units/kg diet.

[2]González-Vega et al. (2015b).

[3]Zhang and Adeola (2017).

[4]Blavi et al. (2017).

[5]Merriman and Stein (2016).

[6]Merriman et al. (2016a).

[7]Unpublished data from the University of Illinois.

[8]Zhang et al. (2016).

[9]Kwon and Kim (2017).

[10]González-Vega et al. (2013).

[11]Bohlke et al. (2005).

[12]Merriman et al. (2016b).

[13]González-Vega et al. (2015a).

Table 2.6. Digestibility of Ca and P in diets without or with microbial phytase fed to gestating sows

Ref.[1]	d of gestation	Main ingredients	Inorganic sources for Ca and P[2]	Dietary level			ATTD	
				Ca	P	Phytase	Ca	P
1	60	Wheat middlings Corn Tapioca meal Peas	CC	0.61	0.49	-	13.4	13.7
				0.63	0.48	500 (Natuphos®)	9.4	20.4
	90	Potato protein Soybean extracted Sunflower meal extracted		0.61	0.49	-	23.8	18.3
				0.63	0.48	500 (Natuphos®)	23.4	33.3
2	70	Barley Tapioca Soybean extract Sunflower seed extract	CC MCP	0.64	0.52	-	16.7	16.3
		Beet pulp Soybean hulls	CC	0.52	0.39	-	18.6	13.9
				0.57	0.39	750 (RONOZYME® P)	23.8	22.3
				0.65	0.40	1,000 (RONOZYME® P)	20.4	22.3
	100		CC MCP	0.64	0.52	-	27.5	26.7
			CC	0.52	0.39	-	28.1	21.7
				0.57	0.39	750 (RONOZYME® P)	34.2	32.5
				0.65	0.40	1,000 (RONOZYME® P)	29.3	32.0
3	48.5	Corn Soybean meal Soybean hulls Wheat bran	CC MCP	0.69	0.47	-	22.3	20.4
				0.71	0.36	500 (Peniophora phytase)	23.9	22.5

Table 2.6. (Cont.)

				0.71	0.36	750 (Peniophora phytase)	26.2	23.1
				0.71	0.36	1,000 (Peniophora phytase)	29.1	29.5
4	90	Corn Soybean meal	CC DCP	1.05	0.63	-	63.1	12.2
				0.60	0.49	-	40.2	19.0
				0.65	0.51	500 (Phyzyme®)	52.3	25.8
				0.74	0.49	1,000 (Phyzyme®)	51.3	21.6
		Wheat Barley Soybean meal Canola meal	CC DCP	0.89	0.66	-	49.4	19.9
				0.83	0.59	-	50.1	16.1
				0.77	0.66	500 (Phyzyme®)	60.6	39.6
				0.69	0.58	1,000 (Phyzyme®)	44.9	24.4
5	71	Corn Oat Sugar beet Soybean meal Rapeseed meal	CC DCP CC	0.78	0.46	-	31.6	42.6
				0.61	0.37	-	23.5	34.2
				0.61	0.37	125 (New Phytase; AB Enzymes)	31.8	45.6
				0.61	0.37	250 (New Phytase; AB Enzymes)	36.8	48.5
				0.61	0.37	375 (New Phytase; AB Enzymes)	37.2	48.3
				0.61	0.37	10,000 (New Phytase; AB Enzymes)	40.2	48.7
6	70	Barley Tapioca Soybean extract Corn gluten meal	CC MCP	0.58	0.52	-	18.5	22.0

Table 2.6. (Cont.)

			CC	0.53	0.40	-	25.8	22.3
				0.40	0.40	125 (OptiPhos®)	25.7	32.2
				0.50	0.40	250 (OptiPhos®)	28.9	31.8
				0.60	0.40	1,000 (OptiPhos®)	26.3	30.3
	100	Sunflower seed extract Dried beet pulp Palm kernel expeller Soybean hulls Rapeseed meal	CC MCP CC	0.58	0.52	-	25.5	29.6
				0.53	0.40	-	28.6	22.1
				0.40	0.40	125 (OptiPhos®)	34.3	38.3
				0.50	0.40	250 (OptiPhos®	36.5	38.2
				0.60	0.40	1,000 (OptiPhos®)	32.9	40.0
7	75	Corn Soybean meal	CC DCP	0.75	0.61	-	26.4	29.6
				0.64	0.51	-	21.2	22.6
				0.64	0.51	500 (Natuphos®)	23.6	27.7
8	108	Barley Wheat Field pea Corn DDGS	-	0.88	0.69	500 (Phyzyme® XP)	25.2	41.4
9	90	Corn Soybean meal	CC MCP	0.78	0.68	-	31.8	40.1
				0.81	0.68	-	21.7	31.4
				0.84	0.69	-	16.4	28.4
10	35	Corn-SBM	CC DCP	0.65	0.60	500 (®quantumblue)	-5.9	20.5

Table 2.6. (Cont.)

	Corn Soybean meal Full-fat rice bran	CC	0.66	0.98	500 (®quantumblue)	4.6	13.9
	Corn Soybean meal Defatted rice bran	CC	1.16	1.09	500 (®quantumblue)	20.7	19.1

[1]Ref. list = 1. Kemme et al. (1997a); 2. Jongbloed et al. (2004); 3. Männer and Simon (2006); 4. Nyachoti et al. (2006); 5. Hanczakowska et al. (2009); 6. Jongbloed et al. (2013); 7. Jang et al. (2014); 8. Nasir et al. (2014); 9. Darriet et al. (2017); 10. Lee et al., 2018.

[2]CC = Ca carbonate; DCP = dicalcium phosphate; MCP = monocalcium phosphate.

Table 2.7. Digestibility of Ca and P in diets without or with phytase fed to lactation sows

Ref.[1]	d of lactation	Main ingredients	Inorganic sources for Ca and P[2]	Dietary level			ATTD	
				Ca	P	Phytase	Ca	P
1	10	Wheat middlings Corn	CC	0.61	0.49	-	29.7	19.4
		Tapioca meal Peas		0.63	0.48	500 (Natuphos®)	26.4	40.8
	24	Potato protein Soybean extracted		0.61	0.49	-	31.2	19.2
		Sunflower meal extracted		0.63	0.48	500 (Natuphos®)	36.7	41.2
2	11	Barley Tapioca meal	CC	0.30	0.40	-	31.1	29.0
		Soybean meal Peas		0.42	0.40	400 (Natuphos®)	45.2	46.2
	18	Rapeseed meal Sunflowerseed meal		0.30	0.40	-	39.8	31.7
				0.42	0.40	400 (Natuphos®)	47.6	45.1
	11	Barley Corn	CC MCP	0.35	0.37	-	40.7	32.9
		Soybean meal		0.64	0.50	-	38.5	38.9
	18			0.35	0.37	-	42.5	34.5
				0.64	0.50	-	42.0	41.9
3		Corn Soybean meal	CC DCP	0.82	0.74	-	-	59.0
			CC DCP	0.77	0.54	500 (Natuphos®)	-	72.0
4	14	Barley Tapioca Soybean extract Peas Corn gluten feed	CC MCP	0.80	0.68	-	24.6	34.9

Table 2.7. (Cont.)

			CC	0.54	0.51	-	19.5	21.4
				0.62	0.50	750 (Peniophora phytase)	29.4	36.8
				0.69	0.50	1,000 (Peniophora phytase)	28.8	38.1
				0.82	0.50	10,000 (Peniophora phytase)	29.0	44.9
5	17	Barley	CC	0.91	0.59	-	58.8	47.9
		Corn Soybean meal	MCP	0.95	0.35	-	59.6	40.7
				0.95	0.35	500 (Consensus phytase)	60.6	55.0
				0.95	0.35	1,000 (Consensus phytase)	63.0	62.6
6	18	Corn Soybean meal	CC DCP	0.71	0.50	-	47.1	25.1
				0.71	0.45	-	46.9	23.3
				0.78	0.44	500 (Phyzyme®)	59.1	38.1
				0.64	0.45	1,000 (Phyzyme®)	47.2	40.3
		Wheat Barley Corn SBM	CC DCP	0.57	0.71	-	54.0	30.3
				0.56	0.58	-	49.8	32.6
				0.44	0.58	500 (Phyzyme®)	43.0	42.1
				0.55	0.54	1,000 (Phyzyme®)	50.4	46.7
7	23	Barley Oat	CC DCP	0.84	0.64	-	51.3	45.3
		Wheat Rapeseed extract	CC	0.82	0.45	-	50.4	42.3
		Soybean extract Dried grass	CC	0.83	0.46	500 (RONOZYME® P)	52.6	49.7

Table 2.7. (Cont.)

		Triticale	CC	0.86	0.61	-	53.4	50.8
		Oat	DCP					
		Rye	CC	0.83	0.42	-	54.1	47.1
		Rapeseed extract						
		Garden pea	CC	0.83	0.42	500 (RONOZYME® P)	56.9	56.4
		Soybean extract						
		Dried grass						
8	17.5	Barley	CC	0.50	0.45	-	38.6	28.6
		Tapioca						
		Soybean extracted	CC	0.50	0.46	125 (OptiPhos®)	43.4	46.6
		Peas						
		Corn gluten meal	CC	0.59	0.46	250 (OptiPhos®	41.0	47.6
		Sunflower seed extracted						
		Rapeseed meal	CC	0.67	0.45	500 (OptiPhos®)	42.5	48.6
			CC	0.80	0.46	1,000 (OptiPhos®)	38.4	48.3
			CC	0.77	0.63	-	32.9	35.8
			MCP					
9	11	Corn	CC	0.64	0.54	-	27.3	34.8
		Soybean meal	DCP					
				0.64	0.54	500 (Natuphos®)	37.2	46.3
				0.75	0.65	-	32.4	35.4
10	20	Corn	CC	0.82	0.62	-	43.9	34.7
		Wheat bran	DCP					
		Full fat soybean, Soybean meal, Fish meal						
11	8	Wheat	CC	1.17	0.78	-	31.6	34.9
		Soybean meal	DCP					
		Field pea	CC	1.05	0.53	-	23.4	36.1
		corn DDGS						
		Canola meal		1.05	0.53	500 (RONOZYME® P)	24.1	42.1
	15		CC	1.17	0.78	-	30.9	29.8
			DCP					

Table 2.7. (Cont.)

	CC	1.05	0.53	-	26.2	34.0
		1.05	0.53	500 (RONOZYME® P)	28.6	46.0

[1]Ref. list = 1. Kemme et al. (1997a); 2. Kemme et al. (1997b); 3. Baidoo et al. (2003); 4. Jongbloed et al. (2004); 5. Männer and Simon (2006); 6. Nyachoti et al. (2006); 7. Grela et al. (2011); 8. Jongbloed et al. (2013); 9. Jang et al. (2014); 10. Liu et al. (2014); 11. Nasir et al. (2014).

[2]CC = Ca carbonate; DCP = dicalcium phosphate; MCP = monocalcium phosphate.

Table 2.8. Concentrations (%) of Ca, P, and phytate-bound P and percentage of phytate-bound P relative to total P in plant feed ingredients

Ingredient[1]	Ca	P	Phytate-bound P	Phytate-bound P, % of total P
Corn	0.02	0.26	0.21	81
Soybean meal	0.33	0.71	0.38	54
Barley	0.06	0.35	0.22	63
Canola meal	0.69	1.08	0.65	60
DDGS[2], corn	0.12	0.73	0.26	36
Rice bran, full fat[3]	0.11	2.58	2.38	92
Rice bran, defatted	0.22	2.16	7.74	81
Wheat	0.06	0.39	0.22	56

[1]Values from NRC (2012).

[2]DDGS = distiller's dried grains with solubles.

[3]Values from Stein et al. (2016).

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CHAPTER 3: STANDARDIZED TOTAL TRACT DIGESTIBILITY OF CALCIUM VARIES AMONG SOURCES OF CALCIUM CARBONATE, BUT NOT AMONG SOURCES OF DICALCIUM PHOSPHATE, BUT MICROBIAL PHYTASE INCREASES CALCIUM DIGESTIBILITY IN CALCIUM CARBONATE

ABSTRACT

Two experiments were conducted to test the hypothesis that standardized total tract digestibility (**STTD**) of Ca and the response to microbial phytase is constant among different sources of Ca carbonate and that the STTD of Ca is constant among different sources of dicalcium phosphate (**DCP**) when fed to growing pigs. In Exp. 1, 80 pigs (initial BW: 19.0 ± 1.9 kg) were randomly allotted to 10 diets and 2 blocks with 4 pigs per diet in each block. Four sources of Ca carbonate were used and each source was included in a diet without microbial phytase and a diet with microbial phytase (500 units/kg diet). Two Ca-free diets without or with microbial phytase were also formulated. Feed allowance was 2.7 times the maintenance energy requirement for ME and daily feed allotments were divided into 2 equal meals. The initial 4 d of each period were considered the adaptation period to the diets followed by 4 d of fecal collection using the marker to marker procedure. Pigs fed diets containing exogenous phytase had lower ($P < 0.05$) basal endogenous loss of Ca compared with pigs fed diets containing no phytase. There were no interactions between phytase and source of Ca carbonate. Values for STTD of Ca were greater ($P < 0.05$) for diets containing microbial phytase (77.3 to 85.4%) compared with diets without exogenous phytase (70.6 to 75.2%), and values for STTD of Ca differed ($P < 0.05$) among the 4 sources of Ca carbonate. In Exp. 2, 40 pigs (initial BW: 14.9 ± 1.3 kg) were allotted to a completely randomized design with 5 diets and 8 replicate pigs per diet. A basal diet in which all

Ca was supplied by Ca carbonate was formulated. Three diets were formulated by adding 3 sources of DCP to the basal diet and a Ca-free diet was also used. Feeding and collection methods were as described for Exp. 1. Results indicated that values for STTD of Ca and apparent total tract digestibility of P were not different among diets, indicating that under the conditions of this experiment, the digestibility of Ca and P in DCP appears to be constant regardless of origin of DCP. In conclusion, use of microbial phytase reduces the basal endogenous loss of Ca and increases Ca digestibility in Ca carbonate. The STTD of Ca varies among sources of Ca carbonate, regardless of phytase inclusion, but that appears not to be the case for the STTD of Ca in different sources of DCP.

Key words: calcium, calcium carbonate, dicalcium phosphate, digestibility, phytase, pigs

INTRODUCTION

The concentration of Ca in most plant feed ingredients is low compared with the requirement for pigs, and Ca carbonate and dicalcium phosphate (**DCP**) are often used in diets for pigs to provide additional Ca. Although Ca is relatively inexpensive compared with other nutrients, excess dietary Ca may decrease P digestibility resulting in reduced feed intake and growth performance (Stein et al., 2011; González-Vega et al., 2016; Merriman et al., 2017; Blavi et al., 2018). Reduced digestibility of P may also result in increased excretion of P and possibly increase environmental pollution (Knowlton et al., 2004). Results of recent research indicate that provisions of P and Ca are most correctly assessed as a ratio between digestible Ca and digestible P (González-Vega et al., 2016; Merriman et al., 2017). However, whereas the digestibility of P in most feed ingredients has been reported (NRC, 2012), the number of experiments in which the digestibility by pigs of Ca in feed ingredients was determined is limited (Stein et al., 2016).

Therefore, determination of digestibility of Ca in dietary sources of Ca is needed.

Absorption of Ca may be estimated by the total tract digestibility procedure (González-Vega et al., 2014; Zhang et al., 2016), but Ca of endogenous origin is excreted in the feces along with undigested dietary Ca (González-Vega et al., 2013; 2014). Therefore, the concept of standardized total tract digestibility (**STTD**) of Ca that provides values for digestibility of Ca that are corrected for the basal endogenous loss of Ca has been introduced (Stein et al., 2016).

Values for STTD of Ca in one source of Ca carbonate and in one source of DCP were reported by González-Vega et al. (2015a) and it was demonstrated that microbial phytase increased the STTD of Ca in Ca carbonate, but not in DCP. However, different suppliers of inorganic sources of Ca may use different raw materials and different production processes and the concentration of Ca in Ca-containing ingredients may vary among suppliers (Petersen and Stein, 2006). It is, however, not known if differences in raw materials and production procedures among suppliers of inorganic Ca influence the STTD of Ca or the response to microbial phytase. Therefore, the objective of this work was to test the hypotheses that STTD of Ca and the response to microbial phytase is constant among sources of Ca carbonate and that the STTD of Ca is constant among sources of DCP when fed to growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for 2 experiments. Pigs used in both experiments were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Animals and Diets

Exp. 1. Standardized Total Tract Digestibility of Ca in Ca Carbonate. Four sources of Ca carbonate (A = Calpro, ILC Resources, Alden, IA; B = Fre-Fro, ILC Resources, Alden, IA; C = GMC-Shelter Limestone, Selter Creek Quarry LLC, Maplehill, NC; D = Alix-US Lime, US Lime Co-St Clair, Marble City, OK) were procured from commercial companies in the United States (Table 3.1). Each source was included in 2 diets; i.e., one diet without microbial phytase and one diet with microbial phytase (500 phytase units/kg; Quantum Blue®, AB Vista, Marlborough, UK). Eighty growing pigs with an initial BW of 19.0 ± 1.9 kg were randomly allotted to 10 diets and 2 blocks with 4 pigs per diet in each block for a total of 8 replicate pigs per diet. All diets were based on corn and potato protein concentrate (Table 3.2). Two Ca-free diets without or with microbial phytase (500 phytase units/kg) were also formulated. All diets were based on corn and potato protein concentrate. Amino acids calculated as standardized ileal digestible amino acids, vitamins, and minerals other than Ca in the Ca-free diets were included in all diets to meet current nutrient requirement estimates (NRC, 2012). The vitamin and mineral premix that was used did not contain Ca.

Exp. 2. Standardized Total Tract Digestibility of Ca in DCP. Forty growing pigs with an average initial body weight of 14.9 ± 1.3 kg were allotted to a randomized complete block design with a total of 8 replicate pigs per diet. Five diets were used and all diets were formulated without exogenous phytase (Table 3.2). A basal diet in which all Ca was supplied by Ca carbonate was formulated. Three sources of commercial DCP were procured from 3 commercial suppliers in the U.S. (A = PCS, PCS Sales USA, Northbrook, IL; B = Ultra-Phos, Kay Dee, LLC, Sioux City, IA; C = Simphos, J. R. Simplot Company, Boise, ID; Table 3.3) and 3 diets were formulated using each source of DCP. Calcium carbonate (same as the Ca carbonate source

A in Exp. 1) was also included in those diets to obtain a total Ca:STTD P ratio of 2.0:1.0, which is close to the requirement estimates for pigs (NRC, 2012). A Ca-free diet was used to determine the basal endogenous loss of Ca. Amino acids calculated as standardized ileal digestible amino acids, vitamins, and, with the exception of Ca in the Ca-free diet, minerals were included to meet current nutrient requirements (NRC, 2012).

Housing, Feeding, and Sample Collection

Pigs were housed individually in metabolism crates that were equipped with fully slatted floors, a feeder, and a cup waterer. A screen floor was installed below the slatted floor of the crates. Feed allowance was 2.7 times the maintenance energy requirement for ME for pigs (i.e., 197 kcal ME/kg $BW^{0.60}$). Water was available at all times. Daily feed allotments were divided into 2 equal meals that were provided at 0800 and 1600 h.

The initial 4 d of each period were considered the adaptation period to the diets followed by 4 d of fecal collection using the marker to marker procedure (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., ferric oxide) appeared (Adeola, 2001). Fecal samples were stored at –20 °C as soon as collected.

Chemical Analyses

At the conclusion of the experiments, fecal samples were dried at 65 °C in a forced air oven in Exp. 1, but fecal samples were lyophilized in Exp. 2. The dried fecal samples then were finely ground through a 1-mm screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ). Based on the methods described in AOAC Int. (2007), Ca and P in feed ingredients, 4 samples of each diet, and fecal samples were analyzed by inductively coupled plasma spectroscopy (Method 985.01 A, B, and C) after wet ash sample preparation [Method

975.03 B(b)]. Concentrations of Ca and P in all samples were analyzed at Missouri Analytical Laboratories (St. Louis, MO, USA) in duplicate. Diets were analyzed for phytase activity (ESC, Ystrad Mynach, UK) by the ELISA method using Quantiplate Kits for Quantum Blue® and feed ingredients were also analyzed for phytate-P (Megazyme method; ESC, Ystrad Mynach, UK). Feed ingredient, diet, and fecal samples were analyzed for dry matter (**DM**; AOAC Int., 2007; method 930.15). Feed ingredient and diet samples were also analyzed for ash (AOAC Int., 2007; method 942.05). Particle size of Ca carbonate and DCP was measured (ASABE, 2008), and the in vitro solubility of the 4 sources of Ca carbonate was determined using the procedure described by Zhang and Coon (1997b).

Calculations

The ATTD of Ca and P in experimental diets was calculated using Eq. [3.1] (Almeida and Stein, 2010):

$$\text{ATTD} = \frac{\text{intake - output}}{\text{intake}} \times 100, \qquad [3.1]$$

where ATTD is in % and intake and output in feces are expressed as gram per day. Because all Ca in the Ca carbonate-containing diets was from Ca carbonate in Exp. 1, the ATTD of Ca in the Ca carbonate-containing diets was considered the ATTD of Ca in Ca carbonate.

The basal endogenous loss of Ca that was estimated as the total tract flow of Ca from pigs fed the Ca-free diets was expressed as mg/kg of DM intake (**DMI**) and was calculated using DMI in kilogram per day and Ca output in feces that was expressed as gram per day. The daily basal endogenous loss of Ca was calculated by multiplying the basal endogenous loss by the DMI of each pig.

Values for STTD of Ca (%) were calculated using Eq. [3.2] (Almeida and Stein, 2010):

$$\text{STTD} = \frac{\text{intake - (output - daily basal endogenous loss)}}{\text{intake}} \times 100, \qquad [3.2]$$

where intake, output, and daily basal endogenous loss are in gram per day.

To calculate the ATTD and STTD of Ca in 3 sources of DCP used in Exp. 2, the contributions of Ca from Ca carbonate to the DCP-containing diets was calculated and the ATTD and STTD of Ca in each source of DCP were calculated using the difference procedure (Adeola, 2001).

Statistical Analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC) and outliers were identified as values that deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range (Tukey, 1977). An outlier was found for each experiment and the outliers were excluded for further statistical analysis. The pig was the experimental unit for all analyses. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC). In Exp. 1, the statistical model included Ca source, phytase, and the Ca-source × phytase interaction as fixed effects and block and replicate within block as random effects. Mean separation was conducted by the PDIFF option with the Tukey's adjustment if an interaction was significant. In Exp. 2, the statistical model included diet or source of DCP as fixed effects and replicate as random effect. Mean separation was conducted by the PDIFF option with the Tukey's adjustment. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

Pigs remained healthy during both experiments and very little feed refusals were observed. Analyzed Ca and P in some diets in Exp. 1 varied slightly from calculated values, but

analyzed Ca and P in the DCP-containing diets used in Exp. 2 were close to calculated values (Tables 3.4 and 3.5). To avoid confounding effects due to analytical discrepancies, calculated values for all diets were used for calculations of Ca and P digestibility values.

Exp. 1. Standardized Total Tract Digestibility of Ca in Ca carbonate

Values for ATTD of DM did not differ between pigs fed Ca-free diets without or with microbial phytase (Table 3.6). However, fecal Ca excretion from pigs fed the Ca-free diet with microbial phytase was less ($P < 0.05$) than from pigs fed the Ca-free diet without phytase, which resulted in lower ($P < 0.05$) basal endogenous loss of Ca from pigs fed the diet containing phytase. The ATTD of P tended ($P = 0.061$) to be greater in the Ca-free diet with phytase than in the Ca-free diet without phytase.

There were no interactions between phytase and source of Ca carbonate (Table 3.7). Feed intake and Ca and P intakes were not affected by use of microbial phytase or by the source of Ca carbonate included in the diet. However, fecal Ca excretion, daily basal endogenous Ca loss, and fecal P excretion were less ($P < 0.001$) from pigs fed diets containing Ca carbonate with microbial phytase compared with pigs fed the diets without microbial phytase. Therefore, absorbed Ca, ATTD of Ca, STTD of Ca, absorbed P, and ATTD of P increased ($P < 0.01$) if microbial phytase was included in the diets compared with diets without microbial phytase, regardless of the source of Ca carbonate. Fecal Ca excretion from pigs fed diet containing Ca carbonate source D was less ($P < 0.05$; data not shown) compared with Ca carbonate sources A, B, and C. Values for ATTD and STTD of Ca in Ca carbonate source A was greater ($P < 0.05$) than in Ca carbonate source D, but there was no difference between Ca carbonate source A and Ca carbonate sources B and C or between the sources B and C and source D (data not shown). The ATTD and STTD of Ca did no differ among Ca carbonate sources B, C, and D (data not

shown). The ATTD of P was not influenced by the source of Ca carbonate in the diet.

Exp. 2. Standardized Total Tract Digestibility of Ca in DCP

Feed intake, basal endogenous Ca loss, and P intake tended to be less ($P < 0.10$) for pigs fed the basal diet compared with pigs fed the DCP-containing diets (Table 3.8). Fecal excretion from pigs fed the diet containing DCP source B was greater ($P < 0.05$) than from pigs fed the basal diet. Values for the ATTD of Ca and STTD of Ca did not differ among the 3 sources of DCP (Table 3.9). Apparent total tract digestible Ca and standardized total tract digestible Ca were also not affected by source of DCP.

DISCUSSION

Based on the total molecular mass, analytical grade Ca carbonate ($CaCO_3$) contains 40.0% Ca. The average concentration of Ca in the 4 commercial sources of Ca carbonate used in Exp. 1 was 39.7%, which indicates very little impurity in the Ca carbonate sources used. For DCP ($CaHPO_4$), based on the total molecular mass, the expected Ca concentration is 29.5%, and the expected P concentration is 22.8%. However, the 3 sources of DCP used in Exp. 2 had an average concentration of 19.1% P, and the average concentration of Ca was 19.5%, which indicates some impurity in these sources of DCP. The P concentration in a commercial DCP is usually lower than 22.8% because all sources of feed grade DCP in reality are mixtures of DCP, monocalcium phosphate, and unreacted Ca carbonate (Baker, 1989; Petersen and Stein, 2006). In addition, Ca fluoride, silica, Mg oxide, Mg phosphate, and Fe phosphate are often present in commercial sources of feed grade DCP (Baker, 1989), which is the reason feed grade DCP does not contain 22.8% P and 29.5% Ca.

The calculated concentration of Ca in the 8 Ca carbonate-containing diets used in Exp. 1

was between 0.67 to 0.69%, depending on the source of Ca carbonate used. Six of the 8 diets with Ca carbonate were within 0.03 percentage units of this value, but 2 diets analyzed only 0.62 and 0.56% Ca. Those values were the average of 4 separate diet samples that were analyzed for Ca. It has been demonstrated that analyzed dietary Ca values often vary greatly (Wu et al., 2018), but because calculated Ca values were used in all calculations, these analytical discrepancies did not impact digestibility data that were calculated for Exp. 1. In contrary, only one fecal sample was used for the Ca analysis because the fecal samples that have been dried and ground thoroughly were considered relatively homogenous compared with diet samples that have more possibility to have segregations of ingredients, resulting in variations in the analysis.

Values for the ATTD of P in the Ca-free diets that were formulated based on corn and monosodium phosphate without and with phytase were in agreement with expected values (NRC, 2012; González-Vega et al., 2015a; Blavi et al., 2017). However, the ATTD of P in the Ca carbonate-containing diets was less than the ATTD of P in the Ca-free diets. This indicates binding of Ca and P in the intestinal tract of pigs if both minerals are included in the diet, which has also been reported in the past (Stein et al., 2011). However, the observation that the ATTD of P in the Ca-free diet is greater than in a diet adequate in Ca indicates that the digestibility of P in growing pigs is not downregulated by the lack of Ca in the diet although the lack of Ca likely prevented the use of absorbed P for bone tissue synthesis. This observation is in agreement with recent data from gestating sows that also indicated that the ATTD of P is greater in a Ca-free diet than in a diet containing Ca carbonate (Lee et al., 2019).

The basal endogenous loss of Ca that was determined in the 2 experiments was within the range of reported values (González-Vega et al., 2015b; Merriman, 2016; Merriman and Stein, 2016; Blavi et al., 2017). The observation that use of microbial phytase decreased the basal

endogenous loss of Ca is different from data for total endogenous loss of Ca determined in pigs fed diets based on canola meal (González-Vega et al., 2013). However, if phytase was added to a Ca-free corn-based diet fed to gestating sows, there was also a decrease in the basal endogenous loss of Ca (Lee et al., 2019). This indicates that a Ca-phytate complex is formed in the intestinal tract between phytate in corn and endogenous Ca and that phytase addition to the diet results in release of this endogenous Ca from the complex, which in turn decreases the excretion of endogenous Ca in feces. Likewise, the negatively charged phytate may chelate Ca ions from feed ingredients in the intestine of pigs, resulting in formation of non-digestible Ca-phytate complexes (Nelson and Kirby, 1987; Selle and Ravindran, 2008). As a consequence, if phytase is used in diets, both Ca and P are released from the complex and the digestibility of Ca and P will increase (Almeida et al., 2013; Rodríguez et al., 2013; González-Vega et al., 2015a). The observation that the ATTD and STTD of Ca and the ATTD of P in the corn-Ca carbonate diets increased if phytase was added to the diets concur with previous data (González-Vega et al., 2015a). These observations support the hypothesis that the Ca from Ca carbonate may bind to phytate in corn and that phytase reduces chelation, resulting in an increase in the digestibility of Ca and P.

Values for ATTD and STTD of Ca in Ca carbonate were in line with previous data (González-Vega et al., 2015a; Merriman and Stein, 2016; Blavi et al., 2017; Kwon and Kim, 2017). The small, but significant, difference in the digestibility of Ca among the 4 sources of Ca carbonate demonstrates that differences among commercial sources of Ca carbonate exist. In this experiment, Ca carbonate source D had a lower digestibility than Ca carbonate source A although the particle size was greater in source A. Differences in the digestibility of Ca are not likely a result of differences in particle size because there appears to be no influence of particle

size on digestibility of Ca in Ca carbonate (Merriman and Stein, 2016) or on bioavailability of Ca in Ca carbonate (Ross et al., 1984). Solubility of Ca carbonate is likely to affect Ca utilization in poultry (Zhang and Coon, 1997a; Kim et al., 2018), but both ATTD and STTD of Ca in 4 sources of Ca carbonate used in this experiment were not correlated ($P > 0.10$; data not shown) with solubility.

Values for the ATTD and STTD of Ca in the 3 sources of DCP that were used in this experiment are in agreement with previous data (González-Vega et al., 2015a; Zhang and Adeola, 2017). The observation that there was no difference in the digestibility of Ca and P among the 3 sources indicates that production processes and origins of the 3 sources of DCP used in this experiment did not influence the digestibility of Ca and P.

Values for the STTD of Ca in DCP were greater than the STTD of Ca in Ca carbonate. This may seem surprising because Ca in DCP originates from Ca carbonate (Baker, 1989), but previous data also demonstrated a greater digestibility of Ca in DCP compared with Ca from Ca carbonate (González-Vega et al., 2015a; Zhang and Adeola, 2017). The reason for this observation may be that some of the Ca from dietary Ca carbonate binds to the phytate that is supplied by corn in the diet when the 2 ingredients reach the aqueous environment in the stomach of pigs, which reduces the digestibility of Ca from Ca carbonate. In contrast, it appears that Ca in DCP, which is bound to P in the phosphoric acid, is less likely to solubilize in the stomach and becomes less available for chelation with phytate (Walk, 2016). These possible effects explain why phytase, as demonstrated in Exp. 1 and in previous experiments (González-Vega et al., 2015a; Blavi et al., 2017), results in increased digestibility of Ca from Ca carbonate because phytase may release the Ca that was bound to phytate. In contrast, there is no effect of phytase on the digestibility of Ca in DCP (González-Vega et al., 2015a), which is consistent with the hypothesis that Ca from DCP is not chelated by phytate.

CONCLUSION

Use of microbial phytase reduces the basal endogenous loss of Ca and increases Ca digestibility in Ca carbonate. The STTD of Ca varies among sources of Ca carbonate, but that appears not to be the case for the STTD of Ca in different sources of DCP. The STTD of Ca in DCP is greater than the STTD of Ca in Ca carbonate.

TABLES

Table 3.1. Nutrient composition (as-fed basis) of feed ingredients, and particle size and in vitro solubility of Ca in Ca carbonate, Exp. 1

Item, %	Corn	Potato protein concentrate	Ca carbonate source					
			A	B	C	D	Mean	SD
Nutrient composition[1]								
Dry matter	86.4	91.3	99.8	99.9	99.8	99.9	99.8	0.1
Ash, %	1.1	0.5	94.8	94.5	98.9	97.0	97.2	3.0
Ca, %	< 0.01	0.02	38.9	40.3	40.0	39.7	39.7	0.6
Total P, %	0.22	0.12	< 0.01	< 0.01	< 0.01	< 0.01	-	-
Phytate[2], %	0.69	0.32	-	-	-	-	-	-
Phytate-P, %	0.20	0.09	-	-	-	-	-	-
Non-phytate P[3], %	0.02	0.03	-	-	-	-	-	-
Particle size, μm	-	-	435	469	407	97	352	172
Solubility, %	-	-	40.0	45.1	43.8	42.0	42.7	2.2

[1]All samples were analyzed in duplicate.

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

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

Table 3.2. Ingredient composition of experimental diets (as-is basis), Exp. 1 and 2

Ingredient, %	Exp. 1		Exp. 2		
	Ca carbonate[1]	Ca-free[1]	Basal	DCP[2]	Ca-free
Corn	80.19	82.39	80.15	80.45	81.85
Potato protein concentrate	15.00	15.00	15.00	15.00	15.00
Ca carbonate	1.70	-	1.70	0.70	-
Dicalcium phosphate	-	-	-	1.80	-
Soybean oil	1.00	0.50	1.00	1.00	1.00
$_{L}$-Lys·HCl	0.35	0.35	0.35	0.35	0.35
$_{DL}$-Met	0.05	0.05	0.10	0.10	0.10
$_{L}$-Trp	0.05	0.05	0.05	0.05	0.05
Monosodium phosphate	1.10	1.10	1.10	-	1.10
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Phytase premix[4]	0.01	0.01	-	-	-

[1]Four sources of commercial Ca carbonate were each included in one diet without microbial phytase and in one diet with microbial phytase (500 phytase units/kg; Quantum Blue®, AB Vista, Marlborough, UK); the Ca-free diet was formulated without or with microbial phytase (500 phytase units/kg; Quantum Blue®, AB Vista, Marlborough, UK).

[2]DCP = dicalcium phosphate. Three sources of commercial DCP were used and each source was included in one diet.

[3]The vitamin-mineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as

Table 3.2. (Cont.)

cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[4]The phytase premix (Quantum Blue®, AB Vista, Marlborough, UK) contained 5,000 units of phytase per g; corn starch was used at the expense of the phytase premix in diets without microbial phytase.

Table 3.3. Nutrient composition of ingredients and particle size of dicalcium phosphate (as-is basis), Exp. 2

Item, %	Corn	Potato protein concentrate	Ca carbonate	Dicalcium phosphate source				
				A	B	C	Mean	SD
Nutrient composition[1]								
Dry matter	86.6	91.0	100.0	94.9	94.0	95.1	94.7	0.6
Ash	1.2	0.4	94.2	84.1	81.5	83.3	83.0	1.3
Ca	< 0.01	0.02	39.7	20.5	18.9	18.9	19.5	1.7
Total P	0.23	0.13	0.13	18.5	19.8	19.1	19.1	0.7
Phytate[2]	0.64	0.28	-	-	-	-	-	-
Phytate-P	0.18	0.08	-	-	-	-	-	-
Non-phytate P[3]	0.05	0.05	0.13	-	-	-	-	-
Particle size, μm	-	-	-	508	1,079	465	684	343

[1]All samples were analyzed in duplicate.

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

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

Table 3.4. Analyzed composition of experimental diets (as-fed basis), Exp. 1

Item[1], %	0 phytase units/kg					500 phytase units/kg				
Ca carbonate source:	A	B	C	D	Ca-free	A	B	C	D	Ca-free
Dry matter	88.1	87.9	88.3	88.3	86.8	87.5	87.4	87.4	87.3	87.3
Ash	3.65	4.11	3.74	4.21	2.44	4.18	3.84	4.55	3.87	2.74
Ca	0.68	0.69	0.62	0.69	0.02	0.68	0.67	0.56	0.64	0.01
Total P	0.49	0.51	0.51	0.46	0.46	0.46	0.48	0.48	0.48	0.49
Phytate[2]	0.60	0.60	0.60	0.60	0.61	0.60	0.60	0.60	0.60	0.61
Phytate-P[3]	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Non-phytate P[4]	0.32	0.34	0.34	0.29	0.29	0.29	0.31	0.31	0.31	0.32
Phytase activity[5]	< 50	< 50	< 50	< 50	< 50	572	644	601	546	744

[1]All samples were analyzed in duplicate and there were 4 diet samples to be analyzed.

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

[3]Phytate-P values were calculated from analyzed phytate-P in the ingredients.

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

[5]Phytase activity = phytase units/kg of diet.

Table 3.5. Analyzed composition of experimental diets (as-fed basis), Exp. 2

Item[1], %	Basal	Dicalcium phosphate source			Ca-free
		A	B	C	
Dry matter	88.3	87.7	87.5	87.3	87.6
Ash	4.53	4.06	3.56	3.49	3.42
Ca	0.68	0.69	0.62	0.61	0.03
Total P	0.58	0.58	0.58	0.57	0.61
Phytate[2]	0.55	0.55	0.55	0.55	0.56
Phytate-P[3]	0.16	0.16	0.16	0.16	0.16
Non-phytate P[4]	0.42	0.42	0.42	0.41	0.45
Phytase activity, unit/kg	< 50	< 50	< 50	< 50	< 50

[1]All samples were analyzed in duplicate and there were 4 diet samples to be analyzed.

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

[3]Phytate-P values were calculated from analyzed phytate-P in the ingredients.

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

Table 3.6. Basal endogenous loss (BEL) of Ca and apparent total tract digestibility (ATTD) of dry matter and P in Ca-free diets without or with microbial phytase fed to growing pigs[1], Exp. 1

Item	Phytase, unit/kg		SEM	*P*-value
	0	500		
Feed intake, g/d	688	645	44	0.506
Fecal excretion, g/d	60	66	6	0.539
ATTD of dry matter, %	90.5	88.8	0.7	0.103
Fecal Ca excretion, mg/d	273	165	42	0.013
BEL of Ca, mg/kg dry matter intake	463	304	64	0.037
P intake, g/d	3.2	3.0	0.2	0.521
Fecal P excretion, g/d	1.0	0.8	0.1	0.021
Absorbed P, g/d	2.2	2.2	0.2	0.890
ATTD of P, %	68.4	73.4	2.5	0.061

[1]Each least squares mean represents 8 observations.

Table 3.7. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca and ATTD of dry matter and P in experimental diets containing 4 sources of Ca carbonate fed to growing pigs[1], Exp. 1

Item, %	0 phytase units/kg				500 phytase units/kg					*P*-value		
Ca carbonate source:	A	B	C	D	A	B	C	D	SEM	Phytase	Source	Interaction
Feed intake, g/d	835	778	824	850	840	756	810	898	43	0.886	0.090	0.846
Fecal excretion, g/d	83	76	85	85	83	76	87	90	6	0.665	0.128	0.972
ATTD of dry matter, %	89.5	89.5	89.1	89.4	89.1	89.0	88.4	89.1	0.5	0.206	0.698	0.973
Calcium												
Ca intake, g/d	5.6	5.4	5.7	5.8	5.6	5.2	5.6	6.1	0.3	0.909	0.154	0.853
Fecal Ca excretion, g/d	1.8	1.7	1.9	2.1	1.0	1.3	1.4	1.6	0.1	< 0.001	0.001	0.422
Absorbed Ca, g/d	3.8	3.7	3.7	3.7	4.6	3.9	4.2	4.5	0.3	0.003	0.441	0.663
ATTD of Ca, %	68.7	69.4	66.1	64.6	81.4	74.9	75.0	73.4	1.8	< 0.001	0.007	0.227
Basal endogenous Ca loss[2], mg/d	340	316	336	347	223	200	215	238	15	< 0.001	0.120	0.984
STTD of Ca[3], %	74.8	75.2	72.0	70.6	85.4	78.7	78.8	77.3	1.8	< 0.001	0.006	0.235
Phosphorus												
P intake, g/d	3.8	3.6	3.8	3.9	3.9	3.5	3.7	4.1	0.2	0.908	0.081	0.852

Table 3.7. (Cont.)

Fecal P excretion, g/d	1.9	1.7	2.0	1.9	1.2	1.3	1.4	1.5	0.1	< 0.001	0.064	0.351
Absorbed P, g/d	1.9	1.9	1.8	2.0	2.6	2.2	2.3	2.6	0.2	< 0.001	0.127	0.618
ATTD of P, %	49.5	51.9	47.0	51.6	67.8	62.9	62.1	64.0	2.3	< 0.001	0.230	0.295

[1]Each least squares mean for experimental diets from growing pigs represents 8 observations, respectively, with the exception for the diet containing source C with 500 phytase units/kg diet (n = 7); the outlier deviated from 1st- and 3rd-quartile by 3.5 times the interquartile range within the treatment.

[2]The daily basal endogenous Ca loss (mg/d) was calculated by multiplying the basal endogenous Ca loss (mg/kg dry matter intake) by the daily dry matter feed intake (kg/d) of each diet.

[3]The STTD of Ca in diets was calculated by correcting the ATTD of Ca for basal endogenous Ca loss that was obtained from pigs fed the Ca-free diets; basal endogenous Ca loss from pigs fed the Ca-free diet without microbial phytase = 463 mg/kg dry matter intake; basal endogenous Ca loss from pigs fed the Ca-free diet with microbial phytase = 304 mg/kg dry matter intake.

Table 3.8. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca and ATTD of dry matter and P in experimental diets fed to growing pigs[1], Exp. 2

Item	Basal	DCP source[2]			SEM	*P*-value
		A	B	C		
Feed intake, g/d	565	625	739	660	43	0.063
Fecal excretion, g/d	60[b]	74[ab]	89[a]	80[ab]	7	0.037
ATTD of dry matter, %	88.8	86.8	87.1	87.0	1.0	0.448
Ca intake, g/d	3.8	4.0	4.6	4.1	0.3	0.318
Fecal Ca excretion, g/d	1.1	1.3	1.3	1.1	0.1	0.395
Absorbed Ca, g/d	2.7	2.8	3.3	3.0	0.2	0.398
ATTD of Ca, %	71.5	68.4	71.2	72.9	2.2	0.551
Basal endogenous Ca loss[3], mg/d	389	428	506	451	30	0.070
STTD of Ca[4], %	81.7	79.0	82.3	83.9	2.2	0.473
P intake, g/d	3.3	3.6	4.3	3.7	0.2	0.079
Fecal P excretion, g/d	1.5	1.7	1.9	1.7	0.1	0.194
Absorbed P, g/d	1.8	2.0	2.4	2.1	0.2	0.215
ATTD of P, %	55.7	53.1	56.1	55.1	3.0	0.893

[1]Each least squares mean for experimental diets from growing pigs represents 8 observations, respectively, with the exception for the diet containing DCP source B diet (n = 7); the outlier deviated from 1st- and 3rd-quartile by 3.2 times the interquartile range within the treatment.

[2]DCP = dicalcium phosphate.

[3]The daily basal endogenous Ca loss (mg/d) was calculated by multiplying the basal

Table 3.8. (Cont.)

endogenous Ca loss (mg/kg dry matter intake) by the daily dry matter feed intake (kg/d) of each diet.

[4]The STTD of Ca in diets was calculated by correcting the ATTD of Ca for basal endogenous Ca loss that was obtained from pigs fed the Ca-free diet (basal endogenous loss Ca loss = 782 mg/kg dry matter intake).

Table 3.9. Apparent (ATTD) and standardized total tract digestibility (STTD) of Ca in 3 different sources of dicalcium phosphate (DCP) fed to growing pigs[1], Exp. 2

Item	DCP source			SEM	*P*-value
	A	B	C		
ATTD					
Digestibility, %	66.1	71.0	74.0	4.1	0.393
Digestible Ca, %	14.5	14.1	13.7	0.8	0.811
STTD					
Digestibility, %	77.0	82.8	85.8	4.1	0.314
Digestible Ca, %	16.9	16.4	15.9	0.8	0.716

[1]Each least squares mean for experimental diets from growing pigs represents 8 observations, respectively, with the exception that the diet containing DCP source B only had 7 observations.

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CHAPTER 4: THE ASH IN METACARPALS, METATARSALS, AND TIBIA IS BETTER CORRELATED WITH TOTAL BODY BONE ASH THAN THE ASH IN OTHER BONES OF GROWING PIGS

ABSTRACT

The objective of this experiment was to determine correlations between individual bones in the body and total bone ash to identify the bone or bones that is or are most representative of total body bone ash in growing pigs. Twenty growing pigs (initial body weight: 40.78 ± 3.47 kg) were allotted to 2 diets using a randomized complete block design with sex and body weight as blocks and thus there were ten replicate pigs (5 gilts and 5 barrows) per diet. The 2 experimental diets were formulated to contain 60% or 100% of the requirement for standardized total tract digestible (**STTD**) P. Calcium was included in both diets to maintain a STTD Ca to STTD P ratio of 1.90:1, which is believed to maximize bone ash. Pigs were allowed ad libitum access to feed and water was available at all times. Body weight of pigs and the amount of feed consumed by pigs were recorded on d 14 and d 28. On the last day, pigs were slaughtered and carcass characteristics were determined. Metacarpals, metatarsals, femur, tibia, fibula, ribs, and all other bones from the left half of the carcass were collected separately. Each of the bone samples were defatted and ashed. Overall, pigs fed the diet containing 100% of the requirement for Ca and P had greater ($P < 0.05$) average daily gain and gain to feed ratio compared with pigs fed the diet containing 60% of the requirement for Ca and P, but there was no difference in average daily feed intake between pigs fed the 2 diets. There was no effect of dietary Ca and P on carcass weights of pigs. Weights of bone ash were greater ($P < 0.05$) for pigs fed the diet containing 100% of the requirement for Ca and P compared with pigs fed the diet containing 60% of the

requirement. Concentration of ash in total and all individual bones except femur and fibula were greater ($P < 0.05$) in pigs fed the diet containing 100% of the requirement for Ca and P compared with pigs fed the diet containing 60% of the requirement. There were positive correlations ($P < 0.05$) in weights of bone ash and concentrations of ash between total bone and all individual bones. Correlation coefficients between the weight of ashed metacarpals, metatarsals, and tibia and the weight of total bone ash were greater than 0.950, which was greater than for femur, fibula, or ribs. Unlike bone ash weight, all correlation coefficients between individual bones and total bones for percentage ash were less than 0.750. In conclusion, metacarpals, metatarsals, and tibia were more representative of total body bone ash compared with femur, fibula, and ribs.

Key words: bone ash, calcium, correlation, phosphorus, growing pigs

INTRODUCTION

The most abundant elements in the body are Ca and P and most Ca and P are deposited in bone tissue (Crenshaw, 2001; Létourneau-Montminy et al., 2015). Therefore, bone characteristics of pigs are affected by dietary Ca and P (Crenshaw et al., 1981; Lagos et al., 2019a; Vier et al., 2019). Using X-ray absorptiometry, it was determined that femur ash was a better indicator of total body mineral content in 25 kg pigs compared with fibula ash (Crenshaw et al., 2009). As a consequence, in recent work to determine effects of dietary Ca and P on body bone ash in growing pigs, femur ash has been used as a representative bone (González-Vega et al., 2016; Merriman et al., 2017; Lagos et al., 2019b) although metacarpal ash has also sometimes been measured (González-Vega et al., 2016; She et al., 2017; Vier et al., 2019). However, to our knowledge, no data have demonstrated which bone best predicts total body bone ash when pigs are fed diets containing different levels of Ca and P. Therefore, the objective of

this experiment was to determine correlations between individual bones and total body bone ash to identify the bone that is most representative of total body bone ash in growing pigs.

MATERIALS AND METHODS

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

Animals, Housing, Feeding, and Diets

Twenty growing pigs [initial body weight (**BW**): 40.78 ± 3.47 kg] were allotted to 2 diets using a randomized complete block design with sex and BW as blocks and thus there were ten replicate pigs (5 gilts and 5 barrows) per diet. Pigs were housed individually in fully slatted pens (0.9 × 1.8 m). Room temperature was controlled and each pen had a feeder, a nipple drinker, and a fully slatted concrete floor. Pigs were allowed ad libitum access to feed and water was available at all times. Pigs were weighed on d 14 and at the conclusion of the experiment (d 28). The amount of feed offered was recorded daily and the amount of feed in the feeders was recorded on d 14 and 28.

The 2 experimental diets (Table 4.1) were based on corn and soybean meal and formulated to contain 60 or 100% of the requirement for standardized total tract digestible (**STTD**) P (NRC, 2012). Calcium was included in both diets to maintain a STTD Ca to STTD P ratio of 1.90:1, which is believed to maximize bone ash (Lagos et al., 2019a; Lee et al., 2019a). All nutrients except Ca and P were included in both diets at the requirement for growing pigs (NRC, 2012).

Carcass Characteristics and Bone Measurements

On the last day of the experiment, pigs were transported to the University of Illinois Meat Science Laboratory. Pigs were fasted for approximately 18 h and weighed again to determine ending live weight. Following exsanguination, blood was weighed. Pigs were scalded, dehaired, and singed to remove all hair from the carcass and toenails and tail were removed. The weight of the viscera (i.e., heart, kidneys, liver, gall bladder, spleen, lungs, trachea, reproductive tract, and emptied gastrointestinal tract) was recorded. Weights of bone with fat and muscle tissues on, feet, skin, and soft tissue from the left side of the carcass were recorded the day after pigs were killed. Heads were excluded in all analyses.

Third and 4^{th} metacarpals, 3^{rd} and 4^{th} metatarsals, femur, tibia, fibula, 3^{rd} and 4^{th} ribs, and 10^{th} and 11^{th} ribs from the left half of the carcass were collected separately and stored at −20 °C. All remaining bones from the left half of the carcass were combined, the weight was recorded, and these bones were also stored at −20 °C. Each of the frozen bone samples was thawed and autoclaved at 125 °C for 55 min. Marrow and fat were removed and bones were soaked in petroleum ether under a chemical hood for 72 h. Defatted bone samples were dried for 2 h at 135 °C and weighted and then ashed overnight at 600 °C. The weight of bone ash in each sample was recorded and concentration of ash was calculated as a percentage of bone dry weight. Weight of defatted total body bone ash was calculated by the sum of weight of individual bones and all remaining bones. Because the entire half of the body was used to measure body bone ash, weight of total and individual bone ash was calculated by multiplying the bone ash weight from the left half by 2.

Chemical Analyses

Diet samples were analyzed for dry matter (AOAC Int., 2007; method 930.15) and ash in

diet and bone samples was analyzed (AOAC Int., 2007; method 942.05). The gross energy in diet samples was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Crude protein in diet samples was calculated as N × 6.25 and N was measured by the combustion procedure (method 990.03; AOAC Int., 2007) using a LECO FP628 (LECO Corp., Saint Joseph, MI). Calcium and P in diet samples were analyzed by inductively coupled plasma spectroscopy (AOAC Int., 2007; method 985.01 A, B, and C) after wet ash sample preparation [AOAC Int., 2007; method 975.03 B(b)].

Statistical Analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC) and homogeneity was also confirmed. Outliers were identified as values that deviated from 1st- and 3rd-quartile by more than 3 times the interquartile range within treatment (Tukey, 1977). The pig was the experimental unit. Data were analyzed using MIXED procedures of SAS (SAS Institute Inc., Cary, NC). The statistical model included diet as fixed effect and sex and BW within sex as random effects. Correlation coefficients (r) between total bone and individual bones were determined using the CORR procedure of SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

All pigs remained healthy and had normal feed intake throughout the experiment.

Growth Performance and Carcass Weights

There was no effect of dietary Ca and P on BW at d 14, but pigs fed the diet containing 100% of the requirement for Ca and P tended to have greater ($P < 0.10$) BW on d 28 (Table 4.2). From d 0 to 14, average daily gain (**ADG**) and average daily feed intake (**ADFI**) of pigs were not

affected by dietary Ca and P, but gain to feed ratio (**G:F**) of pigs fed the diet containing 100% of the requirement for Ca and P was greater ($P < 0.01$) compared with pigs fed the diet containing 60% of the requirement for Ca and P. There was no effect of dietary Ca and P on ADG, ADFI, or G:F of pigs from d 14 to 28. Overall, pigs fed the diet containing 100% of the requirement for Ca and P had greater ($P < 0.05$) ADG and G:F compared with pigs fed the diet containing 60% of the requirement for Ca and P, but there was no difference in ADFI between the 2 diets.

There was no effect of dietary Ca and P on carcass weights of pigs (Table 4.3).

Bone Ash

Weights of total and individual bone ashes were greater ($P < 0.05$) for pigs fed the diet containing 100% of the requirement for Ca and P compared with pigs fed the diet containing 60% of the requirement (Table 4.4). The concentration of ash in total and all individual bones except femur and fibula was greater ($P < 0.05$) in pigs fed the diet containing 100% of the requirement for Ca and P compared with pigs fed the diet containing 60% of the requirement. Ash concentration in the fibula tended to be greater ($P < 0.10$) if pigs were fed Ca and P at the requirement compared with pigs fed Ca and P below the requirement.

There were positive correlations ($P < 0.05$) in the weight of bone ash in individual bones and the weight of total body bone ash (Table 4.5). Correlation coefficients between the weight of ashed metacarpals, metatarsals, and tibia and the weight of total bone ash were greater than 0.950, which was greater than for femur, fibula, or ribs. Unlike bone ash weight, all correlation coefficients between individual bones and total body bone ash for percentage ash were less than 0.750.

DISCUSSION

The analyzed Ca and P in both diets were in agreement with calculated values. Results from this experiment illustrated that growth performance and body bone ash of pigs were reduced if dietary Ca and P are below the requirement. However, this negative effect was greater for bone ash than for growth performance. This implies that compared with pigs fed 100% of the requirement for Ca and P, pigs that were fed the diet containing 60% of the requirement utilized a greater proportion of dietary Ca and P for growth of soft tissues and a lower proportion for bone tissue synthesis. Pigs fed the diet with only 60% of the requirement for Ca and P may also have been able to mobilize Ca and P from bones to compensate for dietary deficiencies of Ca and P. These data support the hypothesis that Ca and P requirements to maximize growth performance are less than requirements to maximize bone ash (NRC, 2012).

The observation that bone ash was reduced by lowering Ca and P in diets was in agreement with previous data (Crenshaw et al., 1981; González-Vega et al., 2016; Lagos et al., 2019a; Vier et al., 2019). Whereas differences in percentage of ash in defatted bones were relatively small between pigs fed the 2 diets, the weight of bone ash was dramatically affected by dietary Ca and P in this experiment. This was also observed in previous experiments (Crenshaw et al., 1981; González-Vega et al., 2016; Lagos et al., 2019a), and indicated that differences observed in bone ash weight were likely a result of differences in the size of bones rather than differences in the percentage of ash. This observation indicates that the composition of bone tissue does not change to a great extent, regardless of dietary provisions of Ca and P. However, the size of the bones are affected by diet Ca and P concentrations which is the reason the weight of bone ash changed without changes in bone ash percentage. This observation may also be the reason for the lower correlation coefficients for percentage ash between ash in individual bone

and total body bone ash.

Most Ca and P in the body are present in skeletal tissue (Crenshaw, 2001). Therefore, analyzing bone ash has been a standard procedure for Ca and P-related experiments for many years and different bone parts, mostly from legs and feet, have been used to represent total body bone ash (Crenshaw et al., 1981; Crenshaw et al., 2009). The reason legs or feet are frequently used may be that it is relatively easier to collect these bones compared with bones in other parts of the body. The observation that correlation coefficients between bone ash weight of metacarpals, metatarsals, and tibia and total bone ash weight were greater than 0.950 whereas ribs had the least coefficients is in agreement with data indicating that physical characteristics of metacarpals and metatarsals from pigs at 3 to 5 months of age were more sensitive to different levels of dietary Ca and P than other individual bones (Crenshaw et al., 1981). However, femur was a better indicator of total mineral contents or physiological characteristics of bone in pigs at early age compared with other individual bones (Crenshaw et al., 1981; Crenshaw et al., 2009). It is possible that the most representative bone for total bone ash depends on the age of pigs, but more research is needed to test this hypothesis by analyzing total bone ash from pigs at different age groups.

CONCLUSION

Providing dietary Ca and P below the requirement negatively affected growth performance of pigs and reduced total body bone ash in growing pigs. As weight of total body bone ash increased, weight of all ashed individual bones also increased. Total ash weight of bones was better correlated with dietary Ca and P than was percentage ash in bones. Metacarpals, metatarsals, and tibia were more representative of total body bone ash in growing pigs compared with femur, fibula, and ribs.

TABLES

Table 4.1. Ingredient and nutrient composition of diets fed to growing pigs (as-fed basis)

	% of the requirement	
Item	60	100
Ingredient, %		
Ground corn	77.91	76.85
Soybean meal	19.00	19.00
Choice white grease	1.00	1.00
Calcium carbonate	0.81	1.11
Dicalcium phosphate	0.26	1.02
$_{L}$-Lys·HCl	0.33	0.33
$_{DL}$-Met	0.05	0.05
$_{L}$-Thr	0.09	0.09
Sodium chloride	0.40	0.40
Vitamin-mineral premix[1]	0.15	0.15
Analyzed nutrient, %		
Dry matter	89.18	89.64
Gross energy	3,906	3,878
Crude protein	14.53	14.45
Ash	3.54	4.24
Calcium	0.49	0.74
Phosphorus	0.39	0.50
STTD Ca to STTD P ratio[2,3]	1.90	1.90

[1]The vitamin-mineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-

Table 4.1. (Cont.)

calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[2]STTD = standardized total tract digestible.

[3]Values for the STTD Ca and STTD P were calculated rather than analyzed (NRC, 2012; Lee et al., 2019b); the ratio between STTD Ca and STTD P was to maximize bone ash of 40 kg pigs (Lagos et al., 2019a; Lee et al., 2019a).

Table 4.2. Growth performance of pigs fed experimental diets (n = 10)

Item[1]	% of the requirement		SEM	*P*-value
	60	100		
BW, kg				
d 0	40.64	40.92	-	-
d 14	52.22	54.14	2.29	0.116
d 28	67.30	70.54	1.46	0.060
d 0 to 14				
ADG, kg/d	0.83	0.94	0.06	0.102
ADFI, kg/d	2.23	2.16	0.07	0.510
G:F	0.37	0.44	0.03	0.001
d 14 to 28				
ADG, kg/d	1.08	1.17	0.11	0.196
ADFI, kg/d	2.54	2.51	0.17	0.881
G:F	0.43	0.47	0.02	0.145
Overall				
ADG, kg/d	0.95	1.06	0.04	0.037
ADFI, kg/d	2.39	2.34	0.12	0.693
G:F	0.40	0.45	0.01	< 0.001

[1]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 4.3. Carcass weights of pigs fed experimental diets (n = 10)

Item	% of the requirement		SEM	*P*-value
	60	100		
Ending live BW, kg	64.89	67.13	1.38	0.162
Hot carcass weight, kg	47.92	49.62	1.11	0.239
Left side				
Side weight, kg	23.43	24.18	0.56	0.235
Bone[1], kg	3.55	3.67	0.08	0.227
Feet, kg	0.74	0.75	0.02	0.741
Skin, kg	2.49	2.40	0.05	0.259
Soft tissue, kg	16.58	17.33	0.46	0.225
Blood, kg	2.86	2.96	0.14	0.570
Viscera[2], kg	6.99	7.09	0.18	0.483

[1]Weights of bone were measured with tissues on and head, tail, and feet were not included.

[2]Visceral weight excluded the weight of digesta in the gastrointestinal tract.

Table 4.4. Bone ash weight and concentration of ash in total bone, metacarpals, metatarsals, femur, tibia, fibula, and ribs from pigs fed experimental diets[1] (n = 10)

Item	% of the requirement		SEM	P-value
	60	100		
Bone ash[2], g				
Total	727.76	946.70	28.10	< 0.001
Metacarpals	11.87	13.84	0.38	0.001
Metatarsals	12.60	15.40	0.48	0.001
Femur	73.66	88.88	4.16	0.025
Tibia	36.91	46.29	1.51	0.001
Fibula	5.51	6.60	0.20	0.001
3rd and 4th ribs	8.87	12.09	0.75	0.001
10th and 11th ribs	8.76	11.44	0.32	< 0.001
Bone ash, % defatted and dried bones				
Total	54.61	57.17	0.39	< 0.001
Metacarpals	59.23	61.53	0.36	< 0.001
Metatarsals	59.24	61.32	0.37	< 0.001
Femur	56.89	58.17	0.53	0.118
Tibia	60.25	61.73	0.39	0.020
Fibula	59.81	61.18	0.65	0.089
3rd and 4th ribs	55.04	57.65	0.59	0.002
10th and 11th ribs	55.60	58.10	0.51	0.003

[1]All bones were collected from the left half of the carcass. Therefore, weight of bone ash was calculated by multiplying by 2. Total bones represent the sum of metacarpals, metatarsals, femur, tibia, fibula, ribs, and miscellaneous bones, but head, tail, and feet were not included.

Table 4.4. (Cont.)

[2]Bone ash (g) was calculated by multiplying defatted and dried bone weight by percentage ash in the defatted and dried bone.

Table 4.5. Correlation coefficients (r) between total bone and individual bones[1] (n = 20)

	Total bone	
Item	Bone ash[2], g	Bone ash, %
Metacarpals	0.956***	0.741***
Metatarsals	0.971***	0.735***
Femur	0.846***	0.722***
Tibia	0.957***	0.644**
Fibula	0.929***	0.658**
3rd and 4th ribs	0.817***	0.704**
10th and 11th ribs	0.876***	0.539*

*P < 0.05; **P < 0.01; ***P < 0.001.

[1]All bones were collected from the left half of the carcass. Therefore, weight of bone ash was calculated by multiplying by 2. Total bones represent the sum of metacarpals, metatarsals, femur, tibia, fibula, ribs, and miscellaneous bones, but head, tail, and feet were not included.

[2]Bone ash (g) was calculated by multiplying defatted and dried bone weight by percentage ash in the defatted and dried bone.

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CHAPTER 5: COMPARATIVE DIGESTIBILITY AND RETENTION OF CALCIUM AND PHOSPHORUS IN NORMAL- AND HIGH-PHYTATE DIETS BY GESTATING SOWS AND GROWING PIGS

ABSTRACT

The objective of this experiment was to test the hypothesis that standardized total tract digestibility (**STTD**) of Ca and P and retention of Ca and P are not affected by the physiological state of pigs. A total of 32 gestating sows (d of gestation = 40) and 32 barrows (body weight = 19.8 kg) were placed in metabolism crates. Two diets were formulated to contain 9.8 or 29.4 g/kg of phytate. Diets were formulated based on corn, soybean meal, Ca carbonate, and dicalcium phosphate and high-phytate diets also contained 400 g/kg full-fat rice bran. A Ca-free diet and a P-free diet were formulated to determine the basal endogenous losses of Ca and P, respectively. Feces and urine were collected for 4 d after 4 d of adaptation. The basal endogenous losses of Ca and P (g/kg DM intake) from gestating sows were greater ($P < 0.05$) than from growing pigs. The digestibility of DM was not affected by physiological state, but was greater ($P < 0.001$) in the normal-phytate diet than in the high-phytate diet. Phytate level did not affect the STTD of Ca or Ca retention by gestating sows, but the STTD of Ca and Ca retention were greater if growing pigs were fed the normal-phytate diet than if they were fed the high-phytate diet (physiological state × phytate level interaction; $P < 0.001$). The STTD of P was greater for the normal-phytate diet than the high-phytate diet, but the difference was greater for growing pigs than for gestating sows (physiological state × phytate level interaction; $P = 0.002$). Phosphorus retention by growing pigs fed the normal-phytate diet was greater than if they were fed the high-phytate diet, but P retention by gestating sows was not affected by phytate level

(physiological state × phytate level interaction; $P < 0.001$). In conclusion, gestating sows have reduced digestibility and retention of Ca and P, but increased basal endogenous loss of Ca and P, compared with growing pigs. Response to dietary phytate is different for Ca and P balance between gestating sows and growing pigs. It may, therefore, not always be accurate to formulate diets for gestating sows using digestibility values for Ca and P that were obtained in growing pigs.

Key words: calcium, digestibility, endogenous loss, phosphorus, retention, sow

INTRODUCTION

Digestibility of energy and some nutrients may be affected by age, weight, and physiological state of the animal and gestating sows usually have greater digestibility of energy than growing pigs (Le Goff and Noblet, 2001; Casas and Stein, 2017). Coefficients for digestibility of Ca and P are most correctly determined as standardized total tract digestibility (**STTD**; NRC, 2012; Stein et al., 2016). Data for the STTD of P in most feed ingredients have been published (NRC, 2012), and the digestibility of Ca has also been determined in many feed ingredients (Stein et al., 2016; Zhang et al., 2016). Most values for STTD of Ca and P in feed ingredients have been determined in growing pigs (González-Vega et al., 2015b; Zhang and Adeola, 2017; Lee et al., 2019b). It is, however, not known if the STTD of Ca and P is different between gestating sows and growing pigs, but in practical diet formulation, values for STTD of Ca and P obtained in growing pigs are also applied to sows. Therefore, the objective of this experiment was to test the hypothesis that standardized total tract digestibility (**STTD**) and retention of Ca and P are not affected by the physiological state of pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Camborough sows (PIC, Hendersonville, TN) were used and growing pigs were the offspring of Line 359 boars and Camborough females that were not used in the experiment (Pig Improvement Company, Hendersonville, TN, USA).

Diets and Feeding

The same batches of corn, soybean meal, rice bran, Ca carbonate, and dicalcium phosphate were used to prepare all diets (Table 5.1). Four diets were used in the experiment (Table 5.2). Two diets were formulated to contain a normal or high amount of phytate. Diets were formulated based on corn, soybean meal, Ca carbonate, and dicalcium phosphate, and the high-phytate diet also contained 400 g/kg full-fat rice bran. A Ca-free diet and a P-free diet were also formulated to determine the basal endogenous losses of Ca and P, respectively. All vitamins and minerals except Ca and P were included in all diets to meet current requirements (NRC, 2012). Concentrations of Ca and P in the normal- and high-phytate diets met the requirement estimates for growing pigs, whereas the Ca and P in the 2 diets exceeded the requirement estimates for sows in less than 90 d of gestation by about 1.3 times (NRC, 2012). Daily feed allotments were provided in 2 equal meals that were provided at 0700 and 1600 h. The daily feed allowance for gestating sows was 1.5 times the maintenance energy requirement calculated based on the body weight (**BW**) of sows (i.e., 100 kcal of metabolizable energy/kg of $BW^{0.75}$; NRC, 2012), and growing pigs were provided feed in an amount that was calculated as 3 times the maintenance energy requirement (i.e., 197 kcal of metabolizable energy/kg of $BW^{0.60}$; NRC, 2012). Orts were collected after feeding to calculate total feed intake by growing pigs and sows. Water was available at all times.

Analysis Animals and Housing

A total of 32 gestating sows (BW = 248.8 ± 20.7 kg; parity = 2.48 ± 1.26; d of gestation = 40 ± 5 d) were allotted to a randomized complete block design with 4 diets and BW and group as the blocks. There were 4 groups with 2 sows per diet in each group for a total of 8 replicate sows per diet. Before the start of the experiment, sows were fed a standard gestation diets that contained 0.72% Ca and 0.53% P. Sows were housed individually in metabolism crates that were equipped with fully slatted floors, a feeder, and a cup waterer. A screen floor and a urine pan were installed below the slatted floor.

Thirty-two growing barrows (initial BW = 19.8 ± 1.0 kg) were also housed individually in metabolism crates and allotted a randomized complete block design with 4 diets and BW and group as blocks. There were 16 pigs per group and 4 replicate pigs per diet based on the BW of pigs within each group and, therefore, there were a total of 8 replicate pigs per diet.

Method of Collection

The experimental period lasted 10 d with the initial 4 d being the adaptation period to the diets followed by 4 d of total collection of feces and urine using the marker to marker procedure (Adeola, 2001). A 4-d adaptation period was used to reduce the length of feeding the Ca-free and P-free diets as much as possible to prevent bone damage to the pigs. Feces and urine were collected separately. Fecal collection was initiated when the first marker (i.e., indigo carmine) that was supplemented in the morning meal on d 5 appeared in the feces and ceased when the second marker (i.e., ferric oxide), which was added to the morning meal on d 9, appeared (Adeola, 2001). Feces were stored at –20 °C as soon as collected.

Urine collections were initiated on d 5 at 0900 h and ceased on d 9 at 0900 h. Urine was collected in buckets placed under the metabolism crates with 50 mL of 3*N* HCl. Buckets were

emptied daily, the weight of the collected urine was recorded, and 10% of the collected urine was stored at –20 °C until subsampling.

Chemical Analysis

At the conclusion of the experiment, fecal samples were pooled within animal, dried at 65 °C in a forced air oven, and finely ground using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ) through a 1-mm screen. Urine samples were thawed and mixed within animal and subsamples were collected for analysis.

Calcium and P in ingredients, diets, feces, and urine samples were analyzed by inductively coupled plasma spectroscopy (AOAC Int., 2007; method 985.01 A, B, and C) after wet ash sample preparation [AOAC Int., 2007; method 975.03 B(b)]. Feed ingredients and diets were also analyzed for phytate-bound P (Megazyme method; ESC, Ystrad Mynach, UK). All ingredient and diet samples were analyzed for dry matter (**DM**; AOAC Int., 2007; method 930.15), ash (AOAC Int., 2007; method 942.05), and gross energy using an isoperibol bomb calorimeter (Model 6300; Parr Instruments, Moline, IL). Fecal samples were also analyzed for DM. Crude protein in feed ingredients and diets was analyzed by combustion (AOAC Int., 2007; method 990.03) using an Elementar Rapid N-cube Protein/Nitrogen Apparatus (Elementar Americas Inc., Mt Laurel, NJ). Acid-hydrolyzed ether extract in ingredient and diet samples were analyzed by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). Ingredients and diets were also analyzed for acid detergent fiber and neutral detergent fiber using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY). All chemical analyses were performed in duplicates with the exception that Ca in diets were analyzed in quadruplicates.

Calculations

The ATTD of Ca and P in experimental diets was calculated using Eq. 5.1 (Almeida and Stein, 2010):

$$\text{ATTD of Ca or P} = \frac{\text{intake of Ca or P - output of Ca or P}}{\text{intake of Ca or P}} \quad [5.1]$$

where Ca and P intake and output in feces are expressed as gram per day.

Basal endogenous losses of Ca and P that were estimated as the fecal flow of Ca and P from animals fed the Ca- and P-free diets were expressed as gram per kilogram of DM intake (**DMI**) were used to calculate the STTD of Ca and P (Eq. 5.2; Almeida and Stein, 2010):

$$\text{STTD of Ca or P} = \text{ATTD of Ca or P} + \frac{\text{basal endogenous loss of Ca or P}}{\text{intake of Ca or P}}. \quad [5.2]$$

Basal endogenous losses of Ca and P expressed as gram per day from pigs fed the normal- and high-phytate diets were calculated by multiplying the respective values for basal endogenous losses of Ca and P by the daily DMI of pigs.

The retention of Ca and P expressed as gram per day was calculated using Eq. 5.3 (Petersen and Stein, 2006):

$$\text{Retention of Ca or P} = \text{intake of Ca or P - (fecal + urinary output of Ca or P)} \quad [5.3]$$

where Ca and P intake and output in feces and urine are expressed as gram per day.

The retention of Ca and P was calculated using Eq. 5.4 (Petersen and Stein, 2006):

$$\text{Retention of Ca or P} = \frac{\text{retention of Ca or P}}{\text{intake of Ca or P}} \quad [5.4]$$

where intake of Ca and P and retention of Ca and P are expressed as gram per day.

Statistical Analysis

Normality of data was verified using the PROC UNIVARIATE of SAS (SAS Inst. Inc., Cary, NC). Outliers were identified as values that were plotted outside 'inner fences' within treatment (Tukey, 1977). The animal was the experimental unit for all analyses. Data were analyzed using PROC MIXED of SAS that provided residual maximum likelihood estimates of variance and covariance components in the model. The statistical model included physiological state, phytate level in the diet, and the interactions between physiological state and phytate level as fixed effects, and group and replicate within group as random effects. To explain interactions, mean separation was conducted by the PDIFF option with the Tukey's adjustment. The basal endogenous losses of Ca and P by gestating sows and growing pigs that were fed Ca-free or P-free diets were also compared using a Student's unpaired *t*-test. Statistical significance was considered at $P < 0.05$.

RESULTS

Gestating sows and growing pigs remained healthy during the experiment and very little feed refusals were observed. The analyzed concentrations of Ca, total P, and phytate in all experimental diets were in agreement with expected values, and phytate-bound P and phytate-bound P relative to total P in the high-phytate diet was greater than in the normal-phytate diet (Table 5.3).

Basal Endogenous Losses of Ca and P

The basal endogenous loss of Ca was 1.58 g/kg of DMI and 0.43 g/kg of DMI for gestating sows and growing pigs, respectively, and the basal endogenous loss of Ca from gestating sows was greater ($P < 0.001$) than from growing pigs (Table 5.4). The basal

endogenous loss of P was 0.78 g/kg DMI from gestating sows and this value was greater (P = 0.011) than the basal endogenous loss of P from growing pigs (0.16 g/kg of DMI).

Digestibility and Retention of Ca

Feed intake and Ca intake by gestating sows were greater than by growing pigs (P < 0.001; Table 5.5). The ATTD of DM was not affected by physiological state, but animals fed the normal-phytate diet had greater (P < 0.001) ATTD of DM than animals fed the high-phytate diet. Fecal excretion by gestating sows was greater than by growing pigs and the difference was greater if the high-phytate diet was fed than if the normal-phytate diet was provided (physiological state × phytate level interaction, P < 0.001). Urine excretion by gestating sows was also greater (P = 0.027) than by growing pigs. Gestating sows had greater (P < 0.001) fecal Ca output than growing pigs and animals fed the high-phytate diet had greater (P < 0.001) fecal Ca output than if the the normal-phytate diet was fed. Gestating sows fed the normal-phytate diet had greater urine Ca output than growing pigs fed the normal-phytate diet, but the urine Ca output was not different between gestating sows and growing pigs fed the high-phytate diet (physiological state × phytate level interaction, P = 0.024). Phytate level did not affect the absorbed Ca, ATTD of Ca, STTD of Ca, or Ca retention in gestating sows, but the absorbed Ca, ATTD of Ca, STTD of Ca, or Ca retention were greater if growing pigs were fed the normal-phytate diet than the high-phytate diet (physiological state × phytate level interaction, P < 0.01). Regardless of dietary treatment, gestating sows had reduced (P < 0.001) digestibility and retention of Ca compared with growing pigs.

Daily basal endogenous loss of Ca expressed as gram per day was greater for the high-phytate diet than for the normal-phytate diet if fed to gestating sows, but for growing pigs, no difference between the 2 diet types was observed for the daily basal endogenous loss of Ca

(physiological state × phytate level interaction, $P = 0.031$). However, regardless of dietary treatment, the daily basal endogenous loss of Ca was much greater ($P < 0.001$) in gestating sows than in growing pigs.

Digestibility and Retention of P

Phosphorus intake by gestating sows was greater than by growing pigs and the difference was greater if the high-phytate diet rather than the normal-phytate diet was fed (physiological state × phytate level interaction; $P < 0.001$; Table 5.6). Fecal P output was greater if the high-phytate diet rather than the normal-phytate diet was fed and the difference was greater for growing pigs than for gestating sows (physiological state × phytate level interaction; $P < 0.001$). Gestating sows had less ($P = 0.036$) absorbed P than growing pigs, and the absorbed P was greater ($P = 0.049$) if pigs were fed the normal-phytate diet compared with the high-phytate diet. The ATTD of P was greater if growing pigs were fed the normal-phytate diet rather than the high-phytate diet, but the ATTD of P was not affected by dietary treatment if diets were fed to gestating sows (physiological state × phytate level interaction; $P = 0.001$). Likewise, the STTD of P was greater if pigs were fed the normal-phytate diet rather than the high-phytate diet, but the difference was greater for growing pigs than for gestating sows (physiological state × phytate level interaction; $P = 0.002$). Urine P output was greater ($P < 0.001$) in gestating sows compared with growing pigs and gestating sows had less ($P < 0.001$) P retention compared with growing pigs. Phosphorus retention by growing pigs fed the normal-phytate diet was greater than if pigs were fed the high-phytate diet, but P retention by gestating sows was not affected by phytate level (physiological state × phytate level interaction; $P < 0.001$). Regardless of dietary treatment, gestating sows had reduced ($P < 0.001$) digestibility and retention of P compared with growing pigs.

Daily basal endogenous loss of P by gestating sows expressed as gram per day was greater for the high-phytate diet than the normal-phytate diet, but for growing pigs, no difference between the 2 diet types was observed (physiological state × phytate level interaction, $P = 0.028$). However, regardless of dietary treatment, the daily basal endogenous loss of P was much greater ($P < 0.001$) in gestating sows than in growing pigs.

DISCUSSION

The difference in feed intake between gestating sows and growing pigs was due to differences in BW between gestating sows and growing pigs (NRC, 2012), but both groups of animals were fed close to what is common in practical production.

Digestibility and Retention of Ca and P in Diets Fed to Gestating Sows

Values for the ATTD of Ca and P in the normal-phytate diet obtained in this study were within the range of the values previously reported for sows fed corn, soybean meal, and inorganic Ca and P-based diets at d 75 to 105 of gestation (Nyachoti et al., 2006; Jang et al., 2014; Darriet et al., 2017; Lee et al., 2018). The ATTD of Ca and P in feed phosphates is greater compared with plant feed ingredients fed to gestating sows (Nyachoti et al., 2006; Jang et al., 2014) and the ATTD of Ca in Ca carbonate fed to growing pigs is less compared with dicalcium phosphate or monocalcium phosphate (González-Vega et al., 2015b). It is possible that the day of pregnancy affects the ATTD of Ca and P because late-gestation sows have greater digestibility compared with mid-gestation sows (Kemme et al., 1997; Nyachoti et al., 2006; Lee et al., 2019a). Dietary vitamin D may also affect values for the absorption of Ca and P (Lei et al., 1994), but it is unlikely vitamin D limited Ca absorption in the present experiment because vitamin D inclusion in all diets exceeded the requirement (NRC, 2012).

It is likely that the very low retention of Ca and P and the increases in urine Ca and P that were observed for gestating sows are a result of sows having adequate stores of Ca and P, therefore, sows had no need for retaining additional Ca and P. Ash contents of bones may be greater in sows than in gilts, indicating that sows accumulate Ca and P in bones over time (Giesemann et al., 1998). A model that calculates the P requirement for gestating sows indicates that requirements are very low in multiparous sows until mid-gestation and almost no P is needed by the fetuses (Bikker and Blok, 2017). However, the requirement increases in late gestation and thus coincides with increased digestibility and retention of Ca and P (Lee et al., 2019a).

Digestibility and Retention of Ca and P in Diets Fed to Growing Pigs

Values for the ATTD and STTD of Ca and P in the normal-phytate diet were in agreement with reported values (Almeida et al., 2013; González-Vega et al., 2016b; Stein et al., 2016). The ATTD and STTD of Ca and P in the high-phytate diet were also within the range of reported values (Trujillo et al., 2010; Casas and Stein, 2015; Lucca et al., 2017). The basal endogenous loss of Ca obtained from growing pigs agreed with data from other studies in which a corn-based Ca-free diet was used (González-Vega et al., 2015a; Merriman and Stein, 2016; Blavi et al., 2017) and the basal endogenous loss of P also concurred with reported values for the basal endogenous loss of P (NRC, 2012). Values for the retention of Ca and P that were expressed as gram per day in growing pigs fed all diets also were in agreement with published data (Mroz et al., 1994; González-Vega et al., 2016b). However, the retention of Ca and P (as percentage of Ca intake) varied between diets and also varies among previous experiments, which most likely is because the body will reduce retention if intake exceeds the requirement for Ca and P (Symeou et al., 2014; González-Vega et al., 2016a).

Effect of Phytate on Ca and P Balance

The observation that the digestibility of Ca and P in the high-phytate diet fed to growing pigs was lower than in the normal-phytate diet is likely due to the reduced level of dicalcium phosphate and the greater level of calcium carbonate and phytate in the high-phytate diet compared with the normal-phytate diet. Phytate-P is not likely hydrolyzed and absorbed into the body of pigs because pigs does not secret the endogenous phytase and thus P digestibility in feed ingredients containing higher amount of phytate-P is relatively lower compared with lower phytate-P. The digestibility of Ca in dicalcium phosphate is greater than in calcium carbonate (González-Vega et al., 2015b) and, therefore, an increase in the proportion of Ca from calcium carbonate in the diet may contribute to reduced digestibility of Ca in the high-phytate diet. Phytate binds positively charged ions including Ca^{2+} because of the negatively charged reactive sites on the phytate molecule, which may result in chelated Ca-phytate complexes (Selle et al., 2009). Unlike Ca in monocalcium phosphate or dicalcium phosphate, Ca from calcium carbonate may chelate with phytate molecules (González-Vega et al., 2015b), and, therefore, if more calcium carbonate is used in the diet, the digestibility of not only Ca, but also P, may be reduced.

The observed interactions between the physiological state and phytate level for the STTD of Ca and P and Ca and P retention indicate that growing pigs were more likely to be affected by dietary phytate than gestating sows. A negative correlation between digestibility of Ca and P and dietary phytate by growing pigs was also reported (Almaguer et al., 2014; Lee et al., 2018). Likewise, insoluble fiber or phytate in diets may decrease the absorption of Ca or P due to less transit time in the gut (Nortey et al., 2007; Hill et al., 2008). In this experiment, therefore, it is possible that the insoluble fiber in rice bran decreased the absorption of Ca and P in the intestines of growing pigs. However, it is not clear why gestating sows and growing pigs have different

responses to dietary phytate for Ca and P digestibility and retention in the body, but it is also possible that the response to the insoluble fiber decreases if the passage rate in the gastrointestinal tract of gestating sows is slower compared with growing pigs. Further research investigating factors affecting digestion and retention of Ca and P in gestating sows is needed.

Comparative Digestibility and Retention of Ca and P in Gestating Sows and Growing Pigs

The basal endogenous losses of Ca and P were in agreement with values previously reported for sows in mid-gestation (Bikker et al., 2017; Lee et al., 2019a). The observation that the basal endogenous loss of P which was expressed in miligram per kilogram of DMI was greater in sows compared with growing pigs concurred with the previous data (Bikker et al., 2017). The authors indicated that the difference was from differences in body size of pigs rather than from DMI. This was also demonstrated in this study (data not shown), but values for the basal endogenous loss of P were still different between the 2 groups of pigs in both experiments, which means that the exact reason for the greater basal endogenous loss of P by sows remains to be elucidated.

The observation that the ATTD of Ca and P in growing pigs is greater than in sows concurs with previous data (Kemme et al., 1997; Lee et al., 2018), but to our knowledge, no comparative values for the STTD of Ca and P or the basal endogenous losses of Ca and P between gestating sows and growing pigs have been reported. It is unlikely that the difference in feed intake is the main reason for this difference because feed intake of sows does not affect digestibility of Ca and P (Lee et al., 2018). The greater endogenous losses of Ca and P from gestating sows than from growing pigs may result in a reduced ATTD of Ca and P in sows compared with growing pigs. However, the current results indicate that digestibility of Ca and P in gestating sows is much less than in growing pigs, even if values are corrected for the greater

basal endogenous losses of Ca and P by gestating sows as is the case for calculation of STTD values. Differences in the basal endogenous losses, therefore, do not explain the differences observed for STTD values and the present data indicate that there are physiological differences between sows and growing pigs that result in differences in ATTD and STTD of Ca and P. It is possible that this is related to the fact that gestating sows have a requirement for Ca and P that is close to the maintenance requirement, whereas growing pigs have a requirement for growth and bone development in addition to the requirement for maintenance (NRC, 2012; Bikker and Blok, 2017), but additional research is needed to address this hypothesis.

CONCLUSION

Gestating sows have reduced digestibility and retention of Ca and P, but increased basal endogenous losses of Ca and P, compared with growing pigs. As a consequence, it may not always be accurate to formulate diets for gestating sows using ATTD or STTD values for Ca and P that were obtained in growing pigs.

TABLES

Table 5.1. Analyzed nutrient composition of feed ingredients (as-is basis)

Item	Corn	SBM[1]	Rice bran	Limestone	DCP[1]
Dry matter, %	86.57	90.72	92.47	100.02	93.94
Gross energy, kcal/kg	3,859	4,240	4,697	-	-
Crude protein, %	7.04	50.52	13.84	-	-
Ash, %	0.87	5.89	8.78	90.63	84.10
AEE[2], %	2.90	0.96	18.17	-	-
Neutral detergent fiber, %	8.74	4.94	16.50	-	-
Acid detergent fiber, %	2.46	2.33	6.76	-	-
Ca, %	0.01	0.34	0.05	39.0	22.0
Total P, %	0.24	0.72	1.98	0.01	18.4
Phytate, %	0.74	1.70	6.52	-	-
Phytate-bound P[3], %	0.21	0.48	1.84	-	-
Phytate-bound P, % of total P	87.50	66.67	92.93	-	-
Non-phytate P[4], %	0.03	0.24	0.14	-	-

[1]SBM = soybean meal; DCP = dicalcium phosphate

[2]AEE = acid-hydrolyzed ether extract.

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

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

Table 5.2. Ingredient composition of experimental diets fed to gestating sows and growing pigs (as-fed basis)

Ingredient, %	Phytate level		Ca-free	P-free
	Normal	High		
Ground corn	72.69	38.29	76.75	-
Soybean meal, 48% crude protein	24.00	19.00	-	-
Rice bran, full-fat	-	40.00	-	-
Cornstarch	-	-	-	46.24
Potato protein concentrate	-	-	17.00	-
Gelatin	-	-	-	20.00
Sucrose	-	-	-	20.00
Soybean oil	-	-	4.00	4.00
Cellulose	-	-	-	5.00
$_{L}$-Lys·HCl	0.40	0.37	-	0.45
$_{DL}$-Met	0.10	0.10	-	0.14
$_{L}$-Thr	0.10	0.10	-	0.29
$_{L}$-Trp	-	-	-	0.17
$_{L}$-His	-	-	-	0.23
$_{L}$-Ile	-	-	-	0.32
$_{L}$-Leu	-	-	-	0.58
$_{L}$-Val	-	-	-	0.28
Ground limestone	0.80	1.35	-	1.20
Dicalcium phosphate	1.30	0.18	-	-
Mono sodium phosphate	-	-	1.15	-
Potassium carbonate	-	-	0.40	0.40

Table 5.2. (Cont.)

Magnesium oxide	-	-	0.10	0.10
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.20	0.20	0.20	0.20

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

Table 5.3. Analyzed nutrient composition of experimental diets fed to gestating sows and growing pigs (as-fed basis)

Item, %	Phytate level		Ca-free	P-free
	Normal	High		
Dry matter	88.99	91.68	89.27	94.17
Gross energy, kcal/kg	3,809	4,212	4,217	4,081
Metabolizable energy[1], kcal/kg	3,285	3,150	3,560	3,872
Crude protein	17.25	18.40	18.94	21.75
Ash	4.58	6.61	2.67	2.54
Acid-hydrolyzed ether extract	2.70	8.94	7.16	3.87
Neutral detergent fiber	5.98	10.95	6.22	4.67
Acid detergent fiber	1.43	4.28	1.73	3.95
Ca	0.73	0.72	0.02	0.50
Total P	0.61	1.06	0.50	0.01
Phytate[2]	0.96	2.84	-	-
Phytate-bound P	0.27	0.80	-	-
Phytate-bound P:total P	44.48	75.47	-	-
Non-phytate P[3]	0.34	0.26	-	-

[1] Values for metabolizable energy were calculated based on the metabolizable energy in feed ingredients reported in NRC (2012).

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

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

Table 5.4. Basal endogenous losses of Ca and P from gestating sows and growing pigs fed Ca-free and P-free diets[1]

Item	Gestating sows	Growing pigs	SED	*P*-value
Basal endogenous loss, g/kg dry matter intake				
Ca	1.58	0.43	0.12	< 0.001
P	0.78	0.16	0.17	0.011

[1] Each mean for gestating sows and growing pigs represents 7 observations.

Table 5.5. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) and retention of Ca in experimental diets fed to gestating sows and growing pigs[1]

Physiological state	Gestating sows		Growing pigs			*P*-value[2]		
Item / Phytate	Normal	High	Normal	High	SEM	State (S)	Phytate (P)	S × P
Initial BW, kg	251.94	249.27	19.72	19.77	4.65	< 0.001	0.752	0.743
Feed intake, kg/d	2.67	2.78	1.02	1.05	0.06	< 0.001	0.084	0.308
Fecal excretion, kg/d	0.25^{b}	0.51^{a}	0.10^{c}	0.21^{b}	0.01	< 0.001	< 0.001	< 0.001
ATTD of dry matter, %	90.07	81.62	90.19	80.88	0.48	0.550	< 0.001	0.349
Urine excretion, kg/d	15.84	12.87	4.35	3.94	4.37	0.027	0.701	0.772
Ca intake, g/d	18.66	20.22	7.15	7.65	0.42	< 0.001	0.002	0.063
Fecal Ca output, g/d	17.66	19.02	1.95	4.64	0.74	< 0.001	< 0.001	0.081
Absorbed Ca, g/d	1.00^{b}	1.23^{b}	5.19^{a}	3.01^{b}	0.53	0.001	0.011	0.003
ATTD of Ca, %	5.52^{c}	6.38^{c}	72.76^{a}	39.33^{b}	3.00	< 0.001	< 0.001	< 0.001
Basal endogenous Ca loss, g/d	3.75^{b}	4.01^{a}	0.40^{c}	0.42^{c}	0.08	< 0.001	0.013	0.031
STTD of Ca[3], %	25.59^{c}	26.23^{c}	78.30^{a}	44.80^{b}	3.00	< 0.001	< 0.001	< 0.001
Urine Ca output, g/d	0.80^{a}	0.54^{ab}	0.26^{b}	0.51^{ab}	0.11	0.013	0.976	0.024

Table 5.5. (Cont.)

Ca retention, g/d	0.21[b]	0.64[b]	4.94[a]	2.50[b]	0.58	0.001	0.024	0.003
Ca retention, % of Ca intake	1.22[c]	3.70[c]	69.14[a]	32.68[b]	3.27	< 0.001	< 0.001	< 0.001

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

[1] Each mean for experimental diets from gestating sows and growing pigs represents 8 observations, with the exceptions of the high-phytate diet for gestating sows (n = 7).

[2] State = effect of physiological states; Phytate = effect of phytate level that is normal-phytate or high-phytate in diets.

[3] Basal endogenous loss of Ca from gestating sows = 1.58 g/kg dry matter intake; basal endogenous loss of Ca from growing pigs = 0.43 g/kg dry matter intake.

Table 5.6. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) and retention of P in experimental diets fed to gestating sows and growing pigs[1]

Item \ Physiological state / Phytate	Gestating sows		Growing pigs		SEM	*P*-value[2]		
	Normal	High	Normal	High		State (S)	Phytate (P)	S × P
P intake, g/d	16.09[b]	30.36[a]	6.16[d]	11.46[c]	0.55	< 0.001	< 0.001	< 0.001
Fecal P output, g/d	13.53[b]	28.85[a]	2.43[d]	8.49[c]	0.81	< 0.001	< 0.001	< 0.001
Absorbed P, g/d	2.56	1.55	3.73	2.97	0.49	0.036	0.049	0.762
ATTD of P, %	16.21[bc]	5.24[c]	60.62[a]	25.93[b]	2.98	< 0.001	< 0.001	0.001
Basal endogenous P loss, g/d	1.87[b]	2.00[a]	0.15[c]	0.16[c]	0.04	< 0.001	0.015	0.028
STTD of P[3], %	27.80[b]	11.83[c]	63.02[a]	27.30[b]	2.98	< 0.001	< 0.001	0.002
Urine P output, g/d	2.39	2.07	0.12	0.19	0.17	< 0.001	0.438	0.221
P retention, g/d	0.17	-0.51	3.61	2.78	0.47	< 0.001	0.055	0.832
P retention, % of P intake	1.23[c]	-1.39[c]	58.68[a]	24.25[b]	2.58	< 0.001	< 0.001	< 0.001

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

[1] Each mean for experimental diets from gestating sows and growing pigs represents 8 observations, with the exceptions of the high-phytate diet for gestating sows (n = 7).

[2] State = effect of physiological states; Phytate = effect of phytate level that is normal-phytate or high-phytate in diets.

Table 5.6. (Cont.)

[3] Basal endogenous loss of P from gestating sows = 0.78 g/kg dry matter intake; basal endogenous loss of P from growing pigs = 0.16 g/kg dry matter intake.

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CHAPTER 6: BASAL ENDOGENOUS LOSS, STANDARDIZED TOTAL TRACT DIGESTIBILITY OF CALCIUM IN CALCIUM CARBONATE, AND RETENTION OF CALCIUM IN GESTATING SOWS CHANGE DURING GESTATION, BUT MICROBIAL PHYTASE REDUCES BASAL ENDOGENOUS LOSS OF CALCIUM

ABSTRACT

The objective was to test the hypothesis that the standardized total tract digestibility (**STTD**) of Ca and the response to microbial phytase on STTD of Ca and apparent total tract digestibility (**ATTD**) of P in diets fed to gestating sows are constant throughout gestation. The second objective was to test the hypothesis that retention of Ca and P does not change during gestation. Thirty six gestating sows (parity = 3.3 ± 1.5; d of gestation = 7 d) were allotted to 4 diets. Two diets containing 0 or 500 units of microbial phytase per kilogram were based on corn, potato protein concentrate, and calcium carbonate. Two Ca-free diets were formulated without or with microbial phytase to estimate basal endogenous loss of Ca. Daily feed allowance was 1.5 times the maintenance energy requirement. Sows were housed individually in gestation stalls and fed a common gestation diet, but they were moved to metabolism crates from d 7 to 20 (early-gestation), d 49 to 62 (mid-gestation), and again from d 91 to 104 (late-gestation). When sows were in metabolism crates, they were fed experimental diets and feces and urine were collected for 4 d after 4 d of adaptation. Results indicated that outcomes were not influenced by the interaction between period of gestation and dietary phytase. The basal endogenous loss of Ca was greater ($P < 0.05$) by sows in early-gestation than by sows in mid- or late-gestation, but supplementation of microbial phytase to the Ca-free diet decreased ($P < 0.01$) the basal endogenous loss of Ca and tended ($P = 0.099$) to increase ATTD of P. Supplementation of

microbial phytase did not affect ATTD of DM, STTD of Ca, Ca retention, ATTD of P, or P retention in sows fed the calcium carbonate-containing diet. The ATTD of DM was not affected by period of gestation, but the ATTD of Ca, the ATTD of P, and the retention of Ca were least ($P < 0.05$) in mid-gestation, followed by early- and late-gestation, respectively, and the STTD of Ca in mid-gestation was also reduced ($P < 0.05$) compared with sows in early- or late-gestation. Phosphorus retention was greater ($P < 0.05$) in late-gestation than in the earlier periods. In conclusion, Ca retention was less negative and ATTD of P tended to increase with supplementation of microbial phytase to the Ca-free diet regardless of gestation period. The basal endogenous loss, STTD of Ca, ATTD of P, and retention of Ca and P in gestating sows change during gestation with the greatest digestibility values observed in late gestation.

Key words: calcium, digestibility, phosphorus, phytase, retention, sows

INTRODUCTION

Standardized total tract digestibility (**STTD**) of Ca has been determined for most Ca containing ingredients fed to growing pigs (González-Vega et al., 2015a; Stein et al., 2016; Zhang et al., 2016), but the STTD of Ca by sows in mid-gestation is less than by growing pigs (Lee et al., 2018). As a consequence, if diets for gestating sows are formulated using STTD values determined in growing pigs, provision of digestible Ca will be less than calculated.

Exogenous phytase increases not only P digestibility, but also Ca digestibility, in diets and feed ingredients including Ca carbonate when fed to growing pigs (Almeida et al., 2013; González-Vega et al., 2015a). The efficacy of phytase to release Ca and P is believed to be influenced by the physiological status of the animal with phytase fed to sows in mid-gestation releasing less Ca and P compared with growing pigs or sows in late-gestation (Kemme et al.,

1997; Sulabo, 2004). It is also possible that the digestibility of Ca and P by sows changes during gestation with sows in late-gestation having greater digestibility than sows in mid-gestation (Kemme et al., 1997; Jongbloed et al., 2004; 2013; Nyachoti et al., 2006). However, to our knowledge, possible changes during gestation of the basal endogenous loss, the STTD of Ca, and retention of Ca and P have not been reported and it is, therefore, not known if values for STTD of Ca or retention of Ca and P obtained in a specific time in gestation is representative of the entire gestation period.

Therefore, the objective of this experiment was to test the hypothesis that basal endogenous loss of Ca, the STTD of Ca in Ca carbonate, and the response to microbial phytase on STTD of Ca and ATTD of P in P-adequate-corn-based diets fed to gestating sows are constant throughout gestation. The second objective was to test the hypothesis that retention of Ca and P does not change during gestation.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before the animal work was initiated. Camborough sows (PIC, Hendersonville, TN) were used in the experiment.

Animals, Housing, and Sample Collection

Thirty six gestating sows (initial BW: 219.1 ± 33.4 kg; average parity: 3.3 ± 1.5) that were one week post-breeding were allotted to 3 blocks of 12 sows using a randomized complete block design. Four diets were fed to the 12 sows in each block; thus, there was a total of 9 replicate sows for each treatment.

Experimental diets included a corn-based diet in which Ca carbonate was the sole source

of Ca and a Ca-free diet (Tables 6.1 and 6.2). Each diet was prepared with no microbial phytase and with addition of 500 units of phytase (Quantum Blue®; AB Vista, Marlborough, United Kingdom). All vitamins and minerals except Ca in the Ca-free diet, were included in all diets to meet estimated nutrient requirements (NRC, 2012). Daily feed allotments were provided in 2 equal meals that were fed at 0800 and 1600 h. Daily feed allowance was 1.5 times the maintenance energy requirement for gestating sows (i.e., 100 kcal of ME/kg of $BW^{0.75}$; NRC, 2012). Water was available at all times.

Sows were housed individually in gestation stalls throughout gestation. However, from d 7 to 20 (early-gestation), d 49 to 62 (mid-gestation), and again from d 91 to 104 (late-gestation), sows were moved to metabolism crates, where they were fed 1 of the 4 experimental diets. Sows were fed the same experimental diet every time they were placed in the metabolism crates, but when sows were housed in the gestation stalls, they were fed a common conventional gestation diet that was formulated to meet the requirement estimates for all nutrients (NRC, 2012). Metabolism crates were equipped with a feeder, a nipple drinker, and a fully slatted T-bar floor. A screen floor and a urine pan were installed below the T-bar floor to allow for collection of feces and urine, respectively. The initial 4 d of each period in the metabolism crates, which was considered the adaptation period to the diets. A 4-d adaptation period was used to reduce the length of feeding the Ca-free diet as much as possible to prevent bone damage to the sows. The adaptation period was followed by 4 d of fecal collection using the marker to marker procedure (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., ferric oxide) appeared (Adeola, 2001). Passage of the marker was expected to take up to 4 d, which is the reason sows were kept in the metabolism crates for 13 d to make sure the last marker had time to pass. Urine was

collected in buckets placed under the urine pans and 50 mL of 3*N* HCl was added to each bucket every morning. Buckets were emptied daily, the weight of the collected urine was recorded, and 10% of the collected urine was stored at –20°C until subsampling. At the end of each collection period, sows were moved back to the gestation stalls. All sows were checked for pregnancy using an ultrasound scanner (VSS700 EZ Preg Checker; Veterinary Sales and Service Inc., Elmhurst, IL) on d 28 after breeding. Non-pregnant sows were removed from the experiment.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and collection period and subsamples were collected. Fecal samples were stored at –20°C as soon as collected, and at the conclusion of the experiment, samples were dried at 65°C in a forced air oven, finely ground through a 1-mm screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ), and mixed within sow and collection period. A subsample of the ground feces was then collected.

Chemical Analysis

Calcium and P in corn, potato protein concentrate, calcium carbonate, diets, feces, and urine samples were analyzed by inductively coupled plasma spectroscopy (AOAC Int., 2007; method 985.01 A, B, and C) after wet ash sample preparation [AOAC Int., 2007; method 975.03 B(b)]. Diets were analyzed for phytase activity (ESC, Ystrad Mynach, UK) by the ELISA method using Quantiplate Kits for Quantum Blue® and feed ingredients were also analyzed for phytate-bound P (Megazyme method; ESC, Ystrad Mynach, UK). All ingredient and diet samples were analyzed for DM (AOAC Int., 2007; method 930.15) and ash (AOAC Int., 2007; method 942.05). Crude protein in corn, potato protein concentrate, and diets was calculated as N × 6.25 and N was analyzed by combustion (AOAC Int., 2007; method 990.03) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI). Acid hydrolyzed ether extract in diet

samples was analyzed by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). Diets were also analyzed for acid detergent fiber and neutral detergent fiber using Ankom Technology methods 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY).

Calculations

The ATTD of DM, Ca, and P in experimental diets was calculated as previously outlined using Eq. [6.1] (Almeida and Stein, 2010):

$$\text{ATTD} = \frac{\text{intake - output}}{\text{intake}} \times 100, \qquad [6.1]$$

where nutrient intake and output in feces are expressed as gram per day.

Basal endogenous loss of Ca, which was estimated as the fecal flow of Ca from sows fed the Ca-free diet, was expressed as gram per kilogram of DM intake (**DMI**). The daily basal endogenous loss of Ca from sows fed the 2 diets containing Ca carbonate without or with microbial phytase was calculated by multiplying values for basal endogenous loss of Ca by the daily DMI of sows.

Values for STTD of Ca (%) were calculated from Eq. [6.2] (Almeida and Stein, 2010):

$$\text{STTD} = \frac{\text{intake - (output - daily basal endogenous loss)}}{\text{intake}} \times 100, \qquad [6.2]$$

where intake, output, and daily basal endogenous loss are in gram per day.

Retention of Ca and P (%) in experimental diets was calculated using Eq. [6.3] (Fernández, 1995):

$$\text{Retention} = \frac{\text{intake - (fecal output + urinary output)}}{\text{intake}} \times 100, \qquad [6.3]$$

where intake and fecal and urinary outputs are expressed as gram per day.

Statistical Analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified as values that deviated from 1st- and 3rd-quartile by more than 3 times the interquartile range within treatment. The sow was the experimental unit for all analyses. Data were analyzed as repeated measures using MIXED procedures of SAS. The statistical model included phytase, period of gestation, and the interaction between phytase and period of gestation as fixed effects and block and replicate within block as random effects. However, only a few interactions were observed and the final model, therefore, included only the main effects of phytase and period of gestation. In the few instances where an interaction between phytase and period of gestation was observed, the SLICE option of SAS was used to analyze data. Least square means were separated using the PDIFF option with Tukey's adjustment. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

The analyzed concentrations of Ca, total P, phytate, and phytase were in agreement with formulated values (Table 6.3). Sows remained healthy during the experimental period and very little feed refusals were observed with the exception that one sow refused to consume the assigned diet and had to be removed. One sow was removed from the experiment because of abortion. Four of the 36 sows that were allotted to the experiment were not pregnant and had to be removed. Therefore, there were 30 sows that completed all 3 periods of the experiment. The number of sows per treatment that completed the experiment was 8 for all dietary treatments except for the diet containing calcium carbonate without supplemental phytase (n = 6). A sow in

early-gestation fed the Ca-free diet with phytase was identified as an outlier in most response criteria. Therefore, data from this sow were not included in the final analysis.

Basal Endogenous Loss of Ca and Balance of P by Sows Fed Ca-Free Diets

Interactions between period of gestation and phytase were not observed for sows fed the Ca-free diets (data not shown). Supplementation of microbial phytase reduced ($P < 0.01$) fecal Ca output, which resulted in a reduction ($P < 0.01$) in the basal endogenous loss of Ca (Table 6.4). Calcium retention was less negative ($P < 0.05$) if microbial phytase was used than if no phytase was included in the diet. The ATTD of DM and P retention were not affected by microbial phytase, but the ATTD of P tended to be greater ($P = 0.099$) if sows were fed the diet with supplemental phytase.

Feed intake increased ($P < 0.05$) from early- to mid- to late-gestation and the ATTD of DM was greater ($P < 0.05$) in sows in late-gestation than in early-gestation. Fecal Ca output was reduced ($P < 0.05$) from early- and mid-gestation to late-gestation. The basal endogenous loss of Ca (milligram per kilogram of DMI) was greatest ($P < 0.05$) by sows in early-gestation, followed by sows in mid- and late-gestation periods, respectively. There was no gestation period effect for total urinary excretion, but urine Ca output was greater ($P < 0.05$) in early- or mid-gestation periods than in late-gestation, resulting in less Ca retention ($P < 0.05$) in early- or mid-gestation periods compared with the late-gestation period. The increased feed intake from early- to mid- to late-gestation periods resulted in the greatest ($P < 0.05$) P intake by sows in the late-gestation period, followed by sows in mid- and early-gestation periods, respectively, and fecal P output from sows in the late-gestation period was less ($P < 0.05$) than from sows in the mid-gestation period. Absorbed P and the ATTD of P were greater ($P < 0.05$) in the late-gestation period than in early- or mid-gestation periods. Phosphorus retention (gram per day) increased ($P < 0.05$)

from the early-gestation period to the late-gestation period and P retention (percentage of P intake) was greater ($P < 0.05$) in late- and mid-gestation periods compared with the early-gestation period.

Digestibility of Ca and Calcium Balance by Sows Fed Ca Carbonate-Containing Diets

Interactions between gestation period and phytase were not observed for sows fed the diet containing Ca carbonate (data not shown). Supplementation of microbial phytase did not affect the ATTD of DM, the ATTD of Ca, the STTD of Ca, or Ca retention (Table 6.5). However, supplementation of microbial phytase reduced ($P < 0.001$) daily basal endogenous loss of Ca (milligram per day).

Feed intake was greater ($P < 0.05$) in the late-gestation period, followed by mid- and early-gestation periods, respectively, and fecal excretion from sows in the late-gestation period was greater ($P < 0.05$) compared with sows in the early-gestation period. Nevertheless, the ATTD of DM was not affected by gestation period. Calcium intake was greatest ($P < 0.05$) in the late-gestation period, followed by mid- and early-gestation periods, respectively, but fecal Ca output was greater ($P < 0.05$) in the mid-gestation period than in early- or late-gestation periods. Absorbed Ca was greater ($P < 0.05$) in sows during the late-gestation period compared with earlier gestation periods. The ATTD of Ca was least ($P < 0.05$) in the mid-gestation period, followed by early- and late-gestation periods, respectively, and the STTD of Ca for sows in the mid-gestation period was also lower ($P < 0.05$) than for sows in early- or late-gestation periods. Urine Ca output was greater ($P < 0.05$) in the early-gestation period compared with the later gestation periods and Ca retention (gram per day and percentage of Ca intake) was greater ($P < 0.05$) in the late-gestation period than in earlier gestation periods.

Phosphorus Balance

Interactions between gestation period and phytase were not observed for P balance by sows fed the diets containing calcium carbonate with the exception that urine P output was reduced from sows in late-gestation compared with early- and mid-gestation periods if no phytase was used, but that was not the case if phytase was included in the diet (interaction, $P = 0.005$; Table 6.6). Supplementation of microbial phytase did not affect the ATTD of P or P retention by gestating sows.

Phosphorus intake was greatest ($P < 0.05$) in the late-gestation period, followed by mid- and early-gestation periods, but fecal P output was greater ($P < 0.05$) in the mid-gestation period than in early- or late-gestation periods. Absorbed P was greater ($P < 0.05$) in the late-gestation period compared with earlier periods and the ATTD of P was least ($P < 0.05$) in the mid-gestation period, followed by early- and late-gestation periods, respectively. Phosphorus retention was also greater ($P < 0.05$) in the late-gestation period than in the earlier gestation periods.

DISCUSSION

Concentrations of Ca and P in the corn, potato protein concentrate, Ca carbonate, and monosodium phosphate that were used in this experiment were in agreement with reported values (NRC, 2012; González-Vega et al., 2015a; Merriman and Stein, 2016). Feed intake and Ca and P intake by sows were greater in late-gestation than in the earlier periods because BW of sows increased from early- to mid- to late-gestation and feed intake was calculated based on the initial BW of sows at each period. This approach was used to maintain a constant feed intake relative to the metabolic BW of sows and thus avoid possible confounding effects of supplying

feed at different quantities relative to the maintenance requirement of sows.

Effects of Phytase on Basal Endogenous Loss of Ca and ATTD of P

The basal endogenous loss of Ca in sows during mid-gestation if no phytase was used (1,196 mg/kg of DMI) was close to the value (1,580 mg/kg of DMI) reported by Lee et al. (2018). However, the basal endogenous loss of Ca from growing pigs fed a corn-based Ca-free diet was between 329 (Merriman and Stein, 2016) and 550 mg/kg DMI (González-Vega et al., 2015b; Merriman, 2016; Blavi et al., 2017), and the present result along with the data by Lee et al. (2018) confirm that gestating sows have much greater basal endogenous loss of Ca than growing pigs if measured as milligram per kilogram of DMI.

The efficacy of phytase depends on the feed ingredients used in diets and the P-adequacy of the diets, because Ca and P digestibility in some ingredients, including dicalcium phosphate and monocalcium phosphate are not affected by phytase (González-Vega et al., 2015a). The observation that use of phytase decreases the basal endogenous loss of Ca from gestating sows indicates that phytate in corn may bind to endogenous Ca to form a Ca-phytate complex that is indigestible. However, the present data indicate that when phytase is added to the diet, there is less phytate to bind to Ca with a subsequent increased absorption and reduced excretion of endogenous Ca. The total endogenous loss of Ca from growing pigs was not affected by phytase if diets were based on canola meal (González-Vega et al., 2013). Because sows have greater endogenous loss than growing pigs, more endogenous Ca is available for binding to the phytate molecule in sows, which may be the reason phytase reduces the basal endogenous loss of Ca from sows, but not from growing pigs.

The observation that values for ATTD of P in sows fed Ca-free diets were greater compared with values from sows fed diets containing corn and Ca carbonate illustrates that there

is an interaction between Ca and P with a reduced ATTD of P if Ca in diets increases as has been previously demonstrated in growing pigs (Stein et al., 2011). The fact that sows absorbed P even if there was no Ca in the diet also illustrates that there is no down-regulation of P-absorption if bone tissue cannot be synthesized due to a lack of Ca, which has also been reported for growing pigs (Stein et al., 2006). Absorbed P can be retained in the body only if both Ca and P are available at the same time (Crenshaw, 2001), which is the reason most of the absorbed P from sows fed the Ca-free diets was excreted in the urine. Likewise, the reason retention of P was greater if sows were fed diets containing Ca carbonate compared with sows fed Ca-free diets, despite a lower absorption of P, is that most absorbed P could be used for bone tissue synthesis if diets containing Ca carbonate were fed.

Effects of Phytase on Digestibility and Retention of Ca and P

Chelation of both endogenous and dietary Ca^{++} in the intestine of pigs, which is a result of the negative charge of phytate (Nelson and Kirby, 1987; Selle et al., 2009), is the reason phytase increases both Ca and P digestibility in feed ingredients and diets fed to growing pigs (Almeida et al., 2013; Rodríguez et al., 2013; González-Vega et al., 2015a). Supplementation with exogenous phytase of diets fed to sows in mid-gestation resulted in increased ATTD of Ca and P (Jongbloed et al., 2004), an increase in ATTD of P only (Nyachoti et al., 2006; Jongbloed et al., 2013; Jang et al., 2014), or no effect on ATTD of Ca or P (Kemme et al., 1997; Liesegang et al., 2005). In the case of late-gestation, phytase increased ATTD of P (Kemme et al., 1997; Jongbloed et al., 2004; Nyachoti et al., 2006) or ATTD of Ca and P (Hanczakowska et al., 2009; Jongbloed et al., 2013). In this experiment, phytase had limited effects on the ATTD and STTD of Ca and on the ATTD of P in the P-sufficient-corn-based diet. It is not clear why different responses to phytase fed to gestating sows have been observed, but it is possible that phytase

efficacy is reduced in P-sufficient diets (Rodehutscord, 2016) and because the ATTD of Ca and P is much lower than in growing pigs fed diets without phytase (Lee et al., 2018), there likely are factors other than formation of Ca-P-phytate complexes that limit absorption of Ca and P. Digestibility of Ca and P by growing pigs decreases as the concentration of phytate increases (Almaguer et al., 2014; Lee et al., 2018), but phytate levels did not affect the ATTD of Ca and P in sows in mid-gestation (Lee et al., 2018), which further demonstrates that differences between growing pigs and gestating sows exist. Thus, more research to elucidate factors that affect absorption of Ca and P in gestating sows is warranted.

Effect of Gestation Period on Digestibility and Retention of Ca and P and Basal Endogenous Loss of Ca

To our knowledge, no data for the STTD of Ca in calcium carbonate by sows in different periods of gestation have been published. However, the observation that sows in mid-gestation had reduced ATTD of Ca and P compared with sows in late-gestation is in agreement with previous data (Kemme et al., 1997; Jongbloed et al., 2004; Liesegang et al., 2005; Nyachoti et al., 2006; Jongbloed et al., 2013). Providing Ca and P above requirements may reduce the digestibility and retention of Ca and P in gestating sows (Kemme et al., 1997; Nyachoti et al., 2006; Bikker and Blok, 2017). In this experiment, the same diets were fed to sows in all periods of gestation. The analyzed Ca and P were approximately 0.88 and 0.55%, respectively, which is close to the requirements in late-gestation, but greater than the requirement estimates for sows in early- or mid-gestation (NRC, 2012). The observation that retained Ca and P, expressed in g/d, were not different between early- and mid-gestation indicates that the requirements for Ca and P are similar between these periods. However, the fact that ATTD of Ca and P increased in late-gestation indicates that sows in late-gestation require more Ca and P than sows in earlier periods.

Indeed, very little Ca and P is needed for fetus development in early- to mid-gestation compared with late-gestation (Bikker and Blok, 2017). The data for Ca and P retention that were calculated in this experiment also indicate that more Ca and P are needed in late-gestation compared with previous periods. It is possible that differences in plasma estrogen during gestation influence 1,25-dihydroxyvitamin D_3 metabolism, which affects the digestibility of Ca and P (Heaney, 1990; Ross et al., 2011; Harmon et al., 2016). Estrogen increases during late-gestation (Kensinger et al., 1982), which may contribute to the increased ATTD of Ca and P by sows in late-gestation compared with early- or mid-gestation.

CONCLUSION

Apparent total tract digestibility, basal endogenous loss, STTD, and retention of Ca and P in gestating sows are influenced by the trimester of gestation. To accurately predict Ca and P absorption in gestating sows. Therefore, it may be necessary to assume different digestibility values for Ca in calcium carbonate and P in corn and monosodium phosphate in the late-gestation period compared with early- or mid-gestation periods. Use of microbial phytase decreases the basal endogenous loss of Ca, but the response to microbial phytase on STTD of Ca and ATTD of P in Ca and P-adequate-corn-based diets fed to gestating sows is less predictable. This may be due to an over-supply of both Ca and P by phytase in nutrient adequate diets. Therefore, there is a need to further clarify the Ca and P requirements of sows during the different trimesters of gestation.

TABLES

Table 6.1. Analyzed nutrient composition of feed ingredients (as-is basis)

Item	Corn	Potato protein concentrate	Calcium carbonate
Dry matter, %	89.77	93.57	99.95
Crude protein, %	6.95	79.87	-
Ash, %	1.42	0.65	93.38
Ca, %	< 0.01	0.02	39.63
Total P, %	0.25	0.13	0.01
Phytate[1], %	0.73	0.28	-
Phytate-bound P, %	0.21	0.08	-
Phytate-bound P, % of total P	82.00	62.99	-
Non-phytate P[2], %	0.05	0.05	-

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

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

Table 6.2. Ingredient composition of diets (as-is basis)

Ingredient, %	Calcium carbonate[1]	Ca-free[1]	Conventional diet[2]
Corn	86.19	88.34	74.58
Soybean meal	-	-	14.00
Sugar beet pulp	-	-	7.00
Potato protein concentrate	8.50	8.50	-
Calcium carbonate	2.10	-	1.30
Soybean oil	1.00	1.00	1.20
L-Lys·HCl	-	-	0.12
L-Thr	-	-	0.05
Monocalcium phosphate	-	-	1.20
Monosodium phosphate	1.15	1.10	-
Potassium carbonate	0.40	0.40	-
Magnesium oxide	0.10	0.10	-
Sodium chloride	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.15	0.15	0.15
Phytase premix[4]	0.01	0.01	-

[1]Diets were formulated without or with 500 units of microbial phytase (Quantum Blue®, AB Vista, Marlborough, UK).

[2]Conventional diet was fed to gestating sows before and between collection periods and the conventional diet did not contain any exogenous phytase.

[3]The vitamin-mineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as

Table 6.2. (Cont.)

cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[4]The phytase premix contained 5,000 units of phytase per g; corn starch was used at the expense of phytase premix in diets without microbial phytase.

Table 6.3. Analyzed nutrient concentrations in experimental diets (as-fed basis)

Item, %	Calcium carbonate		Ca-free		Conventional diet[1]
Phytase units/kg	0	500	0	500	
Metabolizable energy[2]	3,318	3,318	3,391	3,391	3,299
Dry matter	87.73	88.05	87.53	87.34	87.39
Crude protein	12.7	12.6	13.0	13.1	12.5
Ash	4.58	5.27	3.34	3.13	5.42
Neutral detergent fiber	6.39	5.65	6.42	7.13	9.96
Acid detergent fiber	1.33	1.25	1.41	2.16	4.44
Acid hydrolyzed ether extract	2.35	2.36	2.68	2.45	2.06
Ca	0.87	0.89	0.02	0.01	0.86
Total P	0.55	0.54	0.52	0.53	0.49
Phytase activity, phytase units/kg	< 50	687	< 50	587	< 50
Phytate[3], %	0.65	0.65	0.67	0.67	0.78
Phytate-bound P[4], %	0.18	0.18	0.19	0.19	0.22
Phytate-bound P, % of total P	34.55	35.19	36.54	35.85	42.65
Non-phytate P[5], %	0.36	0.35	0.33	0.34	0.28

[1]The conventional gestation diet was fed to gestating sows before and between collection periods and this diet did not contain any exogenous phytase.

[2]Values for metabolizable energy were calculated rather than analyzed (NRC, 2012).

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

[4]Phytate values were calculated from analyzed phytate in the ingredients.

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

Table 6.4. Basal endogenous loss (BEL) of Ca and balance of P by sows fed Ca-free diets without or with microbial phytase fed to sows in early-, mid-, and late-gestation periods

Item, %	Phytase units/kg		SEM	*P*-value	Period of gestation			SEM	*P*-value
	0	500			Early	Mid	Late		
Number of observations, n	24	23	-	-	15	16	16	-	-
Feed intake[1], kg/d	2.65	2.64	0.20	0.926	2.43[c]	2.63[b]	2.86[a]	0.19	< 0.001
Dry fecal excretion[1], kg/d	0.27	0.26	0.02	0.472	0.26	0.26	0.26	0.02	0.997
Urinary excretion[1], kg/d	7.32	6.97	1.31	0.856	7.54	6.19	7.70	1.28	0.552
ATTD of dry matter[2], %	89.13	89.34	0.33	0.641	88.49[b]	89.21[ab]	90.01[a]	0.42	0.047
Calcium									
Fecal Ca output[1], g/d	2.64	1.95	0.35	0.003	2.66[a]	2.39[a]	1.83[b]	0.35	< 0.001
BEL of Ca[2], mg/kg DMI	1,141	858	85	0.002	1,225[a]	1,036[b]	737[c]	87	< 0.001
Urine Ca output[1], g/d	0.13	0.22	0.05	0.210	0.23[a]	0.20[a]	0.09[b]	0.04	0.001
Ca retention[1], g/d	-2.52	-1.94	0.37	0.012	-2.68[b]	-2.35[b]	-1.66[a]	0.37	< 0.001
Phosphorus									
P intake[1], g/d	13.76	13.71	1.03	0.922	12.66[c]	13.66[b]	14.89[a]	1.01	< 0.001

Table 6.4. (Cont.)

Fecal P output[1], g/d	6.50	6.16	0.68	0.088	6.38[ab]	6.81[a]	5.80[b]	0.71	0.040
Absorbed P[1], g/d	7.29	7.63	0.38	0.395	6.36[b]	6.89[b]	9.13[a]	0.40	< 0.001
ATTD of P, %	52.37	54.59	1.62	0.099	50.03[b]	49.87[b]	60.54[a]	2.05	< 0.001
Urine P output[1], g/d	6.32	6.56	0.76	0.589	6.85	5.72	6.75	0.79	0.086
P retention[1], g/d	0.91	0.97	0.46	0.852	-0.58[b]	1.10[ab]	2.31[a]	0.60	0.001
P retention, % of intake	6.46	6.93	3.71	0.829	-3.86[b]	7.95[a]	15.99[a]	4.49	0.001

[1]All values for intake, output, or retention are the average values for the 4-d collection period.

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

Table 6.5. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca and retention of Ca in diets containing calcium carbonate fed to sows in early-, mid-, and late-gestation periods

Item, %	Phytase units/kg		SEM	*P*-value	Period of gestation			SEM	*P*-value
	0	500			Early	Mid	Late		
Number of observations, n	18	24	-	-	14	14	14	-	-
Feed intake[1], kg/d	2.73	2.65	0.19	0.242	2.46[c]	2.69[b]	2.92[a]	0.19	< 0.001
Dry fecal excretion[1], kg/d	0.27	0.25	0.02	0.312	0.24[b]	0.27[ab]	0.28[a]	0.02	0.039
Urinary excretion[1], kg/d	8.73	5.74	1.50	0.127	9.40	6.33	5.98	1.47	0.058
ATTD of dry matter, %	88.94	89.38	0.53	0.571	89.18	88.98	89.33	0.48	0.794
Ca intake[1], g/d	24.12	23.37	1.65	0.241	21.74[c]	23.70[b]	25.79[a]	1.63	< 0.001
Fecal Ca output[1], g/d	16.91	16.20	1.88	0.480	15.19[b]	19.62[a]	14.86[b]	1.94	0.001
Absorbed Ca[1], g/d	7.16	7.08	0.65	0.933	6.47[b]	4.01[b]	10.87[a]	0.78	< 0.001
ATTD of Ca, %	28.70	29.84	3.65	0.795	29.44[b]	17.35[c]	41.02[a]	3.80	< 0.001
BEL of Ca[2], mg/d	2,723	1,990	173	< 0.001	2,719[a]	2,463[b]	1,888[c]	171	< 0.001
STTD of Ca[3], %	40.08	38.57	3.65	0.733	41.83[a]	27.72[b]	48.43[a]	3.80	< 0.001
Urine Ca output[1], g/d	0.38	0.33	0.09	0.444	0.55[a]	0.29[b]	0.22[b]	0.09	< 0.001

Table 6.5. (Cont.)

Ca retention[1], g/d	6.74	6.71	0.66	0.982	5.89[b]	3.68[b]	10.60[a]	0.78	< 0.001
Ca retention, % of intake	27.42	28.72	3.81	0.755	27.33[b]	16.43[c]	40.46[a]	4.00	< 0.001

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

[1]All values for intake, output, or retention are the average values for the 4-d collection period.

[2]BEL = basal endogenous loss; the daily BEL of Ca (mg/d) was calculated by multiplying the BEL of Ca (mg/kg dry matter intake) by the daily dry matter intake (kg/d) of each experimental diet.

[3]The STTD of Ca in each diet within each period of gestation was calculated using the basal endogenous Ca loss that was specific for each period; basal endogenous losses of Ca from sows fed the Ca-free diet without microbial phytase = 1,348, 1,196, and 877 mg/kg dry matter intake for early-, mid-, and late-gestation periods, respectively; basal endogenous losses of Ca from sows fed the Ca-free diet with microbial phytase = 1,130, 876, and 596 mg/kg dry matter intake for early-, mid-, and late-gestation periods, respectively.

Table 6.6. Apparent total tract digestibility (ATTD) of P and retention of P in diets containing Ca carbonate fed to sows in early-, mid-, and late-gestation periods

Item, %	Phytase units/kg		SEM	*P*-value	Period of gestation			SEM	*P*-value
	0	500			Early	Mid	Late		
Number of observations, n	18	24	-	-	14	14	14	-	-
P intake[1], g/d	14.87	14.41	1.02	0.240	13.40^{c}	14.61^{b}	15.90^{a}	1.01	< 0.001
Fecal P output[1], g/d	11.26	10.21	1.27	0.138	9.88^{b}	12.52^{a}	9.81^{b}	1.30	0.001
Absorbed P[1], g/d	3.54	4.06	0.49	0.463	3.42^{b}	1.99^{b}	5.99^{a}	0.51	< 0.001
ATTD of P, %	22.65	27.36	3.41	0.349	25.06^{b}	13.66^{c}	36.29^{a}	3.35	< 0.001
Urine P output without phytase[1,2], g/d	-	-	-	-	1.43^{a}	1.12^{a}	0.39^{b}	0.19	< 0.001
Urine P output with phytase[1,2], g/d	-	-	-	-	1.28^{a}	1.03^{a}	0.91^{ab}	0.17	0.037
P retention[1], g/d	2.54	2.99	0.51	0.543	2.08^{b}	0.92^{b}	5.30^{a}	0.53	< 0.001
P retention, % of intake	15.94	20.08	3.47	0.415	15.41^{b}	6.54^{b}	32.08^{a}	3.40	< 0.001

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

[1]All values for intake, output, or retention are the average values for the 4-d collection period.

[2]There was an interaction between supplemental phytase and gestation period ($P = 0.005$). Therefore, values for urine P output were partitioned using the SLICE option of SAS (SAS Inst. Inc., Cary, NC). Each least squares mean represents 6 observations for sows fed the diet without phytase; each least squares mean represents 8 observations for sows fed the diet with phytase.

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CHAPTER 7: INCREASING CALCIUM CONCENTRATION IN DIETS FOR GESTATING SOWS DECREASES DIGESTIBILITY OF PHOSPHORUS, BUT INCREASES THE CONCENTRATION OF SOME BLOOD BIOMARKERS FOR BONE RESORPTION

ABSTRACT

The objective of this experiment was to test the hypothesis that the concentration of Ca in diets fed to late gestating sows affect the apparent total tract digestibility (**ATTD**) and retention of Ca and P, and serum concentrations of Ca and P, hormones, and blood biomarkers for bone formation and resorption. Thirty-six sows (average parity = 2.8) were housed individually in metabolism crates from d 91 to d 105 of gestation and fed one of 4 experimental diets containing 25, 50, 75, or 100% of the requirement for Ca. All diets were formulated to meet the requirement for P. The initial 5 d of each period were considered the adaptation period, which was followed by 4 d of quantitative collection of feces and urine. At the end of the collection period, a blood sample was collected from all sows. Results indicated that feed intake, fecal and urine excretion, and the ATTD of dry matter were not affected by dietary Ca, but ATTD of Ca increased (quadratic, $P < 0.05$) as Ca in diets increased. Urine Ca output was not affected by dietary Ca, but Ca retention increased (quadratic, $P < 0.05$) as Ca intake increased. Fecal P output increased (linear, $P < 0.001$) as dietary Ca increased, which resulted in linear decreases ($P < 0.001$) in the ATTD of P. Urine P output also decreased (linear, $P < 0.001$) as dietary Ca increased, but P retention increased (linear, $P < 0.05$). The slope of the regression equation that regressed the apparent total tract digestible Ca against dietary Ca intake was 0.33, which indicates that true total tract digestibility of Ca in calcium carbonate was 33%. Serum concentrations of Ca and P

and estrogen, calcitonin, and parathyroid hormone were not affected by Ca concentrations in diets. Serum concentration of carboxyterminal cross-linked telopeptide of type I collagen (**CTX-I**) decreased (linear, $P < 0.05$) as dietary Ca increased and serum bone-specific alkaline phosphatase tended to decrease (linear, $P < 0.10$) as Ca in diets increased. The concentration of osteocalcin (**OC**) in serum was not affected by dietary Ca, but the ratio between OC and CTX-I tended to increase ($P < 0.10$) as dietary Ca increased, which indicated that there was more bone formation than resorption in sows as dietary Ca increased. In conclusion, P digestibility in late gestating sows decreased, but retention of P increased as dietary Ca increased from inadequate to adequate levels and blood biomarkers for bone resorption changed as Ca and P retention increased.

Key words: biomarkers, calcium, digestibility, phosphorus, retention, sows

INTRODUCTION

Values for apparent total tract digestibility (**ATTD**) of P in sows fed Ca-free diets are greater than values from sows fed diets containing corn and calcium carbonate (Lee et al., 2019b), and increasing dietary Ca linearly reduces P digestibility in growing pigs (Stein et al., 2011). These observations indicate that there is an interaction between dietary Ca and P, which is likely a result of precipitation of Ca and P in the intestinal tract of pigs.

Relatively more Ca and P are needed for fetus development in late gestation compared with earlier gestation periods (Bikker and Blok, 2017; Lee et al., 2019b), but an assessment of the exact requirements for Ca and P in sows is challenging and expensive. However, biomarkers for bone turnover including carboxyterminal cross-linked telopeptide of type I collagen (**CTX-I**), osteocalcin (**OC**), and bone-specific alkaline phosphatase (**BAP**) have been used in humans, beef

breeder cows, and growing pigs as indicators of Ca and P adequacy in diets (Larsen et al., 2000; Vasikaran et al., 2011; Anderson et al., 2017; Sørensen et al., 2018). Therefore, changes in dietary Ca and P, retained Ca and P in the body, and bone turnover may be estimated from serum concentrations of biomarkers, but this relationship has not been demonstrated in sows, and it is not known if blood biomarkers can be used to estimate Ca and P status of gestating sows.

Blood Ca levels are regulated by several hormones including parathyroid hormone (**PTH**) and calcitonin and, occasionally, estrogen (Heaney, 1990; Crenshaw, 2001). It is also possible that hormone levels are affected by dietary Ca and P, but pig data to demonstrate this are lacking. Therefore, the objective of this experiment was to test the hypothesis that the concentration of Ca in diets fed to late gestating sows affect ATTD and retention of Ca and P, blood Ca and P concentrations, serum hormone levels, and concentrations of serum biomarkers.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before the animal work was initiated.

Animals, Housing, Diets, and Sample Collection

Thirty-six gestating Camborough sows (PIC, Hendersonville, TN; average parity = 2.8) were allotted to 3 blocks of 12 sows using a randomized complete block design. Four diets were fed to the 12 sows in each block with 3 sows per diet; thus, there was a total of 9 replicate sows for each treatment. Sows were housed individually in metabolism crates from d 91 to d 105 of gestation. Metabolism crates were equipped with a feeder, a nipple drinker, and fully slatted tri-bar floors. A screen floor and a urine pan were installed below the tri-bar floors to allow for collection of feces and urine, respectively.

Experimental diets were based on corn and soybean meal (Table 7.1). All diets were formulated to contain P at the requirement for total P (i.e., 0.55%; NRC, 2012), but Ca was included at 25, 50, 75, or 100% of the requirement (Table 7.2). Thus, the 4 experimental diets were formulated to contain 0.18, 0.36, 0.54, or 0.72% Ca and these concentrations were achieved by adding increasing concentrations of calcium carbonate to the diets at the expense of cornstarch. All vitamins and minerals except Ca were included in all diets to meet requirements (NRC, 2012).

Daily feed allotments were provided in 2 equal meals that were fed at 0800 and 1600 h throughout the experiment. The daily feed allowance was 1.5 times the maintenance energy requirement for gestating sows based on the body weight of sows when they were moved to the metabolism crates (i.e., 100 kcal metabolizable energy/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times.

The initial 5 d of each period in the metabolism crates were considered the adaptation period to the diets and this period was followed by 4 d of fecal collection using the marker to marker procedure (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., chromic oxide) appeared (Adeola, 2001). Fecal samples were stored at –20 °C as soon as collected. Urine was collected in buckets placed under the urine pans with 50 mL of 3*N* HCl from d 6 in the morning until d 10 in the morning. Buckets were emptied daily, the weight of the collected urine was recorded, and 10% of the collected urine was stored at –20 °C until subsampling. Following fecal and urine collections, sows were fasted for 24 h (Vasikaran et al., 2011) and a blood sample was collected from the vena cava. Blood samples were immediately centrifuged and serum samples were collected and stored at –20 ºC.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and collection period and subsamples were collected. Urine subsamples were filtered through a 4- to 8-µm P4 filter (Fisher Scientific International, Inc., Hampton, NH). Fecal samples were dried at 65 °C in a forced-air oven and finely ground through a 1-mm screen before analysis using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ).

Chemical Analyses

Calcium and P in ingredients, diets, feces, urine, and serum were analyzed by inductively coupled plasma spectroscopy (AOAC Int., 2007; method 985.01 A, B, and C) after wet ash sample preparation [AOAC Int., 2007; method 975.03 B(b)]. Ingredient, diet, and fecal samples were analyzed for dry matter (**DM**; AOAC Int., 2007; method 930.15) and ash was analyzed in all ingredient and diet samples (AOAC Int., 2007; method 942.05). Crude protein in corn, soybean meal, sugar beet pulp and all diets was calculated as N × 6.25 and N was analyzed by combustion (AOAC Int., 2007; method 990.03) using a LECO FP628 apparatus (LECO Corp., Saint Joseph, MI). Insoluble dietary fiber and soluble dietary fiber in diets were analyzed according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Acid hydrolyzed ether extract in corn, soybean meal, and sugar beet pulp was analyzed by acid hydrolysis using 3*N* HCl (AnkomHCl, Ankom Technology, Macedon, NY) followed by fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY). The GE in corn, soybean meal, and sugar beet pulp was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL).

Serum samples were analyzed for CTX-I using a Pig Cross-Linked C-Telopeptide of Type I Collagen ELISA Kit (Abbexa Ltd., Cambridge, UK). Concentrations of OC in serum were analyzed using an N-MID® Osteocalcin Enzyme-Linked Immunosorbent Assay (**ELISA**)

Kit (Immunodiagnostic Systems Ltd, The Boldons, UK), and BAP was analyzed using an Ostase® BAP Enzyme Immunoassay Kit (Immunodiagnostic Systems Ltd, The Boldons, UK). Serum samples were also analyzed for calcitonin (Porcine Calcitonin ELISA Kit; MyBioSource, San Diego, CA), PTH (Porcine PTH ELISA Kit; MyBioSource, San Diego, CA), and estrogen as estradiol (Porcine Estrogen ELISA Kit; MyBioSource, San Diego, CA).

Calculations

The ATTD of DM, Ca, and P in experimental diets was calculated as outlined by Almeida and Stein (2010) and retention of Ca and P (%) in experimental diets was calculated according to Petersen and Stein (2006). Apparent total tract digested Ca (gram per day) was regressed against dietary Ca intake (gram per day) using Eq. 7.1, which was adopted from Fan et al. (2001):

$$\text{Apparent total tract digested Ca} = -\text{B} + (\text{A} \times \text{dietary Ca intake}), \quad [7.1]$$

where A is the slope of the regression and represents the coefficient for true total tract digestibility (**TTTD**); and B is the intercept of the regression and represents the endogenous loss of Ca (gram per day).

Statistical Analysis

Data were analyzed using the PROC MIXED (SAS Inst. Inc., Cary, NC) and homogeneity of the variance among treatments and normality was confirmed using the PROC UNIVARIATE of SAS. Outliers were identified and eliminated if values deviated from the 1st or 3rd quartiles by more than 3 times the interquartile range (Tukey, 1977). Sow was the experimental unit for all analyses. The statistical model included diet as fixed effect and parity, block, and replicate within block as random effects and LSmeans of each treatment were calculated. Polynomial contrasts were used to test for linear and quadratic effects of increasing

dietary Ca. The PROC REG of SAS was used to estimate the Y-intercept and the slope to determine the endogenous losses of Ca and the TTTD of Ca, respectively. Significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

Calcium and P balance

Feed intake, fecal excretion, urine excretion, and the ATTD of DM by sows were not affected by the level of Ca in the diets (Table 7.3), but Ca intake, fecal Ca output, and absorbed Ca increased (linear, $P < 0.001$) with increasing Ca in diets. Values for the ATTD of Ca increased (quadratic, $P < 0.05$) as Ca in diets increased. Urine Ca output and Ca retention, expressed as percent of absorbed, were not affected by dietary Ca, but Ca retention expressed as gram per day increased linearly ($P < 0.001$), and Ca retention, expressed as percent of intake, increased quadratically ($P < 0.05$).

Phosphorus intake was not affected by dietary Ca because all diets had the same concentration of P. Fecal P output increased (linear, $P < 0.001$) as dietary Ca increased, which resulted in linear decreases ($P < 0.001$) in ATTD of P and absorbed P (g/d). Urine P output also decreased (linear, $P < 0.001$) as dietary Ca increased, whereas P retention expressed as gram per day and as percent of intake and percent of absorbed increased as dietary Ca increased (linear, $P < 0.05$). The slope of the regression line that was developed by regressing apparent total tract digestible Ca against dietary Ca intake was 0.33, which indicates that the TTTD of Ca in calcium carbonate was 33% (Table 7.4). The intercept of the regression line and the vertical axis was at 0.79 indicating that total endogenous loss of Ca was 0.79 g per kg DM intake.

Calcium and P Levels, Hormones, and Biomarkers in Blood Samples

Serum concentrations of Ca and P and estrogen, calcitonin, and PTH in serum samples were not affected by Ca concentration in diets (Table 7.5). However, the concentration of CTX-I decreased (linear, $P < 0.05$) as Ca concentration increased in the diets, indicating that reduced quantities of Ca were mobilized from the bones as dietary Ca increased. In contrast, serum BAP tended to decrease (linear, $P < 0.10$) as Ca in diets increased. The concentration of OC in serum was not affected by dietary Ca, but the ratio of OC to CTX-I tended to increase ($P < 0.10$) as dietary Ca increased, which indicates that bone formation increased more than bone resorption increased.

DISCUSSION

Calcium and P balance

The requirement for Ca in late gestation sows is 0.72% (NRC, 2012). Concentrations of analyzed Ca in the 4 diets were 0.18, 0.36, 0.59, and 0.71%, which is equivalent to 25, 50, 82, and 99% of the requirement, respectively. Thus analyzed Ca in the 4 diets was in agreement with formulated concentrations. The observations that urine Ca output was not affected by dietary Ca intake, whereas Ca retention increased as dietary Ca increased, indicate that sows retained absorbed Ca with the same efficiency regardless of dietary Ca intake. This indicates that even at the greatest level of Ca intake, sows retained almost all absorbed Ca. A similar observation was made in growing pigs that were fed diets with increasing dietary concentrations of Ca (González-Vega et al., 2016a).

The requirement for P is 0.55% (NRC, 2012) and all diets were formulated to meet the requirement. The analyzed P in all diets was 0.02 to 0.06 percentage units greater than

formulated indicating that all diets met the requirement for P as was intended. The reason for the slightly greater analyzed concentrations than formulated was that corn and soybean meal contained slightly more P than expected. Values for the ATTD of Ca and P in the diets were in agreement with previous values for gestating sows (Nyachoti et al., 2006; Jang et al., 2014; Lee et al., 2019b). The quadratic increase in the ATTD of Ca that was observed as dietary Ca increased is probably a result of a greater proportion of endogenous Ca in the feces of sows fed diets with a low concentration of Ca compared with sows fed diets with greater Ca (González-Vega et al., 2013). The negative values for ATTD of Ca and retention of Ca as percent of intake for sows fed the diet with the least concentration of Ca demonstrate that these sows had endogenous losses of Ca that were greater than the daily Ca intake.

The observation that the ATTD of P was reduced by increasing dietary Ca clearly demonstrates that P absorption is reduced by increasing Ca from calcium carbonate in the diets. This is likely due to chelation of phytate from corn, soybean meal, and sugar beet pulp with Ca^{+2} ions, which results in undigestible Ca-P complexes (Stein et al., 2011). It is also possible that dietary P binds directly to Ca ions in the intestinal tract of pigs, which results in precipitation and, therefore, reduction in digestibility (Walk et al., 2012). However, the observations that urine excretion of P decreased and retention of P increased as dietary Ca increased indicates that P was in excess in the low Ca diets because there was not enough Ca to support maximum bone tissue synthesis. However, as dietary Ca increased, more bone was synthesized, which required more P and less P was, therefore, excreted. These observations indicate that Ca was the limiting nutrient for synthesizing bone because Ca and P are needed at the same time in the body to synthesize bone tissue. This observation is in agreement with data demonstrating that bone mineralization in growing pigs increased as dietary Ca increased with a constant concentration of P (González-

Vega et al., 2016a; Merriman et al., 2017; Lagos et al., 2019). However, regardless of dietary treatment, values for Ca and P retention that were calculated in this experiment were less than those observed in a previous experiment for sows in late gestation (Lee et al., 2019b). Thus, it is possible that even though the diet with the greatest concentration of Ca was formulated to meet the requirement for Ca, sows fed this diet were fed below the concentration of Ca that is required to maximize Ca retention.

The TTTD of Ca in calcium carbonate was close to the standardized total tract digestibility of Ca in calcium carbonate fed to late gestating sows (Lee et al., 2019b). The standardized total tract digestibility and TTTD of Ca in calcium carbonate by growing pigs was between 69 and 76% (González-Vega et al., 2015; Merriman and Stein, 2016; Zhang and Adeola, 2017; Lee et al., 2019a). The observation that the TTTD of Ca in calcium carbonate was 33% indicates that sows have much less digestibility of Ca compared with growing pigs, which concurs with previous data (Lee et al., 2018a; 2018b). The calculated ATTD of P in diets containing corn, soybean meal, and monosodium phosphate is expected to be approximately 60% if the diets are fed to growing pigs (NRC, 2012). However, the observation that the ATTD of P in the 4 diets fed to sows was less than 40% further confirms that sows have much lower digestibility of Ca and P than growing pigs. It is not clear why the digestibility of Ca and P in gestating sows is so much lower than in growing pigs. In the diet with the least amount of Ca, sows were fed only 25% of the requirement, which theoretically should have upregulated the transcellular absorption of Ca resulting in a greater digestibility of Ca. However, the fact that this did not happen indicates that sows are not able to regulate the intestinal absorption of Ca, even if Ca is fed well below the requirement. This observation is in agreement with data from growing pigs (Stein et al., 2011) and intestinal absorption of P also appears not to be upregulated if the

provision of P is below the requirement (Stein et al., 2008).

As demonstrated in this experiment, the DM digestibility was in agreement with what is observed in growing pigs and AA digestibility in gestating sows is also close to values observed in growing pigs (Stein et al., 2001). It therefore appears that the low digestibility of Ca and P is specific to these nutrients and not something that is general for all nutrients fed to gestating sows. The digestibility of Ca and P in lactating sows is closer to values obtained in growing pigs compared with gestating sows (Kemme et al., 1997; Jongbloed et al., 2004; Nyachoti et al., 2006). It therefore seems that the very low digestibility of Ca and P that was observed in this experiment is specific to gestating sows. More research is needed to elucidate the reasons for these low digestibility values in gestating sows.

The y-intercept of the regression line indicated that the endogenous loss of Ca was 2.12 g/d and 0.79 g/kg DM intake if corrected for the average DM intake of sows. The total endogenous loss of Ca was in agreement with the value for the basal endogenous loss of Ca by sows in late gestation that was measured in a previous experiment (Lee et al., 2019b). However, the values obtained from sows were much greater compared with the endogenous loss of Ca in growing pigs (González-Vega et al., 2013; Zhang and Adeola, 2017; Lee et al., 2019a). The reason for this observation may be that gestating sows are fed only 1.5 times the energy requirement for maintenance, whereas growing pigs are usually fed 3.0 to 3.4 times the energy requirement for maintenance. The endogenous losses of AA measured as g per kg DM intake increases as feed intake is reduced (Stein et al., 1999; Moter and Stein, 2004), but it is unlikely that is the case for Ca because the level of feed intake does not affect ATTD of Ca and P in gestating sows (Lee et al., 2018a)

Calcium and P Levels, Hormones, and Biomarkers in Blood Samples

Concentrations of Ca and P in serum were in agreement with expected values (Lauridsen et al., 2010; Weber et al., 2014). These results indicate that dietary Ca levels do not affect serum Ca or P in sows, which is in agreement with previous data (Larsen et al., 2000; González-Vega et al., 2016b). Blood concentrations of Ca are regulated by PTH and calcitonin (Crenshaw, 2001). If blood Ca is low, PTH is released from the parathyroid glands, which results in increased Ca absorption, efflux of Ca from bones, and reabsorption of Ca in the kidneys to increase blood Ca concentration (Crenshaw, 2001; Molina, 2013; Blaine et al., 2015). However, calcitonin is released when blood Ca is high, which results in a decrease in blood Ca concentration because of storage of more Ca in bone and reduced reabsorption of Ca from the kidney (Crenshaw, 2001; Molina, 2013). Estrogen concentration in serum is related to Ca metabolism in the body (Heaney, 1990; Ross et al., 2011; Harmon et al., 2016), and estrogen in serum increases during late gestation and during the post-partum period to support development of mammary glands (Kensinger et al., 1982). Therefore, it was expected that estrogen, PTH, and calcitonin concentration in serum would be affected by dietary Ca, but that was not the case. However, it is possible that this is a result of sows being fasted for 24 h before bleeding. Sows were fasted because some bone biomarkers may be affected by food intake (Vasikaran et al., 2011).

Several biomarkers to predict bone turnover have been used in clinical practice for humans (Seibel, 2005; Vasikaran et al., 2011; Smith and Samadfam, 2017) and in some pig experiments (Weber et al., 2014; Sørensen et al., 2018). Most markers are derivatives or byproducts of bone turnover (Weber et al., 2014; Sørensen et al., 2018). The CTX-I is a collagen peptide derived from the bone matrix, which is released in greater quantities as bone break down increases; OC is synthesized by osteoblasts when new bone tissues are formed and BAP is an

enzyme that is involved in calcification of bone tissues by using blood P as a building block for bone tissue synthesis (Vasikaran et al., 2011). When dietary Ca is low, bone tissue breakdown is increased, which results in an increase in serum concentrations of collagen fragments including CTX-I that were parts of the bone matrix as was demonstrated in this experiment. In contrast, when there is sufficient Ca, it is more likely that osteoblasts are activated to increase bone tissue formation, which results in increases in OC and BAP levels. However, in this experiment, the bone formation markers did not change as a result of increasing dietary Ca and serum BAP actually tended to decrease as dietary Ca increased. This observation was not expected, but previous data also indicated that concentrations of serum BAP were reduced when growing pigs were fed high-Ca and P diets compared with low-Ca and P diets (Sørensen et al., 2018). It is possible this is a result of the fact that changes in bone formation takes up to 3 mo whereas only 10 d are need for bone resorption (Seibel, 2005). In this experiment, sows were fed experimental diets for 2 wk and this may explain why only CTX-I concentration differed among dietary treatments. For other markers to show a change as a result of dietary changes in Ca concentration it is possible that a longer period of feeding is required. Nevertheless, the observation that CTX-1 increased and the OC to CTX-1 ratio tended to increase as dietary Ca increased indicates that these biomarkers possibly can be used to estimate Ca status of gestating sows, but more research is needed to verify this hypothesis.

CONCLUSION

Data from this experiment indicate that P digestibility by late gestating sows decreases, but retention of P increases, as dietary Ca increases from below to at the requirement. Blood Ca, P, and hormones are not affected by dietary Ca if Ca and P levels do not exceed the requirement

for Ca. Some blood biomarkers may be useful in predicting bone resorption by late gestation sows, but verification of current results and quantification of bone mass relative to biomarker concentrations are needed for biomarkers to be used in requirement studies for gestating sows.

TABLES

Table 7.1. Analyzed nutrient composition of feed ingredients (as-is basis)

Item	Corn	Soybean meal	Sugar beet pulp	Calcium carbonate	Monosodium phosphate	Vitamin-mineral premix	Sodium chloride
Dry matter, %	86.3	92.0	89.5	99.9	99.7	96.4	99.7
Gross energy, kcal/kg	3,859	4,240	3,630	-	-	-	-
Crude protein, %	7.1	49.8	7.3	-	-	-	-
Acid hydrolyzed ether extract, %	4.3	2.3	3.0	-	-	-	-
Ash, %	1.3	6.6	6.6	93.3	91.4	55.9	99.8
Ca, %	-	0.30	0.82	37.8	0.05	2.04	0.25
P, %	0.29	0.80	0.09	-	26.7	0.12	-

Table 7.2. Composition of experimental diets (as-is basis)

Item	Ca level (% of the requirement[1])			
	25	50	75	100
Ingredient, %				
Corn	76.45	76.45	76.45	76.45
Soybean meal	11.00	11.00	11.00	11.00
Sugar beet pulp	8.00	8.00	8.00	8.00
Calcium carbonate	0.15	0.62	1.09	1.55
Monosodium phosphate	1.10	1.10	1.10	1.10
Cornstarch	1.40	0.93	0.46	-
Soybean oil	1.20	1.20	1.20	1.20
L-Lys·HCl, 78.8% Lys	0.10	0.10	0.10	0.10
L-Thr, 99% Thr	0.05	0.05	0.05	0.05
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.15	0.15	0.15	0.15
Analyzed composition, %				
Metabolizable energy, kcal/kg[3]	3,347	3,328	3,310	3,291
Dry matter	88.1	88.1	87.6	87.9
Crude protein	12.1	11.3	12.0	11.6
Ash	3.2	4.1	4.5	4.8
Total dietary fiber[4]	14.3	13.6	16.3	14.5
Soluble dietary fiber	2.0	1.4	2.6	2.0
Insoluble dietary fiber	12.3	12.2	13.7	12.5

Table 7.2 (Cont.)

Ca	0.18	0.36	0.59	0.71
P	0.61	0.61	0.61	0.57
Ca:P ratio	0.30:1	0.59:1	0.97:1	1.25:1

[1]The requirement estimate is based on the requirement for Ca by gestating sows that are in their third parity and in late gestation (NRC, 2012).

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[3]Values for metabolizable energy were calculated rather than analyzed (NRC, 2012).

[4]Total dietary fiber = soluble dietary fiber + insoluble dietary fiber.

Table 7.3. Calcium and P balances for sows in late gestation fed diets containing different levels of Ca (n = 9)

	Ca level (% of the requirement[1])					*P*-value[2]	
Item	25	50	75	100	SEM	Lin.	Quad.
Feed intake, kg/d	3.05	3.09	3.05	3.08	0.13	0.834	1.000
Fecal excretion, kg dry matter/d	0.29	0.31	0.30	0.32	0.02	0.116	0.884
Urine excretion, kg/d	3.99	4.76	7.87	4.80	1.39	0.380	0.177
ATTD of dry matter[3], %	89.11	88.80	88.82	88.47	0.54	0.156	0.955
Ca balance							
Ca intake, g/d	5.7	10.8	16.0	21.0	0.5	<0.001	0.935
Fecal Ca output, g/d	6.2	9.3	12.8	16.5	1.0	<0.001	0.571
Absorbed Ca, g/d	-0.4	1.6	3.3	4.7	0.8	<0.001	0.601
ATTD of Ca, %	-7.49	14.34	20.59	22.04	5.95	<0.001	0.039
Urine Ca output, g/d	0.1	0.1	0.1	0.1	0.04	0.300	0.581
Ca retention, g/d	-0.5	1.6	3.2	4.6	0.7	<0.001	0.592
Ca retention, % of intake	-9.33	13.53	19.83	21.45	5.87	<0.001	0.034
Ca retention, % of absorbed[4]	-	99.88	99.80	96.08	5.29	0.491	0.705
P balance							
P intake, g/d	18.6	18.8	18.6	18.8	0.8	0.822	0.967
Fecal P output, g/d	11.5	13.1	13.2	14.4	0.9	<0.001	0.673
Absorbed P, g/d	7.0	5.7	5.3	4.3	0.6	<0.001	0.680
ATTD of P, %	39.02	31.26	29.72	23.97	3.01	<0.001	0.677
Urine P output, g/d	4.9	3.8	2.2	1.3	0.3	<0.001	0.770

Table 7.3. (Cont.)

P retention, g/d	2.3	2.0	3.2	3.3	0.5	0.031	0.704
P retention, % of intake	12.33	10.90	17.87	17.86	3.19	0.026	0.755
P retention, % of absorbed	28.39	29.49	50.83	69.03	7.93	<0.001	0.093

[1]The requirement estimate is based on the requirement for Ca by gestating sows that are in their third parity and in late gestation (NRC, 2012).

[2]Lin. = linear effect of Ca level; Quad. = quadratic effect of Ca level.

[3]ATTD = apparent total tract digestibility.

[4]Regardless of calculated value, Ca retention was assumed to be close to zero. Therefore, the first diet was excluded to test the linear and quadratic effects of dietary Ca.

Table 7.4. Regression of apparent total tract digested Ca (g/d) against dietary Ca intake (g/d) of sows fed diets containing 4 levels of calcium carbonate

Item	Calcium carbonate
Regression equation	Y = – 2.1230 + 0.3318X
SE of slope	0.02
SE of intercept	0.27
Coefficient of determination (r^2)	0.994
Endogenous loss of Ca, g/d	2.12
Endogenous loss of Ca, g/dry matter intake	0.79
True total tract Ca digestibility, %	33.18

Table 7.5. Calcium and P concentrations, bone resorption and formation biomarkers, and hormone concentrations in serum samples of late gestation sows fed diets containing different levels of Ca (n = 9)

	Ca level (% of the requirement[1])					*P*-value[2]	
Item	25	50	75	100	SEM	Lin.	Quad.
Ca, mg/L	93	92	91	92	2.1	0.713	0.539
P, mg/L	81	79	79	83	2.8	0.560	0.162
Hormones							
Estrogen, µg/L	2.1	2.0	2.0	2.0	0.16	0.546	0.706
Calcitonin, µg/L	2.7	2.7	2.5	2.5	0.14	0.230	0.935
Parathyroid hormone, µg/L	1.7	1.8	1.6	1.6	0.10	0.348	0.758
Bone resorption biomarker							
CTX-I[3], µg/L	1.5	1.0	1.4	0.2	0.39	0.033	0.296
Bone formation biomarkers							
Bone alkaline phosphatase, µg/L	12.1	10.7	10.5	10.2	1.15	0.091	0.506
Osteocalcin, µg/L	16.6	18.7	18.8	19.0	1.37	0.176	0.446
Osteocalcin/CTX-I	25	42	43	82	21.5	0.055	0.570

[1]The requirement estimate is based on the requirement for Ca by gestating sows that are in their third parity and in late gestation (NRC, 2012).

[2]Lin. = linear effect of Ca level; Quad. = quadratic effect of Ca level.

[3]CTX-I = cross-linked C-telopeptide of type I collagen.

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CHAPTER 8: CONCLUSION

Values for standardized total tract digestibility (**STTD**) are believed to be additive in a complete diet. Therefore, use of STTD values may result in the most accurate diet formulations and values for the STTD of Ca and P in most feed ingredients fed to growing pigs have been reported in recent years. By supplementing exogenous phytase to diets fed to growing pigs, the STTD of both Ca and P in feed ingredients may increase and, therefore, it is necessary to determine the STTD values in feed ingredients without or with phytase.

In practical diet formulation, values for the STTD of Ca and P obtained in growing pigs are also applied to sows. However, gestating sows have reduced digestibility and retention of Ca and P compared with growing pigs, and the impact of microbial phytase on the digestibility of P and Ca is much less in sows than in growing pigs. Applying STTD values for Ca and P obtained in growing pigs to diets for gestating sows, therefore, results in an overestimation of the absorbed Ca and P in sows. Further research, however, indicated that the digestibility of Ca and P in late gestating sows was greater than in sows in early or mid-gestation and retention of Ca and P was greater in late-gestation compared with earlier gestation periods, which indicates that digestion and absorption of Ca and P may be under hormonal control in sows. It was also demonstrated that a wide Ca:P ratio decreased P digestibility in sows in late-gestation, which demonstrates the need for not overfeeding STTD Ca. In follow-up research, it was demonstrated that several serum biomarkers may be used to predict if a sow is in a positive or a negative Ca and P state, but more research is needed to quantify this effect and to determine if biomarkers can be used in Ca and P requirement experiments.

Overall, gestating sows have much lower digestibility of Ca and P than growing pigs, which demonstrates that digestibility values obtained in growing pigs cannot be used to

accurately formulate diets for gestating sows. Likewise, effects of microbial phytase on digestibility of Ca and P are much less predictable in gestating sows than in growing pigs and phytase effects in sows are much smaller than in growing pigs.