Effect of Body Weight and Reproductive Status on Phosphorus Digestibility and Efficacy of Phytase in Pigs

By

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

Effect of Body Weight and Reproductive Status on Phosphorus Digestibility and Efficacy of Phytase in Pigs

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This study was conducted to determine the effect of body weight and reproductive status on apparent ileal digestibility coefficients (AID) and apparent total tract digestibility coefficients (ATTD) of P, and the efficacy of phytase in pigs. The study was divided into three phases. In phase 1, piglets from 10 to 40 kg BW were used; in phase 2, growing pigs from 40 to 130 kg BW were used; and in phase 3, multiparous sows (ave. parity = 5) were used. In each phase, six animals were surgically fitted with a T-cannula in the distal ileum. Two experimental diets were formulated. Diet 1 was a corn-soybean meal-canola meal-based diet containing 0.45% total P without phytase supplementation and diet 2 was identical to diet 1, but supplemented with 500 FYT/kg of *Peniophora lycii* phytase (Ronozyme P®). The Ca to total P ratio was 1.1:1 in both diets. Chromium oxide was included in the diets at 0.25% as an inert marker. In growing pigs, AID and ATTD were determined at 10, 20, 40, 70, 100, and 130 kg BW. In sows, AID and ATTD were determined in each trimester of gestation and in lactation. In phase 1, BW had no effect ($P > 0.05$) on AID or on ATTD of P regardless of the diet being fed. As BW increased from 40 to 130 kg, AID and ATTD of P decreased linearly ($P < 0.05$) regardless of the

diet fed. In phase 1 and phase 2, phytase supplementation improved ($P < 0.05$) both AID and ATTD of P. In phase 3, an increase ($P < 0.01$) in AID and ATTD of P was observed as sows proceeded through gestation and lactation regardless of the diet being fed. Phytase addition increased ($P < 0.05$) AID only during lactation. Phytase improved ($P < 0.05$) ATTD in the last trimester of gestation and in lactation. No differences between AID and ATTD of P were observed ($P > 0.05$). Efficacy of phytase was highest in lactating sows, followed by growing-finishing pigs, piglets and gestating sows. In conclusion, the physiological status of the pig affects apparent digestibility of P and the efficacy of phytase.

Key Words: Pigs, Phosphorus, Digestibility, Phytase

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LIST OF ABBREVIATIONS

AID	Apparent Ileal Digestibility coefficient(s)
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ATTD	Apparent Total Tract Digestibility coefficient(s)
BW	Body Weight
Ca	Calcium
Co	Cobalt
CP	Crude Protein
Crd	Chromium in digesta
Crf	Chromium in feed
CT	Calcitonin
Cu	Cupper
DM	Dry Matter
dP	Digestible Phosphorus
Fe	Iron
H	Hours
HPLC	High Performance Liquid Chromatography
iP	Inorganic Phosphorus
IP6	Phytic Acid
Lys	Lysine

MCP	Mono-calcium Phosphate
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
NRC	National Research Council
P	Phosphorus
Pd	Phosphorus content in the sample
Pf	Phosphorus content in the feed
PTH	Parathyroid Hormone
SDSU	South Dakota State University
SEM	Standard Error of the Mean
SBM	Soybean Meal
Zn	Zinc

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CHAPTER 1

Introduction

Phosphorus (P) nutrition is an issue of phosphate utilization and metabolism (Anderson, 1991). The need for a better understanding of the mechanisms and factors that contribute to the efficiency of P utilization in animal diets is critical to the success of maintaining the sustainability of natural resources and protection of the environment.

The inefficiency of P utilization in monogastric animals can be attributed to numerous factors. The bioavailability of P in feedstuffs is one of the most important issues, as it is directly responsible for the amount of P excreted by the animal. Phosphorus in plant ingredients is bound to complex structures called phytic acid (Plaami and Kumpulainen, 1995; Plaami, 1997). Due to the inherently low activity of endogenous phytases, monogastric animals lack the ability to degrade these phytates resulting in low P availability (Williams and Taylor, 1985, Jongbloed et al., 1991; Kornegay, 2001). With this in mind, the accurate determination of P bioavailability and the formulation of diets on the basis of bioavailable P are essential in maximizing P utilization and reducing P excretion (Cromwell and Coffey, 1991). Developments in biotechnology have also offered novel solutions in improving P utilization. The introduction of microbial phytase in swine and poultry diets has given livestock producers the ability to nutritionally manipulate P excretion (de Lange, 1997).

In pigs, digestibility studies are performed to estimate bioavailability and indirectly measure nutrient utilization (Fan et al., 2001). Phosphorus digestibility values

are usually referred to as apparent P digestibility coefficients. Numerous factors affect P digestibility, most of which are dietary factors that either enhance or decrease P utilization. However, it is also hypothesized that animal factors such as physiological status may influence P digestibility, though this issue remains controversial. Differences in gastric capacity, gastric pH, gastric retention time, and other physiological changes that accompany increasing age may play a role in the pig's ability to digest phytate P. These factors may also affect phytase activity, which may indicate differences in effectiveness among the different stages. Therefore, there is an apparent need to investigate physiological status as a factor affecting P digestibility and phytase efficacy.

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CHAPTER 2

Effect of body weight and reproductive status on phosphorus digestibility and efficacy of phytase in pigs: Literature review

1. Introduction

The imposition of environmental regulations to reduce nutrient excretion in many countries has added a new but important dimension to intensive livestock production, shifting production from focusing solely on efficiency to an environmentally-conscious production system. The need for practical solutions in reducing P in animal manure has led to the development of numerous management techniques and technologies. Phosphorus reduction in animal wastes has been approached by two major schemes: (1) improvement in formulation accuracy by matching dietary P more closely to the animal's requirement and (2) the use of dietary microbial phytase to make phytate P more available. Formulating swine diets based on either total P or apparent P digestibility is widely used, due to the lack of information on the true digestibility of P in pigs. This is further confounded by the lack of understanding of P absorption within the gastrointestinal tract. Therefore, a more accurate determination of P digestibility values would present opportunities to ensure better P utilization, reduce P excretion, and enhance environmental integrity.

2. The importance of P

In terms of both abundance and function, P is regarded as one of the most important minerals required by the body. It is the second most abundant mineral in the body (Waldroup, 1996), with approximately 80% of the total body phosphate being located in the skeletal system and the rest widely distributed throughout the body (Hays, 1976; Cromwell, 1992; Waldroup, 1996; Groff and Gropper, 1999). These phosphate stores act as a reservoir of P, and may be mobilized during periods of dietary inadequacies (Hays, 1976). Structurally, P is present in three main forms: (1) hydroxyapatite in calcified tissues, (2) phospholipids, which are major components of most biological membranes, and (3) nucleotides and nucleic acids (Cashman and Flynn, 1999).

The primary function of P is in the development and maintenance of skeletal structures; however, it is also involved in virtually every metabolic reaction in the body and is considered to be the most versatile of all the mineral elements (Waldroup, 1996). Within cells, P is a major anion and is involved in numerous processes (Groff and Gropper, 1999). First, P plays an important role in intermediary metabolism of carbohydrates, fats and proteins by contributing to the metabolic potential in the form of high-energy phosphate bonds, such as ATP and creatine phosphate. It also serves in the activation and deactivation of many catalytic proteins through alternating phosphorylation or dephosphorylation (Cashman and Flynn, 1999; Groff and Gropper, 1999). Being an important component of DNA and RNA, P is also essential in genetic transmission and control of cellular metabolism (Waldroup, 1996; Groff and Gropper,

1999). Phosphorus is also important in the formation of phospholipids, which form the lipid bilayer of cell membranes (Waldroup, 1996). Phosphates, serving as an intracellular buffer, aid in maintaining osmotic and acid-base balance (Hays, 1976; Waldroup, 1996; Cashman and Flynn, 1999; Groff and Gropper, 1999). Phosphates are also involved in oxygen delivery as a component of 2,3-diphosphoglycerate (2,3-DPG) (Groff and Gropper, 1999) in red blood cells. Synthesis of 2,3-DPG increases the release of oxygen to tissues. Finally, P is involved in the control of appetite, in a manner not yet fully understood, and in the efficiency of feed utilization (Jongbloed, 1987).

3. P metabolism

3.1. P digestion

The mechanism of P digestion is dependent upon the source and form of P in the diet. Phosphorus found intrinsically in plant ingredients is organically-bound while those in mineral supplements, such as calcium phosphates, are inorganic sources of P. Inorganic P usually has higher bioavailability and is readily absorbed in the small intestine. However, organically-bound P has to undergo enzymatic hydrolysis in the lumen of the small intestine before it is released and absorbed as iP (Figure 2.1) (Groff and Gropper, 1999). This is mainly facilitated by intestinal phosphatases such as alkaline phosphatase and intestinal phytase, which function at the brush border of enterocytes to free P from its bound form. Phospholipase C also hydrolyzes phosphate from phospholipids (Groff and Gropper, 1999), although evidence also shows that P present in certain phospholipids may be absorbed in the organic form (Wilkinson, 1976).

3.2. P absorption

In spite of the critical importance of P, the complete mechanism of P absorption has not been fully elucidated (Figure 2.2). However, P absorption appears to occur through the following processes:

3.2.1. Na^+-dependent, carrier-mediated active transport system

Phosphate is transported against a concentration gradient across the brush border of enterocytes through a Na^+-dependent process (Jongbloed, 1987; Groff and Gropper, 1999). Symporters in the brush border membrane facilitate the co-transport of Na^+ and phosphates into the cytosol. These phosphates cross the cytosol without entering the cytoplasmic phosphate pool. These finally diffuse through the basolateral membrane into the lamina propia, possibly through Na^+-independent phosphate transporters (Danisi and Murer, 1992; Civitelli and Avioli, 1994; Peerce, 1997). This Na^+-P co-transport system has been demonstrated in rat and rabbit small intestines (Crenshaw, 2001). The process is also thought to be saturable (Jongbloed, 1987). Fox and Care (1978) demonstrated this saturation process where net absorption of P from a perfusate in the jejunum increased as phosphate concentration was increased; however, further increase reduced the rate of P absorption. Calcitriol, the active form of vitamin D_3 (1,25-dihydroxycholecalciferol), was also found to stimulate phosphate absorption independent of its effect on Ca absorption (Jongbloed, 1987).

3.2.2. Na^+-independent diffusion mechanism

Phosphorus also appears to be absorbed through a linear, concentration-dependent diffusion process across the intestinal lumen (Anderson, 1991; Allen and Wood, 1994;

Crenshaw, 2001). Phosphates move through ion channels down its electrochemical potential gradient across the brush border membrane of the enterocytes until equilibrium is achieved. However, this passive transport mechanism is believed to be less dominant than the carrier-mediated active transport system.

3.2.3. Calcium-transport system

Evidence indicates that specific Ca-binding proteins in the brush border membrane of intestinal cells, which actively transport Ca across the intestinal wall, also appear to facilitate the absorption of P, although the mechanism is not yet well understood (Kornegay, 1996). These Ca-binding proteins called calbindin are synthesized in the intestine in response to calcitriol (Armbrecht et al., 1999).

3.3. Site of P absorption in pigs

In pigs, numerous studies suggest that the principal site of P absorption is the proximal half of the small intestine, primarily in the duodenum and jejunum (Jongbloed, 1987; Kornegay, 1996; Waldroup, 1996; Groff and Gropper, 1999). Similar observations were reported in rats (McHardy and Parsons, 1956; Harrison and Harrison, 1961; Kowarski and Schachter, 1969; Cramer, 1972; Chen et al., 1974; Walling, 1977) and chicks (Hurwitz and Bar, 1972; Wasserman and Taylor, 1973). Between the duodenum and the jejunum, higher rates of active P absorption were found in the jejunum than in the duodenum (160 vs. 40 nmol/cm^2/hr) with very little absorption occurring in the ileum (Crenshaw, 2001).

In experiments with growing pigs using diets incorporated with labeled Ca and P, Moore and Tyler (1955) concluded that absorption of P took place only in the proximal

half of the small intestine and they did not find any secretion of P into the lumen of the large intestine. Later studies with cannulated pigs at the distal ileum confirm this early conclusion, where P was no longer absorbed distal to the cannula (Jørgensen and Fernandez, 1984; Jørgensen et al., 1985; Partridge et al., 1986; Larsen and Sandström, 1993; Fan et al., 2001; Bohlke, 2002). Other studies demonstrated some P absorption post-ileally, but found it to be negligible (Gueguen et al., 1968; Jongbloed et al., 1992; Mroz et al., 1994).

Some researchers, however, contend that the cecum-colon region is significantly involved in P absorption. Drochner (1984) fitted ileal re-entrant cannulas and simple T-cannulas in the cecum of mini-pigs and found considerable amounts of P absorbed in the large intestine. Den Hartog et al. (1985), using pigs with ileo-caecal re-entrant cannulas, found as much as 40% of P absorbed in the large intestine. Numerous studies have also shown significantly higher total tract digestibility compared to ileal digestibility of P (Bruce and Sundstøhl, 1995; Kienzle et al., 1995; Van der Heijden et al., 1995; O' Quinn et al., 1997; Seynaeve et al., 2000a). This suggests absorption of P in the hindgut.

3.4. Transport and mobilization of P

From the small intestine, absorbed P is transported very rapidly into the blood. This was confirmed using orally administered radioisotopes of P, which appeared in the blood within 10 minutes and peaked after about an hour (Groff and Gropper, 1999). Absorbed P mainly exists in the plasma in two forms: (1) phosphate as part of phospholipids, which constitute about 70% of the total P in the plasma and (2) as iP, primarily as HPO_4^{2-} and $H_2PO_4^-$ (Allen and Wood, 1994).

Normally, these phosphates in circulation are in equilibrium with iP deposited in bones, cellular iP, and with organic phosphates formed in intermediary metabolism (Groff and Gropper, 1999). Circulating phosphates are withdrawn for skeletal development while iP deposited in bones can be readily mobilized to maintain normal plasma P levels.

4. Hormonal regulation of P metabolism

It is believed that P metabolism, as in the case of Ca, is tightly regulated by a hormonal system composed of the parathyroid hormone (PTH), calcitonin (CT), and calcitriol (De Luca, 1979). This system acts simultaneously on processes such as intestinal absorption, bone resorption, deposition, and excretion to maintain the required plasma iP concentration (Figure 2.3) (Fernandez, 1995a).

4.1. Parathyroid hormone (PTH)

Parathyroid hormone (PTH), which is produced by the chief cells of the parathyroid glands, functions mainly in the control of Ca homeostasis through its action on the bones, kidney and small intestine (Figure 2.4) (Jongbloed, 1987). Parathyroid hormone is secreted in response to hypocalcemic and possibly, hypophosphatemic conditions, where it stimulates 1α-hydroxylase activity in the kidney (Jongbloed, 1987; Groff and Gropper, 1999). This enhances the conversion of 25-hydroxycholecalciferol (25-OH-D_3) to calcitriol (1,25-$(OH)_2$-D_3), which then stimulates bone resorption, increases both P and Ca absorption in the small intestine, and increases tubular reabsorption of Ca in the kidneys. In hypocalcemic conditions, it increases renal P excretion to prevent precipitation of Ca-P complexes in critical tissues, which may result

from increased bone resorption (Genuth, 1998; Granner, 2000). However, PTH increases P reabsorption in the proximal tubules when P is deprived in the diet (Genuth, 1998).

Some studies, however, demonstrated that the effect of PTH on P absorption to be insignificant (Jongbloed, 1987; Allen and Wood, 1994). In rats, the rate of P absorption was compared between normal and parathyroidectomized rats. The removal of the parathyroid glands did not have any influence on either P absorption or urinary P excretion as compared to normal rats (Carlsson, 1954; Wasserman and Comar, 1961; Clark and Rivera-Cordero, 1974).

4.2. Calcitonin (CT)

Calcitonin (CT), which is produced by the C cells of the thyroid gland, acts contrarily with PTH (Groff and Gropper, 1999). Calcitonin is secreted in response to hypercalcemic conditions where it reduces plasma Ca levels rapidly by inhibiting bone resorption, and reducing reabsorption of Ca in the proximal tubule of the kidneys (Figure 2.5) (McKercher and Radde, 1981; Jongbloed, 1987). It is also thought to inhibit both Ca and P absorption in the small intestine. However, previous research has shown conflicting results on the effect of CT on P absorption in the small intestine. Tanzer and Navia (1973) and Juan et al. (1976) have shown that CT inhibits P absorption in the gut *in vitro*. In *in vivo* experiments with 4-40 day old piglets, absorptive flux rates of labeled Ca in the small intestine were inhibited with physiological concentrations of porcine CT (3.8 ng/mL) while absorptive flux rates of labeled P were not affected by either physiological or pharmacological CT concentrations (120 ng/mL) (McKercher and Radde, 1981). This discrepancy in results between the *in vivo* and *in vitro* experiments is thought to be due to

CT-induced water secretion from the enterocytes. Thus, McKercher and Radde (1981) proposed that CT does not directly influence active P transport but acts more indirectly on the passive transport of the ion.

4.3. Calcitriol (1,25-$(OH)_2D_3$)

Calcitriol (1,25-$(OH)_2$ D_3), the biologically active form of vitamin D, is a steroid hormone that has an important role in regulating plasma Ca and P concentrations (DeLuca, 1980; Groff and Gropper, 1999). In maintaining Ca homeostasis, calcitriol functions with PTH wherein they both impact several tissues such as the intestine, bone and kidney (Figure 2.6). Parathyroid hormone stimulates 1α-hydroxylase activity in the kidney, which in turn converts 25-OH D_3 derived from the liver into its active form, calcitriol (Jongbloed, 1987; Groff and Gropper, 1999). Therefore, 1α-hydroxylase serves as the major regulatory enzyme, which is enhanced not only by PTH, but by low plasma levels of Ca and phosphate. X-linked hypophosphatemia (XLH), the most common hereditary form of rickets in humans, exhibits responses such as enhanced tubular reabsorption of P as well as an induction of 1α-hydroxylase in response to low serum P concentrations (Grieff, 2002). Engstrom et al. (1985) and Sommerville et al. (1985) both demonstrated an induction of renal 1α-hydroxylase and calcitriol activity with pigs fed P-deficient diets.

As one of its primary effects, calcitriol enhances intestinal absorption of both Ca and P (Genuth, 1998). With respect to P, calcitriol is thought to increase the activity of brush border alkaline phosphatase, which hydrolyzes phosphate ester bonds and improves P absorption (Jongbloed, 1987). Calcitriol is also thought to positively modulate the

number of carriers available for Na^+-dependent P absorption at the brush border membrane, increasing transport efficiency (Lawson, 1985).

5. Bioavailability of P sources

Table 2.1 shows the bioavailability and apparent P digestibility of selected feed ingredients commonly used in diets for pigs and poultry. The concept of biological availability or 'bioavailability', which is generally applied to P, is a measure of the degree to which a P source can support the physiological processes of an animal (Waldroup, 1996). This, however, is a difficult concept to measure since numerous factors are involved in both the absorption and utilization of the mineral. Thus, the bioavailability of P in feed ingredients is estimated as (1) percent digestibility, where the difference between the amount of P consumed and excreted (or collected from the distal ileum) is assumed to be available, and (2) relative bioavailability, which estimates the availability of P by comparing to a known standard of a highly available form (Cromwell, 1996).

5.1. Plant sources

Generally, the availability of P from ingredients of plant origin is low for monogastric animals due to two reasons: (1) intrinsic P is mainly bound to a complex called phytic acid (Figure 2.7), and (2) monogastrics lack the enzyme phytase needed to degrade phytate. Until recently, it has been generally assumed that approximately 30-35% of the total P in plant sources is available to pigs and poultry. However, a greater variability has been shown among and within ingredients (Table 2.2) (Jongbloed et al., 1991; Ravindran et al., 1999; Bedford, 2000). Apparent P digestibility of commonly used

plant ingredients varies from 10 to 50% (Jongbloed et al., 1991). The wide range of variability can be attributed to the origin of the ingredient, the amount of phytate P, and the presence of intrinsic phytase (Jongbloed et al., 1991). Jongbloed et al. (1991) noted that the lower the proportion of phytate, the higher is the digestibility of P of the ingredient. Eeckhout and de Paepe (1994) reported that the portion of total P present as phytate in cereals is about 59-70%, 20-46% in legume seeds, and 34-66% in oil seed meals. Phytate P may also constitute 10-25% of the total P in tubers such as tapioca and potato meal (Ravindran et al., 1994). Some ingredients, such as wheat and barley, have higher availability of P (49 and 30%, respectively) due to the presence of natural phytase (Cromwell, 1992), which increases the value of these ingredients in monogastric diets.

5.2. Animal sources

It is known that the availability of P in ingredients of animal origin has a relatively higher availability of P than those from plant sources, which is often assumed to be around 95-100% (Cromwell, 1996). However, evidence has shown that P digestibility of these sources is lower and also has a wider range of variability as previously believed. Apparent P digestibility of ingredients from animal origin ranges from 68 to 91%, with bone meal and skimmed milk powder having the lowest and highest digestibility coefficients, respectively (Table 2.3) (Jongbloed et al., 1991). Jongbloed et al. (1991) suggested that the variability among batches of the same ingredient can be attributed to differences in the manufacturing process, which contributes to the variability of the P content and digestibility. Physicochemical structure

of the ingredient, such as the particle size of bones in meat and bone meal, may also contribute to the differences (Cromwell, 1996).

5.3. Inorganic sources

Due to the poor availability of the larger fraction of P from plant ingredients, mineral sources of P have been widely used to supplement diets for pigs and poultry. However, there are also marked differences in bioavailability of P between types and origin of feed phosphates for both pigs and poultry (Jongbloed et al., 1991; Waldroup, 1996). Relative bioavailability of P for various inorganic phosphates ranges from 30 to 100% (Table 2.4) (NRC, 1998). These bioavailability estimates, however, are generally expressed as a percentage of a reference standard, either monosodium or monocalcium phosphate. Apparent P digestibility of monosodium phosphate is estimated at about 85% (Table 2.5) (CVB, 1999). Thus, diets formulated based on bioavailability values of these sources would be poorly estimated.

6. Phytic acid

Phytic acid (myoinositol hexakisphosphate) or its salts (called phytate), are essential components of plant seeds (Plaami and Kumpulainen, 1995; Plaami, 1997; Kornegay, 2001). They constitute approximately 1 to 2% by weight of many cereals and oilseeds (Plaami, 1997), and about 60 to 90% of the total P in these feedstuffs occurs as phytate (Lolas et al., 1976; Cosgrove, 1980; Frolich and Asp, 1985; Reddy et al., 1989; Plaami and Kumpulainen, 1995). Phytic acid generally accumulates during the ripening period (Asada et al., 1969; Kornegay, 2001). It is widely distributed in the seed; however, its location varies among different sources. In grains such as corn, most of the

phytate can be found in the germ (Kornegay, 2001). In cereals such as wheat, barley, and rye, phytates are mainly found in the aleurone layer and in the germ associated with protein bodies. In soybeans, phytic acid is located in protein bodies distributed throughout the seed (Baker, 1991; Kornegay, 2001).

6.1. Physiological roles of phytic acid in plants

Phytic acid plays a vital role in numerous physiological processes in plants. It serves mainly as a P reservoir, which is released by plant phytases in the dormant seed during germination and used in ATP synthesis (Plaami, 1997; Kornegay, 2001). It also serves as a primary source of inositol and inositol phosphates, which serve both as secondary messengers during signal transduction in plant cells, and in the transport of substances (Kornegay, 2001). It also acts as an inhibitor of metabolism, which induces dormancy in seeds (Plaami, 1997).

6.2. Anti-nutritional effects of phytic acid

Phytic acid is considered an anti-nutritional factor for monogastric animals mainly due to its ability to bind and form crosslinks with various essential nutrients other than P, rendering them unavailable to the animal and impairing proper digestion.

6.2.1. Minerals

Phytic acid acts as a potent chelator of metal ions at a neutral pH, forming a wide variety of insoluble salts (Vohra et al., 1965; Oberleas, 1973; Cheryan, 1980; Kornegay, 2001). Phytic acid has the ability to form complexes with Ca, Zn, Cu, Co, Mn, Fe, and Mg, with Zn and Cu having the strongest binding affinity to phytate (Maddaiah et al.,

1964; Vohra et al., 1965; Kornegay, 2001). Once the phytate-mineral complex is formed, the mineral becomes unavailable for intestinal absorption.

Of these minerals, the availability of Zn is the most adversely affected (Pallauf and Rimbach, 1995; Plaami, 1997; Kornegay, 2001). Lönnerdahl et al. (1989) and Sandström and Sandberg (1992) found that phytate decreases the rate of Zn absorption. Evidence also indicates that the binding of Zn to phytate may be sufficient to decrease Zn utilization and growth rate in the absence of Zn supplementation (O'Dell and Savage, 1960; Oberleas et al., 1962; Davies and Nightingale, 1975).

Phytic acid was also found to markedly reduce Ca bioavailability by forming Ca-phytate complexes (Sandberg et al., 1993; Saha et al., 1994). One mole of phytic acid can bind an average of 3-6 molecules of Ca to form insoluble phytates at the pH found in the small intestine (Plaami, 1997). These Ca-phytate complexes also inhibit the absorption of Fe and Zn (Rao and Rao, 1983; Hallberg et al., 1989; Champagne and Philippy, 1989).

6.2.2. Proteins and amino acids

Phytic acid also possesses the ability to form crosslinks with protein and amino acids (O' Dell and de Borland, 1976; Knuckles et al., 1985; Kornegay, 2001). The basic phosphate groups of phytic acid may bind amino groups from lysine, histidine and arginine under acidic conditions (De Rham and Jost, 1979; Fretzdorff et al., 1995; Kornegay, 2001). Under neutral conditions, the carboxyl groups of some amino acids may also bind to phytate through a divalent or trivalent mineral. Singh and Krikorian (1982) also reported that phytate has the ability to inhibit the activity of proteolytic enzymes such as pepsin and trypsin under gastrointestinal conditions. This, however, may

be an indirect effect of forming Ca-phytate complexes, which has a buffering effect in the stomach and small intestine.

6.2.3. Polysaccharides

The digestibility of starch may also be affected by phytate (Plaami, 1997; Kornegay, 2001). Phytic acid reduces starch digestibility by (1) directly forming phosphate linkages between phytic acid and starch, (2) combining with digestive enzymes required for starch digestion, and (3) binding Ca, which is a catalyst for enzyme activities (Kornegay, 2001). *In vitro* hydrolysis of starch was reduced when Na-phytate was added in a mixture of either wheat or bean starch incubated with human saliva (Yoon et al., 1983; Thompson, 1986; Thompson et al., 1987).

7. Phytase

The ability to commercially synthesize phytase from microbial sources and its subsequent use in animal diets is considered to be one of the most significant developments in the feed industry in recent decades. The use of microbial phytase, especially in swine and poultry diets, has led to significant reductions in P excretion. In spite of the significant amount of work conducted on microbial phytase, opportunities still remain to maximize the effectiveness of its use.

7.1. Sources of phytases

There are four possible sources of phytase for pigs and poultry: (1) intestinal phytase produced by enterocytes, (2) endogenous phytase present in certain plant ingredients, (3) microbial phytase originating from resident bacteria, and (4) the addition of exogenous phytase (Yi and Kornegay, 1996; Kornegay, 2001).

7.1.1. Intestinal phytase

Intestinal cells in the brush border of the small intestine are able to produce and secrete phytase, mainly as alkaline phosphatases, into the intestinal lumen (Davies and Flett, 1978; Cooper and Gowing, 1983; Jongbloed et al., 1991). Davis et al. (1970) were able to isolate these endogenous enzymes from the digestive tract of pigs and chicks. However, numerous researchers have concluded that in pigs and poultry, the contribution of intestinal phytase in phytate hydrolysis is insignificant (Pointillart et al., 1984; Williams and Taylor, 1985, Jongbloed et al., 1991; Kornegay, 2001). Contents of the stomach and small intestine of pigs (Jongbloed et al., 1992; Yi and Kornegay, 1996) and crop, stomach and small intestine of chickens (Liebert et al., 1993) have shown negligible phytase activity. These suggest that either the amount of intestinal phytase is insufficient or the intestinal environment is not conducive to permit efficient phytate hydrolysis (Cromwell, 1992).

7.1.2. Endogenous phytase in plant feedstuffs

Some plant ingredients such as wheat, barley, and rye possess significant amounts of intrinsic phytase in their seed coat (Table 2.6) (Cromwell, 1992). This increases the inherent availability of P in these grains as well as their by-products. Jongbloed and Kemme (1990) demonstrated the value of this intrinsic phytase in increasing P digestibility. The presence of natural phytase improved P digestibility from 27% to 50% in wheat and 19% to 33% in wheat bran. Phosphorus digestibility was also increased from 31 to 49% in a corn-soy diet when wheat phytase was present in the diet. However, the value of the intrinsic phytase was markedly reduced by steam pelleting (at

temperatures around 80°C), where the phytase was inactivated and no beneficial effect on P digestibility was obtained (Jongbloed and Kemme, 1990).

7.1.3. Microbial phytase from resident bacteria

Resident microflora found in the gastrointestinal tract has the capability of hydrolyzing phytate. These microorganisms are able to produce their own phytase, which may contribute in phytate hydrolysis in the animal. In ruminants, several studies have established that ruminal microflora is capable of releasing P from phytate (Reddy et al., 1982; Nys et al., 1996). *In vitro* studies showed that more than 90% of phytate P was released when concentrates were mixed with rumen fluid (Raun et al., 1956; Morse et al., 1992; Nys et al., 1996). Clark et al. (1986) found that 98% of the dietary phytate was hydrolyzed by dairy cows fed 50% grain and 50% corn silage diets. In chicks, the addition of lysed *E. coli* to a P-deficient diet produced normal growth (Warden and Schaible, 1962). This results from the presence of phytase in *E. coli* as demonstrated more recently by Greiner et al. (1993). However, Kornegay (2001) suggested that the significance of phytase produced by resident bacteria in non-ruminants may be negligible, considering the lack of significant absorption of P in the large intestine.

7.1.4. Exogenous phytase

Nelson et al. (1968) pioneered the use of exogenous phytase derived from microbial sources in animal diets. The phytase, obtained from a culture of *Aspergillus ficuum*, was added to liquified soybean meal, incubated for 24 hours at 50°C, and fed to day-old chicks. Results demonstrated a significant increase in bone ash percentage. Subsequently, the same enzymatic preparation was added to a corn-soybean meal diet fed

to chicks and showed an increase in P digestibility (Nelson et al., 1971). Initial studies with pigs were less promising (Cromwell and Stahly, 1978; Chapple et al., 1979; Shurson et al., 1984). Simons et al. (1990) provided the first promising results in pigs. P digestibility was increased from 20 to 46% in growing pigs (35-70 kg BW) fed a corn-soybean meal diet supplemented with an *A. ficuum*-derived phytase. Similar results were obtained by Beers and Koorn (1990) with 10-30 kg pigs, where P digestibility was improved by more than 20% and significantly increased growth rate and feed efficiency. Despite the positive results obtained from these early studies, the use of microbial phytase was initially deemed impractical due to the high costs associated with the production of the enzyme. However, developments in genetic engineering improved the capabilities of producing microbial phytase through recombination techniques, thereby reducing cost of production. This raised the viability of using phytase in the diet.

7.2. Mode of action

Phytase belongs to the phosphatase family of enzymes due to its ability to cleave phosphate-esters from phytate (Gibson and Ullah, 1990; Kies, 1996). There are two forms of phytase: 3-phytase and 6-phytase (Kies, 1996; Kornegay, 2001). These forms differ in both origin and mode of action. The 3-phytase (EC 3.1.3.8) is normally of microbial origin and starts to cleave phosphates at the carbon-3 position of the phytate molecule (Figure 2.8) (Pallauf and Rimbach, 1995; Kies, 1996; Kornegay, 2001). On the other hand, 6-phytase (EC 3.1.3.26) is characteristic of phytase from plant origin and begins cleaving at the carbon-6 position. In addition, Eeckhout and De Paepe (1996) were able to show differences in the optimal activity of the two forms of phytase in relation to

pH. Microbial phytase derived from *Aspergillus* was found to have optimal activity at a pH of 2.5 and 5.5. In contrast, wheat phytase achieved optimal activity only at a pH of 5.2. The authors suggested that due to these differences, a higher efficiency per unit of activity could be achieved with microbial phytase as compared to plant phytase. This also becomes more significant considering the variations in pH along the digestive tract.

Shute et al. (1988), using phosphatases found in the brain of some mammals, proposed a model for the mode of action of phytase. They proposed the "ping-pong" mechanism to describe phytate hydrolysis, where the cleaved phosphate group is transferred from the phytate molecule to the enzyme, and then from the enzyme to water. This suggests that the process occurs only in aqueous solutions.

Other studies have also demonstrated that phytase does not cleave phosphate groups at random (Venekamp et al., 1995; Kies, 1996). Using Nuclear Magnetic Resonance (NMR) techniques, Venekamp et al. (1995) suggested a certain order of the breakdown of phytate by *Aspergillus* phytase. After initially cleaving the phosphate group at the C3 position, phytase consecutively hydrolyzes phosphate groups at the C4, C5, C6 and C1 positions (Figure 2.8). The last phosphate group (the C2 position), however, was not hydrolyzed during the reaction time applied. Kies (1996) also suggested that phytase does not hydrolyze one phytate molecule at a time. Phytase reacts simultaneously with numerous phytate molecules, cleaving off one phosphate group at a time.

7.3. Site of phytase activity

Changes in pH along the gastrointestinal tract have a significant impact on the activity of phytase. Several authors concluded that the stomach is the main site for phytase activity in pigs (Jongbloed et al., 1992; Mroz et al., 1994; Yi and Kornegay, 1996). Jongbloed et al. (1992) first reported that 85 and 65% of added phytase activity was detected in the digesta collected from the duodenum of growing-finishing pigs fed a basal corn-soybean meal diet and a soybean meal, tapioca, hominy feed and sunflower meal diet. Yi and Kornegay (1996) showed that about 50% of the phytase activity was detected in the digesta collected from the stomach of pigs fed soybean meal-based, semi-purified diets supplemented with phytase. Only 30% was observed in the digesta of the upper small intestine. Phytase activity in the digesta of the lower small intestine of pigs was found to be negligible. Studies conducted with poultry showed similar results. Liebert et al. (1993) detected 69 to 86% of phytase activity in the crop and 31 to 38% in the proventriculus. No phytase activity was detected in the small intestine.

These differences in phytase activity may be attributed to differences in pH: the pH in the stomach is lower and more favorable for high phytase activity than the pH in the small intestine (Yi and Kornegay, 1996). Phytase may be broken down by proteolytic enzymes in the small intestine, which may also explain the low activity in this section of the digestive tract (Yi and Kornegay, 1996; Kornegay, 2001).

8. Aspects of P digestibility

Apparent P digestibility values of feed ingredients are used in formulating diets for pigs. However, Fan et al. (2001) enumerated a number of limitations in the use of

apparent P digestibility values in diet formulation: (1) apparent P digestibility values for various ingredients such as corn and soybean meal are highly variable; (2) digestive utilization of P may be underestimated using apparent P digestibility values by as much as 20-25%; and (3) apparent P digestibility values for single ingredients are not always additive when used in diet formulation. With these limitations, diet formulation based on the 'true' digestibility of P may present possibilities in reducing dietary P levels and P excretion as compared to apparent P digestibility.

To estimate true P digestibility, an understanding of the mechanisms of P absorption and excretion as well as the factors affecting the rate thereof, is required. Endogenous P outputs should be accurately quantified. However, these parameters remain unclear and previous results have been conflicting. Thus, these issues need to be resolved before this can be utilized as a valid strategy in effective P management.

8.1. Techniques used in measuring P digestibility

The method used in estimating P digestibility is very important, mainly due to differences in the accuracy of estimation. There are two major methods used in measuring P digestibility in feeds and ingredients: (1) digestibility studies, and (2) slope-ratio assay (Gueguen, 1996).

8.1.1. Digestibility studies

Digestibility studies indirectly estimate availability of P by measuring digestive utilization (Fan et al., 2001). Apparent P digestibility is computed as the percent difference between the amount of P in the diet and the amount in the feces (or digesta

collected at the distal ileum). True digestibility of P may be calculated by correcting for the contribution of endogenous secretions of P.

In terms of efficiency and sensitivity, measuring apparent P digestibility through this method seems to be one of the most desirable methods of measuring the bioavailability of P in pigs (Gueguen, 1996). Results derived from experiments performed by Kornegay and Qian (1996) and Yi and Kornegay (1996) indicate that apparent P digestibility of pigs was a sensitive indicator for assessing the bioavailability of P and the effects of phytase. In the comparison of different techniques, Dellaert et al. (1990) also concluded that apparent P digestibility was the most sensitive indicator to assess the availability of feed phosphates for pigs. Collection of apparent digestibility data for P would be less invasive, but would require more labor and more intensive facilities.

8.1.2. Slope-ratio assay

The slope-ratio assay provides a combined estimation of digestive and post-absorptive utilization of P at the tissue level (Jongbloed et al., 1991; Fan et al., 2001). This method gives relative biological values for a particular ingredient on a conventional scale (Gueguen, 1996). Normally, monosodium phosphate is used as the reference source (relative value = 100). A number of parameters are used as response criteria, but blood and bone parameters are the most commonly used. Blood parameters (plasma P and alkaline phosphatase levels) are less sensitive to levels of absorbed P (Gueguen, 1996). However, bone parameters (ash, P content and bone breaking strength) are more sensitive to increasing availability of P.

Fan et al. (2001) described some limitations of this method in assessing P bioavailability. First, assay results are highly variable and are influenced by the assay criteria used. Ketaren et al. (1993) demonstrated that P availability in soybean meal using bone parameters was significantly underestimated (17%) as compared to estimates using empty body composition or P retention (62%). Secondly, it is not clear if the assay results for individual ingredients are additive in diet formulation. Lastly, biological assay procedures are relatively expensive to perform for routine determination.

8.2. Endogenous losses of P

Endogenous P outputs potentially complicate estimates of apparent P digestibility (Cromwell, 1996). These endogenous P losses mostly originate from the secretion of digestive juices and the continuous renewal of mucosal cells (Jongbloed, 1987; Fan et al., 2001). However, the amount of endogenous P losses seems to be conflicting in a number of studies and the amount secreted is influenced by numerous factors.

The amount of endogenous P losses seems to be dependent on dietary P supply (Jongbloed, 1987; Jongbloed et al., 1991). In experiments with rats, high P intake resulted in a slight increase in fecal endogenous losses of P (Clark, 1968; Whittemore et al., 1973; Cramer and McMillan, 1980). The Ca:P ratio also affects the amount of endogenous losses of P, however, the results are conflicting. Whittemore et al. (1973) found a reduction in endogenous P losses when Ca:P ratio was lowered while Hermes et al. (1983) found an increase in endogenous P losses.

There is wide variability on the current estimates of endogenous P losses. In growing pigs fed under normal feeding conditions, daily fecal excretion of P is estimated

at 9-10 mg of endogenous P/kg liveweight (Guéguen and Perez, 1979; Jongbloed, 1987; Jongbloed et al., 1991). ARC estimates are higher, where it approximates daily endogenous P losses in pigs at about 15-25 mg/kg liveweight (ARC, 1981). In breeding sows, data of endogenous P losses are not well established (Jongbloed et al., 1991).

More recently, Fan et al. (2001) was able to develop a procedure for determining gastrointestinal endogenous P outputs and true P digestibility values in pigs. Using regression analysis technique, linear relationships were obtained between the total P output in the ileal digesta and feces and the dietary P inputs. By using soybean meal as an assay ingredient, endogenous P outputs of pigs between 5 and 20 kg were estimated to be 0.86 ± 0.09 g/kg DM intake in ileal digesta and 0.31 ± 0.06 g/kg DM intake in the feces (Fan et al., 2001). The ileal and fecal endogenous P output was computed to be about 9.5-24.1% and 3.5-8.9% of the pig's daily requirement (5 to 20 kg) for available P, respectively. This clearly indicates that endogenous P output represents a significant portion of the P requirement (Fan et al., 2001).

8.3. Factors affecting P digestibility

8.3.1. Effect of microbial phytase supplementation

Dietary supplementation of microbial phytase is widely accepted as an effective and practical method of improving P digestibility in animal diets. In poultry, Ravindran et al. (1995) summarized that microbial phytase increased P digestibility by 20-45%. A similar range of improvement (40-50%) in P digestibility was observed in pigs fed diets supplemented with microbial phytase (Mroz et al., 2002). The response to increasing levels of supplemental phytase, however, is not linear. Khan and Cole (unpublished data,

Close and Cole, 2000) demonstrated a quadratic response in P digestibility to increasing phytase level in growing pigs. In terms of P equivalency, Harper et al. (1997) estimated that 500 units of phytase/kg releases about 0.87-0.96 g of iP in growing-finishing pigs fed corn-soybean meal based diets. However, little is known if microbial phytase would have similar efficiency in other stages, such as in nursery piglets or breeding sows. There are numerous dietary factors that affect the effectiveness of microbial phytase in improving P digestibility:

8.3.2. Dietary Ca level or Ca:P ratio

High dietary Ca levels or a wide Ca:total P ratio significantly reduces the effectiveness of phytase and impairs the overall ability of pigs to digest dietary P. Ca:total P ratios ranging from 1.5 to 2.0:1 in low-P corn-soybean meal diets supplemented with microbial phytase decreased the utilization of P for weanling (Lei et al., 1994; Qian et al., 1996) and growing-finishing pigs (Liu et al., 1998). Generally, Ca:total P ratios between 1.0-1.3:1 are satisfactory (Close and Cole, 2000).

There are a number of reasons that may explain this relationship between the Ca:P ratio and P digestibility. Due to their strong affinity to phytate, the extra Ca forms an extremely insoluble complex with phytate which could no longer be degraded by phytase (Wise, 1983; Düngelhoef and Rodehutscord, 1995; Seynaeve et al., 1999; Kornegay, 2001). Additional Ca also acts as a pH buffer, which increases the pH of the digesta and reduces phytase activity (Sandberg et al., 1993; Seynaeve et al., 2000). Excess Ca ions may also directly inhibit the activity of the enzyme by competing for their active sites (Qian et al., 1996; Kornegay, 2001).

8.3.3. Dietary phosphate levels

In some species, the level of dietary phosphate affects phytase activity. This was demonstrated in chicks, where sub-optimal levels of dietary phosphate increased the activities of phytase and alkaline phosphatase in their duodenal mucosa (Davies et al., 1970; McCuaig et al., 1972; Nys et al., 1996). Similarly, phytate hydrolysis increased in rats fed a diet lacking in phosphate (Moore and Veum, 1983). This, however, occurred without any changes in the levels of intestinal phytase activity. In pigs, the level of both dietary P and phytate does not appear to have any effect on phytase activity. Moser et al. (1982) and Pointillart et al. (1985) both found that feeding low P diets to pigs did not increase phytate hydrolysis, suggesting that pigs do not adapt. Phytase and alkaline phosphatase activities were also unaffected by increasing phytate levels.

8.3.4. Dietary vitamin D levels

It has been demonstrated that vitamin D improves phytate hydrolysis (Nelson, 1967; Pointillart et al., 1989; Mohammed et al., 1991; Nys et al., 1996). However, its effect on improving P utilization is not through enhancing phytase activity but through indirect means. Pointillart et al. (1989) reported that vitamin D did not have any effect on the intestinal phosphatase activity in pigs. However, Ca absorption was markedly improved by vitamin D, thus lowering the formation of insoluble Ca-phytate complexes (Nys et al., 1996).

8.3.5. Dietary Zn and Mg levels

Sub-optimal levels of Zn reduce both alkaline phosphatase and phytase activities (Davies and Flett, 1978). In contrast, high dietary levels of Mg reduced the activities of both enzymes but to a lower extent than Ca (McCuaig et al., 1972).

8.3.6. Effect of physiological status

It is hypothesized that the physiological status influences P digestibility and effectiveness of microbial phytase, however, little is known in this area. Literature suggests that mineral digestibility decreases with age, as demonstrated in rodents (Armbrecht, 1987; Buchowski and Miller, 1991; McElroy et al., 1991). This may be attributed to age-related changes in hormonal regulation. Differences in gastric pH, gastric retention time, and other physiological changes that accompany increasing age may also play a role in the ability of pigs to digest phytate-P. These factors may also affect phytase activity, which then may indicate differences in effectiveness among the different stages.

A number of studies have been conducted to determine the relationship of P digestibility and age/body weight (BW) in growing pigs (Eeckhout et al., 1995; Fernandez et al., 1995a and b; Kemme et al., 1997a; Rodehutscord et al., 1999). When BW increased from 15 to 35 kg, apparent total tract digestibility (ATTD) of P was unaffected when P originated mainly from corn, barley and soybean meal (Rodehutscord et al., 1999). However, an improved ATTD of P was also found in 65 kg pigs compared with 35 kg pigs (Eeckhout et al., 1995; Fernandez et al., 1995a and b; Kemme et al.,

1997a). Kemme et al. (1997a) reported that ATTD of P remained constant from 60 kg to 100 kg BW. Presently, no data exist on P digestibility in pigs of heavier weights.

In multiparous sows, lactating sows of parity 5 or greater had 3.4% higher total tract P digestibility than pregnant sows (Kemme et al., 1997a). However, the efficiency of P absorption in lactating sows was found to be 6.6% lower than growing-finishing pigs. In terms of the efficacy of phytase in improving the digestibility of P, lactating sows showed the highest efficacy, followed by growing-finishing pigs, sows at late pregnancy and then piglets (Kemme et al., 1997a). Phytase was found to be least efficient in sows at mid-pregnancy.

9. Conclusion

From the above review of literature, there is an apparent need to investigate physiological status as a factor affecting P digestibility. Most studies evaluating P digestibility used ATTD of P as the criteria. To the best of our knowledge, there have been no experiments in which AID of P has been related to BW or physiological state of the animal. Results of this study would indicate whether or not the same digestibility coefficients of P could be used for all pig categories. Moreover, AID and ATTD of P have not been compared in pigs of different physiological stages. These results may also give insights to the possible role of the large intestine in P metabolism. Differences between AID and ATTD of P would also help determine which is the better estimate of P digestibility. Very few studies have been reported to determine P digestibility in sows, especially in different reproductive stages, and its response to phytase supplementation. The use of phytase in multiparous sows, therefore, has not been fully investigated.

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Table 2.1. Comparison of bioavailability and apparent digestibility of P in selected feed ingredients for pigs and poultry.

Ingredient	% Total P[a]	Bioavailability of P, %[b]	% Available P[c]	Digestibility of P, %[d]	% Digestible P[e]
Corn	0.30	14	0.04	20	0.06
Wheat	0.35	49	0.17	47	0.17
Barley	0.35	30	0.10	39	0.14
Soybean meal	0.65	31	0.20	40	0.26
Canola meal	1.10	21	0.23	30	0.30
Meat and bone meal	6.00	67	4.02	80	4.80
Fish meal	2.50	94	2.35	86	2.00
Dried whey	0.80	97	0.78	82	0.66
Dicalcium phosphate	18.00	107	18.00	67	12.00
Monosodium phosphate	22.00	100	22.00	85	18.50

[a]NRC, 1998
[b]Cromwell, 1992, relative to the availability of P in monosodium phosphate, given a value of 100
[c]% available P = % total P x (% bioavailability of P/100)
[d]CVB, 1999
[e]% digestible P = % total P x (% digestibility of P/100)

Table 2.2. Apparent P digestibility coefficients in selected ingredients of plant origin (Jongbloed et al., 1991).

Ingredient	Number of trials	Apparent P digestibility, %	
		Mean	Range
Barley	5	39	34-44
Corn	7	17	12-26
Wheat	5	47	45-51
Peas	4	45	42-51
Rice bran	4	12	9-13
Wheat middlings	6	28	18-35
Corn gluten feed	10	20	12-32
Tapioca meal	3	10	1-24
Coconut expeller	5	34	25-43
Soybean meal, dehulled	3	38	33-41

Table 2.3. Apparent P digestibility coefficients in ingredients of animal origin (Jongbloed et al., 1991).

Ingredient	Technique	Number of trials	Apparent P digestibility, %
Meat meal	B	1	74
Meat meal	B	1	85
Bone meal	B	1	68
Fish meal	B	2	86
Feather meal, hydrolyzed	B	1	75
Skimmed milk powder	B	1	91
Whey powder	B	1	82
Meat and bone meal	S	1	80

B = balance technique; S = slope ratio technique

Table 2.4. Relative bioavailability of P of various inorganic phosphate sources (NRC, 1998).

Phosphate source	% P	Bioavailability of P, %[a]
Bone meal, steamed	12.50	80-90
Dicalcium phosphate	18.50	95-100
Monocalcium phosphate	21.10	100
Deflourinated phosphate	18.00	85-95
Rock phosphate	9.05	30-50
Monosodium phosphate	24.94	100

[a]Bioavailability estimates are generally expressed as a percentage of monosodium phosphate or monocalcium phosphate

Table 2.5. Natural phytase content of common plant ingredients (Eeckhout and de Paepe, 1994).

Ingredient	Phytase units/kg
Rye	5130
Wheat	1193
Wheat bran	2957
Barley	582
Corn	15
Peas	116
Rapeseed meal	16
Soybean meal	40

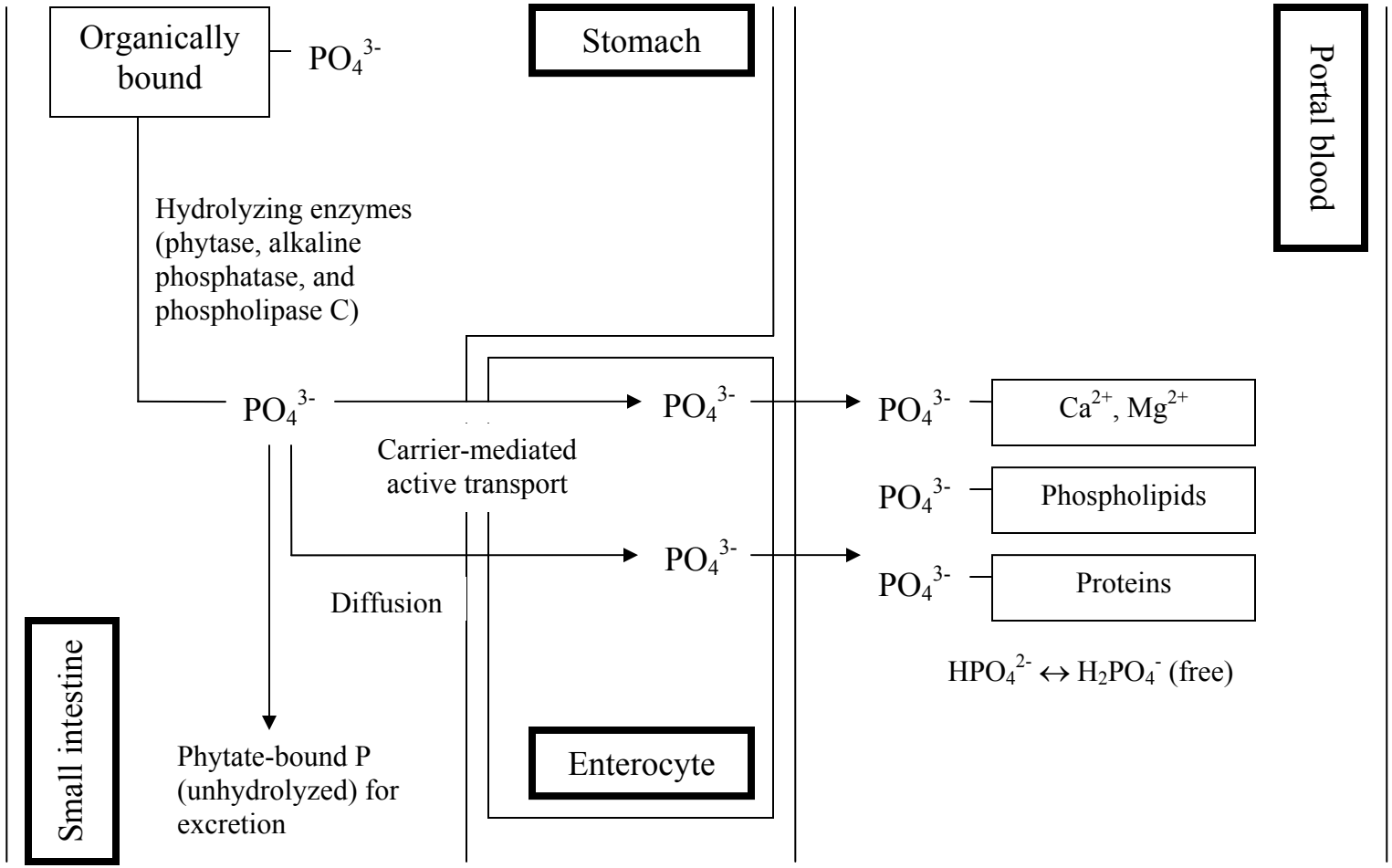


Figure 2.1. Digestion, absorption and transport of phosphorus (adapted from Groff and Gropper, 2000).

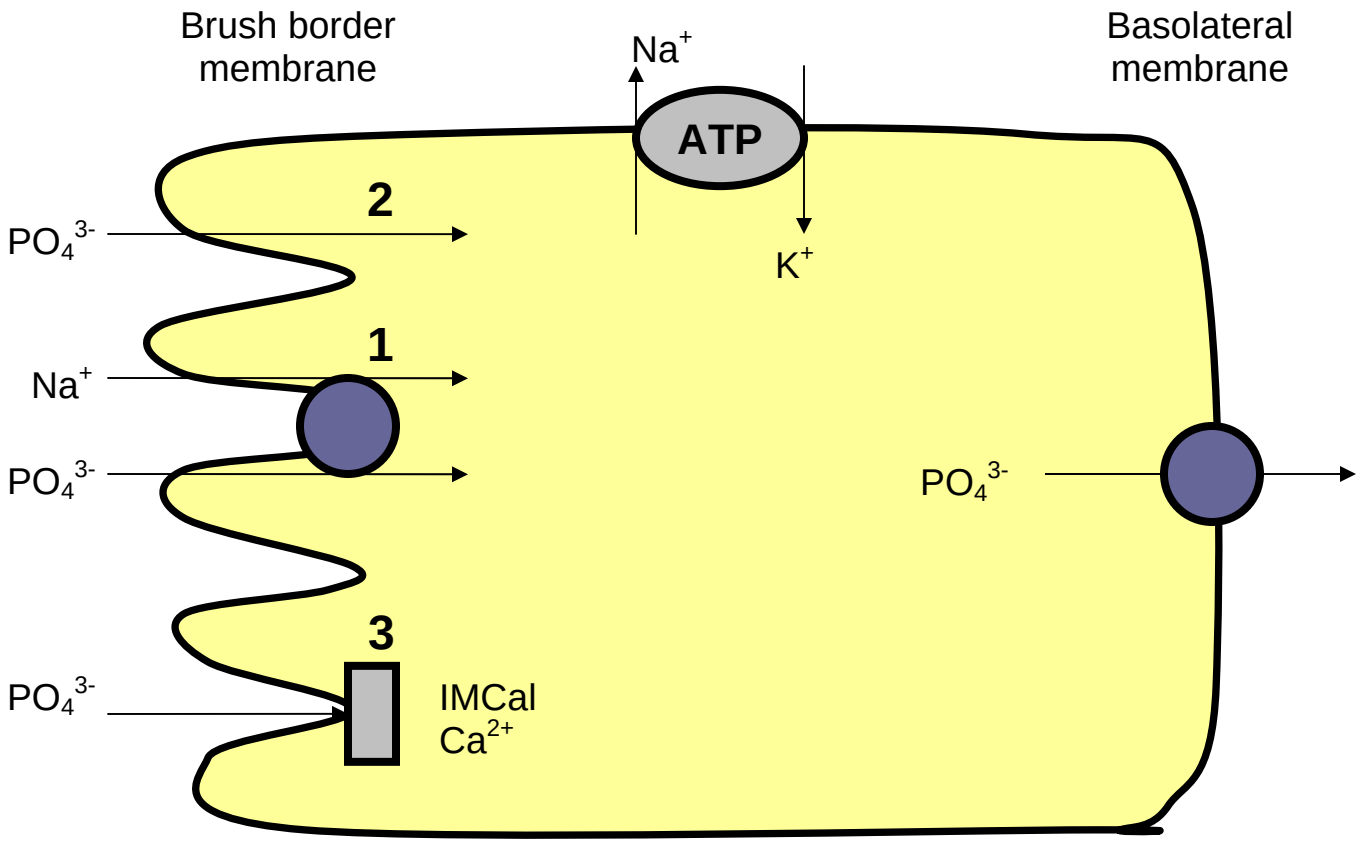


Figure 2.2. Proposed mechanism of P absorption in enterocytes (1 = Na^+-dependent, carrier-mediated active transport system; 2 = Na^+-independent diffusion mechanism; 3 = Ca-binding protein).

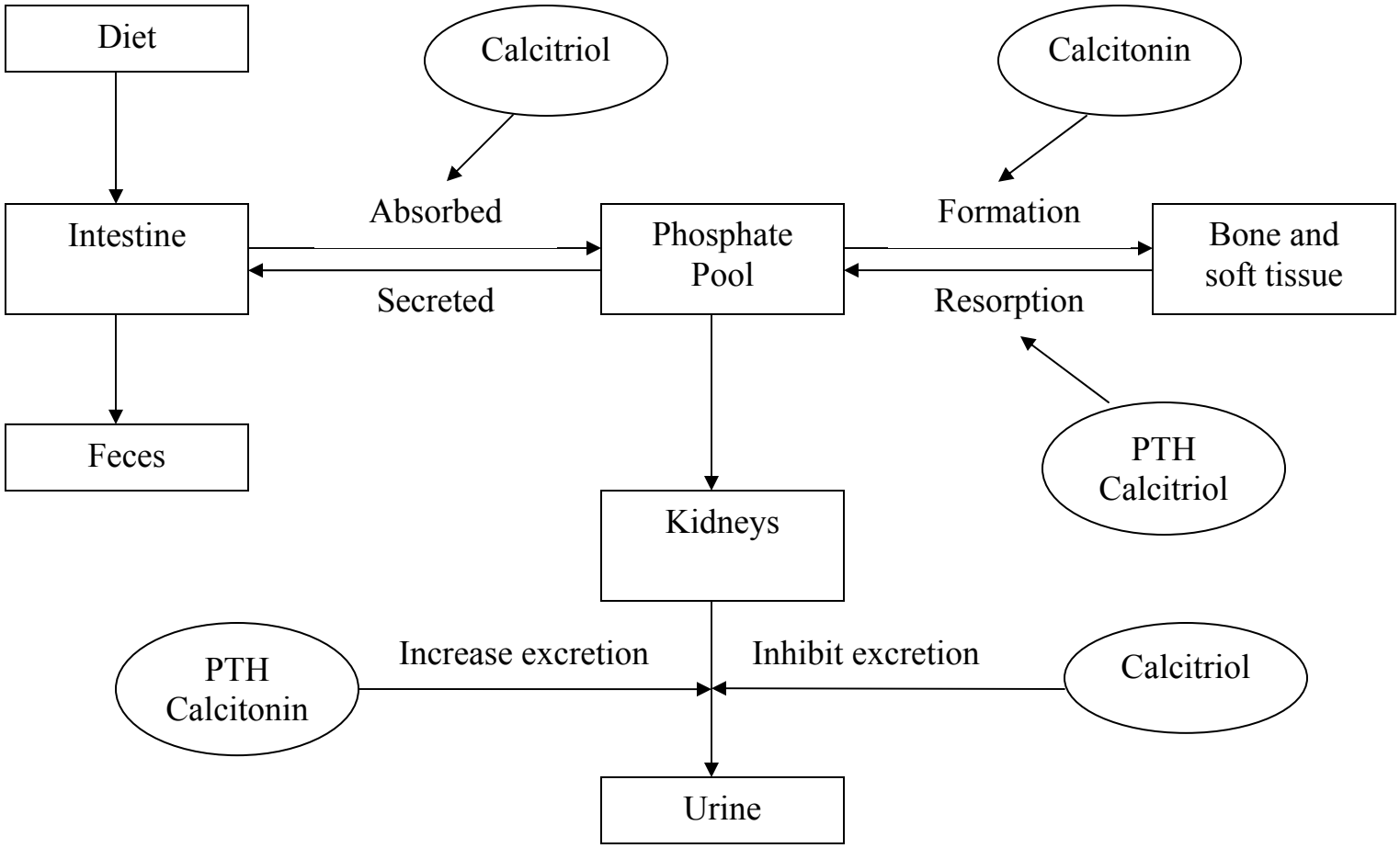


Figure 2.3. Overview of Pi homeostasis (adapted from Stanton and Koeppen, 1998).

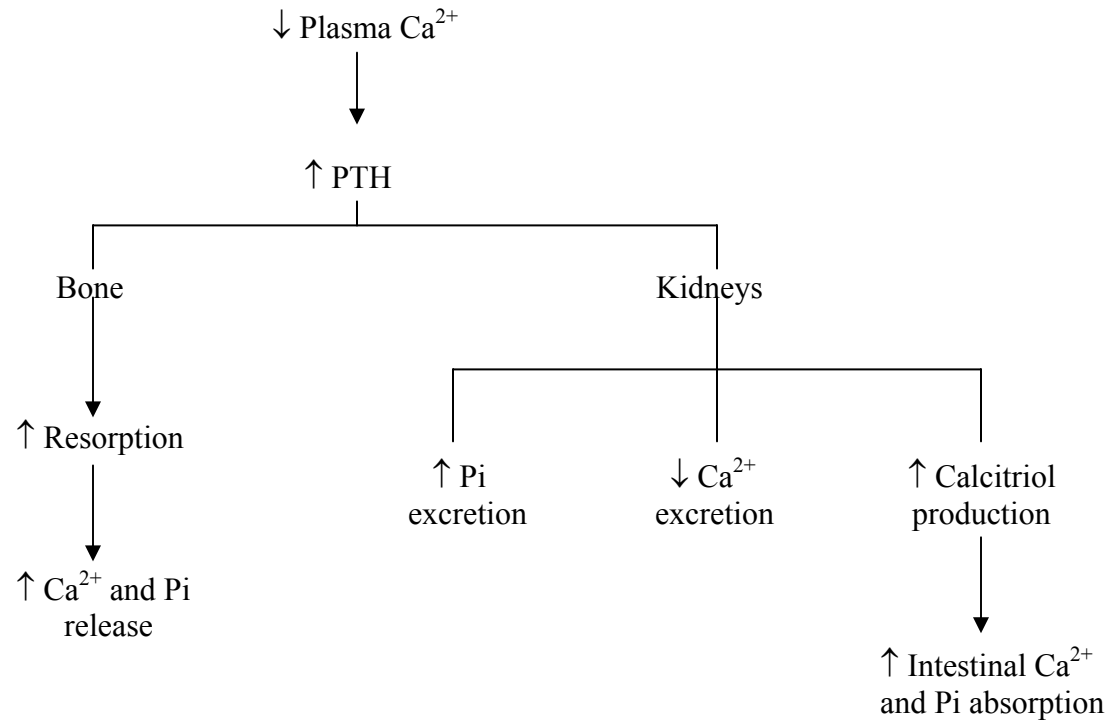


Figure 2.4. Effect of PTH on Ca^{2+} and Pi homeostasis (Stanton and Koeppen, 1998).

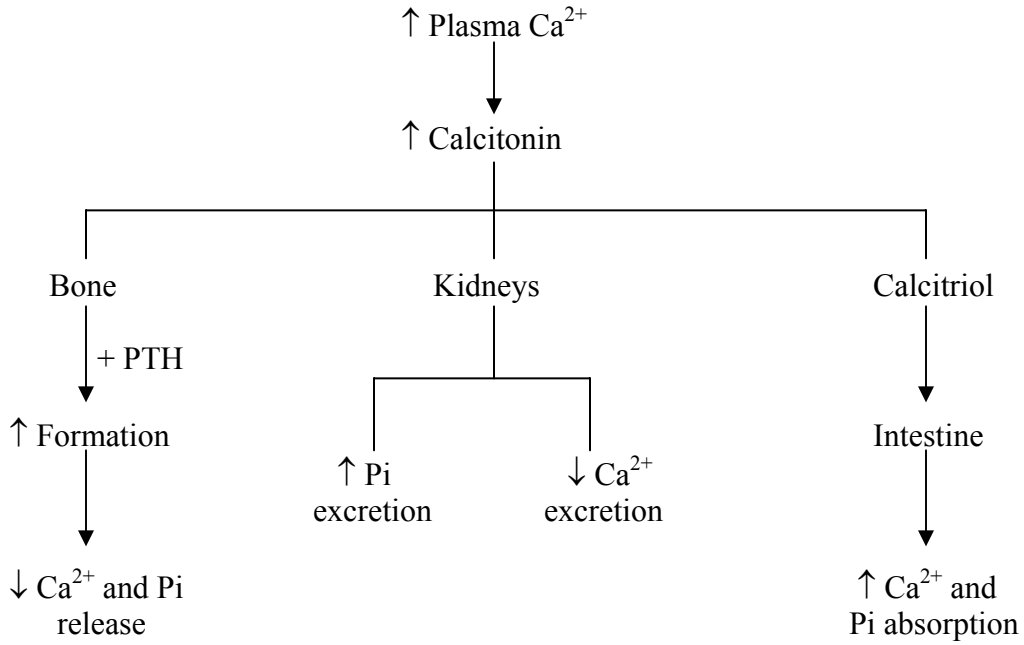


Figure 2.5. Effect of calcitonin on Ca^{2+} and Pi homeostasis (Stanton and Koeppen, 1998).

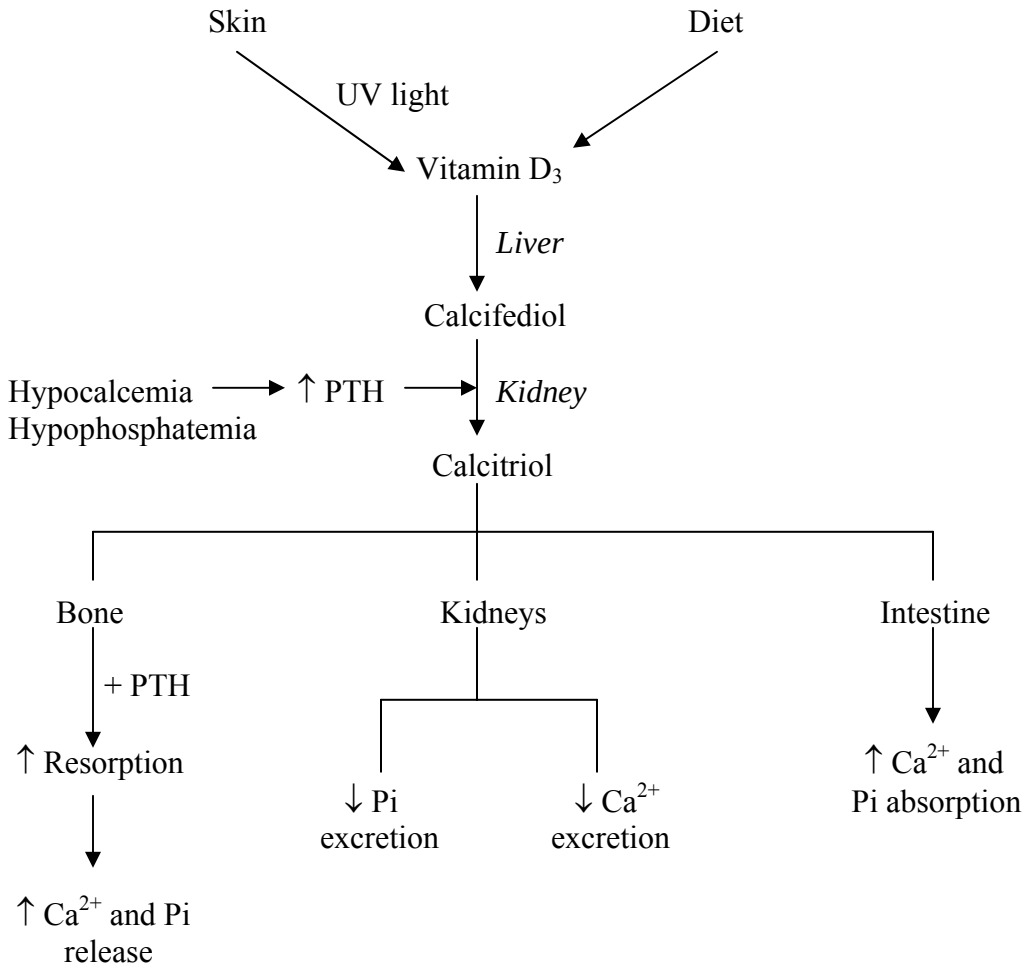


Figure 2.6. Activation of vitamin D_3 and its effect of on Ca^{2+} and Pi metabolism (Stanton and Koeppen, 1998).

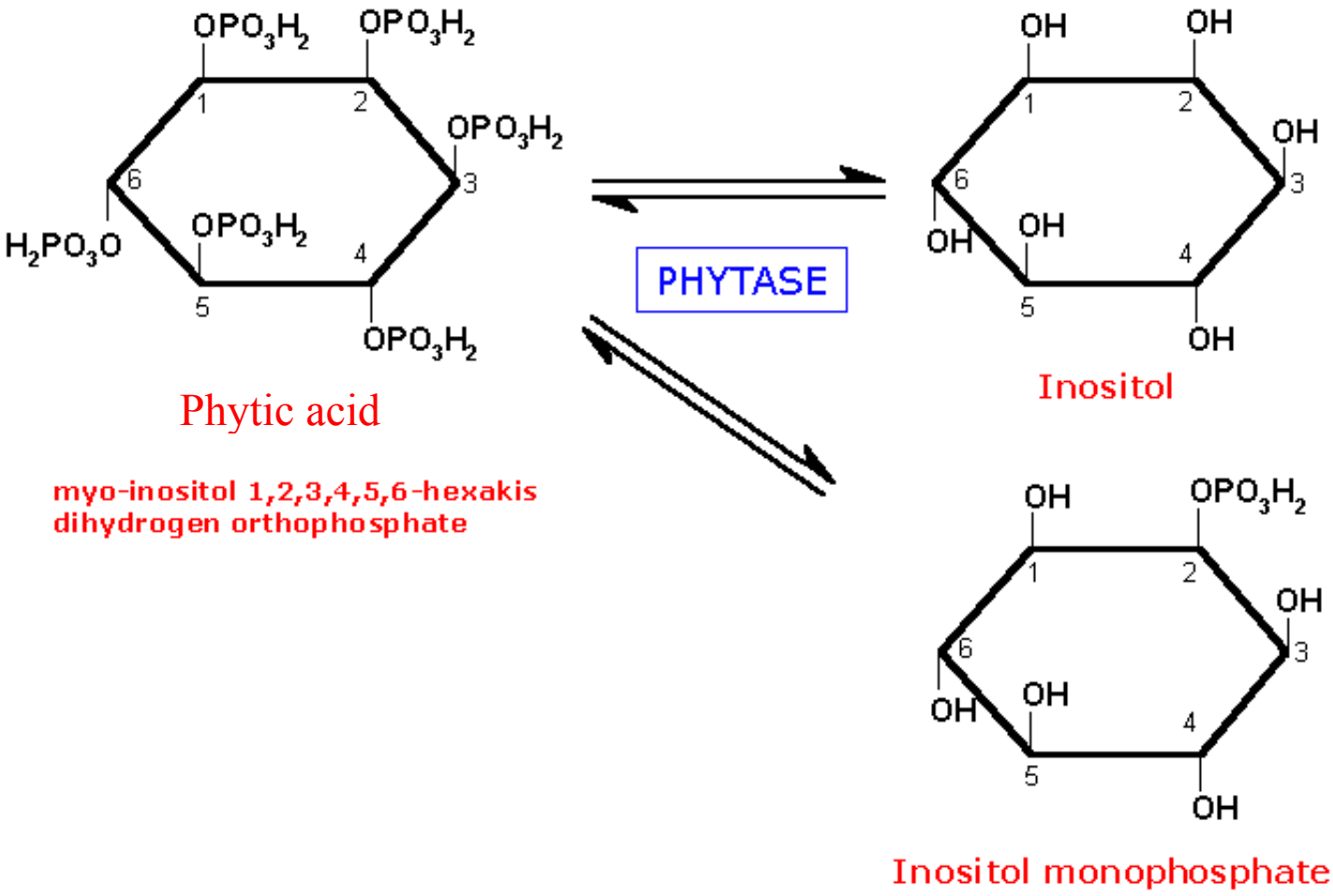
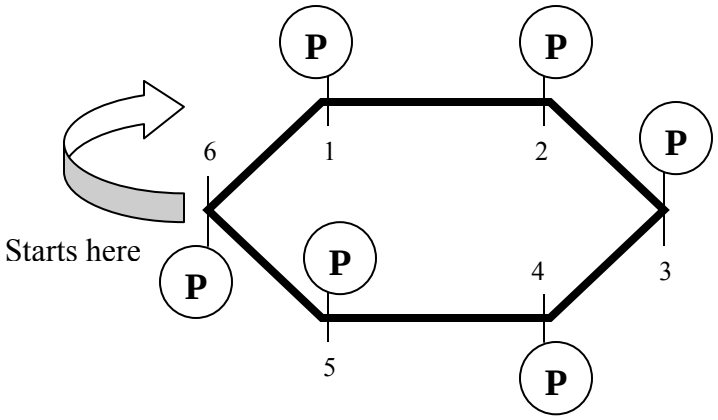
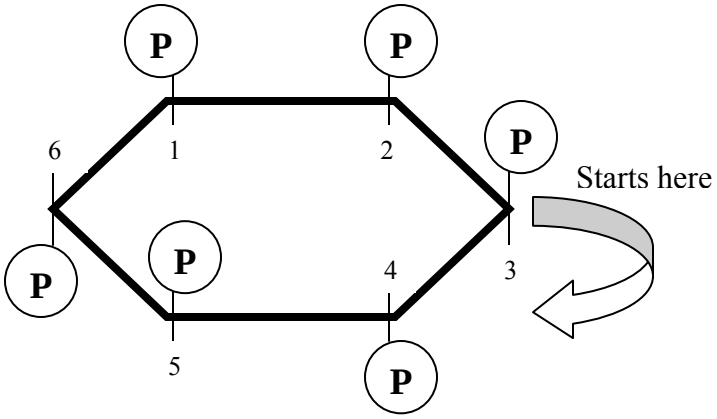


Figure 2.7. Enzymatic hydrolysis of phytic acid by endogenous or exogenous phytase (Cole, 2001).



6-Phytase: EC 3.1.3.26



3-Phytase: EC 3.1.3.8

Figure 2.8. Hydrolysis of phytic acid by 3-phytase and 6-phytase (adapted from Nys et al., 1996).

CHAPTER 3

Effect of body weight and reproductive status on phosphorus digestibility and efficacy of phytase in pigs

ABSTRACT: This study was conducted to determine the effect of body weight and reproductive status on apparent ileal digestibility coefficients (AID) and apparent total tract digestibility coefficients (ATTD) of P, and the efficacy of phytase in pigs. The study was divided into three phases. In phase 1, piglets from 10 to 40 kg BW were used; in phase 2, growing pigs from 40 to 130 kg BW were used; and in phase 3, multiparous sows (ave. parity = 5) were used. In each phase, six animals were surgically fitted with a T-cannula in the distal ileum. Two experimental diets were formulated. Diet 1 was a corn-soybean meal-canola meal-based diet containing 0.45% total P without phytase supplementation and diet 2 was identical to diet 1, but supplemented with 500 FYT/kg of *Peniophora lycii* phytase (Ronozyme P®). The Ca to total P ratio was 1.1:1 in both diets. Chromium oxide was included in the diets at 0.25% as an inert marker. In growing pigs, AID and ATTD were determined at 10, 20, 40, 70, 100, and 130 kg BW. In sows, AID and ATTD were determined in each trimester of gestation and in lactation. In phase 1, BW had no effect ($P > 0.05$) on AID or on ATTD of P regardless of the diet being fed. As BW increased from 40 to 130 kg, AID and ATTD of P decreased linearly ($P < 0.05$) regardless of the diet fed. In phase 1 and phase 2, phytase supplementation improved ($P < 0.05$) both AID and ATTD of P. In phase 3, an increase ($P < 0.01$) in AID and ATTD of

P was observed as sows proceeded through gestation and lactation regardless of the diet being fed. Phytase addition increased ($P < 0.05$) AID only in lactation. Phytase improved ($P < 0.05$) ATTD in the last trimester of gestation and in lactation. No differences between AID and ATTD were observed ($P > 0.05$). Efficacy of phytase was highest in lactating sows, followed by finishing pigs, piglets and gestating sows. In conclusion, the physiological status of the pig affects apparent digestibility of P and the efficacy of phytase.

Key Words: Pigs, Phosphorus, Digestibility, Phytase

Introduction

Phosphorus nutrition is an issue of phosphate utilization and metabolism (Anderson, 1991). A better understanding of the mechanisms and factors that contribute to the efficiency of P utilization in animal diets is needed to reduce the amount of P that is excreted from the animals.

It has been hypothesized that the physiological status of the animal influences P digestibility and the effectiveness of microbial phytase (Kemme et al., 1997a). Previous studies in pigs have shown that apparent total tract digestibility coefficients (ATTD) of P did not change from 15 to 35 kg (Rodehutscord et al., 1999), but increased from 35 to 65 kg (Eeckhout et al., 1995; Fernandez et al., 1995a and b; Kemme et al., 1997a). From 65 to 100 kg BW, a constant ATTD was reported (Kemme et al., 1997a). Gestating and lactating sows of parity 5 or greater had lower ATTD of P than growing-finishing pigs (Kemme et al., 1997a). In terms of the efficacy of phytase in improving the digestibility

of P, the highest efficacy was obtained in lactating sows, followed by growing-finishing pigs, sows at late pregnancy, piglets, and sows at mid-pregnancy (Kemme et al., 1997a). However, none of these studies evaluated the effect of the physiological status of the animal using apparent ileal digestibility (AID) of P. In addition, only a few studies have been conducted to determine the digestibility of P and their response to phytase supplementation in sows in different stages of reproduction. The effect of phytase in diets for sows, therefore, has not been fully investigated.

The objectives of the current experiment were to determine the effect of BW and reproductive status on AID and ATTD of P in growing pigs and sows, and to determine the effect of BW and reproductive status on the efficacy of microbial phytase. It was also the objective to compare AID and ATTD of P within the same animal.

Materials and Methods

Animals and housing

Growing pigs (Phase 1 and Phase 2). The growing period from 10 to 130 kg was divided into two phases. In phase 1, pigs from 10 to 40 kg were used while pigs from 40 to 130 kg were used in phase 2. For phase 1, six Duroc-Hampshire (DH) x Landrace-Yorkshire-Duroc (LYD) newly-weaned barrows (BW: 7.1 ± 0.6 kg) were randomly selected. For phase 2, six DH x LYD barrows (BW: 35.3 ± 1 kg) were also randomly selected. All pigs in both phases were fitted with a T-cannula in the distal ileum using the procedures of Stein et al. (1998). Pigs in phase 1 were cannulated one week after weaning. The dimensions of the T-cannula used in this experiment are shown in Figure

3.1. Following the surgery, pigs were individually housed in 1.2 x 1.4m pens with partially slatted floors. Room temperature was maintained at 20°C. Animal care procedures were approved by the South Dakota State University (SDSU) Animal Care and Use Committee (#02-A019).

Multiparous sows (Phase 3). Six LYD multiparous (average parity: 5) gestating sows were randomly selected. Each sow was fitted with a T-cannula in the distal ileum using the procedures of Stein et al. (1998) on d 7 of pregnancy. After the surgery, sows were individually housed in 1.8 x 2.4m pens with completely slatted concrete floors. At d 107 of gestation, sows were transferred to the SDSU farrowing unit, and housed in farrowing crates in an environmentally-controlled room. Litter size was standardized to nine piglets per sow within 24 h post-partum. Piglets from non-experimental sows were used to replace dead piglets throughout the lactation period.

Diets and feeding

Two experimental diets were used (Table 3.1). Diet 1 (control diet) was a corn-soybean meal-canola meal-based diet without the addition of exogenous phytase while diet 2 was identical to diet 1, but supplemented with 500 FYT/kg of *Peniophora lycii* phytase (Ronozyme P™, Roche Vitamins, Inc., Parsippany, NJ). These two diets were used for all pigs and phases of the experiment. The experimental diets were formulated to contain 0.50% calcium and 0.45% total P to satisfy a calcium:total P ratio of 1.1:1. Chromium oxide was included in both diets as an inert marker at 0.25%. To enhance the palatability of the diet, 3.0% soybean oil was included.

In phases 1 and 2, growing pigs were fed 3 times their maintenance energy requirement. The daily energy requirement for maintenance was calculated using the following equation (NRC, 1998):

$$106 \text{ kcal ME} \times \text{kg BW}^{0.75}$$

Gestating sows were provided 2.0, 2.5 and 2.8 kg feed/day in the first, second, and third trimester of gestation, respectively. Ad libitum access to feed was allowed during lactation, with appropriate amounts of feed added twice per day. For growing pigs and gestating sows, the daily feed allowance was divided into two equal meals, fed at 800 and 1800 h. Water was available at all times.

Data recording, sample collection and experimental design

In phases 1 and 2, pigs were weighed at the beginning of each collection period, and feed allowance was recorded daily. Samples of every batch of the experimental diets were collected after manufacturing. A 0.5 kg sample of feed was taken from 10 different locations in the mixer. The samples were pooled, mixed, and divided into four quarters. Grab samples of feed were taken from the middle of each quarter and placed in a plastic container. This procedure was repeated until 1 kg of feed sample was obtained. This procedure was employed to ensure that the final feed sample was a good representation of each batch. Each feed sample was labeled with the corresponding batch number and production date, and stored in a freezer set at -20°C for subsequent analysis.

Table 3.2 shows the schedule of sample collection of the digesta for each phase. Each collection period lasted 9 d. The initial 5 d were considered an adaptation period to the diet. Grab samples of fresh feces were collected on d 6 and 7 while ileal digesta was

collected for 12 h (from the time of feeding) on d 8 and 9 as described by Stein et al. (1998). A plastic bag was placed at the end of the cannula barrel using a cable tie, allowing digesta to flow into the bag. The bag was then removed and replaced as soon as it was filled with digesta or once every 30 minutes, which ever occurred first. The digesta and the fecal samples were subsequently labeled and stored at –20°C.

The experimental design is illustrated in Table 3.3. A switch back design over 18 days was used in each collection period. At the end of the experiment, pigs were harvested at the Meat Science Laboratory at SDSU.

Chemical analysis

At the end of the experiment, the ileal digesta and the fecal samples were thawed, pooled within animal and diet, mixed, and dried for 24 h at 100°C using a drying oven (Despatch Oven Co., Minneapolis, MN). The samples were finely ground using a sample mill (Cyclone Sample Mill, UDY Corp., Fort Collins, CO) to pass through a 1-mm sieve. Feed samples were also ground to pass through a 1-mm sieve. The feed samples were analyzed for phytase activity according to the procedures of Engelen et al. (1994).

Dry matter analysis for feed, fecal and digesta samples were performed (AOAC, 2000). The chromium content (%) of the diets, ileal digesta, and fecal samples was determined according to Fenton and Fenton (1979). For total inorganic P analysis, fecal samples, ileal digesta, and the feed samples were digested with nitric and perchloric acid (2:1 vol/vol) , and assayed for total P concentrations using a UV-vis scanning spectrophotometer (Model UV-2101 PC Shimadzu Corporation, Kyoto, Japan) (AOAC, 2000).

Calculations and statistical analyses

The AID of P was calculated using the following equation (Stein et al., 1999):

$$AID = (1 - [(Ps/Pf)(Crf/Crs)]) \times 100$$

where:

AID = apparent ileal digestibility of P (%, on DM basis)

Ps = P content in the ileal digesta (%, on DM basis)

Pf = P content in the feed (%, on DM basis)

Crf = chromium content in the feed (%, on DM basis)

Crs = chromium content in the ileal digesta (%, on DM basis)

This equation was also used to calculate ATTD. The effect of BW and reproductive status, phytase, and site of collection were analyzed using Repeated Measures Analysis of the MIXED procedure of SAS (Littell et al., 1996). The model included diet, BW or reproductive status, and the interaction between diet and BW/reproductive status as fixed effects and pig nested within diet as the random effect. Data were pooled for each pig category (piglets, growing-finishing pigs, gestating, and lactating sows) and were also compared using the MIXED procedure of SAS. Least square means were separated using the PDIFF option of SAS.

Results

All pigs remained healthy throughout the experiment and feed intake was normal (SD-NE Swine Nutrition Guide, 2000) within 3 d after the surgeries. However, two piglets in phase 1 lost their cannulas prior to their final collection of ileal digesta. Therefore, only four pigs were collected for digesta in the final collection period in phase

1. In phase 3, one sow had a miscarriage one week prior to farrowing. Therefore, only five sows were collected during lactation.

Calculated and analyzed values of total P were identical (Table 3.2). The control and the phytase-supplemented diets had an average phytase activity of 51 and 652 FYT/kg of diet, respectively.

Effect of BW on apparent P digestibility and efficacy of phytase

In phase 1, there was no effect ($P > 0.05$) of BW on AID or ATTD (Table 3.4). This was true regardless of the diet being fed. There was no interaction ($P > 0.05$) between BW and phytase supplementation. Averaged across BW, phytase supplementation significantly improved AID ($P = 0.04$) and ATTD ($P < 0.01$) (Table 3.4). The improvement in AID with phytase addition was observed only at 10 kg ($P = 0.02$). The improvement in ATTD was observed at 20 ($P = 0.02$) and 40 kg BW ($P < 0.01$).

The interaction between diet and site of collection was not significant ($P > 0.05$). In pigs fed the control diet, AID and ATTD were similar ($P > 0.05$) at 10 and 20 kg BW (Table 3.4). However, ATTD were lower ($P = 0.02$) than AID at 40 kg BW. In pigs fed the phytase-supplemented diet, AID and ATTD were also similar ($P > 0.05$) at 10 and 20 kg, but not at 40 kg BW ($P = 0.05$). There was no interaction ($P > 0.05$) between BW and site of collection. Averaged across BW, ATTD and AID were similar ($P > 0.05$) regardless of the diet fed (Table 3.4).

In phase 2, a linear decrease in AID ($P < 0.01$) and ATTD ($P = 0.05$) was observed in pigs fed the control diet (Table 3.5). Similarly, a linear decrease in AID ($P =$

0.04) and ATTD ($P < 0.01$) was also observed in pigs fed the phytase-supplemented diet. There was no interaction ($P > 0.05$) between BW and phytase supplementation.

Averaged across BW, phytase improved AID ($P < 0.01$) and ATTD ($P < 0.01$) in phase 2 (Table 3.5). With phytase addition, AID was improved at 40 ($P = 0.04$), 70 ($P < 0.01$), 100 ($P = 0.06$) and 130 kg ($P = 0.05$). The addition of phytase ($P < 0.01$) increased ATTD at 40, 70, and 100 kg, but not at 130 kg.

In pigs fed the control diet, AID and ATTD were similar ($P > 0.05$) at 40, 70, and 100 kg BW (Table 3.5). However, ATTD at 130 kg BW was higher ($P < 0.01$) than AID. Except for pigs at 40 kg BW, AID and ATTD were similar ($P > 0.05$) at 70, 100, and 130 kg BW in pigs fed the phytase-supplemented diet. Averaged across BW, average ATTD was higher than AID in pigs fed the control ($P < 0.01$) and the phytase-supplemented diet ($P = 0.02$).

Effect of reproductive status on apparent P digestibility and efficacy of phytase

In phase 3, AID increased linearly ($P < 0.01$) in sows fed the control and the phytase-supplemented diet throughout gestation to lactation (Table 3.6). In sows fed the control diet, ATTD increased quadratically ($P = 0.04$), while ATTD increased linearly ($P = 0.01$) in sows fed the phytase-supplemented diet throughout gestation to lactation.

There was no interaction ($P > 0.05$) between reproductive status and phytase supplementation. Phytase addition improved average AID ($P = 0.02$) and ATTD ($P < 0.01$) in sows, however, the effect of phytase was observed mainly in lactating sows (Table 3.6). No improvements ($P > 0.05$) were observed in AID at 30, 60, and 90 d of gestation, but phytase improved ($P = 0.01$) AID in lactation. There were no

improvements ($P > 0.05$) in ATTD with phytase addition at 30 and 60 d of gestation. However, supplementation with phytase improved ATTD at 90 d of gestation ($P = 0.01$) and in lactation ($P = 0.05$).

The interaction between diet and site of collection was not significant ($P > 0.05$). There were no differences ($P > 0.05$) between average AID and ATTD in sows fed the control and the phytase-supplemented diet regardless of the reproductive status of the sow (Table 3.6).

Comparison of apparent P digestibility in animals of different categories

In Table 3.7, AID and ATTD of P from phase 1, 2 and 3 were compared. The coefficients were summarized into four different categories: piglets (10 – 40 kg), growing-finishing pigs (40 – 130 kg), gestating sows, and lactating sows.

Piglets fed the control diet had a higher ($P < 0.05$) AID than growing-finishing pigs and gestating sows, but it was similar ($P > 0.05$) to sows in lactation. Growing-finishing pigs also had a higher ($P < 0.05$) AID than sows in gestation, but were not different ($P > 0.05$) from lactating sows. The AID of gestating sows were lower ($P < 0.05$) than for lactating sows. In pigs fed the phytase-supplemented diet, piglets, growing-finishing pigs, and lactating sows had similar AID ($P > 0.05$). However, the AID for these three groups were higher ($P < 0.05$) than for gestating sows.

In pigs fed the control and the phytase-supplemented diets, piglets, growing-finishing pigs and lactating sows had similar ATTD ($P > 0.05$). However, gestating sows had ATTD lower ($P < 0.05$) than for the other three groups.

There was no interaction ($P > 0.05$) between diet and animal category. Overall AID was improved significantly ($P < 0.01$) with phytase addition. However, AID was improved in growing-finishing pigs ($P < 0.01$) and lactating sows ($P < 0.01$), but not in piglets and gestating sows. The addition of phytase increased ($P < 0.01$) ATTD of P in all animal categories.

The interaction between diet and site of collection was not significant ($P > 0.05$). Overall AID and ATTD were similar ($P > 0.05$) in pigs fed the control and the phytase-supplemented diet. The AID and ATTD were similar ($P > 0.05$) for all pig categories, except for growing-finishing pigs fed the phytase-supplemented diet ($P = 0.04$).

Discussion

The primary objective of this study was to test the hypothesis that physiological status, i.e. BW and reproductive status, influences P digestibility and phytase efficacy in pigs. The results demonstrated that the physiological state of the animal do affect the digestibility of P and the efficacy of phytase in pigs.

Effect of BW on apparent P digestibility

The AID and ATTD of P remained constant in pigs weighing from 10 to 40 kg. The same response was found in pigs fed the control and phytase-supplemented diet. This conformed with the results of Rodehutscord et al. (1999), which also reported a constant ATTD of P from 15 to 35 kg in pigs fed diets with and without phytase. Results of the current study indicate that the efficiency of P absorption is similar from 10 to 40 kg BW, regardless of the diet being fed. Therefore, a post-weaning pig has the same ability to digest and absorb P compared to a growing pig.

From 40 to 130 kg, AID and ATTD of P linearly decreased in pigs regardless of the diet fed. Results of the current experiment were similar to the conclusions from a review of five experiments (Jongbloed, 1987). In this review, it was demonstrated that ATTD of P decreases with increasing BW. However, a significant increase in ATTD of P from 35 to 65 kg were reported by Eeckhout et al. (1995), Fernandez et al. (1995a and b), and Kemme et al. (1997a), while a constant ATTD of P in pigs from 60 to 100 kg was reported by Kemme et al. (1997a). Our results agree with the conclusions arrived by Jongbloed (1987).

Results of this experiment indicate that increasing BW influences apparent P digestibility, regardless of the site of collection or the diet fed. The linear decline in AID and ATTD of P can be due to (1) increasing proximity of the dietary P concentration to the requirement of the animal, or (2) real animal differences. The diets used in this experiment had the same ingredient composition to exclude the effect of diet composition. As the pig increases in BW, the amount of digestible P intake approaches the requirement. The increasing nearness to the requirement with increasing BW may explain the observed decrease in AID and ATTD of P. However, AID and ATTD of P remained constant from 10 to 40 kg BW, wherein the requirement for digestible P dramatically changes (0.40 to 0.23 g dig P/kg of diet) (NRC, 1998). The lack of response in this period indicates that increasing proximity to the requirement is not the major reason for the response.

In conclusion, increasing BW influences AID and ATTD of P. There is no effect of BW on AID and ATTD of P from 10 to 40 kg; however, AID and ATTD of P decreased linearly from 40 to 130 kg regardless of the diet fed.

Effect of reproductive status on apparent P digestibility

Very few studies have been conducted to determine the digestibility of P in sows, especially in different stages of reproduction. The same is true for the effect of phytase in sows. The results of this study provide insights on the effect of reproductive status on P digestibility.

An increase in ATTD of P as sows proceeded from gestation into lactation has been previously reported (Kemme et al., 1997a). Likewise, a higher P digestibility in lactating sows compared to gestating sows was also reported (Kornegay and Kite, 1983; Kemme et al., 1997a; Giesemann et al., 1998). The results of the current study confirm these findings.

Kemme et al. (1997b) postulated that the high demand for Ca and P during lactation for milk production might have contributed to the increase in P digestibility. This may also be a response to an increase in plasma concentrations of 1,25-$(OH)_2$-cholecalciferol (calcitriol) during lactation (Mahan and Fetter, 1982; Mahan, 1984; Miller et al., 1994). Calcitriol is thought to (1) increase the activity of brush border alkaline phosphatase, which hydrolyzes phosphate ester bonds and improves P absorption, and (2) positively modulate the number of carriers available for Na^+-dependent P absorption at the brush border membrane, increasing P transport efficiency (Jongbloed, 1987).

In conclusion, AID and ATTD of P is affected by the reproductive status of the sow. Both AID and ATTD of P increased as sows proceeded from gestation to lactation, regardless of the diet fed. Gestating sows had a significantly lower AID and ATTD of P compared to lactating sows.

Effect of phytase

The addition of phytase in the diet markedly improved overall AID and ATTD of P. The improvement found in piglets and growing-finishing pigs with phytase was comparable with other reports (Eeckhout and De Paepe, 1992; Pallauf et al., 1992; Cromwell et al., 1993), though the response to phytase has been highly variable among experiments.

There are very few studies conducted in sows to determine the efficacy of phytase. Results of the current study showed an improvement in both AID and ATTD of P in lactating sows with phytase supplementation. However, AID and ATTD in the first and second trimester of gestation showed no improvements with phytase. An improvement in ATTD was observed in the last trimester; while AID was increased but not significantly. These results were comparable to the findings of Kemme et al. (1997a), who found significant improvements in ATTD of P using phytase during the last trimester of gestation and in lactation. A lack of effect in using phytase was also observed during mid-gestation (Kemme et al., 1997a). The reason for the low efficacy of phytase in gestating sows cannot be explained. However, Kemme et al. (1997a) speculated that the sows at mid-pregnancy may have been fed close to or above the requirement.

Differences were also observed between animal categories in terms of phytase efficacy. In this study, phytase efficacy is defined as the degree of improvement (in terms of digestible P/kg DM of diet) after phytase addition to the diet in each animal category. Figure 3.2 illustrates phytase efficacy (in digestible P/kg DM of diet) among different pig categories. Phytase efficacy was numerically highest in lactating sows, followed by sows in late gestation, finishing pigs, piglets, and sows in early to mid-gestation. This was similar to the findings reported by Kemme et al. (1997a). In their study, phytase had the highest efficacy in lactating sows, followed by growing-finishing pigs, piglets, and gestating sows. Since phytase activity is pH-dependent (Eeckhout and De Paepe, 1996) and occurs mainly in the stomach (Yi and Kornegay. 1996), differences in gastric pH and retention time between animal categories may have contributed to the variation in phytase efficacy. However, the relationship between changes in gastric conditions and P digestibility has yet to be investigated.

In conclusion, phytase supplementation improved both AID and ATTD of P, except in sows during the first and second trimester of gestation. Phytase efficacy was numerically highest in lactating sows, followed by sows in late gestation, finishing pigs, piglets, and sows in early to mid-gestation.

Effect of site of collection

Results of the current study showed no differences between overall AID and ATTD of P. Though ATTD was higher than AID in growing-finishing pigs, it was only found at 130 kg pigs fed the control diet and 40 kg pigs fed the phytase-supplemented diet. This agrees with the results of some studies (Jørgensen and Fernandez, 1984;

Jørgensen et al., 1985; Partridge et al., 1986; Larsen and Sandström, 1993; Fan et al., 2001; Bohlke, 2002) but disagree with others (Bruce and Sundstøhl, 1995; Kienzle et al., 1995; O' Quinn et al., 1997; Seynaeve et al., 2000a).

Earlier studies utilizing ileal reentrant cannulas found that the large intestine absorbed considerable amounts of P (Drochner, 1984; Den Hartog et al., 1985). Jongbloed (1987) suggested that the results of these studies lacked validity, as fine-grinding of ingredients in such diets and the possibility of digesta flowing back from the large intestine to the small intestine using this technique may confound the result.

In this study, the lack of difference between AID and ATTD of P suggest no appreciable amount of P absorbed in the distal ileum. This suggests that the large intestine does not play a significant role in P absorption. Therefore, ATTD of P is a suitable measure of digestive utilization of P due to the relatively easier and less costly method of determination compared to AID of P.

Implications

Physiological status, specifically BW and reproductive status, influences apparent P digestibility. Differences were observed in AID and ATTD of P among piglets, growing-finishing pigs, gestating, and lactating sows. This implies that requirements for P should be reconsidered for each specific animal category. Phytase supplementation significantly improved P digestibility. The efficacy of microbial phytase was also different between animal categories. Thus, phytase supplementation can be tailored for each animal category to improve P utilization and more accurately reduce P excretion. No

differences were observed between AID and ATTD of P in all categories. Therefore, ATTD of P is a suitable measure of digestive utilization of P in balance studies due to the relatively easier and less costly method of determination compared to AID of P.

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Table 3.1. Ingredient composition (%) of experimental diets.

Item	Diet	
Ingredient, %	**1**	**2**
Yellow corn	63.64	63.62
Soybean meal, 44%	13.60	13.60
Canola meal	18.10	18.10
Limestone	0.93	0.93
Soybean oil	3.00	3.00
Salt	0.35	0.35
Chromium oxide	0.25	0.25
Vitamin premix[a]	0.03	0.03
Mineral premix[b]	0.10	0.10
Ronozyme P[c]	-	0.02
Total	100.00	100.00
Calculated Analysis, %		
Crude protein	18.0	18.0
Total lysine	0.90	0.90
Calcium	0.50	0.50
Total phosphorus[d]	0.45	0.45
Available phosphorus	0.09	0.09
Phytase activity, FYT/kg[de]	51	652

[a] Vitamin premix provided per kilogram of complete diet: 12,040 IU of vitamin A acetate; 1,191 IU of vitamin D_3 as d-activated animal sterol; 106 IU of vitamin E as α-tocopherol acetate; 6 mg of vitamin K as menadione dimethylpyrimidinol bisulfate; 1.59 mg of biotin; 203 mg of niacin; 99 mg of pantothenic acid; 40 mg of riboflavin; and 0.20 mg of vitamin B_{12}

[b] Trace mineral premix provided per kilogram of complete diet: 23 mg of copper; 110 mg of iron; 0.28 mg of iodine; 23 mg of manganese; 0.28 mg of selenium; and 114 mg of zinc

[c] Roche Vitamins, Inc., Parsipanny, NJ 07054

[d] Analyzed values

[e] FYT = amount of phytase required to release 1 μM of inorganic P from Na phytate/min at pH 5.5 and 37°C

Table 3.2. Schedule of sample collection.

Phase	Start of Feeding Experimental Diet
Phase 1 (10-40 kg)	10, 20, 40 kg BW
Phase 2 (40-130 kg)	40, 70, 100, 130 kg BW
Phase 3 (Sows)	Gestation: d 30, 60 and 90
	Lactation: d 5

Table 3.3. Experimental design.

Pig	Collection Period	
	1	2
1	Diet 1	Diet 2
2	Diet 1	Diet 2
3	Diet 1	Diet 2
4	Diet 2	Diet 1
5	Diet 2	Diet 1
6	Diet 2	Diet 1

Table 3.4. Apparent ileal (AID) and total tract digestibility (ATTD) (%) of P in pigs from 10 to 40 kg BW (Phase 1) fed diets without and with microbial phytase[a]

Item	BW, kg					**Effect of BW**
	10	20	40	Average	SEM	*P*-value
						Linear
n	6	6	4			
AID, %						
- Phytase	28.3	31.4	36.9	32.2	2.69	NS
+ Phytase	40.6	38.6	35.1	38.5	4.48	NS
ATTD, %						
- Phytase	29.1	32.3	24.6	28.7	4.07	NS
+ Phytase	39.1	46.2	47.9	44.4	3.74	NS
Effect of Phytase						
AID						
SEM	4.88	4.88	5.43	2.48	-	-
P-value	0.02	NS	NS	0.04	-	-
ATTD						
SEM	5.52	5.52	5.52	3.16	-	-
P-value	NS	0.02	<0.01	<0.01	-	-
Effect of Collection Site						
- Phytase						
SEM	4.87	4.87	4.87	2.46	-	-
P-value	NS	NS	0.02	NS	-	-
+ Phytase						
SEM	5.58	5.58	6.24	3.27	-	-
P-value	NS	NS	0.05	NS	-	-

[a]AID was calculated as (1 - [(Ps/Pf)(Crf/Crs)]) x 100; where Ps = % P content in the ileal digesta, Pf = % P content in the feed, Crf = % chromium content in the feed, Crs = % chromium content in the ileal digesta all on DM basis; same equation was used to calculate ATTD

Table 3.5. Apparent ileal (AID) and total tract digestibility (ATTD) (%) of P in pigs from 40 to 130 kg BW (Phase 2) fed diets without and with microbial phytase[a]

Item	BW, kg						Effect of BW
	40	70	100	130	Average	SEM	*P*-value
							Linear
n	6	6	6	6			
AID, %							
- Phytase	28.1	23.1	26.2	15.4	23.2	2.60	<0.01
+ Phytase	37.8	35.9	34.8	24.8	33.3	4.02	0.04
ATTD, %							
- Phytase	34.2	24.7	27.6	25.9	28.1	2.17	0.05
+ Phytase	53.0	41.2	37.3	30.9	40.6	2.73	<0.01
Effect of Phytase							
AID							
SEM	4.55	4.76	4.55	4.55	2.76	-	-
P-value	0.04	<0.01	0.06	0.05	<0.01	-	-
ATTD							
SEM	3.46	3.46	3.46	3.46	1.23	-	-
P-value	<0.01	<0.01	<0.01	NS	<0.01	-	-
Effect of Collection Site							
- Phytase							
SEM	3.29	3.45	3.29	3.29	1.42	-	-
P-value	NS	NS	NS	<0.01	<0.01	-	-
+ Phytase							
SEM	4.62	4.62	4.62	4.62	2.63	-	-
P-value	<0.01	NS	NS	NS	0.02	-	-

[a]AID was calculated as (1 - [(Ps/Pf)(Crf/Crs)]) x 100; where Ps = % P content in the ileal digesta, Pf = % P content in the feed, Crf = % chromium content in the feed, Crs = % chromium content in the ileal digesta all on DM basis; same equation was used to calculate ATTD

Table 3.6. Apparent ileal (AID) and total tract digestibility (ATTD) (%) of P in multiparous sows (Phase 3) of different reproductive status fed diets without and with microbial phytase[a]

Item	Reproductive status (days)				Average	SEM	**Effect of Reproductive Status**	
	Gestation			Lactation			*P*-value	
	30	60	90	14			Linear	Quadratic
n	6	6	6	5				
AID, %								
- Phytase	9.3	9.6	19.0	25.5	15.9	4.57	<0.01	NS
+ Phytase	13.8	8.6	32.3	47.5	25.5	6.31	<0.01	NS
ATTD, %								
- Phytase	11.0	5.8	5.9	24.5	11.8	5.58	NS	0.04
+ Phytase	20.5	12.4	25.9	41.5	25.1	5.62	0.01	NS
Effect of Phytase								
AID								
SEM	7.66	7.66	7.66	8.39	3.92	-	-	-
P-value	NS	NS	NS	0.01	0.02	-	-	-
ATTD								
SEM	7.37	7.37	7.37	8.57	3.93	-	-	-
P-value	NS	NS	0.01	0.05	<0.01	-	-	-
Effect of Collection Site								
- Phytase								
SEM	6.97	6.97	6.97	7.60	4.29	-	-	-
P-value	NS	NS	NS	NS	NS	-	-	-
+ Phytase								
SEM	8.03	8.03	8.03	9.28	4.91	-	-	-
P-value	NS	NS	NS	NS	NS	-	-	-

[a]AID was calculated as (1 - [(Ps/Pf)(Crf/Crs)]) x 100; where Ps = % P content in the ileal digesta, Pf = % P content in the feed, Crf = % chromium content in the feed, Crs = % chromium content in the ileal digesta all on DM basis; same equation was used to calculate ATTD

Table 3.7. Comparison of apparent ileal (AID) and total tract digestibility (ATTD) (%) of P in pigs of different categories fed diets without and with microbial phytase[a]

Item	Pig Category					Effect of Category
	Piglets (10 – 40 kg)	G-F Pigs (40 – 130 kg)	Gestation	Lactation	Overall	*P*-value
AID, %						
- Phytase	32.2[b]	23.2[c]	12.7[d]	25.4[bc]	23.4	<0.01
+ Phytase	38.5[bc]	33.3[c]	18.2[d]	47.5[b]	34.4	<0.01
% Improvement	6.3	11.1	5.5	22.1	11.0	
ATTD, %						
- Phytase	28.7[b]	28.1[b]	7.6[c]	24.4[b]	22.2	<0.01
+ Phytase	44.4[b]	40.6[b]	19.6[c]	41.6[b]	36.6	<0.01
% Improvement	15.7	12.5	12.0	17.2	14.7	
Effect of Phytase						
AID						
SEM	2.48	2.76	5.61	8.39	2.29	-
P-value	0.04	<0.01	NS	0.01	<0.01	-
ATTD						
SEM	3.16	1.23	4.33	8.57	1.92	-
P-value	<0.01	<0.01	0.02	0.05	<0.01	-
Effect of Site						
- Phytase						
SEM	2.46	1.42	4.61	7.60	1.74	-
P-value	NS	<0.01	NS	NS	NS	-
+ Phytase						
SEM	3.27	2.63	5.39	9.28	2.44	-
P-value	NS	0.02	NS	NS	NS	-

[a]AID was calculated as (1 - [(Ps/Pf)(Crf/Crs)]) x 100; where Ps = % P content in the ileal digesta, Pf = % P content in the feed, Crf = % chromium content in the feed, Crs = % chromium content in the ileal digesta all on DM basis; same equation was used to calculate ATTD

[b,c,d]Means within a row lacking a common superscript are different ($P < 0.05$)

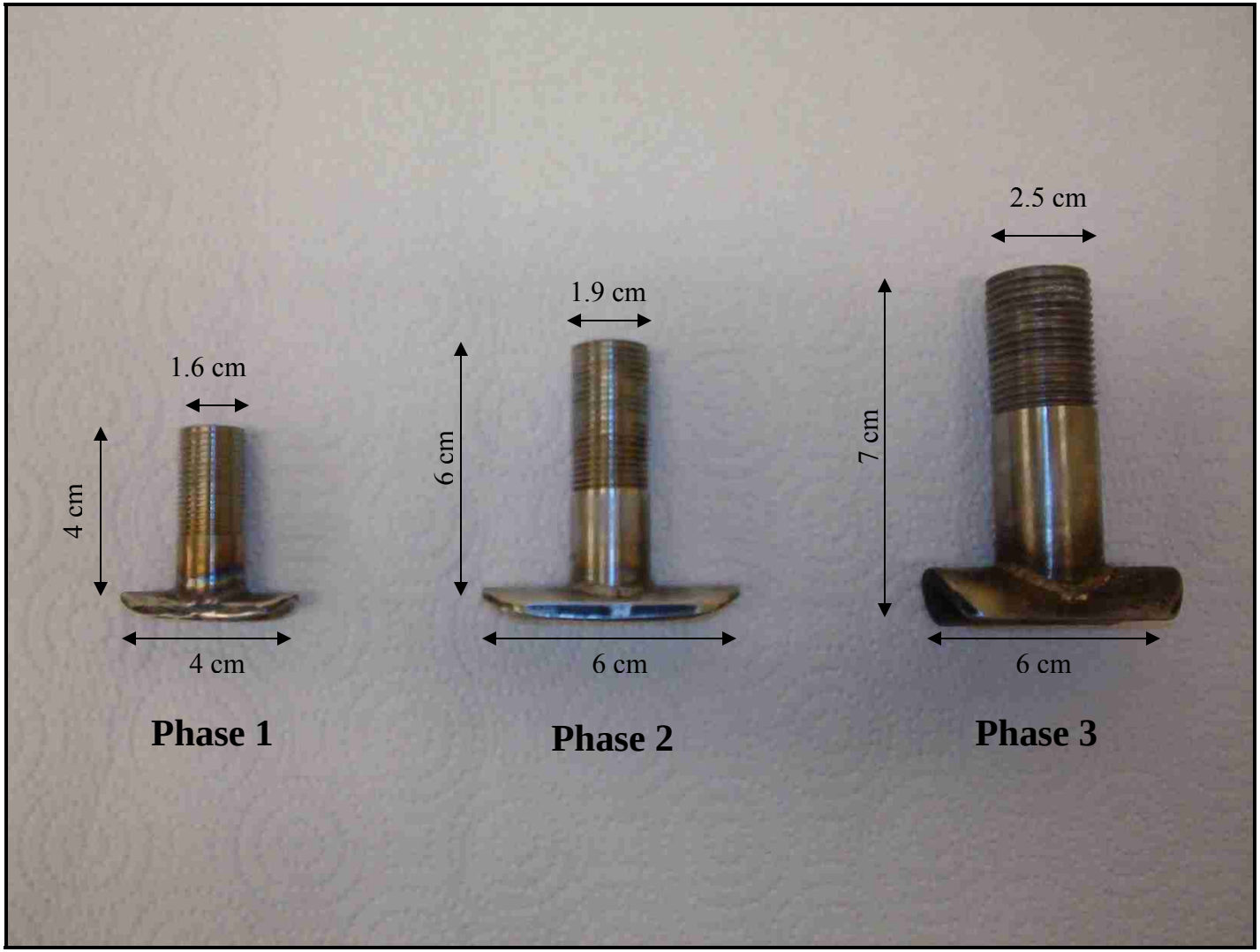

Figure 3.1. Sizes of T-cannulas used in the experiment.

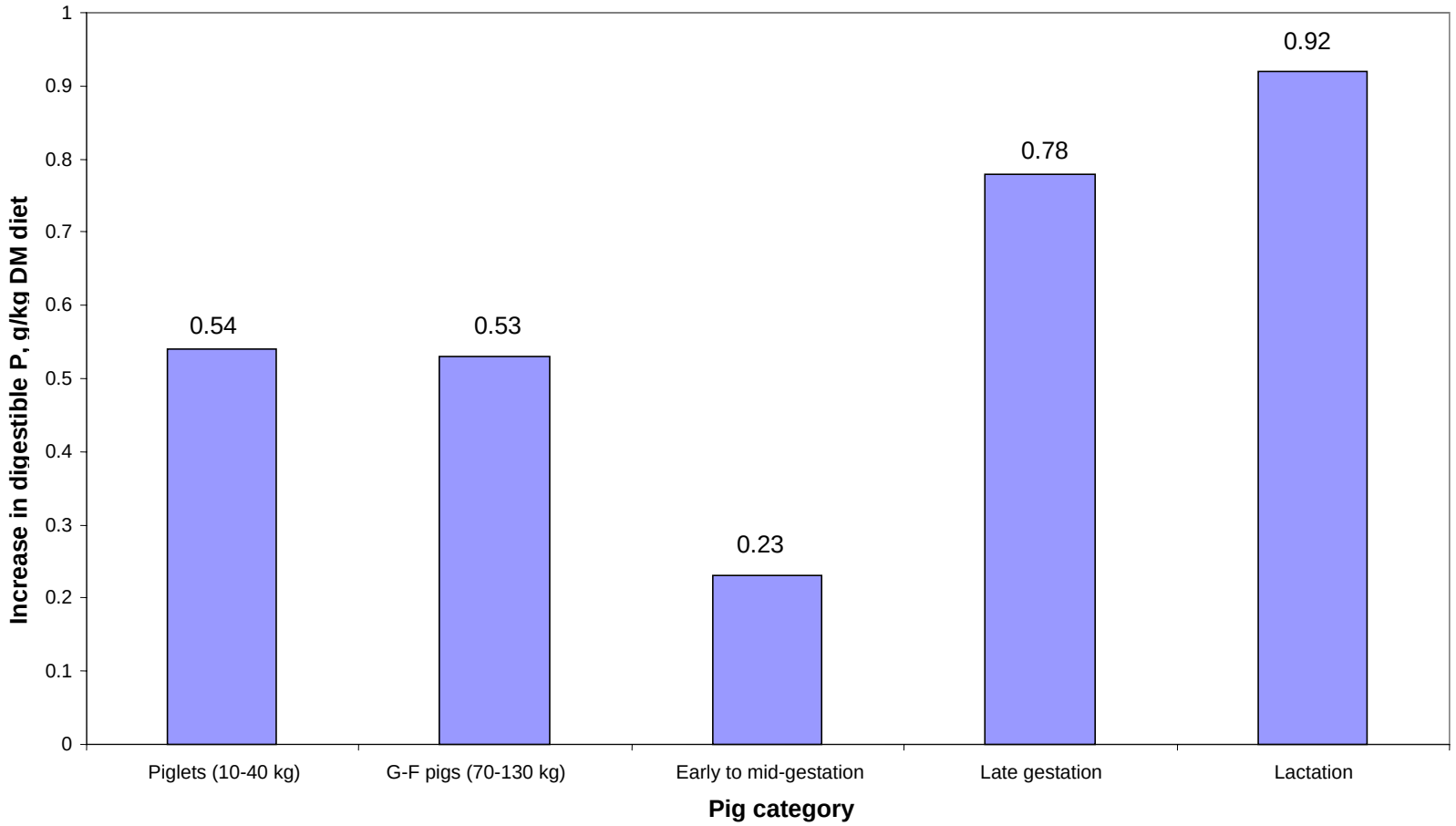

Figure 3.2. Increase in apparent digestible P (g/kg DM diet) with phytase supplementation (500 FYT/kg) in different pig categories.