Quantifying the Effects of Microbial Phytase and Diet Acidity on Ca and P Utilization by Weanling Pigs

by

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(ABSTRACT)

Five experiments were conducted, utilizing 512 crossbred weanling pigs to determine the P (Exp. 1) and Ca (Exp. 2 and 3) equivalency values of microbial phytase based on performance, rib mineralization and P and Ca digestibility estimates, and to investigate the possible interactions of phytase and citric acid (Exp. 4 and 5). In Exp. 1, adding phytase to low P diets linearly increased ADG ($P < .001$), rib shear force ($P < .01$), shear energy ($P < .02$), ash weight ($P < .001$) and ash percent ($P < .001$), Ca ($P < .001$) and P ($P < .001$) digestibility and digestible Ca ($P < .001$) and P ($P < .001$). Added P linearly increased ADG ($P < .003$), rib shear force ($P < .003$) shear energy ($P < .001$), ash weight ($P < .001$) and ash percent ($P < .01$), Ca ($P < .02$) and P ($P < .001$) digestibility and digestible Ca ($P < .02$) and P ($P < .001$). Based on phytase and P linear or nonlinear response equations for ADG, rib shear force, shear energy, and ash weight, P digestibility, and digestible P, the average equivalency of 500 U/kg of phytase was .78 g of P per kg of diet. In Exp. 2, dietary addition of phytase linearly increased rib ash % ($P < .03$), Ca ($P < .001$) and P ($P < .001$) digestibilities, and digested Ca ($P < .001$) and P ($P < .001$), but had no effect ($P > .10$) on ADG and rib shear force and ash weight. Added Ca linearly increased ADG (wk 3-4, $P < .04$), and rib shear force ($P < .001$), ash percentage ($P < .001$) and ash weight ($P < .01$), and digested Ca ($P < .001$), but P digestibility ($P = .07$) and digested P ($P = .08$) were numerically decreased. In Exp. 3, added phytase linearly increased ADG (wk 3-4, $P < .002$), feed efficiency (wk 3-4, $P < .02$), rib ash weight ($P < .001$), Ca total tract digestibility ($P < .001$), and Ca ($P < .001$) and P ($P < .001$) ileal digestibilities. Added Ca linearly increased ADG (wk 3-4, $P < .02$), feed efficiency (wk 3-4, $P < .01$), rib ash percentage ($P < .001$) and ash weight ($P < .001$), shear force ($P < .03$) and energy ($P < .008$), and total tract ($P < .001$) and ileal ($P < .001$) digestible Ca. Based on phytase and Ca linear or nonlinear response equations for ADG in wk 3-4, measurements of rib mineralization, and digestible Ca, 500 U of microbial phytase was estimated to be equivalent to 1.08 g and .78 g of Ca in Exp. 2 and 3, respectively. In Exp. 4 and 5, dietary phytase addition linearly increased rib shear force ($P < .004$ and $P < .02$), shear energy ($P < .001$), dry bone weight ($P < .001$), ash weight ($P < .001$) and ash percent ($P < .001$). Calcium ($P < .001$) and P ($P < .001$) digestibilities were also improved in both experiments when phytase was added. Addition of citric acid in both experiments, reduced dietary pH and stomach digesta pH ($P < .05$). The addition of citric acid improved ADG ($P < .05$), feed efficiency ($P < .04$) and Ca digestibility ($P < .05$) in Exp. 4, but decreased Ca digestibility in Exp. 5 and had no effect on performance. In Exp. 5, the addition of 2.0% citric acid to the diet supplemented with

500 U/kg of phytase caused a decrease ($P < .04$) in the phytase activity recovered in the stomach digesta resulting in a phytase by citric acid interaction ($P < .02$). In summary, the addition of 500 U/kg microbial phytase to weanling pig diets, causes the release of approximately .78 g of P and .93 g of Ca, thus decreasing the need for supplemental P and Ca. The addition of citric acid to phytase supplemented diets does not appear to enhance the efficacy of microbial phytase based on the results of these studies.

Key Words: Pigs, Phytase, Phosphorus, Calcium, Citric Acid, Rib Mineralization

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Chapter I
Introduction

Due to growing environmental concerns, a greater emphasis is now being placed on balancing swine diets for optimal nutrient utilization instead of simply over formulating the diets. One strategy for accomplishing this goal is to breakdown antinutritive compounds within the feed. Phytic acid is one of these antinutritive compounds which is found in all plant ingredients. Approximately 60 to 70% of the P in plant ingredients occurs as phytic acid P (Cromwell, 1992; Ravindran, 1994, 1995) which is unavailable to the pig. In addition, the anionic groups of phytate have been shown to bind other di- and tri-valent cations, including Ca, Mg, Zn, Cu, Fe, Co, and Cr (Maga, 1982; Reddy et al., 1982; Morris, 1986), in the small intestine rendering them unavailable to the pig. It has also been shown that the anioinic phosphate groups of phytate possess the ability to bind proteins (Prattley et al., 1982) and amino acids.

The addition of microbial phytase to the diet has been shown to catalyze the stepwise hydrolysis of the phytate molecule, improving P digestibility, and therefore decreasing P excretion (Simons et al., 1990; Jongbloed et al., 1992). In addition, improvements in Ca digestibility (Mroz et al., 1993; Yi et al., 1996a; Kornegay and Qian, 1996), and Zn, Mg, Fe, and Cu apparent absorption (Pallauf et al., 1992; Nasi and Helander, 1994) have been reported in pigs when diets are supplemented with microbial phytase.

In order for producers to take full advantage of the benefits provided by phytase, accurate equivalency values of phytase for P must be developed. Several studies have attempted to do this in pigs (Kornegay and Qian, 1996; Jongbloed et al., 1996; Yi et al., 1996c; Harper et al., 1997) with ranges in the equivalency of 500 U/kg microbial phytase equal to 0.64 to 2.57 g of P from inorganic sources being reported. Factors which have influenced the wide range in equivalency values reported include: the basal level of P, the response criteria used, the number of levels of both P and phytase fed, and the ratio of Ca to P. In many of the studies, an extremely wide Ca:P ratio was fed which has been shown to decrease phytase efficacy in pigs by Qian et al. (1996a).

Ideally, diets should be formulated to contain an optimal available P to available Ca ratio. In order for this to occur, equivalency values of phytase for Ca must also be developed. Jongbloed et al. (1996) suggested that supplementing pig diets with 500 U/kg of phytase released 0.4 to 0.7 g of digestible Ca. In broilers, Kornegay et al. (1996) reported that 500 U/kg of phytase was equivalent to 0.87 g of total Ca. To further elucidate the Ca equivalency value of microbial phytase, studies must be conducted where multiple levels of Ca and phytase are fed so that response curves to both Ca and phytase can be developed.

Finally, in order for swine producers to begin utilizing phytase on a large scale, it must be cost effective. One possible method for decreasing the cost of microbial phytase is to increase its efficacy, thus decreasing the amount of enzyme which needs to be added to the diet. Microbial phytase has been shown to have two peaks of activity, a smaller peak at pH 2.5 and a larger peak at pH 5.0 (Shieh et al., 1969; Irving and Cosgrove, 1974; Beudecker, 1990). Its primary site of

activity is in the stomach where the pH is between 1 and 4 (Mroz et al., 1997). Therefore, the addition of organic acids to the diet might help to lower the gastric pH if it is above 2.5 to a more optimal pH for phytase activity. Scipioni et al. (1978) demonstrated a lowered gastric pH when citric acid was added to the diet. Jongbloed et al. (1996) reported the results of two experiments in which the possible interactions of organic acids and phytase were investigated. In Exp. 1, they found a lactic acid by phytase interaction on P digestibility. In Exp. 2, they found no interactive effects of lactic or propionic acid, but a synergistic effect of formic acid and phytase on P digestibility was observed. Clearly, organic acids have the potential to increase phytase efficacy when added to the diet, but data in the literature are scarce and inconsistent.

In summary, the addition of microbial phytase to pig diets improves P and Ca digestibility while enhancing the bioavailability of many other di- and tri-valent cations. In addition, the digestibility of amino acids may be enhanced through phytase addition. However, in order for wide scale use by producers, microbial phytase must be cost effective. Organic acids provide one possible route of enhancing phytase efficacy, but data in support of their use are scarce and inconsistent.

Chapter II
Literature Review

Phosphorus

Phosphorus is an essential nutrient in swine diets serving important functions as part of structural compounds in bone and in cell membranes, as a source of high free energy bonds in nucleotides, as a structural component of nucleic acids, as a component of many enzyme cofactors, and as a component in many metabolic pathways. Phosphorus has an atomic number of 15 and an atomic weight of 30.97. It can exist in a trivalent or pentavalent form. However, phosphorus, is commonly found in the body as phosphate (PO_4^{-3}) with a valence of five.

Phosphorus absorption occurs throughout the small intestine with the the largest portion of P absorption occurring in the jejunum (Korwaski and Schachter, 1969; Walling, 1977). Endogenous excretion amounts to about 200 mg/d in rats (Wheeler and Lowenstein, 1979) with approximately two-thirds of this being reabsorbed (Robertson, 1976). There are two primary mechanisms involved in phosphate absorption, an active transport system and a passive transport system. Active transport of PO_4 occurs primarily in the proximal small intestine and it is linearly related to the lumenal Na^+ concentration (Danisi and Straub, 1980). It is blocked by calcitonin (Caniggia et al., 1968; Juan et al., 1976), while 1,25$(OH)_2$-D3 has a stimulatory effect on phosphate absorption (Danisi and Straub, 1980). Passive transport occurs primarily in the jejunum and ileum and is naturally related to the lumenal concentration of PO_4 (Danisi and Straub, 1980). Therefore, when the intake of PO_4 is low, the active transport mechanism is dominant, and when the intake of PO_4 is high, the passive transport mechanism is dominant.

Phosphorus in the body accounts for about 1% of the total body weight. Of the total body P, approximately 85% is found in bone, 14% in soft tissue and muscle, and 1% in blood (Berner, 1997). Phosphorus is stored in bone as a crystalline structure, along with Ca, known as hydroxyappetite ($Ca_{10}(PO_4)_6(OH)_2$). Deficiencies in bone P lead to osteomalacia.

Phosphate metabolism is primarily affected by serum PO_4 concentrations and three hormones: 1,25 dihydroxycholecalciferol (1,25$(OH)_2$-D3), parathyroid hormone (PTH), and calcitonin. 1,25 dihydroxycholecalciferol directly affects PO_4 metabolism by stimulating absorption via active transport mechanisms (Korwaski and Schachter, 1969; Chen et al, 1974; Kabakoff et al., 1982) and by stimulating bone resorption of both PO_4 and Ca (Korwaski and Schachter, 1969; Williams et al., 1989). Indirectly, 1,25$(OH)_2$-D3 causes an increase in bone Ca resorption which leads to higher serum Ca levels which decreases PTH levels, thus causing an increased reabsorption of PO_4 by the kidney (Amiel et al., 1970; Dominguez et al., 1976).

Parathyroid hormone asserts most of its action on the kidney (Bemdt et al., 1990), having an inhibitory effect on PO_4 reabsorption. Its proposed method of action is through a stimulatory effect on adenylate cyclase activity (Bemdt et al., 1990) which leads to an increase in cAMP. It has been proposed that cAMP enhances gluconeogenesis (Dousa, 1981). One of the by-products

of gluconeogenesis is nicotinamide adenine dinucleotide (NAD^+) which has been shown to inhibit the uptake of PO_4 by the brush border membrane of cells lining the kidney tubules (Dousa, 1981).

Calcitonin is released from the parafollicular cells of the thyroid gland in response to high blood Ca. It interacts with a plasma membrane receptor found on osteoclasts (Chambers et al., 1985; Boyde and Jones, 1987) resulting in decreased osteoclastic activity (Malgaroli et al., 1989; Teti and Zallone, 1992) and thus a decreased bone resorption.

Low concentrations of PO_4 in the serum have also been shown to stimulate reabsorption of PO_4 by the tubules of the kidney. In addition, low serum PO_4 concentrations desensitize the kidney tubules to the effects of PTH. In contrast, decreased PO_4 reabsorption occurs when serum PO_4 levels are high. Steroid hormones have also been shown to decrease PO_4 reabsorption.

Calcium

Calcium is an essential nutrient which is necessary for optimal growth and bone development (NRC, 1988). Approximately 99% of the Ca in the body is located in the skeleton. Calcium in the skeleton is found in the structural complex hydroxyappetite along with P. It serves as a Ca reservoir for the rest of the body. Calcium homeostasis in the blood is tightly regulated primarily by PTH, $1,25(OH)_2D_3$, and calcitonin, all of which will be discussed later. In addition, prostaglandins, reproductive steroids, and some other hormones can affect Ca metabolism.

Calcium Absorption. Calcium absorption occurs throughout the small intestine via an active transcellular process or via a paracellular process (Bronner et al., 1986). In order for Ca to be absorbed it must be solubilized within the intestinal lumen. The Ca in a given feedstuff may already be in solution or it may be solubilized through the actions of gastric enzymes and peristalsis. The degree to which dietary Ca can be solubilized in the intestinal lumen is affected by many constituents within the diet. The formation of insoluble Ca-salts, such as Ca carbonate or phosphate, is probably the most detrimental factor affecting Ca absorption. These salts, if present in the diet, dissociate in the stomach as a result of the low pH. However, as the digesta is passed caudally through the small intestine, the pH increases causing the salts to reform and precipitate out of solution. At a neutral pH or higher very little Ca remains in solution (Washburn, 1928). The availability of Ca from limestone is markedly reduced if other Ca-salts are present which are more soluble than $CaCO_3$. In other words, phosphate is more soluble then $CaCO_3$, therefore Ca from $CaCO_3$ will be substantially less available in the presence of phosphate, because less of the $CaCO_3$ will be in solution.

Transcellular Ca transport (Figure 1) occurs primarily in the proximal end of the small intestine (Bronner et al., 1986; Roche et al., 1986). It is an active process which occurs in three steps: uptake of Ca by the brush border membrane, transport of Ca through the cell, and

movement of Ca out of the cell and into the blood stream. Evidence suggests that calcium enters the cell through a Ca channel (Homaidan et al., 1965; Hess and Tsien, 1984; Bronner et al., 1986; Caffrey and Farach-Carson, 1987; Butler and Hillier, 1989; Guggino et al., 1989; Saunders and Isaacson, 1990) traveling down an electrochemical gradient. Once in the cell, it binds to calbindin and diffuses through the cell to the basolateral membrane (Wasserman et al., 1968; Wasserman and Feher, 1977; Wasserman et al., 1978; Thomasett et al., 1982; Feher et al., 1992; Stein, 1992). Facilitated diffusion of Ca attached to calbindin is approximately 70 times faster than if Ca were to diffuse through the cell by itself as described by Fick's law (Stein, 1992). Movement of Ca out of the cell occurs up an electrochemical gradient, requiring energy in the form of ATP to drive the process. Two Ca transporters have been identified. The first is a Ca-ATPase, which utilizes the energy derived from the hydrolysis of ATP to move Ca up its electrochemical gradient and out of the cell (Garrahan and Rega, 1990). Cytoplasmic binding sites for Ca and calmodulin have been identified (Verma et al., 1988; Carafoli et al., 1990). This transport mechanism is thought to be the primary transport mechanism for extrusion of Ca. The second transporter, found in many cells, is a Ca/Na antiport system (Reeves, 1990). Sodium is transported out of the cell via a Na/K-ATPase creating a favorable concentration gradient for Na to move into the cell. The inward movement of Na provides the energy necessary for Ca to be moved out of the cell. This process, however is not thought to play a major roll in Ca extrusion from duodenal cells (Nellans and Popovitch, 1984).

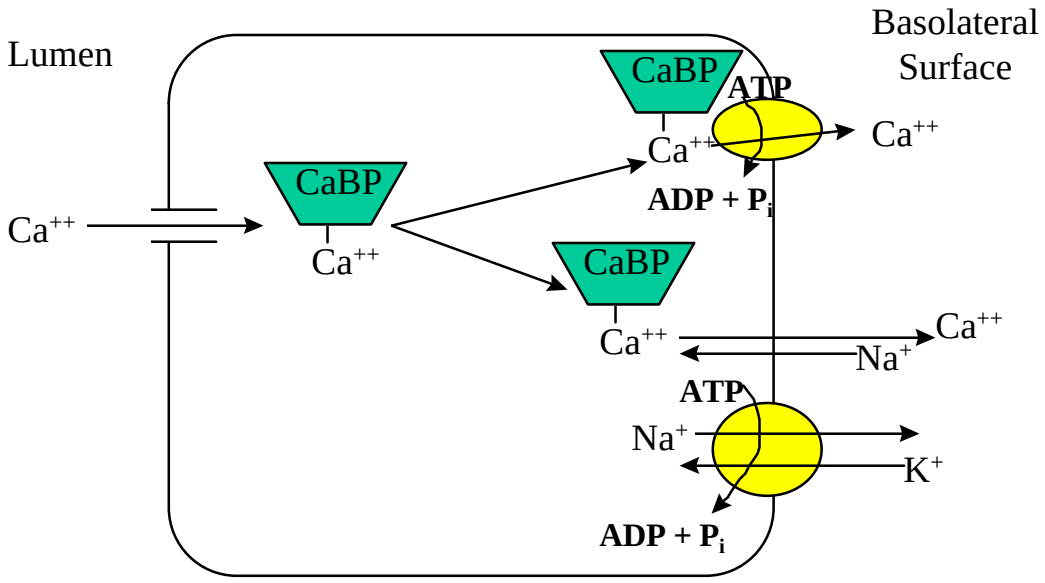

Figure 1. Transcellular Ca transport.

Paracellular transport (Figure 2) is a nonsaturable process which occurs throughout the small intestine. Ca uptake via paracellular transport is enhanced when hyperosmolar substances

relative to body fluids are present in the diet. This causes an increased distention of the gut wall leading to an increased paracellular transport.

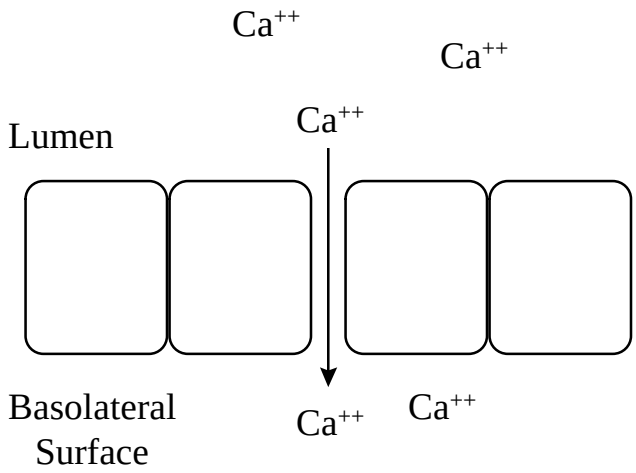

Figure 2. Paracellular Ca transport.

Therefore, Ca transport occurs through two processes, both a saturable transcellular mechanism and a nonsaturable paracellular mechanism. This was described by Wasserman and Taylor (1969) using the following equation:

$$v_a = \frac{V_m \times [Ca]_L}{K_m + [Ca]_L} + b[Ca]_L$$

where
v_a = amount of Ca absorbed per unit time
$V_m = V_{max}$ for the saturable component
$[Ca]_L$ = Ca concentration of the luminal fluid
$K_m = ½ V_{max}$
b = an apparent permeability constant

Regulation of Ca Metabolism. Parathyroid hormone is released from the parathyroid gland in response to low blood Ca. It exerts its primary effects on bone and in the kidney. In both tissues it interacts with a plasma membrane receptor stimulating adenylate cyclase activity which produces the second messenger, cAMP (Aurbach, 1988; Habener and Potts, 1990). In bone, this causes a decrease in osteoblastic activity and an increase in osteoclastic activity

(Bronner, 1996). This has a net result of increasing bone mineral resorption. In the kidney, cAMP concentrations in the urine are increased (Chase and Aurbach, 1967), urinary phosphate excretion is increased, and Ca reabsorption from the distal convoluted tubule is increased (Massry, 1982). Indirectly, PTH increases the intestinal absorption of Ca by stimulating the hydroxylation of 25-OH-D3 to 1,25$(OH)_2$-D3 in the kidney (Norman and Litwack, 1987).

Calcitonin is released from the parafollicular cells of the thyroid gland in response to high blood Ca. It interacts with a plasma membrane receptor found on osteoclasts (Chambers et al., 1985; Boyde and James, 1987) resulting in decreased osteoclastic activity (Malgaroli et al., 1989; Teti and Zallone, 1992) and thus a decreased bone resorption.

Vitamin D is created from the conversion of 7-dehydrocholesterol in the skin in the presence of ultraviolet light. It then undergoes hydroxylation to 25-OH-D3 in the liver, and then hydroxylation to 1,25$(OH)_2$-D3 or 24,25$(OH)_2$-D3 in the kidney. 1,25$(OH)_2$-D3 is the primary metabolically active form of vitamin D having effects in bone, kidney, and intestinal tissue. In the intestine, 1,25$(OH)_2$-D3 increases the transcellular uptake of Ca by enhancing the entrance of Ca into the cell (Miller and Bronner, 1981), by increasing the synthesis of calbindin (Thomasett et al., 1983; Perret et al., 1985, 1988), and finally by increasing the number of transport proteins which move Ca out of the cell (Wasserman et al., 1992). The greatest effect seems to be due to the increased synthesis of calbindin, since neither entry or exit of Ca into the cell have been shown to be rate limiting. In the kidney, 1,25$(OH)_2$-D3 increases Ca reabsorption by the distal convoluted tubule. Finally, in bone, 1,25$(OH)_2$-D3 causes a decrease in the rate of bone turnover. Its method of action in bone is still not clear. Receptors for 1,25$(OH)_2$-D3 have been found only on osteoblasts and not on osteoclasts (Sato and Rodan, 1991). However, increases in both bone formation and resorption have been shown in response to 1,25$(OH)_2$-D3 (Hurwitz et al., 1969). Therefore, the effect on osteoclasts must be indirect.

Response Criteria used to Measure Ca and P Status

Criteria which are often used to measure the response of pigs to varying levels of P or Ca include performance measurements (average daily gain, feed intake and feed efficiency), blood measurements (serum P and Ca concentrations), bone criteria (ash percentage, shear force, bending moment) and digestibility estimates. Pigs have a higher requirement for bone mineralization then they do for growth. Therefore, responses of bone criteria can be seen over a wider range of dietary Ca then responses to growth.

Several studies have found that growth rate, feed intake, and feed efficiency are not sensitive indicators of Ca and P status (Doige et al., 1975; Crenshaw, 1981a; Kornegay, 1981; Koch and Mahan, 1985). However, when Ca and P are fed at a low level, responses in growth rate and feed efficiency have been seen (Miller et al., 1962, 1964; Cromwell et al., 1970, 1972; Doige et al., 1975; van Kempen et al., 1976; Ross et al., 1984). In studies of Kornegay and Qian

(1996) and Yi et al. (1996) where the effects of added phytase and P to a low P basal diet were investigated, growth rate and feed intake were moderately sensitive indicators of the effects of both phytase and P.

Serum Ca and P concentrations, much like performance criteria, are primarily responsive when low levels of Ca and/or P are fed (Doige et al., 1975; Reinhard et al., 1976; Kornegay and Thomas, 1981; Koch and Mahan, 1985). Serum P has been shown to increase while serum Ca decreases as the level of P in the diet is increased (Miller et al., 1964; Harmon et al., 1967; Cromwell et al., 1970; Kornegay and Thomas, 1981). The adverse effects of a wide Ca:P ratio can be seen in serum. As the Ca:P ratio widens, serum P concentrations decrease and serum Ca concentrations increase (Koch et al., 1984; Koch and Mahan, 1985).

Kornegay (1985) and Peo (1991) both concluded that bone parameters were responsive to Ca and P over a wider range of dietary Ca and P than performance or blood parameters. Mechanically, bones are most often measured by shear force and bone bending moment due to the simplicity of these tests (Kornegay, 1985; Peo, 1991). Several studies have found bone bending moment to be a very sensitive test for Ca and P status (Delleart et al., 1991; Cromwell et al, 1993, 1995; Keteran et al 1993a,b). Combs et al. (1991a,b) however, found that bone shear force was even more sensitive than bone bending moment. Bone ash is also a very simple test which is used quite often as a test of bone mineralization. Rutlege et al. (1961) and Miller et al. (1962, 1964) found a high correlation between bone ash and dietary P levels in baby pigs. Several studies have demonstrated the same correlation in weanling pigs (Combs et al., 1962; Hoppe et al., 1992) and grower-finisher pigs (Cromwell et al., 1970; Pond et al., 1975; Mahan et al., 1980).

Typically, metacarpals, metatarsals and femurs have been the bones used to assess Ca and P status. However, in studies by Kornegay and Qian (1996) and Yi et al. (1996) the 10^{th} rib was more highly correlated with the response equations to P and phytase than was the metacarpal.

The use of apparent P digestibility as an estimator of P bioavailability in a feedstuff has been used quite frequently in recent studies where microbial phytase was added to the diet (Pointillart et al., 1984, 1987; Simons, 1990; Eeckhout and de Paepe, 1991; Jongbloed et al., 1992; Dungelhoef et al., 1994; Pallauf et al, 1992, 1994; Mroz et al., 1994; Kornegay and Qian, 1996; Yi et al., 1996). This is supported by the conclusions of Delhaert et al., (1990) who compared eight P supplements added to supply 0.6 to 2.2 g/kg of additional P to a basal diet which contained 3.0 to 3.2 g/kg of P. In addition a comparison of techniques used to evaluate P availability from feedstuffs was made. They concluded that apparent P digestibility was the most sensitive indicator of P bioavailability, followed by bone parameters, with blood parameters showing an insufficient response to P. However, Peo (1991) concluded that apparent Ca and P digestibility were of no use in estimating their bioavailability in feedstuffs. In the studies of Kornegay and Qian (1996) and Yi et al. (1996) where the effects of phytase were investigated, the apparent digestibility of P did serve as a good measure of the bioavailability of P from the feedstuff.

Organic Acids

Organic acids have been added to pig diets at levels of 1.0 to 3.0% in an attempt to improve post-weaning performance and have shown varied effects. Citric acid and fumaric acid have been researched the most, and in general their addition improves postweaning performance. The method of action through which organic acids work is still unclear. It has been proposed that they lower and stabilize the gastric pH making it a less hospitable environment for harmful bacteria. Research reported in the literature is quite variabile.

Effects of Organic Acids on Growth Performance and Nutrient Utilization. In a review of fumaric acid by Kirchgessner and Roth (1982) they reported that the addition of fumaric acid to weanling pig diets improved weight gain, feed intake, feed efficiency, protein digestibility, N retention, and Ca and P balance. Several studies are in agreement with the improvements in growth performance reported by Kirchgessner and Roth (1982), showing increases in body weight gain (Radecki et al., 1988; Falkowski and Aherne, 1984) and feed efficiency (Radecki et al., 1988; Falkowski and Aherne, 1984; Giesting and Easter, 1985) when fumaric acid was added to the diet. However, Scipioni et al. (1978) and Henry et al. (1985) found no beneficial effects when fumaric acid was added to the diet. Burnell et al. (1988) reported an improved daily gain and feed efficiency when citric acid was added to a simple diet for pigs, but no effect on growth performance was seen when citric acid was added to a more complex diet containing dried whey. However, Falkowski and Aherne (1984) found that adding citric acid to a complex diet improved feed efficiency and numerically increased average daily gains. This is in agreement with the findings of Scipioni et al. (1978) and Henry et al. (1985) who reported improvements in average daily gains when diets were supplemented with citric acid. However, Kornegay et al. (1976) and Radecki et al. (1988) found no improvements in performance when citric acid was added to the diet. Risley et al. (1991) reported the results of two experiments in which either 1.5% citric or fumaric acid was added to the diet. In Exp. 1, the addition of 1.5 % citric acid to the diet tended to increase average daily gain and feed efficiency during wk 1-4 postweaning, while the addition of 1.5% fumaric acid had no effect. However in Exp. 2 the opposite effects were observed, with fumaric acid causing increases in average daily gain, while citric acid had no effect.

Effects of Organic Acids on Gastrointestinal pH. Scipioni et al. (1978) reported a decreased gastric and duodenal pH when citric acid was added to the diet. They also found a lower E. coli and anaeorbic bacteria population in the intestine when citric acid was added to the diet. In agreement with these findings, Burnell et al. (1988) reported a decreased intestinal pH when citric acid was included in the diet at a level of 1%.

Phytate

Phytic acid ($C_6H_{18}O_{24}P_6$) is a myoinositol 1, 2, 3, 4, 5, 6 hexa, dihydrogen phosphate (IUPAC-IUB, 1968). Phytate, the salt of phytic acid serves as the primary storage form of P in plants, accounting for 60 to 80% of the total P. Reported values in the literature for the total P content and the phytate-P content of various plant ingredients are shown in Table 1.

Table 1. Total P and phytate P content of various feedstuffs.

Feedstuff	Total P, %	Phytate P, % of total	Reference
Corn	.28		NRC, 1988
Corn	.26	66	Nelson et al., 1968
SBM[a] (44% CP)	.65		NRC, 1988
SBM (48.5% CP)	.64		NRC, 1988
SBM	.61	61	Nelson et al., 1968
SBM (44% CP)	.66	53	Eeckhout and De Paepe, 1994
SBM (48% CP)	.61	52	Eeckhout and De Paepe, 1994
SBM		51-61	Pointillart, 1994
Barley	.34		NRC, 1988
Barley	.34	56	Nelson et al., 1968
Barley	.37	60	Eeckhout and De Paepe, 1994
Barley		51-66	Pointillart, 1994
Wheat	.37		NRC, 1988
Wheat	.30	67	Nelson et al., 1968
Wheat	.33	67	Eeckhout and De Paepe, 1994
Wheat		60-77	Pointillart, 1994

[a]Soybean meal

Monogastric animals are unable to breakdown the phytate molecule, and therefore the P incorporated in the phytate molecule is unavailable to the animal for absorption. Therefore, producers must add large amounts of a highly available inorganic P source to meet the animals P requirements. The unavailable phytate P is excreted which potentially can lead to environmental pollution problems. Estimates of the bioavailability of P from various plant ingredients as reported in the literature are shown below in Table 2.

Table 2. Estimates of P bioavailability from various feedstuffs for pigs.

Source	Bioavailability[a], %	Standard[b]	Reference
Corn	15	MSP	NRC, 1988
Corn	14	MSP	Cromwell, 1992
Corn	15		Pierce et al., 1977
Corn	12		Calvert et al., 1978
Corn	29		Pointillart, 1984
Corn	48		Pointillart, 1987
Corn	29	MSP	Huang and Alle, 1981
SBM[c]	38	MSP	NRC, 1988
SBM (44% CP)	31	MSP	Cromwell, 1992
SBM (44% CP)	27		Tonroy et al., 1973
SBM (44% CP)	36	MSP	Huang and Alle, 1981
Barley	31	MSP	NRC, 1988
Barley	30	MSP	Cromwell, 1992
Barley	28		Calvert et al., 1978
Wheat	50	MSP	NRC, 1988
Wheat	49	MSP	Cromwell, 1992
Wheat	46		Pointillart, 1984
Wheat	51	MSP	Huang and Allee, 1981

[a]Bioavailability is expressed as a percentage relative to the standard which is assumed to have a bioavailability of 100%.
[b]If no standard is listed then bioavailability is expressed as a percentage of apparent absorption.
[c]Soybean meal

Mineral Chelating Ability of Phytic Acid. At a neutral pH phytic acid has been shown to carry one or two negatively charged oxygen atoms in each phosphate group (Erdman, 1979), giving it a total of up to twelve negative charges. Because of these negative charges, phytic acid has the ability to bind with a variety of di and tri-valent cations including Ca, Mg, Zn, Cu, Fe, Co, and Cr (Maga, 1982; Reddy et al., 1982; Morris 1986) in the small intestine of the pig rendering them unavailable for absorption. Figure 3 shows the mineral complexing potential of phytic acid.

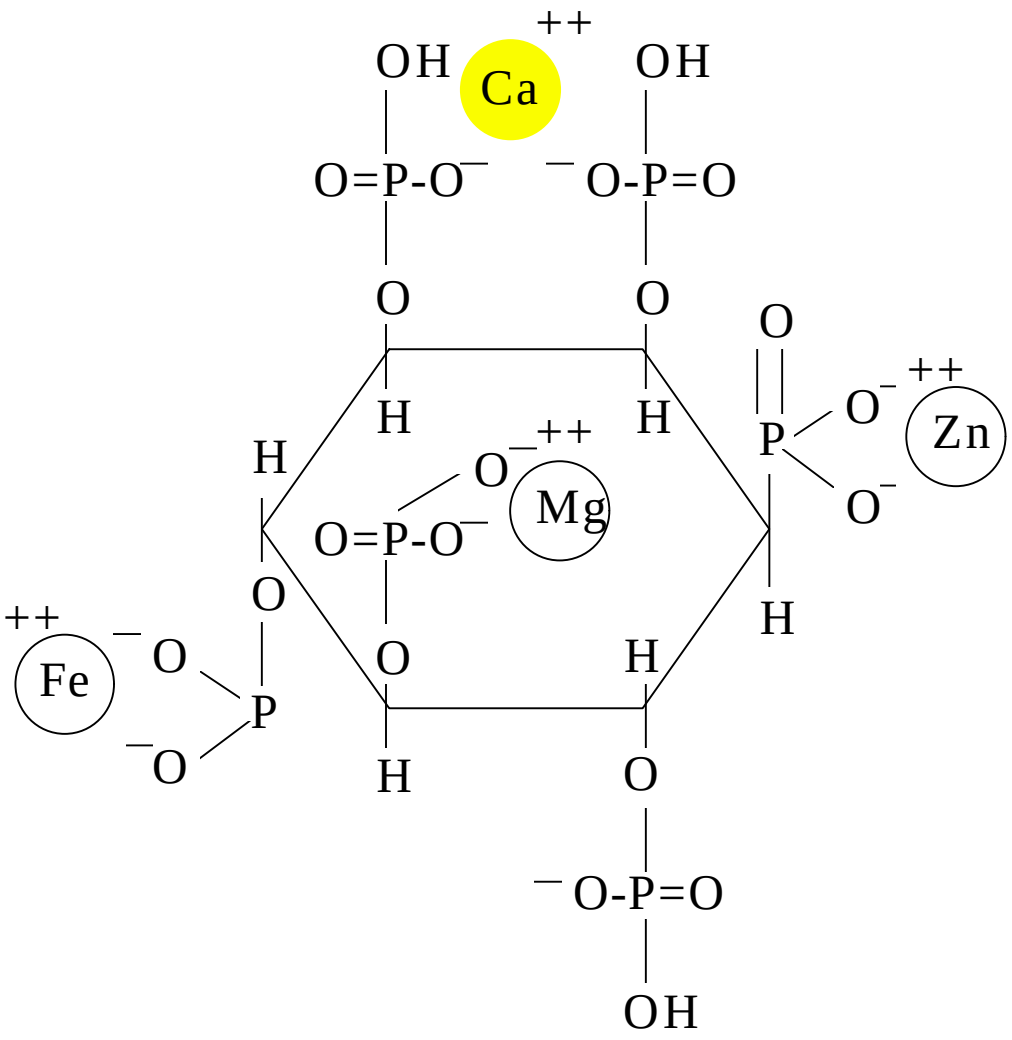

Figure 3. Mineral chelating ability of phytic acid.

Phytic acid has the greatest affinity for Zn and Cu and a fairly low affinity for Ca. However, due to the much larger concentration of Ca in diets fed to pigs relative to Zn and Cu, an effect on Ca is possible. It has also been shown that the anionic phosphate groups of phytate possess the ability to bind proteins (Prattley et al., 1982) and amino acids; having its greatest affinity for the basic amino acids lysine, arginine, and histidine (Reddy et al., 1982).

Phytase

Phytases are a subset of a larger enzyme group known as phosphatases. Phosphatases are enzymes which catalyze the hydrolysis of ester linked phosphates. In this process the bound phosphate is removed from the substrate and in an intermediary step becomes bound to the

enzyme before becoming dissolved in water. Phytases, specifically are phosphatases which catalyze the hydrolysis of phosphate from phytate.

Two functionally distinct phytases have been characterized to date. Both produce similar outcomes, but have different modes of action. The first, 6-phytase, catalyzes the stepwise hydrolysis of phosphate from phytate beginning at position 6 and is primarily found in plants (Kies, 1996). The second, 3-phytase, is produced by many microorganisms and catalyzes the stepwise hydrolysis of phosphate from phytate beginning at position 3 (Kies, 1996). The hydrolysis of phosphate from the phytate molecule is ordered. In other words, 3-phytase begins hydrolyzing phosphate from phytate at position 3 and then continues at positions 4, 5, 6 and 1 producing inositol phosphate-5 (IP-5), IP-4, IP-3, IP-2, and IP-1, respectively (VeneKamp et al., 1995). 3-phytase, does not or only very slowly has the ability to catalyze the hydrolysis of IP-1 to IP plus free phosphate (Kies, 1996).

Plant Phytases. Plant phytases (6-phytase) express maximum activity at approximately pH 5.0 with values reported for barley, bean, corn, peanut, wheat, and wheat bran phytase of pH 5.2, 5.3, 5.6, 5.0, 5.2, and 5.0, respectively. Nayini and Markakis (1986) reported that the optimal pH for soybean phytase occured between pH 4.5 and 4.8. Peak plant phytase activity has been observed to occur at a temperature of 50° C with a range reported in the literature of 45 to 57° C (Irving, 1980). The amount of phytase found in plant sources varies greatly with rye, triticale ,and wheat containing the largest amount (Table 3).

Microbial Phytases. Natuphos® is a commercially available phytase supplement produced by Gist-Brocades and marketed by BASF. It is a 3-phytase produced by *Aspergillus niger* var. van Tieghem. Microbial phytase has been shown to have two optimal pH peaks of activity at pH 2.5 and pH 5.0 to 5.5 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). Simons et al. (1990) using microbial phytase from a crude preparation of *Aspergillus* found that the phytase was 50% more active at pH 2.5 compared to pH 4.5. However, Beudeker (1990) found that Natuphos® phytase was more active at pH 5.5 compared to pH 2.5. At a pH of 7.0 or higher, no activity of microbial phytase has been seen (Simons et al., 1990). The pH of a corn-soybean meal based diet using dicalcium phosphate as the P source is about 6.0.

Table 3. Intrinsic phytase activity in various feedstuffs.

Feedstuff	Phytase, U/kg	Reference
Rye	4900	Pointillart, 1994
Rye	4132-6127	Eeckhout and De Paepe, 1994
Triticale	1500	Pointillart, 1994
Triticale	1475-2039	Eeckhout and De Paepe, 1994
Wheat	700	Pointillart, 1994
Wheat	915-1581	Eeckhout and De Paepe, 1994
Barley	400	Pointillart, 1994
Barley	408-882	Eeckhout and De Paepe, 1994
Wheat Bran	1200	Pointillart, 1994
Wheat Bran	1180-5208	Eeckhout and De Paepe, 1994
Corn	0-46	Eeckhout and De Paepe, 1994
Soybeans (heated)	0-188	Eeckhout and De Paepe, 1994
SBM, 44%	0-120	Eeckhout and De Paepe, 1994
SBM, 48%	0-20	Eeckhout and De Paepe, 1994
SBM	non-detectable	Pointillart, 1994

Site of Phytase Activity. Mroz et al. (1997) reported that 52% of the phytate P was degraded in the stomach and an additional 9% was degraded in the duodenum and jejunum. In addition, no phytase activity could be detected in the ileum. In two experiments by Yi et al. (1996) it was reported that phytase activity in the digesta decreased from the stomach to the upper small intestine to the lower small intestine when measured 3 h after ingestion of a meal. Phytase activity, as a percentage of the dietary phyatse activity, was found to be 51% in the stomach, 31% in the upper small intestine, and 5% in the lower small intestine in experiment 1. In a second experiment values of 41%, 16%, and 5% were observed for the stomach, upper small intestine, and lower small intestine, respectively. The acidity of the stomach lumen ranges from pH 1.0 to 4.5 (Chessen, 1987) and the luminal pH of the gastrointestinal tract increases from the duodenum to the terminal ileum. Mroz et al. (1997) reported that the duodenal pH immediately following a meal was 5.7 and that it gradually decreased to pH 3.3. It is generally accepted that the duodenal pH is approximately 4.8. The jejunum which represents the largest segment of the small intestine (approximately 90% of the total length) has a mean pH of 5.5 to 6.9 and the ileum has a mean pH of 7.0 to 7.4 (Mroz et al., 1997).

Phytase Effects on P Digestibility. Approximately, 60 to 70% of the P found in plant ingredients commonly used for pigs is bound as phytate P, and is therefore unavailable for

absorption (Cromwell, 1992; Ravindran et al., 1994, 1995). Nelson et al. (1968) demonstrated the ability of phytase release this bound P. However, it has not been until recently that a commercially available phytase preparation was approved for use by producers. Natuphos® phytase has been approved for use in Europe since 1991, and was approved for use in the U. S. on November 17, 1995. Addition of microbial phytase has been shown to catalyze the hydrolysis of the phytate molecule, releasing the bound P (Jongbloed et al., 1992; Cromwell et al., 1993; Lei et al., 1993b, Kornegay et al., 1995, 1996; Jongbloed, 1996).

In pigs, microbial phytase supplementation of low P diets has been shown to affect pig performance by increasing average daily gains (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c) primarily due to an increased feed intake (Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996; Yi et al., 1996c). Increases in bone breaking strength or shear force have also been demonstrated in several studies with pigs (Cromwell et al., 1993; Ketaert et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996c). The addition of microbial phytase also decreases P excretion in the range of 25 to 50% (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993a; Lei et al, 1993b; Kornegay and Qian, 1996; Yi et al., 1996c) by increasing P digestibility or retention (Hoppe et al., 1992; Lei et al, 1993a,b; Mroz et al., 1994; Kornegay and Qian, 1996; Yi et al., 1996c).

Similar results to those observed in pigs have been demonstrated in broilers when microbial phytase is supplemented to the diet. An increased BW gain in broilers observed when phytase is added (Denbow et al., 1995; Qian et al., 1996; Yi et al., 1996a,b) can be primarily attributed to an increased feed intake as the level of phytase in the diet was increased (Denbow et al., 1995; Qian et al., 1996; Yi et al., 1996a,b). Increases in tibia shear force (Denbow et al., 1995; Qian et al., 1996) and toe (Denbow et al., 1995; Yi et al., 1996a,b) or tibia (Qian et al., 1996; Yi et al., 1996b) ash have also been observed in broilers fed diets supplemented with microbial phytase. Yi et al. (1996) reported a decreased P excretion in phytase supplemented broilers which could be attributed to an increased P retention (Schöner and Hoppe, 1992; Yi et al., 1996b).

P Equivalency Values of Phytase. In order for producers to efficiently use microbial phytase as a supplement, accurate equivalency values of phytase for P must be developed. Ideally, studies designed to develop equivalency values should feed multiple levels of P without added phytase and multiple levels of phytase at a low level of P in order to develop response equations for both P and phytase. These response equations can then be set equal to one another to determine the P equivalency of phytase. In general, linear ($Y = a + bX$; where Y = response and X = the level of P or phytase) or asymptotic ($Y = a(1 - be^{-kX})$; where Y = response and X = the level of P or phytase) curves have provided the best fits for phytase and phosphorus responses in corn-soybean meal based diets. Jongbloed et al. (1996) reported that a logistic curve provided a better fit to the response of P and phytase in a Dutch practical diet. Several studies have attempted to determine the P equivalency value of phytase in broilers (Table 4; Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996b) and in pigs (Table 5; Kornegay and Qian, 1996,

Jongbloed et al., 1996; Yi et al., 1996c; Harper et al., 1997). The P equivalency value for broilers for 500 U/kg phytase ranges from 0.207 g to 0.458 g of P from inorganic sources. In pigs the range of equivalency values for 500 U/kg phytase is much larger ranging from 0.64 g P to 2.47 g P. Factors which may influence these equivalency value estimates include: the basal level of P, the response criteria used, and perhaps most importantly the ratio of Ca to P. Phosphorus absorption has been shown to be impaired if the Ca:P ratio is too wide (NRC, 1988). In addition, Qian et al. (1996a) reported a detrimental effect of a widening Ca:P ratio in excess of 1.2:1 on phytase efficacy in pigs. In a similar study with broilers, Qian et al. (1996b) reported that widening the Ca:P ratio from 1.4:1 to 2.0:1 decreased the efficacy of microbial phytase. Excess Ca may bind to the phytate molecule, making it insoluble and therefore, unavailable for exposure to phytase in the gastrointestinal tract. In the studies which investigated the equivalency values of microbial phytase for P in broilers which were reported above (Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996b), the Ca:P ratio in all cases was 2:1 except for the positive control diet in the study by Ravindran et al. (1995) where the Ca:P ratio was 1.46:1. In the pig studies of Kornegay and Qian (1996), Jongbloed et al. (1996), and Yi et al. (1996c) only two levels of P were fed, so the response of various criteria to P was assumed to be linear. In addition the Ca:P ratio in the studies of Kornegay and Qian (1996) and Yi et al. (1996c) was 2:1. In the study by Jongbloed et al. (1996) the Ca:P ratio ranged from 1.94:1 to 2.5:1. Harper et al. (1997) in a study with growing-finishing pigs utilized 3 levels of P and maintained a Ca:P ratio of approximately 1.2:1 to 1.4:1 in all diets. They reported that on average 500 U of microbial phytase releases .96 g of P per kilogram of diet.

Phytase Effects on Calcium. Phytic acid, at a neutral pH carries one or two negatively charged oxygen atoms per phosphate group (Erdman, 1979). Therefore, each molecule of phytase carries 6 to 12 negative charges at or near a neutral pH, like that found in the jejunum and ileum. Therfore, cations can be bound by phytate in the small intestine rendering them unavailable for absorption. Addition of microbial phytase to the diet results in the stepwise hydrolysis of PO_4 from the inositol ring of phytate. This prevents the formation of cation-phytate salts in the small intestine.

Calcium was first associated with phytate by McCance and Widdowson in 1935 (Oberleas and Harland, 1996). Nelson et al. (1968) showed that as the level of phytic

Table 4. P equivalency equations reported in the literature for broilers.

Response Criteria	Diet Type[a]	Equation[b]	P Equivalency (g/kg)	Reference
BW Gain	SP	$Y = -9.615Ln(0.9662 + 0.0153e^{-0.0037X})$	.307	Denbow et al., 1995[c]
	SP	$Y = -9.615Ln(0.9643 + 0.0101e^{-0.0057X})$	.344	Denbow et al., 1995[d]
	SP	$Y = -0.112Ln(-0.058 + 0.150e^{-0.0005X})$	.315	Yi et al., 1996b
	CS	$Y = -0.172Ln(0.004 + 0.206e^{-0.0011X)}$	.362	Yi et al., 1996b
	SP	$Y = -0.0559Ln(0.000713 + 0.0073e^{-0.0046})$	.366	Ravindran et al., 1995[d]
	SP	$Y = -0.0559Ln(-0.00098 + 0.00227e^{-0.0007X})$	.413	Ravindran et al., 1995[e]
Toe Ash, %	SP	$Y = -0.4587Ln(0.4538 + 0.1875e^{-0.0013X})$	.273	Denbow et al., 1995[c]
	SP	$Y = -0.4587Ln(0.4419 + 0.1114e^{-0.0032X})$	.352	Denbow et al., 1995[d]
	SP	$Y = -0.126Ln(0.123 - 0.00007X)$	.306	Yi et al., 1996b
	CS	$Y = -0.122Ln(0.069 + 0.039e^{-1.01X})$	.326	Yi et al., 1996b
	SP	$Y = -0.1745Ln(0.08854 + 0.12187e^{-0.0022X})$	.357	Ravindran et al., 1995[d]
	SP	$Y = -0.1745Ln(0.06728 + 0.08505e^{-0.0056X})$	.458	Ravindran et al., 1995[e]

[a]SP = semipurified diet and CS = corn-soybean meal based diet.
[b]Y = digestible P (g/kg) and X = phytase activity (U/kg).
[c]Equations based on a basal diet containing .20% nP.
[d]Equations based on a basal diet containing .27% nP.
[e]Equations based on a basal diet containing .36% nP.

Table 5. P equivalency equations reported in the literature for pigs.

Response Criteria	Diet Type[a]	Equation[b]	P Equivalency (g/kg)	Reference
ADG	SP	$Y = 3.41 - 3.07e^{-.0003X}$	.80	Yi et al., 1996c[c]
	SP	$Y = 1.68 - 2.17e^{-.0016X}$	.70	Yi et al., 1996c[d]
	CS	$Y = 4.062 - 3.865e^{-0.00095X}$	1.66	Kornegay and Qian, 1996[e]
	CS	$Y = 3.362 - 3.380e^{-0.00266X}$	2.47	Kornegay and Qian, 1996[d]
	CS	$Y = 0.0654 - 0.0741e^{-0.00839X}$	.64	Harper et al., 1997
10^{th} Rib Ash %	SP	$Y = 1.03 - 1.00e^{-.0015X}$	.56	Yi et al., 1996c[c]
	SP	$Y = 1.06 - 1.09e^{-0.0014X}$	.52	Yi et al., 1996c[d]
	CS	$Y = 1.848 - 1.926e^{-0.0045X}$	1.65	Kornegay and Qian, 1996[e]
	CS	$Y = 1.629 - 1.806e^{-0.0036X}$	1.33	Kornegay and Qian, 1996[d]
10^{th} Rib Shear Force	CS	$Y = 0.348 - 0.357e^{-0.00082X}$	1.11	Harper et al., 1997
Digestible P	CS	$Y = 1.01 - 1.0013 \times 0.9963^{X}$	.85	Jongbloed et al., 1996
	Dutch	$Y = -0.1786 + 1.31/(1 + e^{(-0.0051 \times (X - 378))})$	.67	Jongbloed et al., 1996
P Digestibility	SP	$Y = 1.30 - 1.21e^{-.0019X}$	.83	Yi et al., 1996c[c]
	SP	$Y = 1.31 - 1.51e^{-.0036X}$	1.10	Yi et al., 1996c[d]
	CS	$Y = 2.631 - 2.965e^{-0.00108X}$	1.19	Kornegay and Qian, 1996[e]
	CS	$Y = 1.564 - 1.735e^{-0.00284X}$	1.14	Kornegay and Qian, 1996[d]
	CS	$Y = -0.087Ln(-6.718 + 7.713e^{-0.000199X})$	1.16	Harper et al., 1997

[a]SP = semipurified diet, CS = corn-soybean meal based diet, and Dutch = Dutch practical diet.
[b]Y = digestible P (g/kg) and X = phytase activity (U/kg)
[c]Equations based on a basal diet containing .05% aP.
[d]Equations based on a basal diet containing .16% aP.
[e]Equations based on a basal diet containing .07% nP.

acid in the diet increased so did the chick's requirement for Ca and that this effect decreased when phytase was added to the diet. Several studies have shown an improved Ca retention in broilers (Schöner et al., 1991, 1993; Kornegay et al., 1996; Yi et al., 1996b) and an improved Ca digestibility in pigs (Mroz et al., 1993; Yi et al., 1996a; Kornegay and Qian, 1996). Jongbloed et al. (1996) suggested that the addition of 500 U/kg of microbial phytase to pig diets was equivalent to 0.4 to 0.7 g of digestible Ca per kg of diet. In broilers, Kornegay et al. (1996) reported that 500 U/kg of microbial phytase was equivalent to .87 g of total Ca. In their study, BW gain provided the highest equivalency estimate (500 U = 1.2 g/kg Ca), followed by gain:feed (500 U = 0.7 g/kg Ca) and digestible Ca (500 U = 0.7 g/kg Ca). Schöner et al. (1994) reported, in broilers, that 500 U of microbial phytase was equivalent to .35 g/kg of total Ca as measured by BW and .56 g/kg of total Ca as measured by phalanx ash.

Phytase Effects on Other Minerals. Phytate has been shown to bind divalent cations in the following preferential order: $Cu^{++} > Zn^{++} > Co^{++} > Mn^{++} > Mg^{++} > Fe^{++} > Ca^{++}$ (Maddaiah et al., 1964; Vohra et al., 1965). Of the trace minerals, Zn has probably received the most attention. Lei et al. (1993c) reported that when phytase was added to the diet, the bioavailability of Zn was increased. Roberson and Edwards (1994) found no effect on Zn retention when microbial phytase was added to broiler diets. Several other studies have shown improvements in Zn status when microbial phytase was supplemented to the diet of pigs (Pallauf et al., 1992; Nasi and Helander, 1994), broilers (Biehl et al., 1995; Yi et al., 1996a) and rats (Rimbach and Pallauf, 1993; Rimbach et al., 1995). Yi et al. (1996) fed four levels of Zn (0, 5, 10, and 20 ppm) with no added phytase and four levels of phytase (150, 300, 450, and 600 U/kg) with no added Zn to broilers so that response equations to phytase and Zn could be developed. The equivalency equation of phytase for Zn was $Y = 0.20 + 0.0082X$; where Y = mg/kg of Zn and X = U/kg of phytase. Based on this equation, 100 U/kg of phytase releases 0.9 mg/kg of Zn. Pallauf et al. (1992) and Nasi and Helander (1994) both reported increases in the apparent absorption of Mg, Fe, and Cu when microbial phytase was added to pig diets.

Phytase Effect on Heavy Metals. The two major elements of interest here are lead and cadmium, both of which have been shown to interact with phytic acid (Wise and Gilburt; 1981; Nolan and Duffin, 1987; Rimbach et al., 1994). Cadmium has received the most attention thus far. Cadmium is a toxic element which builds up over time in the liver and kidney. Dietary phytate has been shown to increase the level of cadmium in the liver and kidney (Rambeck and Walther, 1993; Rimbach et al., 1995). Supplementation of microbial phytase to the diet has been shown to reduce Cd accumulation in rats (Rambeck and Walther, 1993; Rimbach et al., 1995) and Japanese quail (Rambeck and Walther, 1993). However, Rimbach et al. (1996) found that in pigs supplementation of microbial phytase to the diet caused an increase in the concentration of Cd in the liver and kidney. It remains unclear whether the mode of action in pigs is different, or whether the different effects of phytase on Cd accumulation seen in pigs compared to other species is simply due to dietary or study duration differences.

Phytase Effects on Proteins. At a low to neutral pH, the anionic phosphate groups of phytic acid have been shown to possess the ability to bind proteins (Cosgrove, 1980; Prattley and Stanley, 1982; Anderson, 1985; Thompson, 1986) and amino acids; having its greatest affinity for the basic amino acids lysine, arginine, and histidine (Reddy et al., 1982). In addition, phytate may complex with proteases (Singh and Krikorian, 1982) in the gastrointestinal tract, thereby decreasing the activity of these enzymes.

By catalyzing the stepwise hydrolysis of phytate, microbial phytase has the potential to increase protein digestibility by preventing the formation of insoluble phytate-protein complexes in the small intestine. Officer and Batterhan (1992) demonstrated an increased ileal digestibility of crude protein and some amino acids by 7 to 12% when microbial phytase was added to the diet. Khan and Cole (1993) and Mroz et al. (1991) also found an increase in ileal crude protein digestibility of 12.8% and 3.5%, respectively. However, Kemme and Jongbloed (1993a,b,c) and Nasi (1990) found no effect of adding microbial phytase on total tract protein digestibility. More recently, Kemme et al. (1995) found an increase in ileal digestibility of amino acids and Jongbloed et al. (1995) and Christensen and Nielson (1995) demonstrated an increase in apparent total tract digestibility of nitrogen. However, Lantzsch and Drochner (1995) showed no improvement in N digestibility when microbial phytase was added to the diets of breeding sows. Addition of phytase has been shown to improve the apparent N absorption in pigs (Kornegay and Qian, 1996; Yi et al., 1996c) and laying hens (Van der Klis and Versteegh, 1991) and the apparent N retention in broilers (Yi et al., 1996b). Yi et al. (1996d) found that adding 750 U/kg of microbial phytase to the diets of turkey poults increased the ileal N and amino acid digestibility and the apparent retention of N.

Phytase and Organic Acids. Microbial phytase has two optimal pH levels of activity. The first at pH 2.5 and the second at pH 5.0 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). The primary site of phytase activity has been shown to be the stomach where the pH is between 1 and 4 (Mroz et al., 1997). Therefore, it is possible that the pH of the stomach may be greater than the optimal pH level of 2.5 for maximum phytase activity. If this is the case then lowering of the gastric pH might enhance the efficacy of microbial phytase. Scipioni et al. (1978) demonstrated a lowered gastric pH when citric acid was added to the diet. Jongbloed et al. (1996) reported the results of two experiments which they conducted, investigating the effects of adding organic acids to the diet on phytase efficacy. In experiment 1, the effects of lactic acid on phytase efficacy were investigated, and in experiment 2 the effects of lactic, formic, or propionic acid on phytase efficacy were investigated. In experiment 1, they found a lactic acid by phytase interaction on P digestibility. This synergistic effect caused an increase in digestible P of 0.24 g/kg. In experiment 2, no interactive effects of lactic acid or propionic acid were found. However, there was a synergetic effect of formic acid and phytase on P digestibility. This resulted in an increase in digestible P of 0.20 g/kg.

Addition of organic acids to pig diets appears to be capable of causing decreased gastric pH and possibly a decreased intestinal pH. Therefore, if added at the proper level to the diet, organic acids, may provide a means by which the efficacy of microbial phytase can be improved.

However, data in the literature investigating the effects of microbial phytase in conjunction with organic acids are scarce and that which does exist provides inconsistent results.

Chapter III
Phosphorus Equivalency Values of Microbial Phytase in Weanling Pigs Fed a Corn-Soybean Meal Based Diet

ABSTRACT. Ninety-six crossbred pigs (equal barrows and gilts) with an average initial weight of 10.3 kg were used in a 4-wk experiment to investigate the P equivalency value of microbial phytase in weanling pigs using performance, rib mineralization and fecal digestibility measurements. A 19% CP, corn-soybean meal diet low in P and Ca was fed. Diets 1, 2, 3, and 4 contained 3.5 g of total P/kg of diet and 0, 167, 333, and 500 units (U) of added Natuphos® phytase/kg of diet, respectively. Diets 5, 6, and 7 contained no added phytase, and 4.0, 4.5, and 5.0 g of P/kg of diet, respectively. Calcium was maintained in all diets at a level of 5.0 g/kg in an attempt to maintain a more optimal Ca:P ratio. Body weight and pen feed consumption were measured weekly. During wk 4, pen fecal samples were collected twice daily for 5 d for determination of P, Ca and DM digestibilities. At the end of wk 4, the barrow from each pen (n = 48) was killed for collection of tenth ribs for determination of rib shear force, shear energy and ash content. Adding phytase to low P diets linearly increased ADG ($P < .001$), rib shear force ($P < .01$) shear energy ($P < .02$), ash weight (linear effect, $P < .001$; quadratic effect, $P < .03$) and ash percent ($P < .001$), Ca ($P < .001$) and P ($P < .001$) digestibility and digestible Ca ($P < .001$) and P ($P < .001$). Added P linearly increased ADG ($P < .003$), rib shear force ($P < .003$) energy ($P < .001$), ash weight ($P < .001$) and ash percent ($P < .01$), Ca ($P < .02$) and P (linear effect, $P < .001$; quadratic effect, $P < .02$) digestibility and digestible Ca ($P < .02$) and P (linear effect, $P < .001$; quadratic effect, $P < .005$). Based on phytase and P linear or nonlinear response equations for ADG, rib shear force, energy, and ash weight, P digestibility, and digestible P, 500 U/kg of microbial phytase is equivalent to .99, .69, .53, .71, .82, and .96 g of inorganic P, respectively. The average equivalency of 500 U/kg of phytase was .78 g of P per kg of diet.

Key Words: Pigs, Phytase, Phosphorus, Digestibility

Introduction

Sixty to seventy percent of the P in plant ingredients, commonly used in pig diets, is bound as phytate P (Cromwell, 1992; Ravindran, 1994, 1995) which is unavailable to the pig. Several studies have demonstrated that the addition of microbial phytase hydrolyzes the phytate molecule, improving P digestibility and therefore decreasing P excretion by 25-50% (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993a).

In order for commercial swine producers to be able to take advantage of these beneficial effects of microbial phytase, P equivalency values must be developed. Several studies have attempted to do this in broilers (Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996a) and in pigs (Kornegay and Qian, 1996; Jongbloed et al., 1996; Yi et al., 1996b; Harper et al., 1997). However, in many of these studies diets containing wide Ca:P ratios (Denbow et al., 1995; Kornegay and Qian, 1996; Jongbloed et al., 1996; Ravindran et al., 1995; Yi et al., 1996a; Yi et al., 1996b) were fed, which have been shown to decrease the efficacy of microbial phytase (Qian et al., 1996a,b). In addition, several of the studies fed only two levels of P (Kornegay and Qian, 1996; Jongbloed et al., 1996; Yi et al., 1996b) which assumes that the P response is linear. Therefore, the variability seen in P equivalency values is large ranging from .31 g to .46 g per 500 U/kg phytase in poultry and from .52 g to 1.66 g per 500 U/kg phytase in pigs.

This study was designed to better delineate the P equivalency value of phytase by feeding multiple levels of phytase and P, while maintaining a narrow Ca:P ratio, and by focusing on the linear response surfaces of both phytase and P.

Materials and Methods

Ninety-six crossbred pigs (equal barrows and gilts) were used in a 4-wk experiment to investigate the P equivalency value of microbial phytase.

Dietary Treatments. A 19% CP corn-soybean meal diet fortified with vitamins and minerals to meet or exceed NRC (1988) requirements (except Ca and P) was fed in the 4-wk trial. Calcium was fed at a level of 5 g/kg of diet for all treatments in order to maintain a more desirable Ca to P ratio (Table 1).

Diets 1, 2, 3, and 4 contained 3.5 g of total P/kg of diet (no added P) and 0, 167, 333, and 500 U of added Natuphos® phytase/kg of diet, respectively. Diets 5, 6, and 7 contained no added phytase, and 4.0, 4.5, and 5.0 g of total P/kg of diet, respectively (Table 2). Twice as many pigs were fed diet 1 (n = 24) to insure a reliable baseline for phytase and P response curves. A chromic oxide-starch premix (1 part Cr_2O_3:3 parts starch (wt:wt)) was added to all diets during wk 3 and 4 at a level of 0.2% (0.05% Cr_2O_3) as an indigestible indicator.

Table 1. Composition of basal diet.

Ingredients	%
Corn	74.00
Soybean meal, 48.5%	23.20
Vitamin premix[a]	.25
Trace mineral premix[b]	.10
Selenium premix[c]	.05
Limestone[d]	.80
Salt	.30
L-lysine (78%)	.10
Calculated Composition	
CP	17.5
Lysine	.98
P	.356
Ca	.389

[a]Supplied per kilogram of diet: 4400 IU of Vitamin A, 440 IU of Vitamin D_3, 11 IU of Vitamin E, 2.2 mg of Vitamin K, 4.4 mg of riboflavin, 22 mg of calcium pantothenate, 22 mg of niacin, .022 mg of vitamin B_{12}, 440 mg of choline chloride, .44 mg of biotin, 3.9 mg of folic acid, 10 mg of thiamin•HCl, 3.9 mg of pyridoxine•HCl, 82.5 mg of ethoxyquin and 3.6 mg of virginiamycin.
[b]Supplied per kilogram of diet: 44 mg of manganese, 47.5 mg of zinc, 50 mg of iron, 6.25 mg of copper, and 2 mg of iodine.
[c]Supplied .3 mg selenium per kilogram of diet.
[d]Limestone (Limestone Dust Corp., Bluefield, VA).

Table 2. Dietary treatments.

	Phytase,	Calculated		Analyzed	
Treatments	U/kg	Ca, g/kg	P, g/kg	Ca, g/kg	P, g/kg
1	0	5.0	3.5	5.32	3.28
2	167	5.0	3.5	5.32	3.28
3	333	5.0	3.5	5.32	3.28
4	500	5.0	3.5	5.32	3.28
5	0	5.0	4.0	5.32	3.85
6	0	5.0	4.5	5.32	4.35
7	0	5.0	5.0	5.32	4.57

Animal and Feeding Management. The pigs were weaned at an average weight of 7.3 kg and given a 10-d adjustment before treatments were started. During the adjustment period pigs

were fed a diet containing 22% CP (Merrick's Soweena Day 14, Merrick's, Inc., Middleton, WI) for 3 d followed by a 20% CP starter diet containing 10% dried whey and 0.10% mecadox for the remaining 4 d. Following the adjustment period pigs were randomly assigned to treatments (6 replicate pens of one barrow and one gilt each, except diet 1 which had 12 replicate pens) from outcome groups based on gender and body weight. Littermates were balanced across treatments. The average BW was 10.2 kg at the start of treatments.

The pigs were housed in two similar environmentally controlled rooms with 24 pens in each room. Room temperature was initially set at 29° C and was lowered about 2° C per week after the second week. A continuous lighting regimen and recommended air ventilation rates (Murphy et al., 1990) were maintained. Pigs had ad libitum access to feed and water at all times. The care and treatment of the animals followed published guidelines (Consortium, 1988).

Sampling and Analysis. Body weight and pen feed consumption were measured weekly. During wk 4, pen fecal samples were collected twice daily (0700 and 1700) for 5 d and frozen at -20° C in sealed plastic bags until drying at 65° C in a forced air oven. The dried fecal samples and samples of diets were ground to pass through a 1 mm sieve.

Diet and fecal samples were analyzed for Ca, P, and Cr, following nitric-perchloric acid (5:3, vol/vol) wet digestion. Total P concentrations were assayed photometrically by the vanadomolybdate procedure (AOAC, 1990) and Ca and Cr were determined with an atomic absorption spectrophotometer (model 5100 PC, Perkin Elmer, Norwalk, CT) using the manufacturer's recommendations. Dry matter was also determined for diet and fecal samples according to standard AOAC (1990) procedures. Phosphorus, Ca and DM digestibilities were calculated using the indirect method.

At the end of wk 4, the barrow from each pen (6 pigs/treatment except diet 1 which had 12 pigs) was killed for collection of tenth ribs. The tenth ribs were cleaned of all tissues and used for determination of shear force and shear energy as described by Combs et al. (1991). Bone ash was determined in a muffle furnace at 600° C for 12 h.

Statistical Analysis and Calculation of Equivalency Values. Data were separated into two groups, with phytase and with P, and each was analyzed using the GLM procedures of SAS (1990). The model included replicate, phytase or P, and the two-way interactions with replicate. Linear and quadratic contrasts were determined for phytase and P levels. Nonlinear and linear equations of the effects of varying P (no added phytase) and varying phytase levels on various measurements were derived (calculated values of P and phytase were used). The P equivalency values of phytase were obtained by setting the equations for phytase and P equal and solving as described by Yi et al. (1996a).

Results

Phytase Effects. Average daily gain ($P < .001$) during wk 3-4 and overall average daily feed intake ($P < .05$) linearly increased as the level of phytase in the diet increased (Table 3). ADG during wk 1-2 quadratically increased ($P < .04$) as the level of phytase in the diet was increased. The overall linear effect of phytase on ADG in wk 1-4 was significant ($P < .001$) with a 73 g increase in ADG at the highest level of phytase supplementation compared with the basal diet. The addition of phytase tended to linearly decrease ($P = .09$) the amount of P excreted per day (Table 3). Rib shear force ($P < .01$), shear energy ($P < .02$), and ash percent ($P < .001$) linearly increased as phytase was added to the diet (Table 4). Ash weight was quadratically increased ($P < .03$) as the level of phytase added to the diet was increased. Linear increases were also observed for Ca ($P < .001$) and P ($P < .001$) digestibilities and digestible Ca ($P < .001$) and P ($P < .001$) as the level of supplemental phytase in the diet was increased (Table 5). No effect was seen on DM digestibility ($P = .15$).

Phosphorus Effects. Average daily gain quadratically increased in wk 1-2 ($P < .003$) and linearly increased in wk 3-4 ($P < .002$) as the level of P added to the diet increased (Table 3). The overall linear effect of P on ADG in wk 1-4 was significant ($P < .003$) with a 83 g increase in ADG at the highest level of P supplementation compared with the basal diet. The addition of P had no effect on mean daily feed intake ($P = .45$), but the amount of P excreted per day was linearly increased (Table 3; $P < .03$). Rib shear force ($P < .003$), shear energy ($P < .001$), ash weight ($P < .001$), and ash percent ($P < .01$) linearly increased as P was added to the diet (Table 4). The addition of P to the basal diet also linearly improved Ca ($P < .02$) digestibility and digestible Ca ($P < .02$; Table 5). Phosphorus digestibility ($P < .02$) and digestible P ($P < .005$) were quadratically improved with phytase addition. No effects were seen on DM digestibility ($P = .19$).

ADG during wk 1-4, rib shear force, shear energy and ash weight, P digestibility and digestible P were all responsive to the effects of phytase and P; therefore, the nonlinear or linear equation for each main effect with the highest r^2 value was chosen for calculation of P equivalency values of phytase (Table 6). If both linear and nonlinear

Table 3. Effects of microbial phytase and inorganic P additions on ADG, ADFI (wk 1-4), and P excretion of weanling pigs fed a P deficient corn-soybean meal diet[a]

	P	Phytase	ADG, g/d			ADFI	P excreted
Trt.[b]	%	U/kg	wk 1-2	wk 3-4	wk 1-4	g/d	g/d
Phytase effect							
1	.35	0	267	460	363	866	2.58
2	.35	167	322	496	409	906	2.46
3	.35	333	316	517	417	998	2.48
4	.35	500	306	566	436	948	2.33
MSE[c]			37	44	28	.087	.025
C.V., %			12.4	8.8	7.0	9.5	10.0
Probability Values							
Trt.			.03	.002	.001	.047	.32
Lin Trt.			.10	.001	.001	.049	.09
Quad Trt.			.04	.71	.26	.16	.70
P Effect							
1	.35	0	267	460	363	866	2.58
5	.40	0	283	512	398	879	2.65
6	.45	0	366	512	439	915	2.80
7	.50	0	284	605	445	886	2.94
MSE[c]			25	45	31	.093	.024
C.V., %			8.4	8.9	7.7	10.5	9.0
Probability Values							
Trt.			.003	.009	.01	.69	.12
Lin Trt.			.02	.002	.003	.45	.026
Quad Trt.			.003	.30	.27	.50	.78

[a]Each treatment mean represents 6 pens or 12 pigs, except diet 1 that has 12 pens or 24 pigs.
[b]All diets contained 5 g/kg Ca.
[c]SEM = Mean Square Error (MSE)/$\sqrt{n}$, where n = 6 or 12.

Table 4. Effects of microbial phytase and inorganic P additions on tenth rib bone mineralization of weanling pigs fed a P deficient corn-soybean meal diet[a]

Trt.[b]	P %	Phytase U/kg	Ash Weight, g	Bone Ash, %	Shear Force, N	Energy N*mm
Phytase Effect						
1	.35	0	.606	39.5	442	398
2	.35	167	.745	41.2	488	467
3	.35	333	.851	42.0	500	497
4	.35	500	.878	45.4	580	504
MSE[c]			.061	2.36	85.9	77.1
CV, %			8.3	5.7	17.5	16.9
Probability Values						
Trt.			.001	.003	.06	.05
Lin Trt.			.001	.001	.01	.02
Quad Trt.			.03	.37	.62	.33
P Effect						
1	.35	0	.606	39.5	442	398
5	.40	0	.843	43.4	523	508
6	.45	0	.954	45.4	627	651
7	.50	0	1.132	47.3	702	731
MSE[c]			.055	2.65	98.5	74.2
CV, %			6.5	6.1	17.9	13.6
Probability Values						
Trt.			.001	.01	.02	.001
Lin Trt.			.001	.01	.003	.001
Quad Trt.			.25	.44	.98	.61

[a]Each treatment mean represents 6 pens or 12 pigs, except diet 1 that has 12 pens or 24 pigs.
[b]All diets contained 5 g/kg Ca.
[c]SEM = MSE/$\sqrt{n}$, where n = 6 or 12.

Table 5. Effects of microbial phytase and inorganic P additions on P, Ca, and DM digestibility (%), and digested (g/kg) P and Ca of weanling pigs fed a P deficient corn-soybean meal diet[a]

Trt.[b]	P %	Phytase U/kg	Ca dig[c] %	P dig[c] %	DM dig %	DCa[d] %	DP[d] %
Phytase Effect							
1	.35	0	54.2	21.5	87.3	.34	.08
2	.35	167	62.7	28.3	87.2	.38	.11
3	.35	333	64.6	34.3	87.7	.40	.13
4	.35	500	67.3	35.4	86.4	.41	.13
MSE[e]			6.4	4.9	.94	.039	.019
CV, %			10.5	17.3	1.1	10.5	17.3
Probability Values							
Trt.			.003	.001	.13	.003	.001
Lin Trt.			.001	.001	.15	.001	.001
Quad Trt.			.49	.15	.12	.31	.17
P Effect							
1	.35	0	54.2	21.5	87.3	.34	.08
5	.40	0	63.5	32.0	86.1	.39	.14
6	.45	0	68.3	39.1	86.7	.42	.20
7	.50	0	66.5	37.1	85.9	.41	.20
MSE[e]			7.1	4.9	1.26	.044	.019
CV, %			11.5	16.2	1.5	11.6	13.1
Probability Values							
Trt.			.04	.001	.27	.04	.001
Lin Trt.			.02	.001	.19	.02	.001
Quad Trt.			.10	.02	.76	.11	.005

[a]Each treatment mean represents 6 pens or 12 pigs, except diet 1 that has 12 pens or 24 pigs.
[b]All diets contained 5 g/kg Ca.
[c]Digestion coefficient for Ca or P as a percentage
[d]Digestible Ca or P as a percent of the diet.
[e]SEM = MSE/$\sqrt{n}$, where n = 6 or 12.

Table 6. Linear and Non-linear response equations for phytase and phosphorus, and the equivalency values of phytase for phosphorus based on P digestibility (Pdig), digested P (DP), tenth rib ash weight (AshW), shear force, and energy, and mean ADG from weeks 1-4.

	Phytase			Phosphorus			P Equivalency[c] of
Response Criteria	Response Equations[a]	r^2	P value	Response Equations[b]	r^2	P value	500 U Phytase
Linear							
Pdig (%)	$21.4047 + .0275X_1$	.81	.0002	$22.6774 + 11.8847X_2$	.71	.0005	1.05
DP (g/kg)	$.0789 + .0001X_1$	.81	.0002	$.0859 + .02693X_2$	.82	.0001	1.59
AshW (g)	$.6410 + .0005X_1$	.92	.0001	$.6376 + .3320X_2$	.98	.0001	.76
Shear Force (N)	$437.8208 + .2517X_1$	.84	.02	$442.0917 + 175.7667X_2$	.99	.003	.69
Energy (N*mm)	$415.3067 + .2147X_1$	.90	.02	$403.3167 + 226.05X_2$	.99	.0002	.53
Non-Linear							
ADG, wk 1-4	$.9607 - .1519e^{-.0050X_1}$	.98	.0003	$1.067 - .262e^{-.799X_2}$	.97	.003	.99
Pdig (%)	$34.65 - 15.3742e^{-.0053X_1}$	.95	.0002	$38.9953 - 19.8018e^{-2.1556X_2}$	.90	.0005	.60
DP (g/kg)	$.1277 - .0567e^{-.0053X_1}$	.95	.0002	$.2089 - .1395e^{-1.5190X_2}$	.93	.0001	.32
AshW (g)	$.9264 + .3125e^{-.0036X_1}$	.98	.0001	$1.9028 - 1.2791e^{-.3291X_2}$	.98	.0001	.66

[a]Where X_1 = U of phytase

[b]Where X_2 = g of phosphorus

[c]Equivalency expressed as grams of P released per 500 U of added Natuphos® phytase

equations provided good fits ($R^2 > .75$), then they were both used to calculate equivalency values and the average value is reported. The P equivalency values of phytase were obtained by setting the equations for phytase and P equal and solving. Equivalency values for 500 U of phytase/kg of diet based on ADG in wk 1-4, rib shear force, shear energy and ash weight, P digestibility and digestible P were .99, .69, .53, .71, .82, and .95 g P/kg of diet, respectively. On average 500 U of phytase/kg of diet was equivalent to .78 g of P.

Discussion

Phosphorus is an essential element required by pigs for optimal growth, reproduction and bone development. Much of the P in pig diets is unavailable to the pig because it is bound as phytate P. Approximately 66% of the P in corn and 61% of the P in soybean meal is complexed in phytate P (Ravindran et al., 1994, 1995). Therefore, producers have to add large amounts of inorganic P to pig diets in order to meet the needs of the pigs which adds to the cost of the diet and results in increased P excretion which could lead to environmental pollution problems. According to the NRC (1988) a 10-20 kg pig requires an 18% CP diet containing .32% aP. A corn-soybean meal based diet formulated to contain 18% CP contains .38% tP. Theoretically, if this was all available it is enough to meet the pigs requirement for P. However, since approximately 70% of the P is unavailable to the pig, the diet contains only .114% aP and a highly available inorganic source of P must be supplemented to the diet.

Ruminants can utilize phytate P because microbes within the rumen produce the enzyme phytase which hydrolyzes the phytate molecule releasing the bound P. The potential to release P bound by phytate through the addition of phytase to broiler diets was shown nearly 30 years ago (Nelson et al., 1968). However, it has not been until recently, through advancements in genetic engineering, that an affordable method of producing microbial phytase has been available. Many studies have documented the ability of microbial phytase to enhance P digestibility and thus decrease P excretion (reviewed by Kornegay et al., 1995, 1996; Jongbloed, 1996). With commercially produced phytase now available to swine producers it is essential that accurate equivalency values of phytase for P be derived. This needs to be done for two reasons. First to minimize the cost of adding microbial phytase and second to minimize the amount of P excreted in pig manure.

As the level of phosphorus was increased in the diets with no added phytase, the amount of P excreted per day was increased. Phytase supplementation numerically decreased the amount of P excreted per day, but this effect was not significant. However, since phytase increased ADG, the amount of P excreted per unit of gain was decreased. Using the data presented in Table 3, the amount of P excreted per unit of gain can be calculated. Pigs fed the basal diet (diet 1) gained on average 460 g/d in wk 3-4 and excreted on average 2.58 g/d. This represents .561 g of P excreted per 100 g of gain. Pigs fed diet 4 which was supplemented with 500 U/kg of phytase gained on average 566 g/d in wk 3-4 and excreted on average 2.33 g/d. This represents .412 g of P excreted per 100 g of gain. Therefore, P excretion was decreased approximately 26.6% per unit of gain when pigs were fed diets supplemented with 500 U/kg of phytase. The estimated

equivalency value of 500 U/kg phytase for P derived from this study was .78 g. Which means, that pigs fed a diet with approximately 4.3 g of total P should have similar performance to the pigs fed diet 4 which contained 3.4 g total P and 500 U/kg phytase. If we use the data from Table 3 and use the same calculations as above, we estimate that pigs fed 4.0 g/kg of P and no added phytase excreted .518 g P per 100 g BW gain in wk 3-4 and pigs fed 4.5 g/kg of P and no added phytase excreted .546 g P per 100 g BW gain in wk 3-4. Therefore, adding 500 U/kg of phytase in place of this P represents between a 20.5% and a 24.5% decrease in P excretion per unit of BW gain. Similar results have been reported in the literature with many studies reporting a 25 to 50% reduction in P excretion (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993a; Lei et al., 1993b, Kornegay and Qian, 1996; Yi et al., 1996c).

The results of this study clearly demonstrate the beneficial effects of microbial phytase addition to a low P corn-soybean meal based pig diet. Growth performance, rib mineralization and P digestibility were all improved when phytase was added to the diet. This is in agreement with studies in the literature which have demonstrated increased daily gains and feed intakes(Simons et al., 1990; Beers and Jongbloed, 1992; Jongbloed et al., 1992; Kornegay and Qian, 1996, Yi et al., 1996c), increased bone breaking strength or shear force (Cromwell et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996c) and increases in P digestibility or retention (Hoppe et al., 1992; Lei et al, 1993 a,b; Mroz et al., 1994; Kornegay and Qian, 1996; Yi et al., 1996c) when pigs are fed diets supplemented with phytase.

Several studies have attempted to determine the P equivalency value of phytase in broilers (Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996b) and in pigs (Kornegay and Qian, 1996, Jongbloed et al., 1996; Yi et al., 1996c; Harper et al., 1997). The P equivalency value for broilers for 500 U/kg phytase ranges from 0.207 g to 0.458 g. In pigs the range of equivalency values for 500 U/kg phytase is much larger ranging from 0.64 g P to 2.47 g P. Factors which may influence these equivalency value estimates include: the basal level of P, the response criteria used, and perhaps most importantly the ratio of Ca to P. Qian et al. (1996a) reported a detrimental effect of a widening Ca:P ratio in excess of 1.2:1 on phytase efficacy in pigs. In a similar study Qian et al. (1996b) reported that widening the Ca:P ratio from 1.4:1 to 2.0:1 decreased the efficacy of microbial phytase in broilers. In the studies which investigated the equivalency values of microbial phytase for P in broilers which were reported above (Denbow et al., 1995; Ravindran et al., 1995; Yi et al., 1996b), The Ca:P ratio in all cases was 2:1 except for the positive control diet in the study by Ravindran et al. (1995) where the Ca:P ratio was 1.46:1. In the pig studies of Kornegay and Qian (1996), Jongbloed et al. (1996), and Yi et al. (1996c) only two levels of P were fed, so the response of various criteria to P was assumed to be linear. In addition the Ca:P ratio in the studies of Kornegay and Qian (1996) and Yi et al. (1996c) was 2:1. In the study by Jongbloed et al. (1996) the Ca:P ration ranged from 1.94:1 to 2.5:1. Harper et al. (1997) in a study with gowing-finishing pigs utilized 3 levels of P and maintained a Ca:P ratio of approximately 1.2:1 to 1.4:1 in all diets. They reported that on average 500 U of microbial phytase releases .96 g of P per kilogram of diet.

In the experiment reported here, multiple levels of phytase and P were used so that response equations could be derived for various criteria so that equivalency estimates could be made. In addition the Ca:P ratio was maintained in all phytase supplemented diets at 1.42:1 in an attempt to maintain an optimal Ca:P ratio for phytase efficacy. Because of positive linear effects of both phytase and P additions and the goodness of fit ($R^2 > .75$) of the linear or nonlinear equations, ADG, rib shear force, shear energy and ash weight, P digestibility, and digestible P were used to develop equivalency values. The range in equivalency values was from .53 g P to .99 g of P from monocalcium phosphate (MCP) being equivalent to 500 U of phytase per kg of diet with the average being .78 g P equal to 500 U of phytase per kg of diet. The P in MCP is generally considered to be 95-100% available (Baker, 1991; Soares, 1995). Therefore, based on the results of our study 500 U/kg of phytase releases .78 g digestible P. This value seems to be in agreement with the findings of Jongbloed et al. (1996) who found in several studies that 500 to 2,000 U of microbial phytase released between .8 and 1.0 g of digestible P. Equivalency values based on growth data provided the highest equivalency estimates. Digestibility data provided intermediate estimates of equivalency values, and finally bone parameters were the most conservative estimators of P equivalency values.

Results from this study also demonstrate the ability of phytase to increase the digestibility of Ca. Ca digestibility was increased by 8.5, 10.4, and 13.1% with the addition of 167, 333, and 500 U of phytase per kg of diet. Nelson et al. (1968) found that as the level of phytic acid in the diet increased, so did the chicks requirement for Ca. Several studies have reported improved Ca digestibility in pigs when microbial phytase was added to the diet (Mroz et al., 1993; Yi et al., 1996a; Kornegay and Qian, 1996). With the release of Ca in addition to P when phytase is added to pig diets, the optimal Ca:P ratio needs to be reexamined. The NRC (1988) reports that the optimal Ca:P ratio in pig diets is between 1:1 and 1.5:1 for grain-soybean meal based diets. However, when phytase was added to pig diets Qian et al. (1996a) found that P digestibility was decreased when the Ca:P ratio was increased from 1.2:1 to 1.6:1. Ideally, the Ca:P ratio should be based on available Ca and P instead on total Ca and P. However, many more studies are needed to generate a reliable data base for available Ca and P values from various grain sources. In addition, accurate equivalency values of phytase for Ca will need to be developed.

The addition of P also caused a linear increase in Ca digestibility. This may be due in part to a more favorable Ca:P ratio and possibly to a higher availability of Ca from MCP compared to limestone. The optimal Ca:P ratio in the diet of swine is between 1:1 and 1.5:1 (NRC, 1988). As the level of P was increased in the diet from 3.5 g/kg to 5.0 g/kg, the ratio of Ca:P decreased from 1.4:1 to 1:1. The more optimal Ca:P ratio found at the highest level of P supplementation may have increased Ca digestibility. Calcium was maintained in all diets at 5 g/kg. As the level of P in the diet was increased the amount of Ca contributed by MCP was increased and the amount contributed by limestone was decreased. Calcium from limestone is generally considered to be 100% available (Soares, 1995; Baker, 1991). However, the limestone used in our study contained only 33% Ca. Therefore, it contained several impurities. Substances such as Mg which alter the cation:anion ratio have been shown to decrease Ca availability (Soares, 1995). Additionally any anionic substance which can precipitate Ca out of solution as a Ca-salt also decreases Ca

availability (Soares, 1995). These factors in addition to the more optimal Ca:P ratio in the diet containing the highest level of P supplementation may have been responsible for the increased Ca digestibility seen as the level of supplemental P was increased.

Implications

The addition of microbial phytase to P-deficient weanling pig diets causes improvements in growth performance, bone mineralization, P and Ca digestibility, and decreases P excretion. Based on response equations developed in this study, 500 U/kg of phytase releases .78 g of P. Therefore, swine diets can be supplemented with microbial phytase, the amount of inorganic P added can be decreased, and the result will be equivalent performance and decreased P excretion.

Chapter IV
Calcium Equivalency Values of Microbial Phytase in Weanling Pigs Fed Corn-Soybean Meal Based Diets

ABSTRACT. Crossbred weanling pigs (equal barrows and gilts) were used in two 4-wk experiments (Exp. 1, n = 128; Exp. 2, n = 96) to determine the Ca equivalency value of Natuphos® phytase based on performance, rib mineralization and Ca digestibility measurements. A 19% CP, low Ca corn-soybean meal diet was used in both experiments. In Exp. 1, three levels of phytase (200, 400, and 600 U/kg) and three levels of Ca as limestone (4.6, 5.5, and 6.4 g/kg) were added to the basal diet that contained 3.7 g of Ca/kg of diet. In Exp. 2, three levels of phytase (167, 333, and 500 U/kg) and three levels of Ca as $CaCO_3$ (3.3, 4.0, and 4.7 g/kg) were added to the basal diet that contained 2.6 g of Ca/kg of diet. The P level was similar for all diets in Exp. 1 (5.8 g/kg) and in Exp. 2 (6.4 g/kg). Body weight and pen feed consumption were measured weekly, and pen fecal samples were collected twice daily for 5 d during wk 4. At the end of wk 4, the barrow from each pen was killed for collection of tenth ribs and ileal digesta (Exp. 2 only). In Exp. 1, added phytase linearly increased rib ash % ($P < .03$), Ca ($P < .001$) and P ($P < .001$) digestibilities, and digested Ca ($P < .001$) and P ($P < .001$), but had no effect ($P > .10$) on ADG and rib shear force and ash weight. Added Ca linearly increased ADG (wk 3-4, $P < .04$), and rib shear force ($P < .001$), ash percentage ($P < .001$) and ash weight ($P < .01$), and digested Ca ($P < .001$), but P digestibility (P = .07) and digested P (P = .08) were only numerically decreased. In Exp. 2, added phytase linearly increased ADG (wk 3-4, $P < .002$), feed efficiency (wk 3-4, $P < .02$), rib ash weight ($P < .001$), Ca total tract digestibility ($P < .001$), and Ca ($P < .001$) and P (linear effect, $P < .001$; quadratic effect, $P < .04$) ileal digestibilities. Added Ca linearly increased ADG (wk 3-4, $P < .02$), feed efficiency (wk 3-4, $P < .01$), rib ash percentage (linear effect, $P < .001$; quadratic effect, $P < .03$) and ash weight ($P < .001$), shear force ($P < .03$) and energy ($P < .008$), and total tract ($P < .001$) and ileal ($P < .001$) digestible Ca. In summary, based on phytase and Ca linear or nonlinear response equations for ADG in wk 3-4, measurements of rib mineralization, and digestible Ca, 500 U of microbial phytase was estimated to be equivalent to 1.08 g and .78 g of Ca, respectively in Exp. 1 and 2.

Key Words: Pigs, Phytase, Calcium, Digestibility, Ileal, Bone Mineralization

Introduction

Major ingredients in pig and poultry diets are seeds (cereal grains) or products from seeds (oilseed meal and grain by-products). Hence, a large portion of the P occurs as phytate, since 60 to 80% of the P is present in the form of phytic acid (Ravindran et al., 1994, 1996). The phytic acid molecule has a high P content (28.2%) and its six phosphoric acid residues have complexing potential (Figure 1) forming a variety of insoluble salts with di- and trivalent cations (Vohra et al., 1965; Oberleas, 1973).

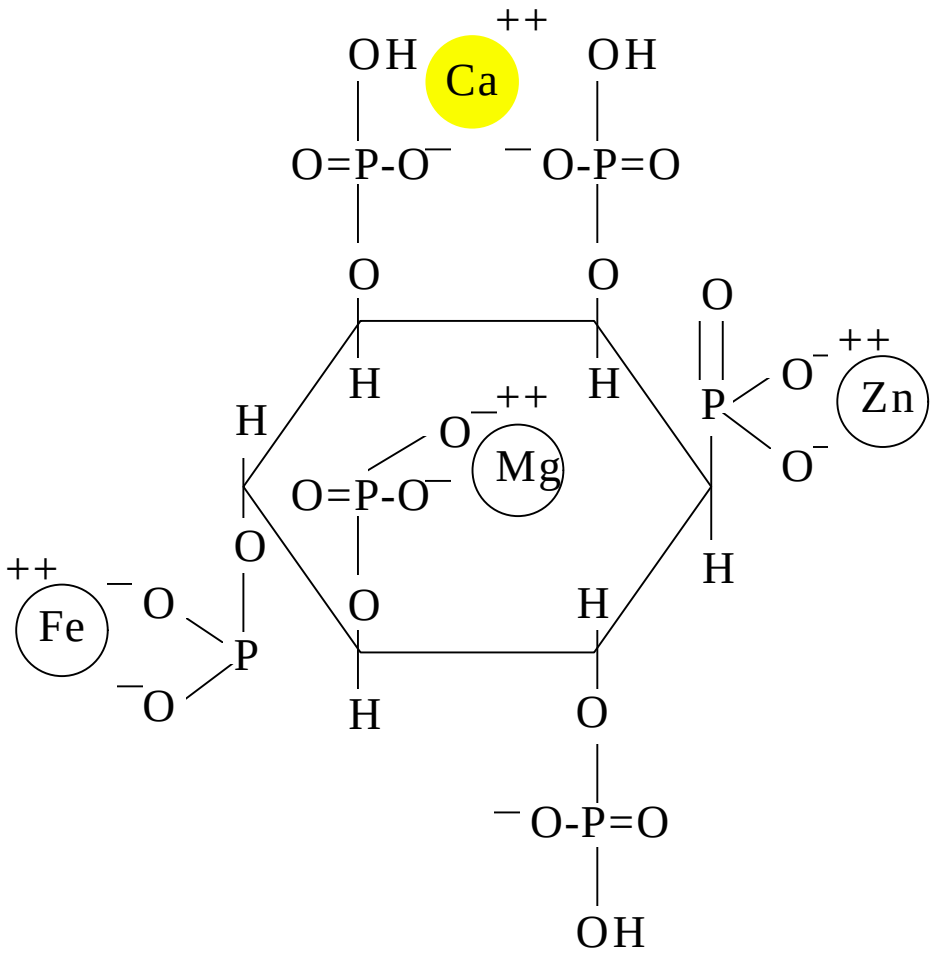

Figure 1. Potential complexing of Ca, Zn, and Mg by phytate.

At a neutral pH each phytic acid phosphate group contains either one or two negatively charged oxygen atoms (Erdman, 1979). Therefore, one mole of phytic acid can bind an average of 3 to 6 moles of Ca to form insoluble phytates at the pH of the small intestine. This binding potentially renders Ca unavailable for intestinal absorption. If a 19% crude protein corn-soybean meal diet contains .1118% or 27.95 mmoles (1,118/40) of Ca, and .27% phytate P (.924%

phytate) or 14.3 mmoles (9,240/648) of phytic acid, the ratio of Ca:phytic acid would be 1.95:1. Theoretically, all of the Ca in the plant ingredients could be bound, and perhaps some of the supplemental Ca.

Improved Ca retention of broilers has been reported when supplemental phytase was added to the diet (Schöner et al., 1991, 1993; Kornegay et al., 1996; Yi et al., 1996b). Mroz et al. (1993) reported enhanced Ca and P digestibility in 30 kg pigs when 300 and 600 U of phytase/kg were added to a basal diet containing suboptimal levels of Ca (.43%) and tP (.43%). Hypophosphaturia and hypercalciuria developed in those pigs fed the basal diet, which was prevented by adding .05% P from KH_2PO_4 or 300 U of phytase/kg. Body weight gain and gain:feed ratios were similar among treatments. Based on this limited number of studies, it does appear that phytase has the potential to make more dietary calcium available for nutritional utilization by the pig. In order for swine producers to feed optimal and not excessive levels of Ca and P and in order to maintain an optimal Ca to P ratio in swine diets, equivalency values of phytase for Ca must be developed. These two experiments were designed to determine the Ca equivalency value of microbial phytase.

Materials and Methods

Two hundred twenty-four (Exp. 1, n = 128; Exp. 2, n = 96) crossbred pigs (equal barrows and gilts) were used in two 4-wk experiments to determine the Ca equivalency value of microbial phytase in weanling pigs fed a corn-soybean meal based diet. Experimental procedures were similar for both experiments, except for some variations in dietary treatments.

Dietary Treatments. A 19 % CP corn-soybean meal based diet fortified with minerals to meet or exceed NRC (1988) requirements (except Ca) was fed in both experiments (Table 1).

Dietary treatments are shown in Table 2. In Exp. 1, diets 1, 2, 3, and 4 contained 3.7 g Ca/kg of diet and 0, 200, 400 and 600 U of added Natuphos® (BASF Corporation, Mt. Olive, NJ) phytase/kg of diet, respectively. Diets 5, 6, and 7 contained no added phytase and 4.6, 5.5, and 6.4 g of total Ca/kg of diet (0.9, 1.8, and 2.7 g/kg of added Ca), respectively. In Exp. 2, diets 1, 2, 3, and 4 contained 2.6 g Ca/kg of diet and 0, 167, 333 and 500 U of added Natuphos® phytase/kg of diet, respectively. Diets 5, 6, and 7 contained no added phytase and 3.3, 4.0, and 4.7 g of total Ca/kg of diet (0.7, 1.4, and 2.1 g/kg of added Ca), respectively. Limestone was used as the Ca source in Exp. 1 and reagent grade $CaCO_3$ was used in Exp. 2. Phosphorus, supplied from dicalcium phosphate, was maintained in all diets at 5.8 and 6.4 g/kg in Exp. 1 and 2, respectively. Twice as many pigs (Exp. 1, n = 32; Exp. 2, n = 24) were fed diet 1 to provide a good baseline. Chromium oxide-starch premix (1 part Cr_2O_3:3 parts starch (wt:wt)) was added at a level of 0.2% (0.05% Cr_2O_3) to all diets as an indigestible indicator.

Table 1. Composition of basal diet for Exp. 1 and 2.

Ingredients	%
Corn	63.60
Soybean meal (44%)	30.90
Vitamin premix[a]	.25
Trace mineral premix[b]	.10
Selenium premix[c]	.05
Dicalcium phosphate[d]	1.20
Salt	.30
Corn starch[e]	3.60
Calculated Composition	
CP	19.0
Lys	1.06
P	.379
Ca	.112

[a]Supplied per kilogram of diet: 4400 IU of Vitamin A, 440 IU of Vitamin D_3, 11 IU of Vitamin E, 2.2 mg of Vitamin K, 4.4 mg of riboflavin, 22 mg of calcium pantothenate, 22 mg of niacin, .022 mg of vitamin B_{12}, 440 mg of choline chloride, .44 mg of biotin, 3.9 mg of folic acid, 10 mg of thiamin•HCl, 3.9 mg of pyridoxine•HCl, 82.5 mg of ethoxyquin and 3.6 mg of virginiamycin.
[b]Supplied per kilogram of diet: 44 mg of manganese, 47.5 mg of zinc, 50 mg of iron, 6.25 mg of copper, and 2 mg of iodine.
[c]Supplied .3 mg selenium per kilogram of diet.
[d]Dynafos (18.5% P, 24% Ca) supplied by Pitman-Moore, Inc., Feed Ingredient Division, Mundelein, IL 60060.
[e]Limestone (Exp. 1, Limestone Dust Corp., Bluefield, VA) or $CaCO_3$ (Exp. 2, Fisher Scientific) replaced corn starch in the appropriate diets.

Table 2. Dietary treatments for Exp. 1 and 2.

	Exp. 1[a]		Exp. 2[a]	
Treatments	Phytase, U/kg	Ca[b,c], g/kg	Phytase, U/kg	Ca[b,d], g/kg
1	0	3.7	0	2.6
2	167	3.7	200	2.6
3	333	3.7	400	2.6
4	500	3.7	600	2.6
5	0	4.6	0	3.3
6	0	5.5	0	4.0
7	0	6.4	0	4.7

[a]Analyzed P level was 5.8 g/kg in Exp. 1 and 6.4 g/kg in Exp. 2.
[b]Values represent total amounts of Ca contained within the diet.
[c]Analyzed Ca levels were 3.6 g/kg for treatments 1, 2, 3 and 4 and 4.7, 5.5, and 6.4 g/kg for treatments 5, 6, and 7, respectively.
[c]Analyzed Ca levels were 2.6 g/kg for treatments 1, 2, 3 and 4 and 3.2, 3.7, and 4.2 g/kg for treatments 5, 6, and 7, respectively.

Animal and Feeding Management. The pigs were weaned at an average BW of 10.4 and 7.2 kg in Exp. 1 and 2, respectively and given a 7-d adjustment prior to the initiation of dietary treatments. During this period they were fed a commercial diet containing 22% CP (Merrick's Soweena Day 14, Merrick's, Inc., Middleton, WI) for 3 d followed by a 20% CP starter diet containing 10% dried whey and 0.10% mecadox for the remaining 4 d. Following the adjustment period, pigs were randomly assigned to dietary treatments from outcome groups based on gender and BW. Littermates were balanced across treatments. The pigs (one barrow and one gilt) were housed in double deck nursery pens (0.6m x 0.9m) with plastic coated, expanded metal floors and a baffle between decks. Each pen was equipped with a nipple waterer and a stainless steel feeder.

The study was conducted in two similar environmentally controlled rooms with 24 pens in each room. Room temperatures were initially set at 29° C and lowered 2° C per wk after wk 2. A continuous lighting regimen and recommended air ventilation rates were maintained (Murphy et al., 1990). Pigs had ad libitum access to feed and water at all times. The care and treatment of pigs followed published guidelines (Consortium, 1988).

Sampling and Analysis. Body weight and pen feed consumption were measured weekly. During wk 4, pen fecal grab samples were collected twice daily (0700 and 1700) for 4 d and frozen at -20° C in plastic bags for subsequent analysis. After thawing, fecal samples were dried in an oven at 60° C. The dried fecal samples along with representative samples of each diet were ground to pass through a 1 mm sieve. Dry matter was determined according to standard AOAC (1990) procedures. Diet and fecal samples were analyzed for Ca, P and Cr following wet

digestion with nitric and perchloric acid (5:3, vol/vol). Total P concentration was determined photometrically by the vanadomolybdate procedure (AOAC, 1990) and Ca and Cr concentrations were determined with an atomic absorption spectrophotometer (model 5100 PC, Perkin Elmer, Norwalk, CT) using the manufacturer's recommendations. The apparent total tract digestibilities (ATTD) and ileal digestibilities of Ca and P were calculated using the indirect method.

At the end of wk 4, the barrow from each pen (Exp. 1, 8 pigs/treatment except Diet 1 which had 16 pigs; Exp. 2, 6 pigs/treatment except Diet 1 which had 12 pigs) was killed for collection of the 10^{th} ribs from both the left and right side. In addition, ileal digesta was taken from the last 20 cm of the ileum in Exp. 2 following the procedures of Donkoh et al. (1994). Rib bones were cleaned of all tissue and used for determination of shear force and shear energy as described by Combs et al. (1991). In addition bone ash was determined in a muffle furnace at 600° C for 12 h.

Statistical Analysis and Calculation of Ca Equivalency Values. Data were separated into phytase and Ca groups. The basal diet was included in each group. The GLM procedures of SAS (1990) were used to analyze each group. Pen was the experimental unit. The model included replicate, phytase or Ca, and the two-way interactions with replicate. Linear and quadratic contrasts were determined for phytase and Ca levels. Nonlinear and linear equations of the effects of varying Ca (no added phytase) and varying phytase levels on various measurements were derived (analyzed values for Ca levels in the feed were used). The Ca equivalency values of phytase were obtained by setting the equations for phytase and Ca equal and solving as described by Yi et al. (1996b).

Results

Experiment 1. During wk 1 and 2 on test, ADG was not influenced by the addition of phytase or Ca to the basal diet containing 3.7 g/kg (.37%) Ca and 5.8 g/kg (.58%) total P (Table 3). During wk 3-4, the addition of phytase numerically ($P = .15$) and Ca linearly ($P < .04$)increased ADG. Overall (wk 1-4), ADG was not significantly influenced by phytase or Ca. Shear force and rib ash weight were not influenced ($P > .10$) by the addition of phytase, but rib ash percentage was linearly increased ($P < .03$) as phytase was added. The addition of Ca linearly increased shear force ($P < .001$), rib ash percentage ($P < .001$) and rib ash weight ($P < .01$).

Calcium and P apparent total tract digestibility (ATTD), and digestible Ca and P were linearly increased ($P < .001$) as phytase was added to the basal diet (Table 4). Calcium ATTD was only slightly increased ($P = .13$) as Ca was added to the basal diet, but the amount of digestible Ca was linearly increased ($P < .001$). The addition of Ca resulted in a small linear decrease in P ATTD ($P < .07$) and digestible P ($P < .05$). Dry matter ATTD was not influenced by phytase or Ca additions.

Table 3. Effects of microbial phytase and dietary calcium on ADG and bone mineralization of weanling pigs fed a Ca deficient corn-soybean meal diet. Exp. 1[a].

	Ca	Phytase	ADG, g/d			Tenth Rib		
Trt.[b]	g/kg	U/kg	1-2	3-4	1-4	Shear, N	Ash, %	Ash, g
Phytase effects								
1	3.7	0	323	514	419	531	45.5	1.03
2	3.7	200	307	526	417	642	46.0	1.10
3	3.7	400	304	524	414	613	47.0	.94
4	3.7	600	324	563	443	531	47.5	1.08
MSE[c]			61	64	51	160	1.9	.214
CV, %			15.4	11.9	11.7	28.5	4.2	20.1
Probability values								
Trt.			.83	.45	.71	.39	.18	.50
Lin Trt.			.98	.15	.37	.90	.03	.88
Quad Trt.			.37	.63	.43	.13	.93	.58
Calcium effects								
1	3.7	0	323	514	419	530	45.5	1.03
5	4.6	0	324	546	435	532	45.7	1.14
6	5.5	0	315	566	441	632	47.4	1.29
7	6.4	0	287	572	430	788	50.1	1.39
MSE[c]			50	58	44	103	2.3	.24
CV, %			15.1	10.4	10.3	17.2	5.0	20.1
Probability values								
Trt.			.40	.11	.72	.001	.01	.03
Lin Trt.			.14	.04	.48	.001	.001	.01
Quad Trt.			.45	.55	.43	.06	.18	.95

[a]Each treatment mean represents 8 pens (16 pigs), except diet 1 that has 16 pens (32 pigs.)
[b]All diets contained 5.8 g of P/kg.
[c]SEM = MSE (Mean Square Error) /$\sqrt{n}$, where n = 8 or 16.

Since ADG in wk 3-4, digestible Ca, and tenth rib ash percentage were responsive to both phytase and Ca, the nonlinear or linear response equations for these variables were derived for estimation of Ca equivalency values of phytase (Table 5). Calcium equivalency values for 500 U of phytase were estimated to be 1.30, 1.32, and .62 g Ca, respectively for ADG in wk 3-4, rib ash percentage and digestible Ca with an average of 1.08 g Ca released per 500 U of phytase.

Table 4. Effects of microbial phytase and dietary calcium on dry matter, Ca and P digestibility of weanling pigs fed Ca deficient corn-soybean meal diets[a]. Exp. 1.

Trt.[b]	Ca g/kg	Phytase U/kg	Ca dig[c] %	DCa[d] %	P dig[c] %	DP[d] %	DM dig %
Phytase effects							
1	3.7	0	65.1	.259	52.8	.341	82.4
2	3.7	200	68.6	.272	55.3	.358	81.5
3	3.7	400	73.7	.293	60.7	.392	82.8
4	3.7	600	72.6	.288	60.3	.390	81.4
MSE[e]			3.9	.016	3.9	.025	1.7
CV, %			5.6	5.7	6.8	6.8	2.0
Probability values							
Trt.			.001	.001	.001	.001	.28
Lin trt.			.001	.001	.001	.001	.44
Quad trt.			.09	.08	.28	.29	.68
Calcium effects							
1	3.7	0	65.1	.259	52.8	.341	82.4
5	4.6	0	67.5	.353	50.7	.327	82.6
6	5.5	0	67.2	.411	49.5	.320	82.4
7	6.4	0	68.0	.481	50.0	.323	82.7
MSE[e]			3.9	.021	3.7	.024	1.8
CV, %			5.9	5.9	7.1	7.1	2.2
Probability values							
Trt.			.29	.001	.15	.16	.98
Lin trt.			.13	.001	.07	.08	.85
Quad trt.			.55	.10	.30	.29	.96

[a]Each treatment mean represents 8 pens or 16 pigs, except diet 1 that has 16 pens or 32 pigs.
[b]All diets contained 5.8 g of P/kg.
[c]Digestion coefficient for Ca or P as a percentage.
[d]Digestible Ca or P as a percent of the diet.
[e]SEM = MSE/$\sqrt{n}$, where n = 8 or 16.

Table 5. Response equations for Ca and phytase. Exp. 1.

	Phytase (X_1)	r^2	Calcium (X_2)	r^2
MDG 3-4, kg	$=.516 + .000093X_1$	.98	$=.432 + .0256X_2$	.91
Ash, %	$=45.4 + .00319X_1$	.99	$=37.9 + 1.806X_2$	.82
DCa Fecal, %	$=.296 - .03629e^{-.0038X1}$	.82	$=.855 - 1.1173e^{-.158X2}$	.99

Experiment 2. ADG in wk 1-2 was quadratically increased by phytase (P < .03), but Ca addition had no effect. In wk 3-4, ADG was linearly improved by both phytase (*P* < .001) and Ca (*P* < .02). Overall ADG showed no improvement to added phytase (*P* < .19) while a quadratic improvement to added Ca (*P* < .02) was observed. Additions of phytase (*P* < .02) and Ca (*P* < .01) also improved the feed:gain ratio in wk 3-4 (Table 6). Rib ash weight was linearly improved by both phytase (*P* < .001) and Ca (*P* < .001), while quadratic improvements in rib ash percentage (*P* < .03) and linear increases in rib shear force (*P* < .03) were observed only with the addition of Ca to the diet (Table 6).

The addition of phytase linearly improved Ca ATTD (*P* < .001) and the amount of Ca digested (*P* < .001) as a percentage of feed intake, and quadratically improved the amount of P digested (P < .04; Table 7). A quadratic effect of phytase on DM ATTD (*P* < .04) was observed when phytase was added to the diet. The addition of Ca caused a linear increase in the amount of Ca digested (*P* < .001) as a percentage of feed intake, but the ATTD of Ca, P, and DM was not consistently affected.

The addition of phytase linearly increased the apparent ileal digestibility of Ca (*P* < .001) and the amount of Ca digested (*P* < .001) upon reaching the ileal-cecal junction as a percentage of feed intake. Phytase addition quadratically improved P digestibility (*P* < .04) and the amount of P (*P* < .04) digested upon reaching the ileal-cecal junction as a percentage of feed intake (Table 8). The addition of Ca linearly (*P* < .001) increased the amount of ileal Ca digested as a percentage of feed intake, but addition of Ca did not affect the apparent ileal digestibility of Ca and P.

Calcium equivalency estimates were calculated for ADG in wk 3-4, rib ash and shear force and digestible total tract and ileal Ca, using response equations derived for phytase and Ca (Table 9). Calcium equivalency values for 500 U of phytase were estimated to be 1.43, .85, .42, .36, and .85 g of Ca, respectively for ADG in wk 3-4, rib ash weight, rib shear force, digestible total tract Ca, and digestible ileal Ca, respectively. The average value was .78 g Ca for 500 U of phytase.

Table 6. Effects of microbial phytase and dietary calcium on ADG and bone mineralization of weanling pigs fed a Ca deficient corn-soybean meal diet[a]. Exp. 2.

Trt.[b]	Ca g/kg	Phytase U/kg	ADG, g/d 1-2	ADG, g/d 3-4	ADG, g/d 1-4	F:G wk 3-4	Tenth Rib Shear, N	Tenth Rib Ash, %	Tenth Rib Ash, g
Phytase effects									
1	2.6	0	385	599	492	2.04	518	46.0	.90
2	2.6	167	465	618	542	1.97	560	46.8	.87
3	2.6	333	413	615	514	1.93	585	46.7	1.02
4	2.6	500	375	691	533	1.80	600	47.2	1.09
MSE[c]			61	38	37	.15	104	1.6	.10
CV, %			15.1	6.2	7.2	7.5	18.9	3.5	10.1
Probability values									
Trt.			.08	.007	.07	.08	.40	.48	.002
Lin Trt.			.48	.001	.19	.02	.13	.19	.001
Quad Trt.			.03	.09	.34	.64	.74	.86	.19
Calcium effects									
1	2.6	0	385	599	492	2.04	518	46.0	.90
5	3.2	0	443	641	542	2.00	622	47.3	1.05
6	3.7	0	413	673	543	1.88	704	50.7	1.01
7	4.2	0	401	654	527	1.86	669	49.8	1.29
MSE[c]			54	43	31	.13	120	1.1	.10
CV, %			13.3	6.8	6.0	6.6	20.3	2.3	9.5
Probability values									
Trt.			.24	.03	.02	.07	.05	.001	.001
Lin Trt.			.85	.02	.07	.01	.03	.001	.001
Quad Trt.			.14	.11	.02	.96	.19	.03	.16

[a]Each treatment mean represents 6 pens or 12 pigs, except diet 1 that has 12 pens or 24 pigs.
[b]All diets contained 6.4 g of P/kg of diet.
[c]SEM = MSE/$\sqrt{n}$, where n = 6 or 12.

Table 7. Effects of microbial phytase and dietary calcium on dry matter, Ca and P digestibility of weanling pigs fed Ca deficient corn-soybean meal diets[a]. Exp. 2.

Trt.[b]	Ca g/kg	Phytase U/kg	CaDig[c] %	DCa[d] %	PDig[c] %	DP[d] %	DM dig %
Phytase effects							
1	2.6	0	73.5	.216	57.8	.404	85.7
2	2.6	167	76.5	.224	64.4	.460	86.1
3	2.6	333	77.9	.228	63.9	.456	85.5
4	2.6	500	80.8	.238	61.4	.449	83.7
MSE[e]			3.4	.010	6.8	.048	1.3
CV, %			4.4	4.4	11.1	11.1	1.5
Probability values							
Trt.			.002	.01	.18	.07	.02
Lin trt.			.001	.001	.35	.11	.01
Quad trt.			.99	.86	.09	.10	.04
Calcium effects							
1	2.6	0	73.5	.216	57.8	.404	85.7
5	3.2	0	67.5	.237	56.4	.410	83.7
6	3.7	0	76.3	.307	64.2	.466	86.0
7	4.2	0	68.4	.318	57.6	.400	84.6
MSE[e]			4.5	.018	6.1	.043	1.2
CV, %			6.3	6.9	10.4	10.4	1.4
Probability values							
Trt.			.01	.001	.14	.04	.01
Lin trt.			.38	.001	.48	.54	.58
Quad trt.			.58	.45	.27	.04	.54

[a]Each treatment mean represents 6 pens or 12 pigs, except diet 1 that has 12 pens or 24 pigs.
[b]All diets contained 6.4 g of P/kg.
[c]Digestion coefficient for Ca or P as a percentage.
[d]Digestible Ca or P as a percent of the diet.
[e]SEM = MSE/$\sqrt{n}$, where n = 6 or 12.

Table 8. Effects of microbial phytase and dietary calcium on ileal dry matter, Ca and P digestibility of weanling pigs fed Ca deficient corn-soybean meal diets[a]. Exp. 2.

Trt.[b]	Ca g/kg	Phytase U/kg	CaDig[c] %	DCa[d] %	PDig[c] %	DP[d] %	DM dig g/kg
Phytase effects							
1	2.6	0	57.8	.170	45.8	.320	64.9
2	2.6	167	65.9	.193	56.5	.403	66.5
3	2.6	333	70.1	.205	60.8	.434	65.9
4	2.6	500	70.3	.207	60.0	.439	59.6
MSE[e]			6.3	.018	6.5	.046	5.4
CV, %			9.7	9.7	12.1	12.1	8.5
Probability values							
Trt.			.001	.001	.001	.001	.14
Lin trt.			.001	.001	.001	.001	.07
Quad trt.			.11	.133	.04	.036	.07
Calcium effects							
1	2.6	0	57.8	.170	45.8	.320	64.8
5	3.2	0	52.7	.185	44.1	.320	59.5
6	3.7	0	51.7	.208	44.6	.323	62.9
7	4.2	0	56.6	.263	44.8	.311	66.1
MSE[e]			5.8	.022	9.5	.068	8.2
CV, %			10.6	10.9	21.2	21.37	12.9
Probability values							
Trt.			.16	.001	.98	.88	.51
Lin trt.			.63	.001	.88	.84	.60
Quad trt.			.04	.028	.80	.81	.19

[a]Each treatment mean represents 6 pens or 12 pigs, except diet 1 that has 12 pens or 24 pigs.
[b]All diets contained 6.4 g of P/kg.
[c]Digestion coefficients for Ca or P as percentage.
[d]Digestible Ca or P as a percent of the diet.
[e]SEM = MSE/$\sqrt{n}$, where n = 6 or 12.

Table 9. Response equations for Ca and phytase. Exp. 2.

	Phytase (X_1)	r^2	Calcium (X_2)	r^2
MDG 3-4, kg	$=.5916 + .000149X_1$	.99	$=.6699 - 14.3694e^{-2.04X2}$	.85
Ash Wt., g	$=.8560 + .0004X_1$	.75	$=.3265 + .2166X_2$	.79
Force, N	$=669.92 - 153.41e^{-.0016X1}$	.99	$=685.94 - 22814.36e^{-1.885X2}$	.93
DCa Fecal, %	$=.2158 + .000042X_1$	.97	$=.02649 + .07097X_2$	.91
DCa Ileal, %	$=.2105 - .0412e^{-.0054x1}$	.99	$=.0125 + .0566X_2$	.89

Discussion

Calcium was first reported to be associated with phytate by McCance and Widdowson in 1935 (Oberleas and Harland, 1996). The implications of this in broiler diets were documented by Nelson et al. (1968). They found that as the level of phytic acid in the diet increased, so did the chicks' requirement for Ca. This effect was diminished when phytase was added to the diet. Eeckhout and De Paepe (1991) reported a high positive correlation between Ca and P digestibilities when phytase was added to a low P pig diet. They suggested that this relationship could be explained by the fact that phytic acid acts as a Ca binding agent in the proximal small intestine. Therefore, hydrolysis of phytate in the stomach as a result of phytase activity results in increased digestibility, not only of P, but indirectly of Ca. Pointillart (1993) also found in his studies, primarily with cereal phytase, that improved P utilization was generally accompanied by improved Ca retention. Several studies have reported improved Ca retention in broilers when the diets were supplemented with microbial phytase (Schöner et al., 1991, 1993; Kornegay et al., 1996; Yi et al., 1996b). Improvements in Ca digestibility in pig diets supplemented with microbial phytase have also been reported (Mroz et al., 1993; Yi et al., 1996a; Kornegay and Qian, 1996).

Ca digestibility in both Exp. 1 and 2 was improved by the addition of phytase which confirms earlier reports as cited above. Multiple levels of phytase and Ca were used so that response equations could be derived for various criteria and used to calculate equivalency estimates of phytase for Ca. Because of positive linear effects of both phytase and Ca additions and the goodness of fit ($r^2 > .75$) of the linear or nonlinear equation, ADG (wk 3-4; Exp. 1 and 2), ash % (Exp. 1), ash weight (Exp. 2), shear force (Exp. 2), fecal digested Ca (Exp. 1 and 2), and ileal digested Ca (Exp. 2) were used to develop equivalency values. Shear force was used in Exp. 2, despite not showing a significant phytase effect ($P = .13$), because the asymptotic curve provided an excellent fit ($r^2 = .99$). In addition Ca equivalency estimates of phytase based on the equations for shear force were more conservative than those based on the equations for ash weight. The average equivalency estimate was 1.08 g Ca for 500 U of phytase in Exp. 1 when limestone was used and .78 g Ca for 500 U of phytase in Exp. 2 when $CaCO_3$ was used. The difference in equivalency estimates in Exp. 1 and 2 may be due to several factors including, the basal Ca level, the Ca source, and the Ca:P ratio. Bone parameters have a higher dietary

requirement for Ca, compared with growth parameters, in order for a response in bone ash or shear force to be seen (Soares, 1995). Therefore, the responses to phytase and Ca seen in bone ash and shear force may have been depressed due to the extremely low level of Ca in the basal diet. Calcium carbonate was used in Exp. 2 instead of limestone because the limestone used in Exp. 1 was believed to be not of the highest quality. Calcium from limestone is generally considered to be 100% available (Soares, 1995; Baker, 1996). However, the limestone used in Exp. 1 contained only 33% Ca, and therefore, contained many impurities. Substances such as Mg which alter the cation:anion ratio have been shown to decrease Ca availability (Soares, 1995). In addition, any substance which can precipitate Ca out of solution through the formation of a Ca-salt decreases Ca availability (Soares, 1995). Therefore, we expected to see a higher digestibility of Ca from the $CaCO_3$ used in Exp. 2 compared with the limestone used in Exp. 1. However, this was not the case. Using the data for digested Ca in diets 1, 5, 6, and 7, presented in Tables 4 and 7, the digestibility of Ca from both Ca sources was calculated. The Ca digestibilities were 82% and 64%, respectively for limestone and $CaCO_3$. This difference in Ca digestibility may be due to the higher concentration of P in the diets of Exp. 2. Phosphate can bind Ca making it unavailable for absorption (Soares, 1995). The ratio of Ca to P (in the basal diet and phytase treatments) was 0.64:1 for Exp. 1 and 0.41:1 for Exp. 2. This lower ratio of Ca:P in Exp. 2 may have caused a decreased availability of Ca for absorption and therefore a decreased digestibility of Ca relative to that seen in Exp. 1.

If we use a Ca digestibility of 64% for $CaCO_3$ and 82% for limestone, as calculated above, then 500 U/kg of phytase releases between .5 and .84 g of digestible Ca. In a review by Jongbloed et al. (1996) they suggested that the addition of 500 U/kg of microbial phytase would release .4 to .7 g of digestible Ca per kg of diet. Using turkey pullets, Kornegay et al. (1996) reported an average Ca equivalency value of .87 g total Ca being equal to 500 U/kg of microbial phytase. In their study, BW gain provided the highest Ca equivalency estimate (500 U phytase = 1.2 g/kg Ca) followed by gain:feed (500 U phytase = 0.7 g/kg Ca) and digestible Ca (500 U phytase = 0.7 g/kg Ca). Schöner et al. (1994) reported that 500 U of microbial phytase was eqiuvalent to .35 g/kg of total Ca as measured by BW gain and .56 g/kg of total Ca as measrured by phalanx ash. In both of our experiments, fecal digested Ca provided the lowest estimate for equivalency values (Exp. 1, .62; Exp. 2, .36). In Exp. 2, the equivalency value based on ileal digested Ca (500 U = .85 g Ca) was much higher than the one based on fecal digested Ca (500 U = .36 g Ca) suggesting that a significant amount of Ca is excreted into the lumen of the large intestine. Bone parameters (Exp. 1: ash %, 500 U = 1.32 g Ca; Exp. 2: ash wt., 500 U = .85 g Ca; shear force, 500 U = .42 g Ca) tended to give intermediary equivalency values and growth performance (Exp. 1:ADG (3-4), 500 U = 1.30 g Ca; Exp. 2: ADG (3-4), 500 U = 1.43 g Ca) tended to give the highest equivalency values.

In Exp. 1 and 2, the basal diet contained 0.1118% or 27.95 mmoles (1,118/40) of Ca from plant ingredients. In Exp. 1, limestone was added to the diet in order to increase the total Ca concentration in the diet to .37% or 92.5 mmoles (3,700/40). The equivalency value derived in Exp. 1 for 500 U of phytase was 1.08 g or 27.0 mmoles (1,080/40) of Ca/kg of diet. Therefore, approximately 97% of the plant Ca or 29.2% of the total Ca in the diet was bound by

phytate and subsequently released by phytase. In Exp. 2, $CaCO_3$ was added to the diet in order to increase the total Ca concentration to .26% or 65.0 mmoles (2,600/40). The equivalency value derived in Exp. 2 for 500 U of phytase was 0.78 g or 19.5 mmoles (1,080/40) of Ca/kg of diet. Therefore, approximately 70% of the plant Ca or 30.0% of the total Ca in the diet was bound by phytate and subsequently released by phytase. The basal diet for both experiments contained approximately .27% phytate P (.924% phytate) or 14.3 mmoles (9,240/648) of phytic acid. Therefore each mole of phytic acid chelated 1.89 moles (27.0/14.3) of Ca and 1.36 moles (19.5/14.3) of Ca in Exp. 1 and 2, respectively. Erdman (1979) demonstrated that at a neutral pH, each phosphate group contained either one or two negative charges giving each mole of phytic acid the ability to bind three to six moles of Ca. The results of these two experiments indicate that phytic acid binds less Ca than it is theoretically capable of. This is likely due to the cpacity of phytate to chelate minerals other then Ca, thus reducing the total amount of Ca bound.

The Ca:P ratios for the phytase treatments in Exp. 1 and 2 were 0.64:1 and 0.41:1, respectively. Qian et al. (1996) found that the optimal Ca:P ratio for pig diets supplemented with microbial phytase was 1:1. The greatest negative effect of an adverse Ca:P ratio is seen when Ca is supplemented at too high a level while P is supplemented at a marginal level. When this occurs, the high level of Ca causes a decreased absorption of P (NRC, 1988). A high level of phosphate relative to Ca can cause a decrease in Ca absorption due to the formation of insoluble $Ca\text{-}PO_4$ salts (Soares, 1995). This may have contributed to the reduced response to both Ca and phytase additions, particularly in Exp. 2 where the Ca to P ratio was particularly low (0.41:1).

Ross et al. (1984) reported data from five studies investigating the bioavailability of Ca from various Ca supplements in pigs with an average starting weight of 15 kg. They concluded that bone breaking strength was the most sensitive indicator of Ca status as it provided a linear response to Ca over the widest range (.05 to .65% total Ca). However, they found that mortality was high at the lowest level of Ca and that the curve was appearing to plateau at the highest level of Ca. They concluded that the most desirable response surface for Ca occurred between .20 and .50% total Ca. In the other four experiments presented by Ross et al. (1984), they found that bone strength and ash always linearly increased as the Ca level in the diet increased form .20% to .35% to .50%. Performance data gave variable responses, with some experiments showing improvements in weight gain and feed efficiency and others showing no effect as the Ca level in the diet was increased. They found no differences in the bioavailability of Ca from calcitic limestone, oyster shell flour, gypsum, marble dust, and aragonite. Calcium from dolomitic limestone was less available than Ca from any of the other sources.

In Exp. 1 presented in this paper, total Ca levels in the diets were .37, .46, .55, and .64%. Therefore, based on the data of Ross et al. (1984) the highest two levels of Ca may not have provided linear responses. In fact growth rate tended to be depressed at the highest level of Ca. However, the pigs in Exp. 1 weighed approximately 6.5 kg less than the pigs in the study of Ross et al. (1984). Therefore, the pigs used in Exp. 1 would have a slightly higher requirement for Ca than those used by Ross et al. (1984). In Exp. 2, dietary Ca levels fed were .26, .33, .40, and .47%, which are within the range reported by Ross et al. (1984) to provide a linear response

in bone breaking strength. In agreement with the findings of Ross et al. (1984), rib shear and rib ash were linearly improved by adding Ca to the diet in both Exp. 1 and 2. Overall ADG was not influenced by dietary Ca levels, but ADG in wk 3-4 was linearly improved by Ca. This suggests that the effects of Ca on ADG may require more time to be seen as bone stores of Ca are initially used to compensate for Ca needed for growth.

Implications

Supplementing microbial phytase to the diet of weanling pigs increases Ca digestibility in addition to increasing P digestibility. Therefore, when formulating diets with added microbial phytase, approximately .78 to 1.08 g less Ca from limestone can be added to the diet.

Chapter V
The Effects of Microbial Phytase, Citric Acid and Their Interaction in a Corn-Soybean Meal Based Diet for Weanling Pigs

ABSTRACT. Crossbred weanling pigs (equal barrows and gilts) with an average initial weight of 7.4 kg (Exp. 1) or 9.6 kg (Exp. 2) were used in two 4-wk experiments (Exp. 1, n = 96; Exp. 2, n = 96) to investigate the effects of added phytase or citric acid and their interactions on performance, rib mineralization, gastric pH and digestibility measurements. A corn-soybean meal based diet low in Ca and P was used in both experiments. In Exp. 1, three citric acid levels (0.0, 1.5 and 3.0%) and four phytase levels (0, 250, 500, and 750 U/kg) were used in a 3 x 4 factorial arrangement of treatments. In Exp. 2, two citric acid levels (0.0 and 2.0%) and three phytase levels (0, 250, and 500 U/kg) were used in a 2 x 3 factorial arrangement of treatments. Phosphorus was maintained at 3.9 and 3.7 g/kg in Exp. 1 and 2, respectively. Calcium was maintained in a 2.5:1 ratio with available P plus estimated released P in Exp. 1 and at a level of 4.3 g/kg in Exp. 2. Body weight and feed consumption were measured weekly, and pen fecal samples were collected twice daily for 5 d during wk 4. At the end of wk 4, the barrow in each pen was killed following a fast-refeed-fast (22-1-2 h) regimen for collection of tenth ribs and stomach digesta. In Exp 1 and 2, phytase addition linearly increased rib shear force ($P < .004$ and $P < .02$), shear energy ($P < .001$), dry bone weight ($P < .001$), ash weight ($P < .001$) and ash percent ($P < .001$). Calcium ($P < .001$) and P ($P < .001$) digestibilities were also improved in both experiments when phytase was added. Addition of citric acid in both experiments, reduced dietary pH and stomach digesta pH ($P < .05$). The addition of citric acid improved ADG ($P < .05$), feed efficiency ($P < .04$) and Ca digestibility ($P < .05$) in Exp. 1, but decreased Ca digestibility and had no effect on performance in Exp. 2. In Exp. 2, the addition of 2.0% citric acid to the diet supplemented with 500 U/kg of phytase caused a decrease ($P < .04$) in the phytase activity recovered in the stomach digesta resulting in a phytase by citric acid interaction ($P < .02$). In summary, the additions of citric acid and phytase to weanling pig diets were both beneficial, but no synergistic effects were observed.

Key Words: Pigs, Phytase, Acidity, Digestibility, Bone Mineralization

Introduction

Phosphorus is a key element in swine diets that is necessary for optimal growth, reproduction and bone development (NRC, 1988). However, 60 to 70% of the P in plant ingredients, commonly used in swine diets, occurs as phytate P which is unavailable to the pig (Cromwell, 1992; Ravindran, 1994, 1995). Addition of microbial phytase to swine diets has been shown to hydrolyze the phytate molecule, releasing the bound P (Jongbloed et al., 1992; Cromwell et al., 1993; Lei et al., 1993, Kornegay and Qian, 1996). Several studies have shown that the optimal pH of microbial phytase occurs at two peaks, the highest activity was observed at a pH of 5.0 to 5.5 and the second highest activity was at a pH of 2.5 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). A typical corn-soybean meal based diet for pigs using dicalcium phosphate as the inorganic P source, has a pH of about 6.0. The additions of organic acids, such as citric, formic, and fumaric acid are known to lower diet acidity. Since the site of phytase activity is primarily in the stomach (Yi and Kornegay, 1996), lowering the dietary pH might reduce the pH of the stomach and thereby increase the effectiveness of microbial phytase. Improving the efficiency of phytase could lead to reduced feed costs, and greater use of phytase which would be of environmental importance. Therefore, the objective of this study was to determine the effectiveness of reducing diet acidity as a means to improve phytase efficacy in corn-soybean meal diets fed to young pigs.

Materials and Methods

One hundred ninety-two (Exp. 1, n = 96; Exp. 2, n = 96) crossbred pigs (equal barrows and gilts) with an initial weight of 7.4 kg (Exp. 1) or 9.6 kg (Exp. 2) were used in two 4-wk experiments to investigate the effects of microbial phytase and citric acid addition to weanling pig diets and their interaction on performance, bone parameters, stomach digesta pH and phytase activity, and fecal digestibilities of Ca and P. Experimental procedures were similar for both experiments except for variations in dietary treatments.

Dietary Treatments. A 19% and an 18% CP corn-soybean meal based diet fortified with minerals to meet or exceed NRC (1988) requirements (except Ca and P) was fed in Exp. 1 and 2, respectively (Table 1). In Exp 1., three citric acid (citric acid anhydrous, White Crane Brand, Nanntong, China) levels (0.0, 1.5, or 3.0%) and four phytase (Natuphos®, BASF Corp., Mount Olive, NJ) levels (0, 250, 500, or 750 U/kg) were used in a 3 x 4 factorial arrangement of treatments (Table 2). Phosphorus was maintained at .39% total P with only .013% added as defluorinated phosphate (DFP). A constant Ca to total available P (included estimated released P) was maintained at 2.5:1 in all diets. In Exp. 2, two citric acid levels (0.0 and 2.0% citric acid) and three phytase levels (0, 250, and 500 U/kg) were used in a 2 x 3 factorial arrangement of treatments (Table 3). Phosphorus and Ca were maintained in all diets at 3.7 and 4.3 g/kg, respectively. Limestone was used as the Ca source in Exp. 1 and $CaCl_2$ was used in Exp. 2. A Cr_2O_3-starch premix (1 part Cr_2O_3:3 parts starch (wt:wt)) was added at a level of 0.2% (0.05% Cr_2O_3) to all diets as an indigestible indicator.

Table 1. Composition of basal diet for Exp. 1 and 2.

	% of Diet	
Ingredient	Exp. 1	Exp. 2
Corn	63.6	67.2
Soybean Meal (44% CP)	30.9	28.2
Vitamin premix[a]	.25	.25
Trace Mineral premix[b]	.10	.10
Selenium premix[c]	.05	.05
Salt	.30	.30
Deflourinated phosphate	.07	--------
Calcium Chloride (76% pure)	--------	1.18
Chromic oxide premix	.20	.20
Corn Starch[d]	4.53	2.52
Calculated Composition		
CP	19.0	18.1
Lysine	1.06	.99
P	3.92	3.70
Ca	1.27	4.28

[a]Supplied per kilogram of diet: 4400 IU of Vitamin A, 440 IU of Vitamin D_3, 11 IU of Vitamin E, 2.2 mg of Vitamin K, 4.4 mg of riboflavin, 22 mg of calcium pantothenate, 22 mg of niacin, .022 mg of vitamin B_{12}, 440 mg of choline chloride, .44 mg of biotin, 3.9 mg of folic acid, 10 mg of thiamin•HCl, 3.9 mg of pyridoxine•HCl, 82.5 mg of ethoxyquin and 3.6 mg of virginiamycin.
[b]Supplied per kilogram of diet: 44 mg of manganese, 47.5 mg of zinc, 50 mg of iron, 6.25 mg of copper, and 2 mg of iodine.
[c]Supplied .3 mg selenium per kilogram of diet.
[d]Corn starch was replaced with citric acid (Citric Acid Anhydrous, White Crane Brand, Nanntong, China) and/or phytase (Natuphos®, BASF Corp., Mount Olive, NJ) in appropriate diets. Limestone (Limestone Dust Corp., Bluefield, VA) was added to diets in Exp. 1 in replace of corn starch to maintain a constant Ca:aP ratio.

Table 2. Dietary treatments, estimated total available P, and Ca:P ratios. Exp. 1

Diet	Phytase, U/kg	Acid[a], %	rP[b], %	arP[c], %	Ca[d,e], %	Ca:arP	Ca:tP[f]
1	0	0	0	.115	.288	2.50	.74
2	250	0	.049	.164	.410	2.50	1.05
3	500	0	.086	.201	.500	2.50	1.28
4	750	0	.123	.238	.593	2.50	1.52
5	0	1.5	0	.115	.288	2.50	.74
6	250	1.5	.049	.164	.410	2.50	1.05
7	500	1.5	.086	.201	.500	2.50	1.28
8	750	1.5	.123	.238	.593	2.50	1.52
9	0	3.0	0	.115	.288	2.50	.74
10	250	3.0	.049	.164	.410	2.50	1.05
11	500	3.0	.086	.201	.500	2.50	1.28
12	750	3.0	.123	.238	.593	2.50	1.52

[a]Citric acid replaced corn starch in appropriate diets.
[b]Estimated 20, 40, and 60% release of phytate P (.24% calculated total) for 250, 500, and 750 U of phytase/kg diet.
[c]arP = total available P plus estimated released P (see footnote b) with the addition of phytase.
[d]Limestone replaced corn starch in appropriate diets.
[e]Analyzed Ca levels were .280, .383, .431, .568, .301, .469, .458, .549, .246, .355, .506, and .608 % for treatments 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, respectively.
[f]Total P level was calculated to be .39% for all treatments. Total P level was analyzed to be .329% for all treatments.

Table 3. Dietary treatments and calculated P and Ca concentrations. Exp. 2.

Diet[a]	Phytase, U/kg	Citric Acid, %	P[b], %	Ca[c], %
1	0	0	3.7	4.3
2	250	0	3.7	4.3
3	500	0	3.7	4.3
4	0	2.0	3.7	4.3
5	250	2.0	3.7	4.3
6	500	2.0	3.7	4.3

[a]The Ca:P ratio was maintained at 1.16:1 in all diets.
[b]Analyzed P level was 3.4 g/kg.
[c]Analyzed Ca level was 4.4 g/kg.

Animal and Feeding Management. The pigs were given a 7-d postweaning adjustment period prior to the start of treatments during which they were fed a diet containing 22% CP

(Merrick's Soweena Day 14, Merrick's, Inc., Middleton, WI) for 3 d followed by a 20% CP starter diet containing 10% dried whey and 0.10% mecadox (0.55% carbadox) for the remaining 4 d. Following the adjustment period, pigs were randomly assigned to dietary treatments from outcome groups based on gender and BW. Littermates were balanced across treatments.

The pigs (one barrow and one gilt) were housed in double deck nursery pens (0.6m x 0.9m) with coated, expanded metal floors and a baffle between decks. Each pen was equipped with a nipple waterer and a stainless steel feeder. Pens were located in two similar environmentally controlled rooms with 24 pens in each room. Room temperature was initially set at 29° C and was lowered about 2° C per week after the second week. A continuous lighting regimen and recommended (Murphy et al., 1990) air ventilation rates were maintained. Pigs had ad libitum access to feed and water at all times. The care and treatment of the animals followed published guidelines (Consortium, 1988).

Sampling and Analysis. Body weight and pen feed composition were measured weekly. During wk 4, pen fecal grab samples were taken twice daily for 4 d and frozen at -20° C for subsequent drying at 65° C to a constant weight and mineral analysis. The dried fecal samples and samples of diets were ground to pass through a 1 mm sieve.

At the end of wk 4, the barrow from each pen (Exp. 1, four pigs/treatment; Exp. 2, eight pigs/treatment) was killed for collection of 10th ribs and stomach digesta. Prior to slaughter, pigs were fasted for 22 h, then given access to feed for 1 h At the end of the hour any remaining feed was removed, and the pigs were slaughtered 2 h later. Immediately upon slaughter the stomach contents were removed for immediate determination of pH. Stomach digesta pH was determined after mixing 5 g of digesta in 50 ml of deionized water for 1 min using an electromagnetic mixer and stir bar.

Diet and fecal samples were analyzed for Ca, P and Cr following a nitric-perchloric acid (5:3, vol/vol) digestion. Calcium and Cr were analyzed using an atomic absorption spectrophotometer (model 5100 PC, Perkin Elmer, Norwalk, CT) using the manufactutrer's recommendations. Total P concentration was analyzed photometrically by the vanadomolybdate procedure (AOAC, 1990). Tenth ribs were cleaned of all tissues and used for determination of bone shear force and shear energy as described by Combs et al. (1991). In addition, bone ash was determined in a muffle furnace at 600° C for 12 h. Diet samples and stomach digesta samples that had been freeze dried were analyzed for phytase activity (Exp. 2, only) using the procedures of Engelen et al. (1994).

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (1990). The model included replicate, phytase, citric acid, and the two-way interaction of phytase and citric acid. Linear and quadratic contrasts were determined for phytase and linear contrasts were determined for citric acid levels in Exp. 1. Linear contrasts were determined only for phytase levels in Exp. 2 using the orthogonal contrast procedures of SAS (1990).

Results

The only phytase interactions with citric acid were on stomach digesta phytase activity and rib shear energy in Exp. 2. Therefore, with the exception of these two criteria, the main effects of added phytase or citric acid are only discussed separately.

Experiment 1.

Phytase Effects. Stomach pH appeared to be linearly increased ($P < .05$) as phytase was added to the diet (Table 4). The addition of phytase did not affect growth

Table 4. The effects of microbial phytase and citric acid on diet and stomach pH. Exp. 1[a,b].

Citric	Phytase, U/kg				
Acid, %	0	250	500	750	**Mean**
Diet pH					
0	6.49	6.57	6.48	6.49	**6.51**
1.5	4.95	5.02	4.99	5.03	**5.00**
3.0	4.26	4.32	4.44	4.38	**4.35**
Mean	**5.23**	**5.30**	**5.30**	**5.30**	
Stomach pH[c,d]					
0	3.88	3.84	3.70	3.84	**3.81**
1.5	2.81	3.46	4.05	3.59	**3.48**
3.0	2.55	3.51	3.60	3.63	**3.31**
Mean	**3.08**	**3.61**	**3.79**	**3.68**	

[a]Each treatment mean represents four pens (two pigs/pen).
[b]The pooled SEM for a single mean was 0.345 for stomach pH.
[c]Phytase linear effect ($P < .05$).
[d]Citric acid linear effect ($P < .05$).

performance (Table 5), but rib shear force ($P < .004$), shear energy ($P < .001$), dry bone weight ($P < .001$), ash weight ($P < .001$) and ash percentage ($P < .001$) were linearly increased as the level of phytase in the diet increased (Table 6). Phytase addition linearly improved DM digestibility ($P < .01$) and quadratically improved Ca ($P < .001$) and P ($P < .02$) digestibilities (Table 7).

Citric Acid Effects. Addition of 1.5 and 3.0% citric acid caused a 1.51 and a 2.16 unit decrease in diet pH which resulted in a linear decrease ($P < .05$) in the pH of the stomach digesta post-slaughter (Table 4). Average daily gain ($P < .05$) and the feed to gain ratio ($P < .001$) in wk 1-2 were both linearly improved as the level of citric acid in the diet was increased (Table 5). The overall effect (wk 1-4) of added citric acid on the feed:gain ratio ($P < .04$) and ADG ($P < .05$)

remained significant. No effect of diet acidity on bone parameters was observed (Table 6). Calcium digestibility ($P < .05$) was improved by the addition of citric acid, but no effects were seen on DM and P digestibilities (Table 7).

Table 5. Effects of microbial phytase and citric acid on daily gain and feed:gain ratios after two and four weeks on test. Exp. 1[a,b].

Citric	Phytase, U/kg				
Acid, %	0	250	500	750	**Mean**
ADG wk 1-2[c]*, g*					
0.0	222	247	236	223	**232**
1.5	243	250	247	245	**246**
3.0	244	282	286	274	**271**
Mean	**236**	**258**	**256**	**247**	
ADG wk 1-4[c]*, g*					
0.0	307	321	330	294	**313**
1.5	330	336	336	341	**336**
3.0	329	330	353	346	**340**
Mean	**321**	**329**	**340**	**327**	
F:G wk 1-2[d]					
0.0	2.35	2.28	2.48	2.44	**2.39**
1.5	1.97	2.05	2.25	2.39	**2.17**
3.0	2.01	1.98	1.76	1.98	**1.93**
Mean	**2.11**	**2.11**	**2.16**	**2.27**	
F:G wk 1-4[e]					
0.0	2.13	2.12	2.22	2.35	**2.21**
1.5	1.92	2.07	2.14	2.24	**2.09**
3.0	1.96	2.15	1.90	2.03	**2.00**
Mean	**2.01**	**2.11**	**2.09**	**2.21**	

[a]Each treatment mean represents four pens (two pigs/pen).
[b]The pooled SEM for a single mean was 0.030 for ADG in wk 1-2, 0.015 for ADG in wk 1-4, 0.150 for F:G in wk 1-2, and 0.265 for F:G in wk 1-4.
[c]Citric acid linear effect ($P < .05$).
[d]Citric acid linear effect ($P < .001$).
[e]Citric acid Linear effect ($P < .04$).

Table 6. Effects of microbial phytase and citric acid on rib shear force, energy, dry bone weight, ash weight and ash percentage. Exp. 1[a,b].

Citric	Phytase, U/kg				
Acid, %	0	250	500	750	**Mean**
Shear force[c]*, N*					
0.0	512	436	549	571	**517**
1.5	455	586	540	657	**560**
3.0	463	497	646	533	**537**
Mean	**477**	**507**	**579**	**587**	
Shear energy[d]*, N*mm*					
0.0	400	424	498	538	**465**
1.5	418	475	487	659	**509**
3.0	366	536	612	563	**518**
Mean	**395**	**473**	**532**	**586**	
Dry bone weight[d]*, g*					
0.0	1.33	1.37	1.49	1.58	**1.44**
1.5	1.26	1.68	1.43	1.82	**1.55**
3.0	1.11	1.45	1.79	1.63	**1.50**
Mean	**1.23**	**1.50**	**1.57**	**1.68**	
Ash weight[d]*, g*					
0.0	.498	.582	.667	.738	**.621**
1.5	.514	.692	.623	.831	**.665**
3.0	.455	.645	.923	.747	**.695**
Mean	**.489**	**.639**	**.737**	**.772**	
Ash[d]*, %*					
0.0	37.40	42.53	45.18	47.11	**43.05**
1.5	41.17	40.99	43.39	45.94	**42.87**
3.0	41.20	44.45	51.86	46.12	**46.00**
Mean	**39.92**	**42.49**	**46.81**	**46.39**	

[a]Each treatment mean represents four pens or 8 pigs.

[b] The pooled SEM for a single mean was 48.6 for shear force, 50.8 for energy, 0.145 for dry bone weight, 0.065 for ash weight, and 2.21 for ash %.

[c]Phytase linear effect ($P < .004$).

[d]Phytase linear effect ($P < .001$).

Table 7. Effects of microbial phytase and citric acid on DM, Ca and P digestibilities. Exp. 1[a,b].

Citric	Phytase, U/kg				
Acid, %	0	250	500	750	Mean
DM digestibility[c], *%*					
0	80.4	79.8	84.1	80.9	81.3
1.5	79.9	79.6	81.1	83.6	81.1
3.0	81.2	79.4	80.4	82.7	81.0
Mean	80.5	79.6	81.8	82.4	
Ca digestibility[d,e], *%*					
0	51.9	70.3	71.9	74.7	67.2
1.5	56.2	74.5	78.0	82.3	72.7
3.0	54.9	73.4	79.0	81.1	72.0
Mean	54.3	72.7	76.3	79.4	
P digestibility[f], *%*					
0	22.9	35.3	50.4	49.9	39.6
1.5	21.7	40.9	51.4	61.4	43.9
3.0	25.5	38.2	49.9	58.4	43.3
Mean	23.4	38.1	50.6	56.6	

[a]Each mean represents four pens (two pigs/pen).
[b] The pooled SEM for a single mean was 1.11 for DM Dig, 3.05 for Ca Dig, and 2.84 for P Dig
[c]Phytase linear effect ($P < .01$).
[d]Phytase linear effect ($P < .001$), quadratic effect ($P < .001$).
[e]Citric acid linear effect ($P < .05$).
[f]Phytase linear effect ($P < .001$), quadratic effect ($P < .02$).

Experiment 2.

Phytase Effects. Diet and stomach pH were unaffected by the addition of phytase (Table 8). Added phytase did not affect growth performance or feed efficiency (Table 9), but rib shear force ($P < .02$), shear energy ($P < .001$), dry bone weight ($P < .001$), ash weight ($P < .001$) and ash percentage ($P < .001$) were linearly increased as the level of phytase in the diet increased (Table 10). Phytase additions linearly improved Ca ($P < .001$) and P ($P < .001$) digestibilities (Table 11), but DM digestibility was unaffected.

Table 8. The effects of microbial phytase and citric acid on stomach and dietary acid levels and phytase activity in the stomach. Exp. 2[a,b].

Citric	Phytase, U/kg			
Acid, %	0	250	500	**Mean**
Diet pH[c]				
0.0	5.45	5.32	5.39	**5.39**
2.0	3.78	4.18	4.07	**4.01**
Mean	**4.62**	**4.75**	**4.73**	
Stomach pH[d]				
0.0	3.48	3.66	3.66	**3.60**
2.0	3.30	3.38	3.32	**3.33**
Mean	**3.39**	**3.52**	**3.49**	
Stomach digesta phytase activity[e]*, U/Kg*				
0.0	48	133	211	**131**
2.0	44	137	148	**110**
Mean	**46**	**135**	**179**	

[a]Each treatment mean represents eight pens (two pigs/pen).
[b] The pooled SEM for a single mean was 0.159 for stomach pH and 11.81 for stomach digesta phytase activity
[c]Citric acid effect ($P < .001$).
[d]Citric acid effect ($P < .05$).
[e]Phytase*Citric acid effect ($P < .02$), Phytase linear effect ($P < .001$), Citric acid effect ($P < .04$).

Citric Acid Effects. The addition of 2.0% citric acid resulted in a 1.38 unit decrease in diet pH which resulted in a 0.26 pH unit decrease ($P < .05$) in the pH of the stomach digesta post-slaughter (Table 8). Adding citric acid to the diet had no effect on performance data (Table 9) or bone parameters (Table 10). Dry matter Ca and P digestibilities ($P < .04$) were unaffected by the addition of citric acid (Table 11).

Phytase Interactions with Citric Acid. The addition of phytase with no added citric acid linearly increased ($P < .001$) the amount of phytase activity recovered in the stomach digesta post-slaughter (Table 8). Adding citric acid to the diet had no effect on stomach digesta phytase activity when no phytase was added to the diet or when 250 U/kg of phytase was added to the diet. However, the addition of 2.0% citric acid to the diet supplemented with 500 U/kg of

phytase caused a decrease ($P < .04$) in the phytase activity recovered in the stomach digesta resulting in a phytase by citric acid interaction ($P < .02$). Rib shear energy was linearly increased ($P < .02$) as phytase was added to the diet while no effect of citric acid was seen (Table 10). However, when no phytase was added to the diet citric acid numerically decreased rib shear energy, and when phytase was supplemented to the diet citric acid numerically increased rib shear energy. This resulted in a phytase by citric acid interaction ($P < .03$) on rib shear energy.

Table 9. Effects of microbial phytase and citric acid on daily gain and feed:gain ratios. Exp. 2[a,b].

Citric	Phytase, U/kg			
Acid, %	0	250	500	**Mean**
ADG wk 1-2, g				
0.0	626	694	562	**627**
2.0	591	587	663	**613**
Mean	**608**	**640**	**612**	
ADG wk 1-4, g				
0.0	781	810	799	**797**
2.0	748	799	812	**786**
Mean	**765**	**805**	**805**	
F:G wk 1-2				
0.0	1.98	1.50	1.73	**1.74**
2.0	1.85	1.82	1.69	**1.79**
Mean	**1.92**	**1.92**	**1.71**	
F:G wk 1-4				
0.0	2.34	1.99	2.10	**2.14**
2.0	2.18	2.17	2.16	**2.17**
Mean	**2.26**	**2.07**	**2.13**	

[a]Each treatment mean represents eight pens (two pigs/pen).
[b] The pooled SEM for a single mean was 0.021 for ADG in wk 1-2, 0..032 for ADG in wk 1-4, 0.138 for F:G in wk 1-2, and 0.010 for F:G in wk 1-4.

Table 10. Effects of microbial phytase and citric acid on rib shear force, energy, dry bone weight, ash weight and ash %. Exp. 2[a,b].

Citric	Phytase, U/kg			
Acid, %	0	250	500	**Mean**
Shear force[c]*, N*				
0.0	420	416	428	**421**
2.0	383	486	486	**468**
Mean	**401**	**451**	**451**	
Shear energy[d]*, N*mm*				
0.0	415	450	497	**454**
2.0	359	514	571	**481**
Mean	**387**	**482**	**534**	
Dry bone weight[e]*, g*				
0.0	2.25	2.37	2.66	**2.43**
2.0	2.06	2.49	2.57	**2.37**
Mean	**2.16**	**2.43**	**2.62**	
Ash weight[e]*, g*				
0.0	.772	.884	1.041	**.899**
2.0	.732	.964	1.012	**.902**
Mean	**.752**	**.924**	**1.027**	
Ash[e]*, %*				
0.0	34.40	37.39	39.38	**37.05**
2.0	35.73	38.50	39.43	**37.89**
Mean	**35.06**	**37.95**	**39.40**	

[a]Each treatment mean represents eight pens (two pigs/pen).
[b] The pooled SEM for a single mean was 31.04 for shear force, 24.93 for energy, 0.110 for dry bone weight, 0.046 for ash weight, and 0.011 for ash %.
[c]Phytase linear effect ($P < .02$).
[d]Phytase*Citric acid effect ($P < .03$), Phytase linear effect ($P < .001$).
[e]Phytase linear effect ($P < .001$).

Table 11. Effects of microbial phytase and citric acid on calcium, phosphorus, and dry matter digestibilities. Exp. 2[a,b].

Citric	Phytase, U/kg			
Acid, %	0	250	500	**Mean**
DM dig, %				
0.0	80.0	79.1	79.4	**79.5**
2.0	78.7	79.0	78.7	**78.8**
Mean	**79.3**	**79.0**	**79.1**	
Ca Dig[c]*, %*				
0.0	56.7	61.5	68.9	**62.3**
2.0	50.5	65.2	65.2	**60.3**
Mean	**53.5**	**63.4**	**67.0**	
P Dig[c]*, %*				
0.0	16.4	20.5	31.6	**22.8**
2.0	15.7	25.9	35.6	**25.7**
Mean	**16.1**	**23.2**	**33.6**	

[a]Each mean represents eight pens (two pigs/pen).
[b] The pooled SEM for a single mean was 0.975 for DM Dig, 2.97 for Ca Dig, and 3.69 for P Dig.
[c]Phytase linear effect ($P < .001$).

Discussion

Sixty to 70% of the P in plant ingredients, commonly used in swine diets, occurs as phytate P which is unavailable to the pig (Cromwell, 1992; Ravindran, 1994, 1995). Microbial phytase has been used as a dietary supplement to improve the availability of phytate P (Simons et al., 1990; Lei et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996).

Microbial phytase has been shown to have two optimal pH peaks of activity at pH 5.0 to 5.5 and pH 2.5 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). Shieh et al. (1969) and Irving and Cosgrove (1974) reported that phytase was 50% less active at pH 2.5 than at pH 5.0. Whereas, Simons et al. (1990) reported that microbial phytase was 50% less active at pH 5.5 than at pH 2.5. In agreement with Shieh et al. (1969) and Irving and Cosgrove (1974), Beudeker (1990) found that Natuphos® phytase was more active at pH 5.5 than at pH 2.5.

Acidification of starter diets using organic acids, primarily fumaric and citric acids, lowers diet acidity and has generally improved postweaning performance (Kirchgessner and Roth, 1982; Falkowski and Aherne, 1984; Giesting and Easter, 1985; Giesting et al., 1991; Risley et al., 1992). Jongbloed et al. (1996a) conducted two experiments to investigate the effects of adding organic acids to the diet on phytase efficacy. In Exp. 1, they reported a lactic acid by phytase interaction on P digestibility; the addition of lactic acid increased digestible P by 0.24 g/kg. In Exp. 2, there were no interactive effects of lactic acid or propionic acid observed, but there was an increase in digestible P of 0.20 g/kg when formic acid was added in the presence of phytase.

In our experiments reported here, both phytase and citric acid had beneficial effects when added to weanling pig diets, but few interactions between phytase and citric acid were found. At the highest level of phytase supplementation (500 U/kg) in Exp. 2, citric acid caused a decrease in the amount of phytase activity recovered from the stomach digesta post-slaughter resulting in a significant interactive effect. In Exp. 1, the stomach digesta of pigs fed diets containing 750 U/kg of phytase and 0, 1.5 or 3.0% citric acid were tested for phytase activity and is reported elsewhere (Yi and Kornegay, 1996). In agreement with the results of Exp. 2, a decreased recovery of phytase in the stomach digesta was observed when 1.5% or 3.0% citric acid was added to a diet supplemented with 750 U/kg of phytase compared to a diet containing 750 U/kg phytase and no added citric acid. Interestingly, this decrease in stomach digesta phytase activity does not seem to have any carry over affects on performance, bone mineralization, or Ca and P digestibility as no additional interactions were found in either Exp. 1 or 2 with the exception of rib shear energy in Exp. 2. Rib shear energy, has been shown to be variable and not the best measure of Ca and P status so it remains uncertain if the phytase by citric acid interaction on rib shear energy in Exp. 2 is important or not. Stomach digesta was collected at only one point in time (3 h after consumption of a meal) so the mechanism by which citric acid decreased stomach digesta phytase activity is unclear. The citric acid may have lowered the stomach pH below the pH optima of the phytase and thereby decreased the activity of the phytase, or it may have affected the transit time of the digesta and as a result affected the phytase remaining in the stomach 3 h after meal consumption. A lowered gastric acidity may have also provided a more optimal environment for endogenous proteases which increased the degradation of phytase.

The effects of adding microbial phytase are due to its ability to hydrolyze the phytate molecule releasing the bound P. This caused increases in bone mineralization and P digestibility and improved performance as has been reported earlier (reviewed by Kornegay, 1995, 1996; Jongbloed, 1996b). In addition, phytase caused an increased digestibility of Ca. In Exp. 1, this result was confounded by the fact that the Ca level in the diet was increased as the level of phytase was increased to maintain the Ca to available plus estimated released P (arP) ratio. This was done in an attempt to optimize the Ca:arP ratio. According to the NRC (1988) the optimum Ca:total P ratio is between 1:1 and 1.5:1. However, in a study by Qian et al. (1996) they found P digestibility decreased as the Ca:total P ratio was increased from 1.2:1 to 1.6:1. Ideally, the Ca:P ratio should be based on available Ca and P instead of total Ca and P. In Exp. 1, as the level of phytase added to the diet was increased, more P became available. Therefore, we increased the level of Ca to try and maintain an equal Ca:arP in all diets. However, since the Ca in limestone is

highly available (Soares, 1995; Bakker, 1996) the observed Ca digestibility naturally increased as did the level of limestone. In addition, the $CaCO_3$ from the limestone has a high buffering capacity. Therefore, in Exp. 1 it appears that adding phytase increased the pH of the stomach digesta. This however, may have been due to the added limestone in the phytase treatments and not the phytase since the $CaCO_3$ in limestone can act as a buffer. At a low pH, like that found in the stomach, much of the $CaCO_3$ in the limestone would be in its ionic form where it could actively bind hydrogen ions and therefore increase the pH of the stomach digesta. These effects were not seen in the diet pH because at the higher pH levels found in the diets very little ionic $CaCO_3$ would be found and therefore the buffering capacity of the $CaCO_3$ would be much less. In agreement with this, Christiansen and Webb (1990) found a numerical increase in the abomasal pH of lambs when limestone was added to the diet. For this reason, the Ca level was left constant in all diets in Exp. 2 and $CaCl_2$ was used as the Ca source instead of limestone. The Ca level was left quite low (4.3 g/kg) in an attempt to maintain a near optimal Ca:total P ratio (1.2:1) in all diets. In Exp. 2, an increased Ca digestibility of 13 percentage units and 17 percentage units was observed when 250 U/kg and 500 U/kg of phytase were added to the diet, respectively. This clearly demonstrates that phytase can release Ca bound to phytic acid when it hydrolyzes phytate as has been previously reported in broilers (Schöner et al., 1991, 1993; Kornegay et al., 1996; Yi et al., 1996b) and pigs (Mroz et al., 1993; Yi et al., 1996; Kornegay and Qian, 1996, Radcliffe and Kornegay, 1997).

The effects of adding organic acids to weanling pig diets have been primarily attributed to their ability to lower or stabilize the gastric pH, making it a less hospitable environment for harmful bacteria to proliferate. In agreement with previous work (Kirchgessner and Roth, 1982; Falkowski and Aherne, 1984; Giesting and Easter, 1985; Giesting et al., 1991; Risley et al., 1992), pigs fed diets with added citric acid grew faster and had more efficient feed to gain ratios in Exp. 1. However, these results were not seen in Exp. 2. If the effects of adding organic acids are due to a decreased gastric pH which provides a less hospitable environment for harmful bacteria, then pigs which are bacterially challenged would be more responsive to adding organic acids than pigs which were not. The pigs in Exp. 2 grew faster and utilized their feed far more efficiently then the pigs in Exp. 1. This indicates that the pigs in Exp. 1 may have been under more stress, or they may have been less healthy due to disease challenges than the pigs in Exp. 2 which would explain why the response in growth performance to added citric acid was seen only in Exp. 1. In Exp. 1, citric acid addition improved Ca digestibility. Significant decreases in stomach digesta pH were also observed in both experiments. This decrease in pH may have slowed gastric emptying, causing an increased transit time through the gastrointestinal tract and allowing more time for Ca absorption. In addition Ca has a greater apparent absorption in an acidic environments. Scipioni et al. (1978) reported a decreased duodenal pH when citric acid was added to the diet. Burnell et al (1988) also observed a lowered intestinal pH when citric acid was included in the diet at a level of 1%. The lowered pH of the stomach digesta may also have provided a more optimal environment for endogenous enzymes to work, thus leaving less substrate for Ca to bind with, forming insoluble salts which are not easily absorbed.

Implications

Addition of citric acid to weanling pig diets lowers the gastric pH, and pig performance may be improved. This lowered gastric pH did not enhance the efficacy of microbial phytase. In fact, when high levels of phytase were supplemented to the diet, there was a decrease in the amount of phytase activity recovered from the stomach post-slaughter. However, this does not seem to affect phytase efficacy since no citric acid interaction with phytase was observed on growth performance, feed efficiency, DM, Ca and P digestibility, or bone parameters.

Chapter VI
General Summary

Adding microbial phytase to weanling pig diets low in P or Ca will result in improvements in growth, bone mineralization and Ca and P digestibility. Therefore, less inorganic Ca and P needs to be added to the diet, and as a result the amount of Ca and P being excreted per unit of growth is significantly decreased.

Response criteria which were sensitive to both P and phytase addition and which provided good linear or non-linear fits were used to determine P equivalency values, and included ADG, rib shear force, shear energy and ash weight, P digestibility and digestible P. Equivalency values based on growth data provided the highest equivalency estimates, and bone parameters provided the most conservative estimates. Digestibility data provided intermediate estimates of equivalency values. The P equivalency values of 500 U/kg microbial phytase ranged from .53 g P to .99 g P.

Response criteria which were sensitive to both Ca and phytase addition and which provided good linear or non-linear fits were used to determine Ca equivalency values, and included ADG, rib shear force, ash percent and ash weight, and fecal and ileal digestible Ca. Growth parameters provided the highest estimates of the Ca equivalency of microbial phytase, and estimates based on the fecal digestibility of Ca provided the lowest estimates. Calcium equivalency estimates based on ileal digestible Ca or bone parameters provided intermediate estimates of Ca equivalency. The Ca equivalency values of 500 U/kg microbial phytase ranged from .36 g P to 1.43 g Ca.

Addition of citric acid to weanling pigs diets caused a decrease in gastric pH measured 3 h after meal consumption. A decreased recovery of phytase activity in the stomach was also observed at the highest level of phytase supplementation. This however, did not appear to affect phytase efficacy as no phytase by citric acid interactions were observed on growth performance, feed efficiency, DM, Ca and P digestibility, or bone parameters. Citric acid alone may improve weanling pig performance, although results seen were variable.

In summary, the addition of phytase to low P or Ca weanling pig diets causes improvements in growth performance, bone mineralization and Ca and P digestibility. When 500 U/kg of phytase was added to the diet, approximately 0.78 g less P and .93 g less Ca was required in the diet. The efficacy of microbial phytase does not seem to be affected when 1.0 to 3.0% citric acid is added to the diet.

Chapter VII
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