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Supplemental Microbial Phytase Improves Utilization
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Xingen Lei

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SUPPLEMENTAL MICROBIAL PHYTASE IMPROVES UTILIZATION OF
PHYTATE PHOSPHORUS AND OTHER MINERALS BY YOUNG PIGS

By

Xingen Lei

A DISSERTATION

Submitted to
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ABSTRACT

SUPPLEMENTAL MICROBIAL PHYTASE IMPROVES UTILIZATION OF PHYTATE PHOSPHORUS AND OTHER MINERALS BY YOUNG PIGS

By

Xingen Lei

The low bioavailability of phytate-P in plant feeds to pigs contributes to diet expense of supplemental P and to P pollution in areas of intensive animal agriculture. The objectives of this research were to determine effects of supplemental Aspergillus niger phytase on utilization of phytate-P, Zn, and Ca, and interactions of phytase, Ca, Zn, and vitamin D in swine diets. Four major consecutive experiments were conducted with 258 weanling pigs. Variable levels of phytase, Zn, Ca, and vitamin D were incorporated into corn-soybean meal basal diets (BD) without added inorganic forms of P and(or) Zn in seven different trials. Response criteria including growth performance, plasma mineral concentrations and alkaline phosphatase (AP) activity, and mineral balance were repeatedly measured over time. Linear increases in weight gain, feed intake, and plasma inorganic P (P) concentrations were observed with increased phytase activity from 0 to 750 phytase units

Xingen Lei

(PU)/g BD. Responses of weight gain, gain/feed, and plasma AP activity were maximized at 1,200 PU/g BD. Concentrations of plasma P continued to increase and plasma Ca decreased linearly with increases in enzyme activity from 750 to 1,350 PU/g BD. Supplementing the BD with 750 PU/g increased P retention by 50% and reduced fecal P excretion by 42%, and 1200 or 1350 PU/g BD improved these two measures further. One thousand PU were equal in effect to .91 mg P from calcium phosphate. Pigs receiving 1,350 PU/g BD maintained normal concentrations of plasma Zn and P and normal rates and efficiency of gain. Supplemental phytase improved dietary Ca utilization, but did not affect plasma concentrations of Mg, Cu, or Fe. Only a reduced dietary Ca (.5%) level allowed supplemental phytase to produce all the above improvements. Normal to high dietary Ca (.9%) markedly decreased the efficacy of phytase, which was partially alleviated by raising dietary vitamin D. In conclusion, supplementing corn-soybean meal diets of weanling pigs with A. niger phytase at 1,200 PU/g appeared to maximize phytate-P utilization at a reduced diet Ca level, and essentially obviated the need for inorganic P addition.

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LIST OF ABBREVIATIONS

ADFI	average daily feed intake
ADG	average daily gain
AP	alkaline phosphatase
BD	basal diet
PU	phytase unit

INTRODUCTION

Over 60 to 70% of P in food of plant origin is bound with myo-inositol phosphates as phytate that is poorly available to simple-stomached animals such as pigs and poultry. The low bioavailability of plant P to these animals results in the need for addition of inorganic P to the diets to meet their P requirements. Because inorganic P deposits are limited, this supplementation not only increases feed cost, but also sometimes causes feed P shortage, which was the case in the 1970s in the USA. Moreover, the unutilized dietary P excreted in the manure contributes to P pollution in areas of intensive animal agriculture.

More than 20 years ago, attempts were made to use phytase, an enzyme that hydrolyzes phytate, in the feed to improve phytate-P utilization. However, because of high anticipated phytase production cost compared to the relatively low price of inorganic P sources, the potential for phytase never received adequate attention. Recent heightened environmental awareness of P and nitrogen pollution originating from animal manure in some European countries as well as in the USA has renewed the interest in

phytase. Modern biotechnology has facilitated the development of commercial phytases. Currently, a limited supply of microbial phytase is available in Europe, and effects on phytate-P from plant feeds for pigs and poultry have been examined. However, the data reported on pigs has been mainly generated from digestion or balance trials with single or double doses of phytase in the diets. The effects of supplemental microbial phytase on P status in plasma and performance of pigs were not studied.

This research was undertaken with weanling pigs supplemented with a series of levels of microbial phytase in typical corn-soybean meal diets. The objectives were to determine the effectiveness, optimal dietary activity, and inorganic P equivalents of supplemental phytase. In addition, the improvement in dietary Ca and Zn utilization by added phytase, and the effect of dietary concentrations of Ca, Zn, and vitamin D on the efficacy of phytase were determined.

LITERATURE REVIEW

Phosphorus

1. Phosphorus has versatile functions in animal body

Phosphorus accounts for approximately 1% of the body weight of mature pigs (Peo, 1991). Although this is only 25% of the total mineral in the body and ranks second to that of Ca (Cromwell and Coffey, 1991), P is probably the most protean of all the mineral elements. About 80% of P is found as hydroxyapatite in the bones and teeth, so that P with Ca plays vital roles in the development and maintenance of the skeletal tissues (Underwood, 1981). The remaining 20% of P is widely distributed in the fluids and soft tissues where it functions in almost all processes and aspects of metabolism (Peo, 1991). As a part of RNA and DNA, P is essential for cell reproduction. As a part of organic phosphate such as ATP, P is necessary for energy transduction. As a part of phospholipid, P is essential for maintaining membrane integrity and is involved in fat transport. As an anion, P cooperates with other mineral elements to maintain osmotic and acid-base balance. In addition, P is involved in the control of appetite and in

the efficiency of feed utilization (Underwood, 1981).

Deprivation of dietary P results in an initial fall in plasma inorganic P concentration (hypophosphatemia), a rise in plasma Ca concentration (hypercalcemia), and a rise in plasma alkaline phosphatase (AP) activity (Miller et al., 1964; Underwood, 1981; Engstrom et al., 1985; Pointillart et al., 1987; Pointillart, 1991). Hypophosphaturia, hypercalciuria, and hyperhydroxyprolinuria often follow (Pointillart et al., 1987; Nasi, 1990; Pointillart, 1991). Renal 1-alpha-hydroxylase and plasma 1, 25-(OH)₂D₃ levels are increased (Littledike and Goff, 1987). If sufficiently severe or prolonged, clinical signs of P deficiency appear. These include depressed appetite, poor rate and efficiency of gain, rickets in the young and osteomalacia in the old, and impaired fertility (Underwood, 1981, NRC, 1988). However, these clinical signs are almost indistinguishable from those resulting from deficiencies of dietary Ca or vitamin D (Peo, 1991).

2. Phosphorus metabolism depends on Ca, vitamin D, and other factors

Phosphorus is absorbed mainly in the proximal end of the duodenum in the orthophosphate ($\text{PO}_4^{=}$) form (Bartter, 1964; Irving, 1964), so that P absorption in the large intestine of pigs appears negligible (Jongbloed, 1987). Although the mechanism of P absorption is still unclear, the

factors influencing the amount of dietary P absorbed have been elucidated (Peo, 1991). These factors include 1) dietary level and source of P, 2) dietary Ca and vitamin D level, 3) dietary ratio of available P and Ca, 4) intestinal pH, and 5) other minerals that are antagonistic to P absorption (Underwood, 1981; Peo, 1991). Normal dietary levels of Ca enhance P absorption (Fox et al., 1978), but high levels of dietary Ca generally decrease P absorption (Jongbloed, 1987).

The P concentration of whole blood ranges from 35 to 45 mg/dL (Peo, 1991), while plasma inorganic P concentrations are about 7 to 10 mg/dL (Miller et al., 1964; Ullrey et al., 1967; Hancock et al., 1986; Miller and Ullrey, 1987; Friendship and Henry, 1992). Low plasma P concentrations result in poor performance, probably due to the impairment of the extensive metabolic functions associated with P (Hays, 1976). Phosphorus is excreted from the body via the feces and urine (Moore and Tyler, 1955a, b; Pike and Brown, 1984). Thus, digestibility determinations are apparently incomplete estimates of the biological value of a P supplement (Peo, 1991). Various response criteria have been used to determine dietary P availability by pigs. Peo (1991) reviewed a number of studies conducted from 1962 to 1986 and classified the sensitivity of the commonly used criteria as 1) high: physical traits of bone and percent bone ash (for the young); 2) good: gain, feed conversion, and serum AP

activity; 3) low: serum Ca and P concentrations and percent bone ash (for the adults); and 4) very low: percent composition of bone ash.

3. Phosphorus supplements in diets are very costly

Corn-soybean meal diets for swine contain approximately .3% of total P, which would be sufficient to meet the P requirement of growing pigs if it was completely bioavailable (Simons et al., 1990; Cromwell., 1991). However, the average bioavailability of P in these diets is only 15% (Cromwell and Coffey, 1991). Thus, supplements of an inorganic P source (e.g. dicalcium phosphate or defluorinated phosphate) or animal products (e. g. meat and bone meal or fish meal) in the diets are necessary to prevent deficiencies of P in pigs. Indeed, supplementation with inorganic P effectively meets the P requirement of pigs and thereby maximizes overall performance (NRC, 1988).

However, there are three major problems associated with inorganic P supplementation. First of all, inorganic P is quite expensive and ranks next to corn and soybean meal as the major expense of swine diets. At least \$2 or 3 for each marketing pig would be saved if inorganic P supplementation could be obviated (NAS, 1974). Next, inorganic P is a non-renewable P resource. Continuing use of inorganic P supplements at the current rate for animal production will lead to exhaustion of these limited deposits (NAS, 1974).

Finally, and probably most important, part of the supplemental inorganic P, and most of the dietary phytate-P ends up in swine manure and results in excessive P entering the environment (Cromwell and Coffey, 1991). Over 100 million tons of manure are excreted by livestock and poultry in the United States annually, with approximately 18% from swine and poultry (Sommers and Sutton, 1980; Gilbertson et al., 1984). Because P concentration in non-ruminant manure is twice as high as that in manure of ruminants, wastes of swine and poultry contribute one-third of the total P excreted in animal waste (Sommers and Sutton, 1980; Gilbertson et al., 1984). The amount of P applied to soils in animal waste, together with chemical fertilizer, often exceeds the amount needed for plant growth. As a result, P tends to accumulate in the soil and becomes a major pollutant of the environment (Tunney, 1990; Lenis, 1989). Phosphates move into surface water from surface run-off and soil erosion and then stimulate growth of algae and other aquatic plants there (Sharpley and Menzel, 1987). This eutrophication decreases the quality of fresh water, diminishes its oxygen content, and thereby creates an undesirable environment for fish and other wildlife (Cromwell and Coffey, 1991; Swick and Ivey, 1992). In the Netherlands, Denmark, Belgium, and some parts of France, pollution of the environment with P and nitrogen originating from animal manure is becoming a major problem (Lenis,

1989). The same concern is also becoming an important issue in the USA (Cromwell and Coffey, 1991; Swick and Ivey, 1992), and probably in other parts of the world as well.

Phytate

1. Phytate stores phosphate and energy in plant seeds

Phytic acid was discovered by Harting in 1855 (Reddy et al., 1982). Through extensive studies by many generations, it becomes acceptable that phytic acid is myo-inositol 1, 2, 3, 4, 5, 6-hexakis phosphate with an empirical formula of $C_6H_{18}O_{24}P_6$ (Reddy et al., 1982; Gibson and Ullah, 1990). In plants, phytic acids exists as Ca-Mg-K salts called phytin (Cosgrove, 1980). The structure and conformation of phytic acid has been a subject of controversy (Reddy et al., 1982). Weingartner and Erdman (1978) proposed a partially dissociated Anderson-based structure for phytic acid that might occur at neutral pH, which explains cation bindings of phytic acid.

Phytate rapidly accumulates in seeds during the ripening period (Nahapetian and Bassiri, 1975). Possible physiological roles for phytic acid include its role as a storage of phosphate, an energy source, an inhibitor of dormancy, and as a mineral storage site (Gibson and Ullah, 1990). In addition, phytic acid prevents aflatoxin production in soybean seeds by making Zn unavailable to the

mold (Gupta and Venkatasubramanian, 1975). Phytic acid has been shown to form an Fe chelate that inhibits Fe-catalyzed hydroxyl radical formation and lipid peroxidation, thus could be used as a food antioxidant (Graf and Eaton, 1990; Empson et al., 1991). Phytate may protect the seed against oxidative damages during storage (Hernandez-Unzon and Ortega-Delgado, 1989). Furthermore, phytic acid affects cooking quality in legumes (Bhatta and Slinkard, 1989).

2. Phytate represents most of phosphorus in cereals and legumes

Concentrations of phytate and phytate-P in various foods have been summarized by Nelson et al. (1968) and Reddy et al. (1982). In general, the amount of phytate varies from .50% to 1.89% in cereals, from .40% to 2.06% in legumes, from 2.00% to 5.20% in oil seeds, and from .40% to 7.50% in protein products (Reddy et al., 1982). In cereals such as wheat, rice, barley and rye, the majority of phytate locates near the outside of the seed coat, whereas in corn, phytate concentrates in the germ (O'Dell et al., 1972b). In legumes, phytate is distributed throughout the entire protein complex of the seed (Reddy et al., 1982). The uneven distribution of phytic acid in different morphological components or parts of cereal and legume seeds allows milling to selectively remove or reduce phytate contents in certain products, which will be discussed later.

3. Phytate is fairly indigestible by simple-stomached animals

Phytate is a nutrient source because it could provide animals with P. However, the biological availability of phytate-P in feed to ruminants greatly differs from that to nonruminants. Due to the rumen microorganisms, cattle and sheep hydrolyze naturally-occurring or pure phytate fairly efficiently (Reid et al., 1947; Raun et al., 1956). Nelson et al. (1976) studied the hydrolysis of natural phytate-P from soybean meal, sorghum grains, and corn meal in calves and steers. They found that the initial phytate hydrolysis occurred in the rumen and was complete before the feed reached the other parts of the digestive tract. Clark et al. (1986) fed 30 Holstein cows with diets of 50% grain and 50% of corn-silage through the first 18 wk of lactation. Their results indicated that cows ingested approximately 40 g phytate-P daily and hydrolyzed 98% of it to inorganic P. In general, biological availability of dietary phytate-P to ruminants are 50% or greater (Reddy et al., 1982).

In contrast, pigs can utilize only a small portion of dietary phytate-P. The biological values of phytate-P in four earlier studies ranged from 25% to 40% (Reddy et al., 1982). Calvert et al. (1978) demonstrated that phytate-P from barley and corn was about 17% and 8% digestible, respectively, for growing pigs when phytate in the diets was the major source of P. Recently, Jongbloed et al. (1992)

reported that the typical corn-soybean meal diets for swine contained approximately .33% of total P, of which 78.8% was as phytate-bound P. They found that the apparent digestibility of phytate was 21.5% for duodenal digesta and 12.9% for overall. Comparable availabilities of phytate-P from similar corn-soybean basal or control diets have been shown in other studies (Pointillart et al., 1984, 1985, 1987; Nasi, 1990; Simons et al., 1990; Pointillart, 1991). These values generally agree with the estimates of Cromwell and Coffey (1991).

Phytate-P from cereal grains and legumes is also poorly available to poultry (Nelson, 1967; Reddy et al., 1982). Gillis et al. (1957) fed chicks and turkeys ^{32}P -labeled Ca-phytate and ^{32}P -labeled monosodium orthophosphate and measured the amount of radioactivity retained in the tibia. They found that chicks and turkey used P from Ca-phytate only 10% and less than 2%, respectively, as effectively as P from monosodium orthophosphate. Employing the chromic oxide balance method, Nelson (1976) demonstrated that 4- and 9-wk-old chicks and laying hens (single comb white leghorn) respectively, hydrolyzed 0, 3, and 8% of dietary phytate-P from corn which was the only grain source. However, other researchers have reported relatively high biological values of phytate-P in the diets for poultry (Reddy et al., 1982).

The bioavailability of dietary phytate-P from plant foods to rats appears in the same range as that to swine and

poultry (Moore and Veum, 1982, 1983; Williams and Taylor, 1985). In a limited number of studies with humans, the bioavailability of dietary phytate-P from wheat bran varies from 40% to 60% (Sandberg et al, 1982, 1986, 1987; Sandberg and Andersson, 1988).

4. Phytate utilization varies with dietary factors and age

Species is not the only determining factor of availability of dietary phytate-P. Dietary ingredients, dietary concentrations of phytate, P, Ca, and vitamin D, and age of animals also influence the utilization of phytate-P. Replacing corn in part by wheat (Pointillart et al., 1984), triticale (Pointillart et al., 1987), or rye bran (Pointillart, 1991) in diets for growing pigs significantly enhanced phytate-P utilization. Improvement in phytate hydrolysis was also shown in chicks and laying hens by substituting 50% of the corn with wheat in the diets (Nelson, 1976). Ranhotra et al. (1974) found that the amount of phytate hydrolyzed increased with elevated dietary phytate levels in rats. In contrast, Pierce et al. (1977) reported that increasing dietary phytate-P level from .30% to .38% resulted in impairment in overall performance and development in young pigs (11-14 kg). Moore and Veum (1982) demonstrated that phytate-P was more available to rats given diets with low inorganic-P than diets with supplements of P. This adaptation by rats appeared to be related to enhanced

phytase or alkaline phosphatase synthesis by the gastrointestinal microflora stimulated by the low level of P in the digesta (Moore and Veum, 1983). Ballam et al. (1985) fed 3-wk-old broiler chicks corn-soybean meal diets containing varying amounts of Ca and P. They found that increasing dietary inorganic P level from .12% to .45% improved phytate hydrolysis in the absence of added Ca (dietary Ca level: .09%). Where all diets contained 1.0% Ca, the addition of all levels of inorganic P (up to .80%) decreased phytate hydrolysis by chicks. It is believed that calcium binds phytate in the gastrointestinal tract by forming an insoluble Ca-phytate complex, and thus rendering both Ca and P in the molecule largely unavailable to absorption (Wise, 1983; Nelson and Kirby, 1987). When there is a low Ca to phytate ratio in the diet, the majority of the dietary phytate could be hydrolyzed (Ballam et al., 1985) and metabolized (Nahapetian and Young, 1980). However, high levels of Ca in the diets of rats (Taylor and Coleman, 1979; Nahapetian and Young, 1980) and poultry (Edwards and Veltmann, 1983; Ballam et al., 1985; Sheideler and Sell, 1987) were consistently shown to decrease the availability of phytate-P. Mohammed et al. (1991) fed 1-d-old male broiler chicks either a control diet containing recommended levels of P, Ca, and cholecalciferol or experimental diets low in P and with variable levels of Ca (normal or low) and cholecalciferol (normal or high). They concluded that

lowering of Ca or raising cholecalciferol alone improved phytate-P utilization and simultaneously applying both in low-P diets restored all variables to the levels for the control diet. Pointillart et al. (1989) found that increasing Ca level from .9% to 1.4% in diets containing 0.5% total P (all plant origin) intensified the harmful effects of P deficiency secondary to phytate feeding in pigs, but not via reduced phytate-P availability. Generally, the proportion of dietary phytate-P hydrolyzed increases with age in poultry (Peeler, 1972) and pigs (Newton et al., 1983). However, the opposite occurred as hens progressed through the egg laying period (Scheideler and Sell, 1987). Likewise, Nelson and Kirby (1979) observed significant decreases in phytate hydrolysis during each of the first 4-wk periods in rats. They also noted that mature rats hydrolyzed only half of the dietary phytate utilized by weanling rats.

5. Phytate is an antinutritional factor

The ability of phytate to form complexes with metal ions and proteins has been studied extensively (Wise, 1983, Champagne et al., 1990). At neutral pH, phosphate groups of phytate have either one or two negatively charged oxygen atoms (Reddy et al., 1982). It is apparent that various cations could strongly chelate between two phosphate groups or weakly within a phosphate group (Erdman, 1979). Maddaiah

et al. (1964) found the following decreasing order of stability of metal-phytate complex: $Zn^{++} > Cu^{++} > Co^{++} > Mn^{++} > Ca^{++}$. In the intestine, phytate is able to bind to protein via divalent cation bridges (Wise, 1983). Thus, numerous studies have proven that phytate in plant foods reduces bioavailability of essential mineral elements (Oberleas, 1973; O'Dell, 1979, Reddy et al., 1982) and protein (Rojas and Scott, 1969; Prattley and Stanley, 1982). Of great importance in both human and animal nutrition is the adverse effect of phytate on Zn utilization.

Tucker and Salmon (1955) first discovered Zn deficiency in pigs fed a diet composed of corn and soybean meal. Later, O'Dell and Savage (1960) found that the poor Zn availability in the soy protein, and in other plant foods as well, resulted from the formation of a complex between Zn and phytate, which prevented Zn from absorption. Oberleas et al. (1962, 1966) demonstrated the same adverse effect of phytate on Zn metabolism in pigs and rats. More importantly, Oberleas et al. (1962) observed that the addition of Ca to phytate-supplemented diets further reduced utilization of dietary Zn, but high levels of dietary Ca had no effect on Zn utilization in the absence of phytate. It has been assumed that the high amount of Ca in common diets may result in coprecipitation of trace metals with Ca-phytate, and may break the phytate-protein complex in the intestine (Wise, 1983).

It is well established that Zn from plant origin foods is less available than from animal origin (Reddy et al., 1982, Bobilya et al., 1991), thus pigs receiving corn-soybean meal diets require higher dietary Zn level than those consuming casein-glucose diets (Shanklin et al., 1968; NRC, 1988). Newton et al. (1983) reported that incorporating 10% or 20% wheat bran in diets for growing pigs decreased Zn absorption. Sandstead et al. (1990) showed that as little as 26 g of wheat bran added to the daily bread of men fed omnivorous diets impaired retention of both Zn and Ca. Direct demonstration of inhibitory effect of phytate in humans on a stable isotope Zn tracer absorption and on a radioactive Zn tracer retention was given by Turnland et al. (1984) and Sandstrom et al. (1987), respectively. These findings provided the basis for the thesis that Zn deficiency among humans in Middle Eastern countries was in part caused by the high phytate contents of the breads that are the staple food of the poor (Prasad et al., 1963).

6. Phytate contents in foods can be reduced by processing

Several methods are shown to reduce or remove considerable amounts of phytate in cereals and legumes. Kumar et al. (1978) reported that cooking decreased both water- and acid-soluble phytate-P in green gram, cowpea, and chickpea. de Boland et al. (1975) found that autoclaving inositol hexaphosphate and isolated soy protein for 2 hr

resulted in substantial loss of phytate. As discussed earlier, phytate is stored as a source of P for seed germination in plants. Thus, germination reduces or eliminates considerable amounts of phytate from the seeds or grains (Reddy et al., 1982). In addition, fermentation, soaking, and autolysis may also appreciably reduce phytate contents of plant foods (Reddy et al., 1982). Phytate contents in common bean seeds (Phaseolus vulgaris L.) substantially decrease after prolonged storage (Hernandez-Unzon and Ortega-Delgado, 1989).

It has been mentioned in the above section that phytate unequally distributes in different morphological components or parts of cereal grains. Therefore, selective removal or reducing of phytate contents in certain products by mechanical processes is possible. Milling followed by germ separation can remove 89% of the phytic acid in corn in which phytate concentrates in germ (O'Dell et al., 1972). Milling should also remove appreciable amounts of phytate from wheat, barley, rice, rye, and triticale, those grains have phytate in the surface layer (Reddy et al., 1982).

Various chemical methods have also been used to remove phytate from soybeans (Reddy et al., 1982). All the procedures are based on the different solubility of phytate and protein depending on pH. However, neither this approach nor other operations described above are economical on an industrial scale.

Phytase

1. Phytase initiates phytate degradation

Phytases, myo-inositol hexaphosphate phosphohydrolases, comprise a family of enzymes that catalyze the stepwise removal of inorganic orthophosphate from phytate. In addition, phytases hydrolyze a variety of natural and synthetic phosphorylated substrates accepted by nonspecific acid phosphatase as well (Gibson and Ullah, 1990). Thus, phytases are also special kinds of phosphatase by nature. Two types of phytases are recognized (Gibson and Ullah, 1990). One is called 3-phytase (E. C. 3. 1. 3. 8) which initiates the removal of phosphate groups attached to position 1 or 3 of myo-inositol. The other is called 6-phytase (E. C. 3. 1. 3. 26) which first frees the phosphate at 6-position (Nayini and Markakis, 1984). Both enzymes eventually deesterify phytic acid completely. Phytases characterized from microorganisms and the filamentous fungi belong to 3-phytase. The seeds of higher plants typically contain 6-phytase. However, in the majority of examples, the type of phytase activity is not described because of the difficulty in characterization and/or the existence of mixed intermediate products (Gibson and Ullah, 1990). In general, both types of activity are referred to as phytase. The unit of phytase activity described in this thesis is defined as the amount of enzyme that liberates 1 nmol of inorganic P

from sodium phytate per minute at pH 5 and 37°C. Different units used in other studies are converted when discussed herein.

Phytase activity from rice bran was one of the first enzymes exhibiting phosphomonoesterase activity to be characterized (Suzurk et al., 1907). But, the current best characterized phytase is produced by a strain or variety of Aspergillus niger, A. ficuum NRRL 3135. Soybean phytase has also been well studied. Gibson and Ullah, with their associates have been actively involved in the biochemical characterization of these two phytases and the cloning of the genes for these enzymes. Most of their work, plus that of others, has been summarized (Gibson and Ullah, 1990). From these data, we understand that A. ficuum phytase is a 3-phytase and a glycoprotein with a molecule weight between 85-100 KDa. Soybean phytase is a 6-phytase with a molecular weight approximately 50 KDa. Both enzymes are most active around pH 5.0, with the fungal enzyme having a broader pH profile in the acidic range. Both possess optimal activity at 55-58°C. Both are inhibited competitively by phosphate, but soybean phytase is more sensitive to the inhibition. The fungal phytase has much higher turnover number for phytic acid than the soybean phytase. Other properties of these two enzymes have been described by Gibson and Ullah (1990). The enzymatic properties of other phytases from certain cereals and legumes have also been studied (Reddy et al., 1982).

2. Phytase activity occurs in microorganisms, plants, and animal tissues

Although phytase is formed during germination and may play key roles in this process (Reddy et al., 1982), it is also widely distributed in microorganisms and animal tissues (Bitar and Reinhold, 1972; Cosgrove, 1980; Nayini and Markakis, 1986). The exact numbers of microorganisms capable of producing phytase is unknown. But it is known that many microorganisms produce intracellular phytase (Cosgrove, 1980; Nayini and Markakis, 1986). Shieh and Ware (1968) conducted a painstaking survey of more than 2000 microorganisms and observed that 30 of the isolates produced extracellular phytase activity. All the extracellular phytase producers were of fungal origin, among which A. ficuum NRRL 3135 generated the highest amount of enzyme on a corn-starch-based medium with limited inorganic P. Their findings have been extremely important because A. ficuum or its mutants has been extensively used as a rich source of microbial phytase. Lopez et al. (1983) found that 16 bacteria strains isolated from a natural lactic fermentation of corn meal had active phytase. Kang et al. (1988) isolated 67 strains from the fermenting corn and soybean meals with phytase activity. They identified Bacillus licheniformis and Enterobacter cloacae as the highest phytase producers. Phytase activity was also found in culture filtrates of Bacillus subtilis (Powar and Jangannathan, 1967). In

addition, phytase activity was also detected in a number of soil microorganisms (Cosgrove, 1980), yeasts (Nayini and Markakis, 1984; Lambrechts et al., 1992), molds used in oriental food fermentation (Wang et al., 1980), and rumen (Raun et al., 1956).

Phytase has been reported in a wide range of seeds of higher plants containing phytic acid (Reddy et al., 1982; Gibson and Ullah, 1990). Among the commonly used grains and legumes, wheat (Ranhotra and Loewe, 1975), triticale (Pointillart et al., 1987), and rye (Pointillart, 1991) are relatively rich in phytase. These grains and their by-products are used as sources of supplemental dietary phytase in both animal and human diets, which will be discussed later.

Phytase activity was detected in the intestinal mucosa of rat, chicken, calf, and man (Bitar and Reinhold, 1972). However, phytase activity in the intestinal mucosa of pigs is negligible if it is really there (Pointillart et al., 1984). Mucosal production of phytase is induced by low dietary P in chicks (McCuaig et al., 1972) but not in rats (Moore and Veum, 1983). Zinc deficiency markedly reduces production of endogenous phytase (Davies and Flett, 1978). In summary, phytase in the gastrointestinal tract of animals may originate from ingested plant ingredients, the gut microflora, and endogenous production by the intestinal mucosa. However, the total phytase activity in the

gastrointestinal tract of pigs fed ordinary corn-soybean meal diets is negligible (Jongbloed et al., 1992).

3. Phytase releases phytate-phosphorus from plant foods in vitro

Incubating synthetic or natural phytate in phytase activity bearing microorganism cultures results in substantial hydrolysis of phytate. Raun et al. (1956) found that washed suspensions of rumen microorganisms were able to hydrolyze appreciable amounts of Ca-phytate as measured by the presence of inorganic P following incubation in an artificial rumen system. Lopez et al. (1983) found that natural lactic acid fermentation of corn significantly reduced phytate contents and thus increased the amount of free P in the culture. Similar hydrolysis of phytate was also seen in fermented peanut press cake (Fardiaz and Markakis, 1981) and in fermented and germinated pearl millet (Khetarpaul and Chauhan, 1990).

Nelson et al. (1968a) added A. ficuum phytase to a liquid soybean meal and incubated the mixture for 2-4 h at 50 °C. After drying, the treated soybean meal was fed to 1-d-old chicks. The birds utilized this hydrolyzed phytate-P as efficiently as they did inorganic P. Under the same light, Zhu et al. (1990) found that 81% of phytate-P was converted to orthophosphate in a feed mixture of soybean meal, corn and wheat bran when treated with native phytase

in wheat bran and ground corn by a 2-step process. The first step consisted of two successive 2-h conditioning periods at 55°C for corn and soybean meal; one at pH 3.5 with citric acid followed by another at pH 5.1. In the second, or hydrolysis step, wheat bran (.25 parts), a rich source of phytase, was mixed with the wet, citric acid-conditioned corn and soybean meal, and the mixture was held for 8 h at 45°C and pH 5.1. After the enzymatic reaction, the increase in orthophosphate accounted for 78% of the phytate-P released and the mixture contained .42% inorganic P in dry matter. A 17-d chick feeding trial showed that tibia bone ash weights tended to be higher for chicks consuming the treated, low-phytate feed than for those fed on the control feed. Han and Wilfred (1988) applied crude culture filtrate of A. ficuum to soybean and cottonseed meals for 24 h at 37°C. They found that 85% phytate in soybean meal and 67% phytate in cottonseed meal was hydrolyzed. They also noted that treatment at higher temperature (50 °C), pH 4-5.5, and heating the substrates 1 h at 121 °C prior to the enzyme treatment facilitated the hydrolysis of phytate by microbial phytase. Simons et al. (1990) further characterized the in vitro effects of A. ficuum phytase on degradation of phytate in plant feeds. They demonstrated that the enzyme activity maximized at both pH 5.5 and pH 2.5, and confirmed the thermal stability of the enzyme resistant to heat of pelleting previously shown (Jongbloed and Kemme, 1990).

Supplementing the microbial phytase at 1000 PU/g substrate, they observed that hydrolysis of phytate in ground corn or soybean meal was complete after an incubation of 1 h at 40 °C and was 80% in a corn-soybean meal diet for growing pigs after 4 h at room temperature. In addition to orthophosphate liberation, an increase in amount of protein in a solid stable fermentation of canola meal by A. ficuum phytase was also shown (Nair and Duvnjak, 1990). Based on the above results, we can see the possibility of preparing low-phytate diets for poultry and swine by treating plant feeds with either microbial or cereal phytase in vitro. However, this process seems uneconomical (Zhu et al., 1990). Han (1989) estimated that feeding the phytase treated feed would be approximately 17 times more expensive than the addition of fertilizer-grade P, without crediting other benefits associated with phytase application.

4. Phytase supplementation in diets improves phytate-phosphorus bioavailability to poultry

Attempts to add phytase to chick diets were dated back to 1944, and supplements of sun-cured alfalfa meal, wheat meal, barley meal, and lysed E. Coli. cellular material to the diets promoted phytate-P utilization by chicks (Harms and Damron, 1977). Nelson et al. (1971) supplemented a corn-soybean meal diet for chicks with preparations of A. ficuum phytase. They found that the supplemental microbial phytase

was completely effective in hydrolyzing the dietary phytate-P in the alimentary tract of the chick. Twenty years later, the effectiveness of A. ficuum phytase in improving phytate-P availability to poultry was consistently confirmed by a number of researchers (Kiiskien and Piironen, 1990; Saylor, 1991; Schoner et al., 1991; Simons et al., 1990; Swick and Ivey, 1990). However, the amounts of dietary microbial phytase needed to match the effect of recommended levels of supplemental inorganic P in these studies were variable.

Simons et al. (1990) supplemented graded levels of A. ficuum phytase in diets composed of corn, soybean meal, sorghum meal, and sunflower meal for broilers. The results indicated that supplementing phytase increased dietary P availability to 60% and decreased the amount of P in the dropping by 50%. Though phytase up to 1500 PU/g diet further improved weight gain and P availability, phytase at 750 PU/g diet resulted in body weight gain to be equivalent to that obtained with diets supplemented with inorganic P. Schoner et al. (1991) reported that supplementing the microbial phytase at 800 PU/g diet resulted in a higher total P retention than that of control diets with added inorganic P. Moreover, Kiiskien and Piironen (1990) reported that phytase added to a barley-oat diet at 500 PU/g gave performance equivalent to that of inorganic P supplemented diet. In contrast, Saylor (1991) found that supplemental phytase at 1000 PU/g of a corn-soybean meal diet was insufficient to

release the phytate-P completely. Swick and Ivey (1990) demonstrated that chicks supplemented with phytase at 450 PU/g had a 20% improvement in dietary P retention but a slower gain, compared with that fed inorganic P. The variations of phytase activity to maximize the utilization of dietary phytate-P in these studies may have been attributed to many factors (Swick and Ivey, 1992). However, differences in diet composition and P concentration could be one of the major reasons. As previously discussed, dietary phytase activity originating from ingredients, dietary Ca and other nutrients affect phytate utilization and in turn phytase efficacy. Improvements of phytate-P utilization was linearly related to supplemental phytase at low-P levels (Schoner et al., 1991). But, the improvements diminished with increased inorganic P concentrations in the diets (Swick and Ivey, 1991). The inorganic P equivalent of phytase was proposed as 700 PU to 1.0 g P from dicalcium phosphate (Schoner et al., 1991).

5. Phytase supplementation in diets improves phytate-phosphorus bioavailability to swine

Recently, two sources of phytase have been shown to effectively improve phytate-P utilization in swine diets. One is cereal phytase. Using corn-soybean meal diets as the control, Pointillart and associates investigated the effects of cereal phytase from wheat (Pointillart et al, 1984),

triticale (Pointillart et al., 1987), and rye bran (Pointillart, 1991) on dietary phytate-P availability to growing pigs. The actual determined phytase activity in the experimental diet incorporated with 90% wheat, 80% triticale, or 20% rye bran was 160, 440, and 1200 PU/g, respectively. In contrast, the phytase activity in the control corn-soybean meal diets was negligible (0 to 20 PU/g). The measured criteria included P and Ca balance, bone and plasma concentrations of P and Ca, plasma vitamin D metabolites and parathyroid hormone, bone bending moments and intestinal phosphatase activities. The responses of these measures consistently indicated that all three cereal phytases significantly improved dietary phytate-P utilization and reduced, as expected, fecal P excretion. Meanwhile, dietary Ca utilization was also enhanced. Besides, Newton et al. (1983) reported that adding 10% or 20% of wheat bran in the diets for growing pigs elevated dietary P absorption.

The other source of phytase is microbial phytase, almost exclusively from A. ficuum. Simons et al. (1990) supplemented the enzyme at 1000 PU/g of a corn-soybean meal diet and a practical Dutch diet for growing pigs. Similar improvement in apparent P digestibility (24%) and reduction in fecal P excretion (35%) was shown in both diets by supplemental phytase. Nasi (1990) found that supplementing the enzyme at 500 PU/g of corn-soybean meal diets improved P

digestibility to the same level of inorganic P supplemented control diets. But, a larger proportion of dietary P was retained in phytase-supplemented diets than in the control diets, indicating that utilization of dietary P was improved by supplemental phytase as well. Leunissen and Young (1992) demonstrated that supplemental phytase at 500 PU/g diet for weanling pigs increased the availability of dietary P equivalent to that of adding .17% of inorganic P. They did not show further benefit of supplemental phytase by doubling the dose. However, the basal diet used by them contained, in addition to corn and soybean meal, 17.0% canola meal, 10% whey, and .04% calcium phosphate which resulted in a high dietary P level (.55%). Cromwell (1991) and associates supplemented corn-soybean meal diets for growing-finishing pigs with the microbial phytase at 0, 500, and 1000 PU/g and measured the responses of performance and bone traits. Based on the results, Cromwell (1991) proposed that supplemental phytase at 1000 PU/g diet would release sufficient available P from phytate in corn-soybean meal diets to almost meet the P requirement of finishing pigs (NRC, 1988).

In contrast, earlier attempts to feed Saccharomyces cerevisiae or other phytase-containing yeast culture to pigs failed to show improvements in phytate-P availability (Cromwell and Stahly, 1978; Chapple et al., 1979; Shurson et al., 1983). However, these researchers used phytase products with undefined activity, and(or) used mainly weight gain as

the response measure. Thus, the ineffectiveness of yeast phytase may have been attributed to a possible activity insufficiency of the products, a possible incompatibility of these enzymes with the low pH in the stomach of pigs, and a possible incompleteness or insensitivity of the response measures. The A. ficuum phytase resulting in positive response in the above discussed trials was acid and heat resistant over a broad pH and temperature (pH 2.0 to 6.0, and up to 60 °C, Simons et al., 1990). The effectiveness of the enzyme in vivo in hydrolysis of phytate was confirmed by Jongbloed et al. (1992). Using two simple T-cannulas in the duodenum and terminal ileum of pigs, they demonstrated that supplementing A. ficuum phytase at 1500 PU/g in the diets degraded a substantial portion of dietary phytate in the gastroduodenal section.

6. Phytase supplementation in diets improves phytate degradation in humans

Dietary fiber is advocated increasingly for humans for its purported health benefits. Increasing dietary consumption of cereal fibers, legumes, and soy protein isolates has become a trend and may result in an increased intake of phytate. The major concern in human nutrition is the adverse effect of phytate on mineral metabolism, particularly on trace elements, rather than P bioavailability (Reinhold et al., 1976; Davis, 1979; Wise,

1983; Sandstrom et al., 1987; Sandstead et al., 1990; Sandstead, 1992). However, endogenous phytases in bran have a major effect on phytate hydrolysis in humans and the amount of activity varies with the method of processing of the fiber (Sandberg et al., 1982, 1986, 1987; Sandberg and Andersson, 1988). Sandberg et al. (1987) fed the raw or extruded wheat bran to seven adult patients with ileostomies and found a significant reduction in phytate digestibility in the extruded bran compared to the unprocessed bran. The deactivation treatment of the bran did not make it resistant to hydrolysis, and the differences in phytate hydrolysis between these types of bran were directly related to the phytase activity (Sandberg and Andersson, 1988).

Summary

Phosphorus is the most protean mineral element in the animal body. Supplementing inorganic P to a corn-soybean meal diets for pigs and poultry is necessary but increases feed cost and manure P load on the environment. Though the structure and physiological roles of phytate in plants are still unclear, distribution and bioavailability of phytate in the commonly used cereal and legume foods are well elucidated. Phytase can degrade phytate and the activity occurs widely in microorganisms, plants, and animal tissues. A. ficuum phytase is the currently best characterized and

extensively used. Supplemental microbial or cereal phytases effectively hydrolyze phytate-P and thus improve its bioavailability to simple-stomached animals. The improvements have been shown both in vitro and in vivo. As expected, animals supplemented with dietary phytase or fed phytase-treated diets excrete less P in the manure. Besides, utilization of dietary Ca was also improved by supplemental phytase. Nevertheless, supplemental dietary phytase in swine and poultry production still remains uneconomical.

EXPERIMENTAL SERIES I

LINEAR IMPROVEMENTS IN PHYTATE PHOSPHORUS
BIOAVAILABILITY BY SUPPLEMENTAL DIETARY PHYTASE

(Submitted to J. Anim. Sci.)

ABSTRACT

Two experiments were conducted with weanling pigs to determine the effectiveness of a dietary supplement of A. niger phytase in improving the availability of phytate-P in corn-soybean meal diets without supplemental inorganic P. Exp. 1.1 consisted of two P and Ca balance trials and two feeding trials. Twelve pigs ($8.18 \pm .44$ kg BW) were housed individually in stainless steel metabolism cages. Six pigs received 750 or 687 phytase units (PU)/g of basal diet and the other six pigs received the basal diet without supplemental phytase as control. In Exp. 1.2, ninety-six pigs ($8.81 \pm .75$ kg BW) were allotted to 16 partially-slotted floor pens and were supplemented with 0, 250, 500 or 750 PU/g of basal diet for 4 wk. Individual pig weights and pen feed consumption were measured weekly. Blood samples were taken from all pigs at the end of each trial in Exp. 1.1 and from three pigs per pen weekly in Exp. 1.2 to measure serum (plasma) inorganic P (P) and Ca concentrations, and alkaline phosphatase (AP) activities. The results of Exp. 1.1 indicated that dietary phytase increased P retention by 50% ($P < .0001$) and decreased fecal P excretion by 42% ($P < .0001$). Pigs receiving dietary

phytase had serum P and Ca concentrations, and serum AP activities that were near normal, whereas control pigs had values indicative of a moderate P deficiency. Favorable effects of phytase disappeared when removed from the diet. The results of Exp. 1.2 indicated a linear increase in plasma P ($P < .001$), ADG ($P < .07$), and ADFI ($P < .01$) with increased dietary phytase activity. Plasma AP activity decreased linearly with increased dietary phytase activity up to 500 PU/g of diet. Gain/feed and plasma Ca concentration were measures least affected by dietary phytase activity. In conclusion, supplements of A. niger phytase up to 750 PU/g of feed in corn-soybean meal diets of weanling pigs resulted in a linear improvement in utilization of phytate-P.

Key words: Pigs, Phytase, Phosphorus, Plasma Ca, P, and
Alkaline Phosphatase

Introduction

More than 60% of P in corn and 50% of P in soybean meal is in the form of phytate, which is poorly available to pigs and other simple-stomached animals (Reddy et al., 1982). Attempts have been made to use microbial phytases to improve the availability of phytate-P in these feeds. Nelson et al. (1971) fed day-old chicks with an acetone-dried preparation of phytase from A. niger and demonstrated an improvement in

availability of phytate-P from corn and soybean meal. Recently, positive effects of dietary supplemental phytase produced by the same fungal species on phytate-P utilization in broilers and pigs have been demonstrated by Simons et al. (1990) and Nasi (1990). In contrast, feeding Saccharomyces cerevisiae or other phytase-containing yeast cultures to pigs failed to show improvements in phytate-P availability (Cromwell and Stahly, 1978; Chapple et al., 1979; Shurson et al., 1983). However, these researchers used phytase products with undefined activity, and(or) used mainly weight gain as the response measure. Therefore, the present two experiments were conducted with weanling pigs to determine 1) the efficacy of supplements of A. niger phytase to a corn-soybean meal diet without supplemental inorganic P on P and Ca balance, serum inorganic P (P) and Ca concentrations, and alkaline phosphatase (AP) activity; 2) the possible carry-over effects of phytase feeding on serum P and Ca concentrations, and AP activity after phytase withdrawal; and 3) the statistical relationship between supplemental dietary phytase activity and the resultant improvement in dietary phytate-P utilization as measured by performance and plasma P status.

Materials and Methods

Phytase. The microbial phytase used in this study was produced by A. niger (var. ficuum). The enzyme product was kindly provided by Alko Ltd., Rajamaki, Finland and the activity was approximately 500,000 phytase units (PU)/g (one PU is defined as the amount of enzyme that liberates 1 nmol of inorganic P from sodium phytate per minute at pH 5 and 37 °C). Actual phytase activity was confirmed by the assay method of Han et al. (1987) before the product was mixed with other feed ingredients in the preparation of the complete diet.

Animals and Treatments. All pigs used in the two experiments were 4-wk old crossbred (Landrace-Yorkshire-Hampshire) weanling pigs. In Exp. 1.1, twelve pigs ($8.18 \pm .44$ kg BW) were selected and allotted equally into two groups receiving supplemental phytase (+ phytase) or no supplemental phytase as control (- phytase). Pigs were housed in individual stainless steel metabolism cages and fed the low-P, basal diet (Table 1.1) for 2 wk to adjust and deplete P reserves before the formal trials began. Four consecutive trials were then conducted as follows: Trial 1, first P and Ca balance for 7 d; Trial 2, free feeding for 10 d; Trial 3, second P and Ca balance for 7 d; and Trial 4, free feeding basal diet for 14 d. Pigs in the + phytase group received 750, 750, and 687 PU/g of basal diet in

Trials 1, 2, and 3, respectively. Supplemental phytase activity in Trial 3 was reduced to approximately 90% of that in Trials 1 and 2 based on the increase in age and in feed intake of pigs. In Trial 4, these pigs were fed the same basal diet as fed to the control group to determine if there was any carry-over effect of phytase feeding on blood serum P status.

In Exp. 1.2, ninety-six pigs ($8.81 \pm .75$ kg BW) were split equally into heavy and light blocks based on body weight. Within each block, 48 pigs were allotted to eight pens with six pigs each. Four dietary levels of supplemental phytase activity, 0, 250, 500, and 750 PU/g of basal diet were assigned randomly to pens twice in each block and four times in the whole experiment. Pigs were reared in partially-slotted floor pens and given ad libitum access to feed and water. Experimental housing was maintained at 22-25 °C, with a 12 h light:dark cycle. All pigs were fed the low P, basal diet for 1 wk to deplete P reserves before the formal trial.

Basal Diets. The basal diets were fortified corn-soybean meal diets without supplemental inorganic P (Table 1.1). The diets provided adequate levels of all nutrients (NRC, 1988) with the exception of P, Ca, and lysine in Exp. 1.1 and P and Ca in Exp. 1.2. Calcium carbonate was added to provide a calculated Ca/P ratio of approximately 1.5 in the basal diets. Concentrations of Ca and P in all experimental

Table 1.1. Composition and nutritive values of basal diets

Item	Experiment 1.1	Experiment 1.2
Ingredient	----- g/kg -----	
Corn (ground, shelled)	780.0	777.4
Soybean meal (44% CP)	200.0	200.0
Calcium carbonate (38% Ca)	10.0	10.0
L-Lysine HCl		2.6
Salt (NaCl)	3.5	3.5
Vitamin-trace mineral premix ^a	3.0	3.0
Vitamin E-Se premix ^b	3.0	3.0
Antibiotic premix ^c	.5	.5

Calculated nutritive values (as fed)

ME, MJ/kg	13.8	13.8
CP, g/kg	155.0	155.0
Lysine, g/kg	7.8	10.2
Ca, g/kg	4.4	4.4
P, g/kg	3.2	3.2

^a Supplied the following amounts per kilogram diet: vitamin A, 1,980 IU; vitamin D₃, 396 IU; menadione, 3.3 mg; riboflavin, 2 mg; niacin, 11 mg; d-pantothenic acid, 8 mg; choline, 66 mg; vitamin B₁₂, 12 µg; Zn, 45 mg; Fe, 35 mg; Mn, 20 mg; Cu, 6 mg; I, .12 mg.

^b Supplied 10 IU of vitamin E and .2 mg of Se per kilogram diet.

^c Supplied 55 mg of chlortetracycline per kilogram diet.

diets were analyzed (Table 1.2). Synthetic lysine was not incorporated into the diets in Exp. 1.1. Because we were uncertain initially of the effectiveness of phytase in releasing phytate-P from the basal diets, we considered that it might be important to reduce dietary Ca and lysine concentrations proportionately with the low P concentration and to keep the ratios among these three nutrients close to that of NRC (1988).

Table 1.2. Analyzed dietary Ca and P concentrations of experimental diets^a

Item		Phytase, PU/g	Ca --- g/kg	P -----
Experiment 1.1				
Trial 1	- Phytase	0	6.8	3.1
	+ Phytase	750	5.8	3.2
Trial 2	- Phytase	0	6.8	3.1
	+ Phytase	750	5.8	3.2
Trial 3	- Phytase	0	7.1	3.5
	+ Phytase	687	5.8	3.3
Trial 4		0	8.9	3.7
Experiment 1.2				
Diet 1, basal		0	5.0	2.8
Diet 2		250	5.5	3.2
Diet 3		500	5.0	3.1
Diet 4		750	5.0	3.3

^a As fed basis.

Sample Collection and Measurements. In Exp. 1.1, P and Ca balance trials were conducted as previously described (Ilori et al., 1984). Collection period was 4 d and 3 d in Trials 1 and 3, respectively. Feces of individual pigs were collected daily and air-dried. Urine was collected into 2-L plastic containers daily, and a 10% well-mixed sample was stored at -20 °C for P and Ca analyses. At the end of each trial, including the beginning and the end of the first wk of Trial 4, blood samples were taken from each pig and serum was prepared for assay of P and Ca concentrations, and serum AP activity. Body weight also was recorded at each bleeding. In Exp. 1.2, individual pig weights and pen feed consumption were measured weekly. Blood samples were taken weekly from three pigs per pen for assay of plasma P and Ca concentrations, and plasma AP activity.

Assays. Concentrations of P in feed, feces, urine, and blood serum or plasma were determined by a colorimetric method (Gomori, 1942), and Ca concentrations were determined by flame atomic absorption spectrophotometry (Instrumentation Laboratory, Inc., Model IL 951). Serum or plasma AP activity was determined on the day that blood samples were drawn by the method outlined by Sigma Chemical (1987).

Statistics. Differences in blood serum measures and ADG between pigs fed diets supplemented with or without phytase in Exp. 1.1 were analyzed statistically by simple t -test

rather than time repeated measurement because of the switch in feeding method and the difference in supplemental phytase activity in the diets between different trials. However, P and Ca balance data in Trials 1 and 3 were pooled and analyzed by time repeated measurements because percentage of improvement and significance level of the improvement by supplemental phytase in the two trials were almost identical. There was also no interaction of trial (time) by treatment. The results of Exp. 1.2 were analyzed as a randomized complete block design with 4 treatments (4 phytase levels) in 2 blocks (heavy and light) with time repeated measurements and the pen was the experimental unit. Orthogonal polynomials of dietary phytase activity with different measures were developed by procedures outlined by Gill (1978). Bonferroni t -test was used for treatment mean comparisons. Significance level was set as $P < .05$ unless indicated otherwise. All analyses were conducted with the SAS program (SAS, 1988).

Results

Experiment 1.1

Balance of P and Ca. The pooled P and Ca balance data in the two trials are presented in Table 1.3. With similar daily P intake, pigs fed phytase retained 50% more P daily ($P < .0001$) than control pigs. Meanwhile, daily fecal P

output was reduced by 42% ($P < .0001$) in pigs fed phytase. Daily urinary P loss of pigs was extremely small compared with their fecal P loss regardless of dietary phytase supplementation. This resulted in an almost complete retention of absorbed P in both control and phytase-fed pigs and an almost identical increase of 23 percentage units ($P < .0001$) in apparent digestibility of P and percentage of P retained/intake in pigs fed phytase.

Daily Ca intake of the control pigs was 14% higher ($P < .01$) than that of pigs fed phytase, due to the somewhat higher Ca concentration of the control diet. But, neither daily Ca absorbed nor daily Ca retained in control pigs was increased. In contrast, pigs fed phytase retained slightly more Ca and had 13 ($P < .0001$) and 14 ($P < .02$) percentage unit increases in apparent digestibility of Ca and percentage of Ca retained/intake, respectively. Daily fecal Ca output in pigs fed phytase was reduced by 52% ($P < .0001$) whereas daily urinary Ca output was not different ($P < .33$) from that of control pigs. In addition, fecal P and Ca concentrations in pigs fed phytase were reduced by 45% (1.1 vs 2.0%, $P < .0001$) and 50% (1.1 vs 2.2%, $P < .0001$), respectively.

Table 1.3. Balance of P and Ca in pigs fed diet with or without supplemental microbial phytase^a

	- Phytase	+ Phytase	SED ^b	<u>P</u> <
----- Phosphorus, mg/d -----				
Intake	1,801	1,809	95	.93
Fecal	966	561	67	.0001
Urinary	4.0	3.5	1.0	.64
Absorbed	835	1,248	84	.0001
% of intake	46.4	69.0	2.9	.0001
Retained	831	1,245	84	.0001
% of intake	46.2	68.8	2.9	.0001
% of absorbed	99.5	99.7	.2	.09
----- Calcium, mg/d -----				
Intake	3,753	3,214	182	.014
Fecal	1,092	526	79	.0001
Urinary	615	460	153	.33
Absorbed	2,661	2,688	172	.88
% of intake	70.9	83.6	2.0	.0001
Retained	2,046	2,228	250	.48
% of intake	54.5	69.3	5.1	.02
% of absorbed	76.9	82.9	5.8	.32

^a Data presented here were pooled from the two balance trials.

^b Standard error of difference of two means (df of error, 10).

Serum Inorganic P and Ca Concentrations, and Alkaline Phosphatase Activity. Effects of supplemental dietary phytase on serum P and Ca concentrations, and AP activity are summarized in Table 1.4. The initial concentrations of serum P in pigs of the two treatment groups were essentially the same. However, serum P concentrations in pigs fed phytase increased to 7.0 mg/dL at the end of Trial 1 and stayed above this level in Trials 2 and 3. In contrast, serum P concentration in control pigs initially increased, but then gradually decreased from 6.6 mg/dL at the end of Trial 1 to 4.5 mg/dL at the end of Trial 3. The difference in serum P between the two treatment groups was 2.3 mg/dL at the end of Trial 2 ($P < .0007$) and 3.0 mg/dL at the end of Trial 3 ($P < .0001$). Serum Ca concentration of pigs fed phytase was lower ($P < .001$) than that of the control pigs within these three trials. Serum AP activity of pigs fed phytase was lower ($P < .03$) than that of control pigs at the end of Trial 3.

The significant differences in serum P and Ca concentrations, and AP activity between control and phytase-fed pigs disappeared in Trial 4 when phytase-fed pigs were switched to the same low-P, basal diet as control pigs. Serum P concentrations in the previously phytase-treated pigs decreased and serum Ca increased to the levels of control pigs. Serum AP activity was also similar to that of

Table 1.4. Serum inorganic P and Ca concentrations, and serum alkaline phosphatase activity of pigs fed diet with or without supplemental microbial phytase in experiment 1.1

Trial	- Phytase	+ Phytase	SED ^a	P <
----- Serum inorganic P, mg/dL -----				
Initial	5.4	5.2	.54	.84
Trial 1	6.6	7.0	.33	.22
Trial 2	5.1	7.4	.48	.0007
Trial 3	4.5	7.5	.48	.0001
Trial 4, wk 1	5.7	5.5	.41	.72
wk 2	5.8	5.7	.40	.94
----- Serum Ca, mg/dL -----				
Initial	12.3	12.9	.24	.05
Trial 1	12.3	11.0	.30	.001
Trial 2	13.9	10.8	.41	.0001
Trial 3	14.3	11.8	.33	.0001
Trial 4, wk 1	17.0	16.2	1.00	.43
wk 2	14.8	15.0	.83	.86
----- Serum alkaline phosphatase, U ^b /dL -----				
Initial	22.3	23.4	4.03	.78
Trial 1	25.1	22.5	2.24	.27
Trial 2	18.1	16.8	1.54	.44
Trial 3	22.5	16.5	2.37	.03
Trial 4, wk 1	17.4	15.4	2.44	.45
wk 2	17.0	15.9	3.07	.74

^a Standard error of difference of two means (df of error, 10).

^bOne unit of activity is defined as that amount of enzyme that produces 1 umole of P-nitrophenol per minute under the conditions of the assay (Sigma Procedure No. 425, 1987).

control pigs after phytase was removed from the diet, but it did not change as much as the concentrations of serum P and Ca. Compared with that at the end of Trial 3, serum AP activity and serum P concentration in control pigs in Trial 4 decreased approximately 5 units (U)/dL and increased 1 mg/dL, respectively. These changes may have been due to the switch from restricted feeding in Trial 3 to free feeding in Trial 4 and an increase in age.

Weight Gain. Pigs fed phytase had a greater ADG ($P < .06$) than control pigs in Trial 2, when all pigs were allowed to consume their diets on an ad libitum basis. The ADG of pigs and the difference between control and treated groups was relatively small in the two balance trials. In the final week of Trial 4, control pigs grew faster than pigs previously fed phytase, probably due to compensatory growth. However, the ADG of pigs was really measured only to indicate that pigs in both groups were in good health and were gaining weight in the experiment (Table 1.5).

Table 1.5. Daily gains of pigs fed diet with or without supplemental microbial phytase in experiment 1.1

Trial	- Phytase	+ Phytase	SED ^a	P <
Initial	65	71	20	.80
Trial 1	205	219	18	.48
Trial 2	370	436	32	.06
Trial 3	212	252	35	.28
Trial 4, wk 1	338	354	57	.79
wk 2	411	275	101	.21

^aStandard error of difference of two means (df of error, 10), unit = gram.

Experiment 1.2

Plasma Inorganic P and Ca Concentrations, and Alkaline Phosphatase Activity. Effects of dietary phytase activity on plasma P and Ca concentrations, and AP activity are presented in Table 1.6. Plasma P concentrations increased linearly with increase in dietary phytase activity. The responses could be represented by the following four orthogonal polynomials:

$$\text{Wk 1. } Y = 4.10 + .00292X \text{ (}\underline{P} < .001, \underline{r} = .97\text{)};$$

$$\text{Wk 2. } Y = 4.02 + .00322X \text{ (}\underline{P} < .001, \underline{r} = .98\text{)};$$

$$\text{Wk 3. } Y = 3.69 + .00377X \text{ (}\underline{P} < .001, \underline{r} = .96\text{)};$$

$$\text{Wk 4. } Y = 3.23 + .00346X \text{ (}\underline{P} < .001, \underline{r} = .99\text{)};$$

Where X = dietary phytase activity, PU/g; Y = plasma P concentration, mg/dL. There was no maximum break point of plasma P concentrations among the three phytase-supplemented groups of pigs. Compared with suggested normal values (7 to 8 mg/dL, Ullrey et al., 1968), plasma P concentrations of pigs receiving no phytase were less than 50% of norm and pigs receiving the highest phytase activity (750 PU/g) were slightly below the norm.

Plasma AP activity of pigs receiving no supplemental phytase increased from wk 1 to wk 4 and eventually exceeded ($\underline{P} < .05$) that of the three groups of pigs receiving supplemental phytase at wk 4. Plasma AP activity decreased linearly ($\underline{P} < .06$) with increase in dietary phytase activity

Table 1.6. Plasma inorganic P and Ca concentrations, and plasma alkaline phosphatase activity of pigs receiving graded dietary levels of supplemental microbial phytase activity in experiment 1.2

Time	----- Phytase, PU/g of diet -----			
	0	250	500	750
----- Plasma inorganic P, mg/dL -----				
Initial	5.2	5.0	5.4	5.6
Wk 1	4.1 ^w	5.0 ^x	5.2 ^x	6.5 ^y
Wk 2	3.9 ^w	5.1 ^x	5.5 ^x	6.4 ^y
Wk 3	3.4 ^w	5.1 ^x	5.4 ^x	6.5 ^y
Wk 4	3.2 ^w	4.1 ^x	5.0 ^y	5.9 ^z
(SED ^a = .31, df of error = 45)				
----- Plasma Ca, mg/dL -----				
Initial	11.6	12.2	12.0	11.6
Wk 1	12.3	12.4	12.5	11.9
Wk 2	13.0	12.6	12.4	12.4
Wk 3	12.6	12.2	12.3	11.8
Wk 4	12.6	12.4	12.2	11.9
(SED = .42, df of error = 28)				
----- Plasma alkaline phosphatase, U ^b /dL ---				
Initial	18.4	20.7	16.6	18.8
Wk 1	16.6	20.6	16.6	18.1
Wk 2	20.7	21.2	18.9	17.4
Wk 3	21.3 ^w	20.8 ^{wx}	16.6 ^{wx}	16.0 ^x
Wk 4	26.9 ^w	19.7 ^x	15.7 ^x	16.6 ^x
(SED = 1.96, df of error = 48)				

^a Standard error of difference of means between any two dietary levels of phytase activity at a given wk.

^b See Table 4 for enzyme unit definition.

^{w, x, y, z} Means within a row lacking a common superscript letter differ ($P < .05$).

from 0 to 750 PU/g of feed at wk 3 but only up to 500 PU/g of feed at wk 4. The relationships between plasma AP activity (Y, U/dL) and dietary phytase activity (X, PU/g) fit the following orthogonal polynomials:

$$\text{Wk 3. } Y = 21.68 - .0008X \text{ (}\underline{P} < .06, \underline{r} = .94\text{)};$$

$$\text{Wk 4 } Y = 26.94 - .0383X + .0000326X^2 \text{ (}\underline{P} < .05, R^2 = .99\text{)}.$$

There was no effect of dietary phytase activity on plasma Ca concentration of pigs.

Weight Gain, Feed Intake, and Gain/Feed. Effects of dietary phytase activity on ADG, ADFI, and gain/feed are presented in Table 1.7. Pigs receiving supplemental phytase grew faster than pigs receiving no phytase, and differences were significant at wk 3 and 4. There was no significant difference in ADG among the three groups of pigs fed phytase, but a linear increase in ADG with increase in dietary phytase activity was evident. The relationships between ADG (Y, g/d) in wk 4 and over the entire period and dietary phytase activity (X, PU/g) can be described by the following orthogonal polynomials:

$$\text{Wk 4. } Y = 358 + .159X \text{ (}\underline{P} < .06, \underline{r} = .98\text{)};$$

$$\text{Overall. } Y = 327 + .112X \text{ (}\underline{P} < .07, \underline{r} = .95\text{)}.$$

Feed intake (Y, g/d) increased linearly as dietary phytase activity (X, PU/g) increased and the responses at wk 3 and wk 4 can be described by the following orthogonal polynomials:

$$\text{Wk 3. } Y = 867 + .253X \text{ (}\underline{P} < .01, \underline{r} = .95\text{)};$$

Table 1.7. Daily gain, feed intake, and gain/feed of pigs receiving graded dietary levels of supplemental microbial phytase activity in experiment 1.2

Time	----- Phytase, PU/g of diet -----			
	0	250	500	750
----- Daily gain, g/d -----				
Initial	108	139	133	156
Wk 1	242	295	307	294
Wk 2	300	326	365	365
Wk 3	371 ^x	452 ^y	436 ^{xy}	462 ^y
Wk 4	351 ^x	411 ^{xy}	433 ^y	475 ^y
Overall ^a	316 ^x	371 ^{xy}	385 ^y	405 ^y

(SED^b = 27 at a given wk and 22 for overall,
df of error = 46)

----- Feed intake, g/d -----				
Wk 1	557	666	679	667
Wk 2	762	784	842	835
Wk 3	872 ^x	985 ^{xy}	984 ^{xy}	1,084 ^y
Wk 4	992 ^x	1,122 ^x	1,144 ^{xy}	1,292 ^y
Overall ^c	796 ^x	890 ^{xy}	912 ^{xy}	961 ^y

(SED = 54 at a given wk and 47 for overall,
df of error = 21)

----- Gain/feed, g/kg -----				
Wk 1	433	435	448	458
Wk 2	390	423	433	435
Wk 3	424	463	443	433
Wk 4	355	368	380	383
Overall	403	418	420	425

(SED = 28 at a given wk and 15 for overall,
df of error = 48)

^a Overall mean from wk 1 to wk 4.

^b Standard error of difference of means between any two dietary levels of phytase activity.

^c Comparisons of overall means may not be used because of an interaction ($P < .001$) of time by treatment on ADFI.

^{x, y} Means within a row lacking a common superscript letter differ ($P < .05$).

Wk 4. $Y = 999 + .369X$ ($P < .0001$, $r = .97$).

Overall ADFI also responded linearly to the increase in dietary phytase activity: $Y = 812 + .208X$ ($P < .01$, $r = .97$). However, this equation should be interpreted with caution because of the interaction ($P < .001$) of treatment with time on ADFI.

Gain/feed appeared improved by the increase in dietary phytase level, but the effect was nonsignificant.

For all the measures taken in Exp. 1.2, there was no interaction between dietary phytase activity and block (weight). Consequently, block effect was not included in Tables 1.6 and 1.7.

Discussion

Results of the balance studies strongly indicate that addition of A. niger phytase at 750 PU/g of corn-soybean meal diet improved phytate-P utilization in weanling pigs. Given similar daily intake of P, pigs receiving supplemental phytase had a 23 percentage unit increase ($P < .0001$) in apparent digestibility of P than pigs receiving no supplemental phytase. Correspondingly, daily fecal P excretion was reduced by 42% ($P < .0001$) in these pigs. Simons et al. (1990) have reported that addition of A. niger phytase at 1000 PU/g feed (phytase units are converted to the unit as defined in our study when other studies are discussed) to diets of growing pigs (35 to 70

kg) increased apparent digestibility of P by 24 percentage units and decreased the amount of P in the feces by 35%. Nasi (1990) used the same source of phytase as in this study at 500 PU/g of corn-soybean meal diet for 95-kg pigs and obtained an improved apparent digestibility of P that increased to the same level as an inorganic P supplemented diet and was 24 percentage units higher than that of diet unsupplemented with phytase. Leunissen and Young (1992) also demonstrated that supplements of the same phytase as used in this study at 500 PU/g of diet for weanling pigs improved apparent digestibility of P to the same level as their positive control diet. However, the improvement over the diet unsupplemented with phytase was 11 percentage units and accounted for only 50% of that obtained in studies of Nasi (1990) and Simon et al. (1990), and in this study. The low improvement may be explained by the relatively high dietary concentration of total P (.55% with .04% calcium phosphate supplementation) and high apparent digestibility of P in the basal diet (60%). In addition, both Nasi (1990) and we have shown the same amount of increase in percentage of P retained/intake as that of apparent digestibility of P. An increase of 10 percentage units in the percentage of P retained/absorbed due to 500 PU/g of diet has been found in the study of Nasi (1990). A marginal effect of phytase on P retained/absorbed was also demonstrated in this study, but the improvement may be too small to consider. Phytase has

also been shown to improve bone strength and P concentration (Cromwell et al., 1991; Leunissen and Young, 1992).

Therefore, A. niger phytase appears to improve phytate-P utilization as well as digestion.

Supplemental microbial phytase seems to improve Ca utilization in addition to P utilization as apparent digestibility of Ca and percentage of Ca retained/intake were both increased by 13 percentage units in pigs fed supplemental phytase. However, this improvement may be confounded with an effect of the lower daily Ca intake. Nevertheless, comparable improvement in digestion and utilization of Ca by dietary phytase has been previously demonstrated (Nasi, 1990; Simons et al., 1990). Dietary phytase may improve Ca utilization indirectly by improving P utilization because dietary Ca will be well utilized for skeletal growth only as dietary P is simultaneously utilized.

The effectiveness of phytase in improving phytate-P availability has also been shown by the responses in performance and measures of blood P status. In Exp. 1.1, pigs receiving supplemental phytase maintained serum P and Ca concentrations, and serum AP activity near to the normal range (Miller et al., 1964; Ullrey et al., 1967) whereas pigs receiving no supplemental phytase developed a moderate P deficiency. In Exp. 1.2, plasma P concentration, ADG, and ADFI increased linearly as dietary phytase activity

increased. More convincingly, favorable effects of dietary phytase on serum P and Ca concentrations, and AP activity in pigs fed phytase in the first 3 trials of Exp. 1.1 disappeared when phytase was withdrawn from the diet. This rapid and consistent change not only indicates no carry-over effect of phytase feeding on the measures of blood P status, but also confirms the effectiveness of phytase on phytate P utilization. The ineffectiveness of yeast phytase on phytate P availability in pigs (Cromwell and Stahly, 1978; Chapple et al., 1979; Shurson et al., 1983) may be attributed to 1) the possibility that activity of the yeast phytase products may have been insufficient to produce a response, and (or) 2) a possible incompatibility of these yeast phytases with the low pH in the stomach of pigs. The A. niger phytases used in this study and by other researchers who obtained positive results (Nasi, 1990; Simons et al., 1990; Cromwell et al., 1991, Leunissen and Young, 1992) are acid and heat resistant over a broad pH and temperature range (pH 2.0 to 6.0, and up to 60 °C). Therefore, we may reasonably expect these enzymes to be active and to function in the stomach of pigs under physiological conditions. This hypothesis has been confirmed by Jongbloed et al. (1992). Using two simple T-cannulas in the duodenum and terminal ileum of pigs, they demonstrated that lowering of dietary A. niger phytase activity (1500 PU/g) by 415 PU after gastroduodenal degradation resulted in an increase in phytic acid

degradation of 70 percentage units. Subsequently, liberated ortho-phosphates were absorbed in the small intestine, and fecal P excretion was greatly reduced. Hence, supplemental phytase may not only improve utilization of phytate-P in cereal-plant protein diets, but also alleviate or eliminate P pollution by reducing P in swine manure applied to the land, a severe problem facing the swine industry today (Cromwell, 1991).

Plasma P concentration seems to be the most sensitive and convenient measure of dietary phytase on phytate-P utilization in this study. Supplements of dietary phytase at 750 PU/g of feed produced comparable improvement in P concentrations and AP activities in serum of pigs in Exp. 1.1 and in plasma of pigs in Exp. 1.2. However, significant differences in serum Ca concentrations between pigs receiving no phytase and 750 PU/g of diet shown in Exp. 1.1 was not seen in plasma Ca concentrations in Exp. 1.2. This inconsistency may be partly accounted for by the differences in dietary Ca concentrations in the two studies.

The linear effects of dietary phytase activity up to 750 PU/g of diet on most of the measures of phytate-P utilization taken in this study were very consistent. This was reflected by the extremely high repeatability of the strong correlations and significance shown in the developed orthogonal polynomials. Similar response patterns in chicks to supplemental dietary phytase activity have been reported

by Nelson et al. (1971) and Schöner et al. (1991). However, phytase dose-related responses in pigs appear to vary in different studies. A linear relationship ($P < .01$) between supplementing dietary phytase at 0, 500, and 1000 PU/g of diet and ADG and bone strength in growing pigs was shown in one study (Cromwell, 1991), but the higher level of supplemental dietary phytase (1000 PU/g) was more effective in improving only bone strength in another study (Cromwell et al., 1991). Similarly, there was no further beneficial effect on any of the response criteria by doubling supplemental phytase activity from 500 to 1,000 PU/g of diet in weanling pigs in the study of Leunissen and Young (1992). In comparison to the results of this study, the lack of further improvement in phytate-P utilization by dietary phytase activity higher than 500 PU/g of diet in the studies discussed above may be explained by 1) the growing-finishing pigs used by Cromwell et al. (1991) had a lower P requirement than the weanling pigs we used; and 2) the basal diet used by Leunissen and Young (1992) had a higher total and available P concentration than our basal diets, due to .04% supplemented calcium phosphate and different feed ingredients, as mentioned above. Lower P requirement and higher dietary available P concentration would lessen the amount of phytate-P needed to meet the requirement of P in pigs, thereby making the higher dietary phytase activity unnecessary to release just this portion of P from phytate.

Cromwell et al. (1991) did not observe any effect of phytase supplemented in the diets with adequate inorganic P. In addition, inorganic P is a strong inhibitor of phytase in vitro (Shieh and Ware, 1968; Gibson and Ullah, 1988).

Among all the measures made in this study, it seems that only the response of plasma AP activity was maximized at wk 4 of Exp. 1.2 at dietary phytase activity of 587 PU/g of diet. However, this level of phytase activity may not be the optimal dose that could maximize the improvement of phytate-P utilization because other response measures still showed linear increases up to 750 PU/g of diet. Even 750 PU/g of diet may still be inadequate for pigs to positively maintain normal plasma P concentrations (Ullrey et al., 1967). If plasma P concentrations are to be maintained at approximately 7.5 mg/dL, pigs may need supplemental phytase activity of 1200 PU/g of diet based on the orthogonal polynomials developed. Therefore, higher dietary levels of phytase activity should be tested in future studies to determine the phytase activity at which response measures are maximum.

Implications

The A. niger phytase used in this study was very effective in improving the availability of phytate-P in a corn-soybean meal diet for weanling pigs and may greatly reduce the need for supplemental inorganic P, the third largest expense in cereal grain-plant protein swine diets. Supplementary phytase up to 750 units per gram of feed resulted in a linear improvement in phytate-P utilization as measured by plasma inorganic P concentration, average daily gain and feed intake. Furthermore, supplements of 750 phytase units per gram of feed increased absorption and retention of P by 23 percentage units and reduced fecal P excretion by 42%. Higher levels of dietary phytase activity may be needed to maximize this improvement and to eliminate the need for dietary inorganic P supplementation for weanling pigs.

EXPERIMENTAL SERIES II
MAXIMAL IMPROVEMENTS IN PHYTATE PHOSPHORUS
BIOAVAILABILITY BY SUPPLEMENTAL DIETARY PHYTASE
(Submitted to J. Anim. Sci.)

ABSTRACT

Two experiments were conducted with crossbred weanling pigs to determine the optimal dietary supplement of A. niger phytase activity to maximize utilization of phytate-P in a corn-soybean meal basal diet (BD) without added inorganic P. In addition, the inorganic P equivalent of phytase supplementation was determined. In Exp. 2.1, fifty pigs ($7.61 \pm .56$ kg BW) were allotted to 10 pens and were supplemented with 750, 1,050, 1,250, or 1,350 phytase units (PU)/g BD, or were fed the BD plus .21% P as mono-dibasic calcium phosphate (MDCaP) for 4 wk. In Exp. 2.2, twelve pigs ($6.39 \pm .74$ kg BW) were individually housed in metabolism cages and received BD, BD plus the optimal phytase activity (1,200 PU/g), or BD plus .21% P as MDCaP for 2 wk. Weekly measures included ADG, ADFI, plasma concentrations of P (inorganic), Ca, Zn, Mg, Cu, and Fe and plasma alkaline phosphatase (AP) activity in 3 pigs per pen in Exp. 2.1 and in all pigs in Exp. 2.2. Feces and urine were collected from all pigs individually for 4 d at the end of Exp. 2.2 to determine P (total) and Ca balances. No improvements ($P > .05$) in ADG, ADFI, gain/feed or plasma AP activity beyond 1,050 PU/g BD were seen in Exp. 2.1. Quadratic relationships

between dietary phytase activity and these measures consistently predicted maximum breakpoints at approximately 1,200 PU/g. Estimated maximum responses of these measures were 90% or more of those in pigs receiving MDCaP. In addition, 1,250 PU/g appeared adequate to maintain plasma P and Ca concentrations in the normal range. Effects of dietary phytase activity on plasma Mg, Cu, Fe, and Zn concentrations were not significant. In Exp. 2.2, pigs receiving 1,200 PU/g utilized dietary P more effectively ($P < .05$) than pigs fed the BD or the BD plus MDCaP. Although consuming 44% less P per day, these pigs retained only 7% less P ($P > .05$) than pigs receiving MDCaP. One thousand units of phytase activity supported retention of 1.1 mg P from the BD and were equivalent in effect to .91 mg P from MDCaP. Concentrations of plasma P were lower and plasma Zn were higher in pigs fed 1,200 PU than in pigs fed MDCaP, but other measures were not different between these two groups. Supplements of A. niger phytase at 1,200 PU/g of a corn-soybean meal diet for weanling pigs appeared to maximize utilization of phytate-P and obviate the need for almost all of an inorganic P supplement.

Key words: Pigs, Phytase, Phytate, Phosphorus, Plasma Ca, P, and Alkaline Phosphatase

Introduction

The effectiveness of A. niger phytase in improving the utilization of phytate-P in corn-soybean meal diets for weanling pigs has been shown previously (Lei et al., 1991; Leunissen and Young, 1992). The effect was linear over a range of dietary phytase activity from 0 to 750 phytase units (PU)/g of corn-soybean meal diet (Lei et al., 1992a). However, the relationship between dietary phytase activity beyond 750 PU/g and further improvements in phytate-P utilization, and the possibility of eliminating the need for supplemental inorganic P, were not studied. Furthermore, weanling pigs receiving the highest phytase activity (750 PU/g) did not sustain plasma P (inorganic) concentrations within the normal range suggested by Ullrey et al. (1967). By using orthogonal polynomials developed from our previous study (Lei et al., 1992a), we predicted that the phytase activity in a corn-soybean meal diet required to maintain normal plasma P concentrations for weanling pigs would be 1200 PU/g. Therefore, higher levels of supplemental dietary phytase activity, arrayed around this predicted value, were used in two subsequent experiments. The objectives of these studies were 1) to determine the breakpoint relating supplemental dietary phytase activity to maximum improvements in phytate-P utilization, and 2) to determine the inorganic P equivalent of optimal phytase activity.

Materials and Methods

Phytase and Diets. The microbial phytase (*A. niger*, Alko Ltd., Rajamaki, Finland) and the process of phytase incorporation into diets were the same as described previously (Lei et al., 1992a). The basal diet (BD) is shown in Table 2.1 and was the same fortified corn-soybean meal diet without supplemental inorganic P as used in Exp. 1.2 in our previous study (Lei et al., 1992a). Mono-dibasic calcium phosphate (MDCaP) was added to the basal diet as a positive control. The analyzed concentrations of P, Ca, Mg, Cu, Fe, and Zn in the experimental diets are presented in Table 2.2.

Animals and Treatments. All pigs used in the two experiments were 4-wk old weanling crossbreds (Landrace-Yorkshire-Hampshire). In Exp. 2.1, fifty pigs ($7.61 \pm .56$ kg BW) were split equally into heavy and light blocks based on body weight. Within each block, 25 pigs were allotted into five pens of five pigs each. The BD was supplemented with microbial phytase at 750, 1,050, 1,250, or 1,350 PU/g (one PU is defined as the amount of enzyme that liberates 1 nmol of inorganic P from sodium phytate per minute at Ph 5 and 37 °C) or with .21% inorganic P (MDCaP) as the positive control. Selected dietary levels of phytase activity were based on the predicted optimal activity (1,200 PU/g; Lei et

Table 2.1. Composition and nutritive values of the basal diet and basal diet supplemented with mono-dibasic calcium phosphate (MDCaP)^a

Item	Basal	+ MDCaP
Ingredient	----- g/kg -----	
Corn (ground, shelled)	777.4	769.4
Soybean meal (44% CP)	200.0	200.0
MDCaP	-	10.0
Calcium carbonate (38% Ca)	10.0	7.0
L-Lysine HCl	2.6	2.6
Salt (NaCl)	3.5	3.5
Vitamin-trace mineral premix ^b	3.0	3.0
Vitamin E-Se premix ^c	3.0	3.0
Antibiotic premix ^d	.5	.5

Calculated nutritive values (as fed)

ME, MJ/kg	13.8	13.7
CP, g/kg	155.0	153.4
Lysine, g/kg	10.2	10.2
Ca, g/kg	4.6	5.6
P, g/kg	3.3	5.4

^a MDCaP contains not less than 21% P and 15% Ca and no more than 18% Ca.

^b Supplied the following amounts per kilogram diet: vitamin A, 1,980 IU; vitamin D₃, 396 IU; menadione, 3.3 mg; riboflavin, 2 mg; niacin, 11 mg; d-pantothenic acid, 8 mg; choline, 66 mg; vitamin B₁₂, 12 µg; Zn, 45 mg; Fe, 35 mg; Mn, 20 mg; Cu, 6 mg; I, .12 mg.

^c Supplied 10 IU of vitamin E and .2 mg of Se per kilogram diet.

^d Supplied 55 mg of chlortetracycline per kilogram diet.

Table 2.2. Analyzed dietary concentrations of P, Ca, and other elements^a

Diet	P	Ca	Mg	Zn	Cu	Fe
	-----	g/kg	-----	-----	mg/kg	-----
Experiment 1						
Basal	2.9	6.5	1.5	61	12	178
+ 750 PU	3.4	7.3	1.6	69	14	205
+ 1050 PU	3.3	6.3	1.5	58	12	163
+ 1250 PU	3.1	6.5	1.6	62	13	184
+ 1350 PU	3.2	6.6	1.6	78	13	188
+ MDCaP	6.2	6.7	1.6	65	11	183
Experiment 2						
Basal	3.3	5.8		73	18	
+ Phytase	3.2	5.6		73	13	
+ MDCaP	5.3	5.3		72	15	

^a As fed basis.

al., 1992a). Housing, management, P-depletion procedures, and the experimental periods were the same as in the previous study (Lei et al., 1992a).

In Exp. 2.2, twelve pigs ($6.39 \pm .74$ kg BW) were allotted equally into three groups receiving the BD, the BD plus the optimal phytase activity (1,200 PU/g) determined in Exp. 2.1 (+ Phytase), or the BD plus .21% inorganic P (+ MDCaP). Pigs were housed in individual stainless steel metabolism cages and fed the low-P, BD (Table 2.1) for 2 wk to adjust P reserves before the actual experimental period. Pigs were then fed their designated diets for 2 wk.

Sample Collection and Measurements. In Exp. 2.1, individual pig weights and pen feed consumption were measured weekly. Blood samples were taken weekly from three pigs per pen for assay of plasma P, Ca, Mg, Cu, Fe, and Zn concentrations, and plasma alkaline phosphatase (AP) activity. In Exp. 2.2, P and Ca balance trials were conducted as previously described (Ilori et al., 1984; Lei et al., 1992a). Total collections of feces and urine from individual pigs were made during the last 4 d of the trial. Blood samples were taken from each pig initially (Wk 0), at the end of the first wk (Wk 1), and at the end of the trial (Wk 2) for assay of plasma P, Ca, and Zn concentrations, and plasma AP activity. Body weights also were recorded at each bleeding.

Assays. Concentrations of P and Ca in feed, feces,

urine, and plasma and activity of plasma AP were determined by the methods previously outlined (Lei et al., 1992a). Concentrations of other elements (Mg, Cu, Fe, and Zn) in plasma and feed were determined by flame atomic absorption spectrophotometry (Instrumentation Laboratory, Inc., Model IL 951). Plasma AP activity was determined on the same day that blood samples were drawn by the method outlined by Sigma Chemical (1987).

Statistics. Results of Exp. 2.1 were analyzed as a randomized complete block design with 5 treatments (4 phytase levels plus inorganic P as positive control) in 2 blocks (heavy and light BW) with repeated measurements (4 wk). Pen was considered the experimental unit. Regression equations between dietary phytase activity and various response measures were developed by the GLM procedure of SAS (1988). Breakpoints of dietary phytase activity for different measures and their inorganic P equivalents were determined as outlined by Gill (1978). Results of P and Ca balance in Exp. 2.2 were analyzed as a randomized complete block design with 3 treatments. Plasma measures in Exp. 2.2 were analyzed as the same model with repeated measurements (2 wk). Relationships between plasma and urinary measures were also determined by the GLM procedure of SAS (1988). The Bonferroni t-test was used for treatment mean comparisons. The significance level was set at $P < .05$, unless indicated otherwise.

Results

Experiment 2.1

Daily Gain, Daily Feed Intake and Gain/Feed. Effects of dietary phytase activity on ADG, ADFI, and gain/feed are presented in Table 2.3. Average daily gains of pigs receiving MDCaP were greater ($P < .05$) than those of pigs receiving the three lowest levels of phytase activity at wk 3 and greater ($P \leq .05$) than those of pigs receiving the lowest and highest levels of dietary phytase activity at wk 4. Among the four groups of pigs receiving graded levels of phytase, daily gains tended to increase with dietary phytase activity at wk 3, but there was no linear relationship ($P > .05$). Daily gains of pigs at wk 4 appeared to respond to dietary phytase activity quadratically (second degree polynomials, $P < .06$, Table 2.6). Overall, pigs receiving 750 PU/g feed had the lowest and pigs receiving MDCaP had the highest ADG. The other three groups of pigs receiving phytase had very similar ADG. Pigs receiving 1250 PU/g feed had ADG closest to those of pigs receiving MDCaP.

Significant differences in ADFI between different treatment groups of pigs were observed only at wk 4. Pigs receiving 750 PU/g feed had lower intakes ($P < .05$) than pigs receiving 1250 PU/g feed or MDCaP.

Pigs receiving MDCaP tended to have a higher gain/feed

Table 2.3. Daily gain, feed intake, and feed efficiency of pigs receiving graded levels of supplemental microbial phytase activity or supplemental mono-dibasic calcium phosphate (MDCaP) in the diet in experiment 2.1

Time	----- Phytase, PU/g of diet -----				
	750	1050	1250	1350	MDCaP
----- Daily gain, g -----					
Wk 1	172	203	226	161	221
Wk 2	242	288	247	212	320
Wk 3	285 ^x	307 ^x	320 ^x	381 ^{xy}	453 ^y
Wk 4	395 ^x	557 ^{yz}	603 ^{yz}	547 ^y	660 ^z
Overall ^a	273	339	349	325	414
(SED ^b = 34.7, df of error = 18)					
----- Feed intake, g/d -----					
Wk 1	339	325	376	315	373
Wk 2	594	597	617	550	610
Wk 3	760	696	784	773	909
Wk 4	994 ^x	1115 ^{xy}	1268 ^y	1138 ^{xy}	1243 ^y
Overall	672	683	761	694	784
(SED = 70.7, df of error = 11)					
----- Gain/feed, g/kg -----					
Wk 1	520	619	599	511	590
Wk 2	413	477	396	385	523
Wk 3	410	452	409	498	499
Wk 4	401	498	476	483	533
Overall	419	496	458	471	528
(SED = 56.0, df of error = 14)					

^a Comparison of overall means may not be used because of interaction of treatment by time on ADG ($P < .0001$) and ADFI ($P < .06$).

^b Standard error of differences between any two treatment means at a given wk.

^{x, y, z} Means within a row lacking a common superscript letter differ ($P < .05$).

than other groups of pigs, but there was no significant difference between any two treatment groups. However, the relationship between gain/feed and dietary phytase activity at wk 1 and 4 fit two second degree polynomials (Table 2.6).

Plasma Inorganic P and Ca Concentrations and Alkaline Phosphatase Activity. Effects of dietary phytase activity on plasma P and Ca concentrations and AP activity are presented in Table 2.4. Plasma P concentrations increased linearly with dietary phytase activity at all weeks of the study. The responses could be represented by four orthogonal polynomials (Table 2.6). During the last 2 wk, pigs receiving 1250 or 1350 PU/g feed had plasma P concentrations near or in the proposed normal range (Ullrey et al., 1967), but the values were only 70% of those of the pigs fed supplemental MDCaP.

In contrast, plasma Ca concentrations of pigs decreased linearly with increasing dietary phytase activity. Likewise, there were four polynomials that represented these responses (Table 2.6). However, the response of plasma Ca to dietary phytase activity at wk 3 could be fit better by a quadratic regression (Table 2.6). This may have resulted from the random oscillation of a few observations, which often happens in relatively small samples. Pigs receiving supplemental MDCaP had lower ($P < .05$) plasma Ca concentrations than pigs receiving 750 PU/g feed.

Table 2.4. Plasma inorganic P and Ca concentrations, and alkaline phosphatase activity of pigs receiving graded levels of supplemental microbial phytase activity or supplemental mono-dibasic calcium phosphate (MDCaP) in the diet in experiment 2.1

Time	----- Phytase, PU/g of diet -----				
	750	1050	1250	1350	MDCaP
----- Plasma inorganic P, mg/dL -----					
Wk 0	6.2	6.4	5.5	5.4	5.8
Wk 1	5.0 ^x	5.2 ^x	5.4 ^x	6.0 ^x	8.6 ^y
Wk 2	4.6 ^x	5.9 ^{xy}	6.0 ^{xy}	6.7 ^y	9.5 ^z
Wk 3	4.8 ^x	6.0 ^{xy}	6.3 ^y	6.5 ^y	10.3 ^z
Wk 4	4.5 ^x	5.9 ^{xy}	6.7 ^y	7.1 ^y	10.2 ^z
(SED ^a = .48, df of error = 16)					
----- Plasma Ca, mg/dL -----					
Wk 0	9.7	10.1	10.3	10.2	12.4
Wk 1	12.8 ^x	11.9 ^{xy}	11.1 ^{xy}	11.4 ^{xy}	10.7 ^y
Wk 2	12.9 ^x	11.9 ^{xy}	11.6 ^{xy}	11.0 ^{xy}	10.6 ^y
Wk 3	13.4 ^x	11.0 ^y	11.2 ^y	11.0 ^y	10.6 ^y
Wk 4	13.5 ^x	12.3 ^{xy}	12.0 ^{xy}	11.7 ^{xy}	10.8 ^y
(SED = .58, df of error = 12)					
----- Plasma alkaline phosphatase, U ^b /dL -----					
Wk 0	13.4	13.3	12.1	12.7	12.6
Wk 1	17.1	16.3	16.0	16.2	13.7
Wk 2	19.6	18.5	15.3	17.9	15.6
Wk 3	20.0 ^x	14.4 ^{xy}	12.3 ^y	16.2 ^{xy}	12.5 ^y
Wk 4	19.6 ^x	16.3 ^{xy}	13.9 ^{xy}	16.1 ^{xy}	11.6 ^y
(SED = 1.88, df of error = 16)					

^a Standard error of differences between any two treatment means at a given wk.

^b Sigma unit (Sigma Procedure No. 425, 1987).

^x, ^y, ^z Means within a row lacking a common superscript letter differ ($P < .05$).

Significant differences in plasma AP activity were observed between treatment groups during the last 2 wk of the study. Pigs receiving 750 PU/g feed had higher plasma AP activity ($P < .05$) than pigs receiving either 1250 PU/g feed or MDCaP. Pigs receiving 1050 and 1350 PU/g feed had similar activities, intermediate to those of pigs in the other three groups. A quadratic relationship between plasma AP activity and dietary phytase activity appeared at wk 3 (Table 2.6).

Plasma Mg, Cu, Fe, and Zn Concentrations. There was no consistent effect of dietary phytase activity on plasma Mg, Fe, Cu, or Zn concentrations (Table 2.5). However, the relationship between plasma concentrations of these elements and dietary phytase activity could be represented by second degree polynomials (Table 2.6).

Breakpoints of Dietary Phytase Activity. All the coefficients and levels of significance of the above regressions between dietary phytase activity (X , PU/g) and various response measures (Y) are summarized in Table 2.6. The models for linear and quadratic regressions were as follows:

$$\text{Linear: } Y = a + b_1X;$$

$$\text{Quadratic: } Y = a + b_1X + b_2X^2.$$

Breakpoints, estimates of dietary phytase activity at which maximum responses of designated measures are expected to occur, were derived as $X = -b_1/2b_2$ from the quadratic

Table 2.5. Plasma concentrations of Mg, Cu, Fe, and Zn in pigs receiving graded levels of supplemental microbial phytase activity or supplemental mono-dibasic calcium phosphate (MDCaP) in the diet in experiment 2.1

Time	----- Phytase, PU/g of diet -----				
	750	1050	1250	1350	MDCaP
----- Plasma Mg, mg/dL -----					
Wk 0	2.27	2.23	2.27	2.22	2.20
Wk 1	2.01	2.04	1.96	2.01	1.96
Wk 2	1.97	1.99	1.92	1.96	1.92
Wk 3	2.07 ^y	2.07 ^y	1.92 ^{xy}	1.85 ^x	1.99 ^{xy}
Wk 4	1.92	1.87	1.83	1.90	1.89
(SED ^a = .071, df of error = 20)					
----- Plasma Cu, µg/dL -----					
Wk 0	201	207	201	209	194
Wk 1	159	148	152	169	147
Wk 2	158	150	140	171	138
Wk 3	171	155	153	182	156
Wk 4	137	143	139	128	144
(SED = 12.5, df of error = 14)					
----- Plasma Fe, µg/dL -----					
Wk 0	137	160	141	126	157
Wk 1	183	228	211	205	197
Wk 2	193	217	186	156	170
Wk 3	250	184	181	176	204
Wk 4	175	195	179	175	175
(SED = 31.8, df of error = 20)					
----- Plasma Zn, µg/dL -----					
Wk 0	76	95	90	89	90
Wk 1	75	82	75	74	80
Wk 2	112	106	100	103	98
Wk 3	79	74	64	80	81
Wk 4	106 ^y	106 ^y	106 ^y	107 ^y	83 ^x
(SED = 8.8, df of error = 17)					

^a Standard error of differences between any two treatment means at a given wk.

^{x, y} Means within a row lacking a common superscript letter differ ($P < .05$ for Mg and $P < .15$ for Zn).

regressions, and are shown in Table 2.7. Four major measures, ADG, gain/feed, plasma AP activity, and plasma Ca concentrations appeared to maximize at similar dietary phytase activities (approximately 1,200 PU/g). The estimated maximum responses of these measures were equal to or greater than 90% of those of the pigs fed supplemental MDCaP. Indeed, there was no significant difference between these two categories (Table 2.7). Breakpoints of dietary phytase activity for plasma Mg, Cu, and Fe concentrations deviated slightly from those for the four major measures discussed above.

Experiment 2.2

Balance of P and Ca. Results of P and Ca balance are presented in Table 2.8. With similar daily P intake, pigs fed phytase retained 60% more P and excreted 55% less P in feces than pigs fed the BD ($P < .05$). Daily urinary P loss in pigs on both diets was negligible. Thus, 99% of absorbed P was retained in both groups of pigs. The apparent digestibility of dietary P and the percentage of P ingested that was retained by pigs fed phytase were 40 and 41 percentage units higher ($P < .05$) than those by pigs fed the BD. On the other hand, daily P intakes of pigs fed supplemental MDCaP were 44% higher ($P < .05$) than those of pigs fed phytase.

Table 2.6. Regression coefficients of different measures with dietary phytase activity in experiment 2.1^a

Measure ^b	Week	a	b ₁	b ₂	R ²	P <
Quadratic						
ADG	4	-862	2.45	-.001	.68	.06
Gain/feed	1	-720	2.56	-.00122	.50	.17
	4	-263	1.31	-.00057	.71	.05
Plasma AP	3	74	-.11	.000047	.60	.10
Plasma Ca	3	28	-.029	.000012	.75	.03
Plasma Mg	3	1.2	.0021	-.0000012	.71	.04
Plasma Cu	3	444	-.57	.00027	.54	.15
Plasma Fe	2	-239	.93	-.00047	.67	.06
	3	650	-.77	.00031	.56	.13
Linear						
Plasma inorganic P	1	3.90	.0014		.38	.11
	2	2.30	.0032		.79	.003
	3	2.77	.0028		.41	.08
	4	1.25	.0044		.90	.0004
Plasma Ca	1	14.66	-.0026		.80	.003
	2	15.05	-.0029		.58	.03
	3	15.87	-.0038		.59	.03
	4	15.70	-.0030		.66	.01

^a The model for linear regression is $Y = a + b_1X$, and for quadratic regression is $Y = a + b_1X + b_2X^2$, where Y= response measure, and X= dietary phytase activity (PU/g).

^b Unit is, g for ADG, g/kg for gain/feed, U/dL for plasma AP, mg/dL for plasma inorganic P, Ca, and Mg, and µg/dL for plasma Cu and Fe.

Table 2.7. Breakpoints of dietary phytase activity for different response measures and the comparison of these maximum responses with those of the control pigs receiving supplemental mono-dibasic calcium phosphate in the diet in experiment 2.1

Measure	Wk	Break Point ^a	Maximum Response	Control Response	SED ^b	df	P<
ADG, g/d	4	1183	587	660	42	7	.12
Gain/feed, g/kg	1	1052	629	590	37	5	.34
	4	1156	496	533	37	5	.36
Plasma AP, U/dL	3	1136	13.2	12.5	1.9	6	.72
Plasma Ca, mg/dL	3	1200	10.9	10.6	.47	7	.53
Plasma Mg, mg/dL	3	884	2.1	2.0	.06	9	.13
Plasma Cu, µg/dL	3	1036	150	156	10	7	.56
Plasma Fe, µg/dL	2	985	219	170	21	10	.04
	3	1240	176	204	21	10	.21

^a Dietary phytase activity, PU/g of diet.

^b Standard error of differences between the estimated and control means.

Table 2.8. Balance of P and Ca in pigs fed the basal diet supplemented with or without the optimal dose of microbial phytase or mono-dibasic calcium phosphate (MDCaP) in experiment 2.2

Item	Basal	+ Phytase	+ MDCaP	SED ^a	P < ^b
----- Phosphorus, mg/d -----					
Intake	1,092 ^x	1,126 ^x	1,618 ^y	157	.01
Fecal	792 ^y	359 ^x	763 ^y	109	.006
Urinary	3 ^x	5 ^x	39 ^y	10	.05
Absorbed	300 ^x	767 ^y	855 ^y	109	.001
% of intake	28 ^x	68 ^z	53 ^y	5	.0001
Retained	297 ^x	762 ^y	816 ^y	114	.002
% of intake	27 ^x	68 ^z	50 ^y	6	.0001
% of absorbed	99	99	95	2	.07
----- Calcium, mg/d -----					
Intake	1,920	1,971	1,557	211	.16
Fecal	1,041 ^y	746 ^{xy}	716 ^x	111	.13
Urinary	370 ^y	212 ^{xy}	63 ^x	87	.10
Absorbed	879 ^{xy}	1,225 ^y	841 ^x	130	.03
% of intake	46	62	54	6	.08
Retained	509 ^x	1,013 ^y	778 ^{xy}	132	.01
% of intake	27 ^x	51 ^y	50 ^y	8	.02
% of absorbed	58 ^x	83 ^{xy}	93 ^y	11	.04

^a Standard error of differences between any two treatment means.

^b Significance of main effects.

^{x, y, z} Means within a row lacking a common superscript letter differ ($P < .05$).

However, pigs supplemented with MDCaP retained only 7% more ($P > .05$) P daily because their daily fecal and urinary P excretion was 2.1 and 7.8 times higher ($P < .05$), respectively, than that of pigs fed phytase. Consequently, the apparent digestibility of dietary P and percentage of ingested P that was retained by pigs supplemented with MDCaP was 15 and 18 percentage units lower ($P < .05$), respectively, than that of pigs fed phytase. Based on the differences in daily P intake and retention among these three groups of pigs, 1,000 PU supported retention of 1.1 mg P from the BD which was effectively equivalent to .91 mg inorganic P as MDCaP. In addition, pigs fed MDCaP absorbed the supplemental inorganic P almost completely because these pigs excreted basically the same amount of P in their feces as those pigs fed the BD.

Daily Ca intake of pigs fed the BD or BD plus phytase was essentially the same, but daily Ca retained by the former was only half that of the latter ($P < .05$). Compared with pigs fed phytase, pigs fed supplemental MDCaP had slightly lower daily Ca intake. However, these two groups of pigs retained the same proportion of ingested Ca and shared a similar daily fecal Ca excretion. Pigs fed supplemental MDCaP excreted less Ca in their urine and, thus, utilized absorbed Ca somewhat better than pigs fed phytase.

Plasma Inorganic P, Ca, and Zn Concentrations, and Alkaline Phosphatase Activity. Effects of supplemental dietary phytase and MDCaP on plasma P, Ca, and Zn concentrations, and AP activity are presented in Table 2.9. Initial values of these four measures among the three treatment groups were essentially the same. However, plasma P concentrations were different ($P < .05$) during the 2 wk of study between any two treatment groups. Pigs fed the BD failed to sustain their initial plasma P concentrations and had these values decreased more than 50% at the end of wk 2. In contrast, pigs fed phytase had an increase of 1 mg/dL above their initial plasma P concentrations, and pigs supplemented with MDCaP had an even greater ($P < .05$) increase of plasma P concentrations during the same period. Plasma Ca concentrations of pigs fed the BD were higher ($P < .05$) than those of pigs in the other two groups. Plasma Zn concentrations of pigs fed phytase were higher ($P < .05$) at wk 2 than those of pigs fed MDCaP. Plasma AP activities of pigs fed phytase and MDCaP tended to be lower ($P > .05$) than those of pigs fed the BD.

Relationship between Urinary P and Ca Excretion and Plasma Inorganic P and Ca Concentration. There were moderate correlations ($r = .5$ to $.7$) between urinary P and Ca excretion and plasma P and Ca concentrations. Daily urinary P excretion (Y , mg/d) was positively and negatively related

Table 2.9. Plasma inorganic P, Ca, and Zn concentrations and alkaline phosphatase activity of pigs fed the basal diet supplemented with or without the optimal dose of microbial phytase or mono-dibasic calcium phosphate (MDCaP) in experiment 2.2

Time	Basal	+ Phytase	+ MDCaP
----- Plasma inorganic P, mg/dL -----			
Wk 0	5.12	5.13	4.54
Wk 1	4.19 ^x	5.49 ^y	6.78 ^z
Wk 2	2.54 ^x	6.00 ^y	7.05 ^z
(SED _b = .35, df of error = 8)			
----- Plasma Ca, mg/dL -----			
Wk 0	9.93	9.87	9.41
Wk 1	10.77 ^y	10.07 ^{xy}	8.89 ^x
Wk 2	11.29 ^y	9.32 ^x	8.56 ^x
(SED = .44, df of error = 10)			
----- Plasma Zn, µg/dL -----			
Wk 0	64	66	63
Wk 1	89	73	76
Wk 2	59 ^{xy}	76 ^y	51 ^x
(SED = 7.3, df of error = 10)			
----- Plasma alkaline phosphatase, U ^b /dL -----			
Wk 0	14.88	12.77	11.43
Wk 1	19.53	14.41	12.75
Wk 2	15.98	11.08	12.90
(SED = 2.81, df of error = 10)			

^a Standard error of differences between any two treatment means at a given wk.

^b Sigma unit (Sigma Procedure No. 425, 1987).

^{x, y, z} Means within a row lacking a common superscript letter differ ($P < .05$).

to plasma P and Ca concentrations (X, mg/dL), respectively:

$$Y = -51 + 12.1 X \text{ (X = plasma P at wk 1, } \underline{P} < .06, \underline{r} = .55);$$

$$Y = -19 + 6.6 X \text{ (X = plasma P at wk 2, } \underline{P} < .07, \underline{r} = .55);$$

$$Y = 156 - 14.1 X \text{ (X = plasma Ca at wk 1, } \underline{P} < .05, \underline{r} = .58).$$

In contrast, daily urinary Ca excretion (Y, mg/d) was negatively and positively related to plasma P and Ca concentrations (X, mg/dL), respectively:

$$Y = 888 - 123 X \text{ (X = plasma P at wk 1, } \underline{P} < .01, \underline{r} = .69);$$

$$Y = 492 - 53 X \text{ (X = plasma P at wk 2, } \underline{P} < .07, \underline{r} = .54);$$

$$Y = 109 + 52 X \text{ (X = plasma Ca at wk 1, } \underline{P} < .06, \underline{r} = .56).$$

In addition, a nonsignificant negative correlation ($\underline{r} = -.4$, $\underline{P} > .2$) was found between urinary P and Ca excretion.

Daily Gain. Overall ADG during the 2 wk study was 130, 160, and 166 g for pigs fed the BD, BD plus phytase, and BD plus MDCaP, respectively. There was no significant effect of treatments on ADG. However, daily gain of pigs was measured only to indicate that pigs were in good health and were gaining weight in the experiment.

Discussion

Two questions arose after our earlier demonstration of a significant linear effect of dietary phytase activity (up to 750 PU/g) on ADG, ADFI, and plasma P concentrations in young pigs (Lei et al., 1992a). The first question was how dietary phytase activity beyond 750 PU/g would affect these

response measures, and how much activity would be needed to produce maximum responses. The second was whether or not supplements of the determined optimal dietary phytase activity could obviate the need for inorganic P supplements for weanling pigs fed corn-soybean meal diets. The results of Exp. 2.1 showed no further improvement ($P > .05$) in ADG, ADFI, gain/feed, or plasma AP activity when dietary phytase activity reached 1,050 PU/g. Quadratic relationships between dietary phytase activity and these measures were found and consistently revealed the maximum breakpoint to be approximately 1,200 PU/g. However, breakpoints for dietary phytase activity relating to plasma Mg, Cu, and Fe concentrations were lower and inconsistent.

With all the measures taken, only pigs receiving 750 PU/g were consistently inferior to those pigs fed MDCaP. Differences in these measures between the other three groups of pigs receiving higher levels of phytase activity and MDCaP were not significant in most cases. The equivalency of estimated maximum responses of ADG, gain/feed, plasma AP activity, and plasma Ca concentrations at the respective breakpoints of dietary phytase activity to that of pigs fed MDCaP were at least 90%. Indeed, plasma P concentrations in pigs receiving 1,250 or 1,350 PU/g were only 70% of those of pigs supplemented with MDCaP in Exp. 2.1. Significant differences between pigs receiving 1,200 PU/g and MDCaP were also observed in Exp. 2.2. However, the higher plasma P

concentrations in pigs supplemented with MDCaP may not be particularly crucial because the balance data of Exp. 2.2 indicated that pigs fed MDCaP, ingesting 44% more P daily than pigs fed 1,200 PU/g, retained no more than 7% of additional P. Urinary P excretion, in addition to fecal P excretion, was much greater in these pigs and was positively correlated to the higher plasma P concentrations.

Differences in plasma P concentrations may be offset by the differences in urinary P excretion in these two groups of pigs. Alternatively, the combined improvement in absorption and retention of dietary P in pigs receiving 1,200 PU/g as compared to pigs receiving inorganic P supplementation may minimize the possible adverse effect of low dietary P intake, and hopefully of the lower plasma P concentration as well. Moreover, plasma P concentrations in pigs receiving 1,250 or 1,350 PU/g in Exp. 2.1 were almost within the normal range (Ullrey et al., 1967). Therefore, we may conclude that supplements of dietary phytase activity of 1,200 PU/g could maximize phytate-P utilization by weanling pigs and, thus, nearly, if not completely, eliminate the need for inorganic P supplements in corn-soybean meal diets for weanling pigs.

Cromwell (1991) suggested that supplemental dietary phytase at 1,000 PU/g might almost eliminate the need for inorganic P supplements for finishing pigs. If his results together with that we obtained in this study were applied in

commercial swine production, a large portion of the inorganic P supplements currently used would be saved. Of equal or greater importance is the 50% reduction of P excreted in swine manure, based on the decreases in fecal and urinary P excretion in pigs fed supplemental phytase as compared to those supplemented with MDCaP. However, this depends on the assumption that pigs can sustain low P intake continuously from weaning to finishing. In addition, pigs fed 1,200 PU/g in Exp. 2.2 excreted a larger amount of Ca via urine than pigs fed MDCaP. It is uncertain whether this hypercalciuria was a sign of relative P deficiency (Pointillart et al., 1987) or simply a function of the higher Ca intake. Pigs receiving dietary phytase had relatively higher ADFI than pigs fed MDCaP and thus ingested more Ca. This may require a lower dietary Ca level to balance the low total P concentration of the corn-soybean meal diet in which inorganic P supplements are replaced by dietary phytase. Increased dietary Ca concentration has also been suggested to affect phytate-P utilization adversely (Mohammed et al., 1991). Therefore, future studies should examine both concentrations and ratios of dietary phytase, P, Ca and other nutrients related to P metabolism, such as vitamin D.

The inorganic P equivalent of dietary phytase activity found in this study was lower than that previously reported. Leunissen and Young (1992) supplemented the diets for

weanling pigs with the same sources of phytase as used in this study at 500 to 1,000 PU/g. The responses of all criteria measured by them indicated that 500 PU increased the availability of P equivalent to that of adding .17% of inorganic P from calcium phosphate to diets. In a broiler study, Schöner et al.(1991) reported that 700 PU were equivalent to 1.0 mg P as monocalcium phosphate. The ingredients and the P concentrations of the BD used in these two studies were greatly different from those of the BD that we used in this study. These may have affected the efficiency of supplemental phytase in releasing phytate-P from the diets. Moreover, the efficiency of phytase may be different in pigs than in broilers. However, our estimate of equivalency agrees with the calculations of Cromwell (1991).

The results of this study were comparable to those of the study reported previously (Lei et al., 1992a). However, plasma P concentrations, ADG, and ADFI were lower in the current study. When plasma P concentrations were regressed against dietary phytase activity, slopes at the same week were similar, but intercepts (a) were lower in the current study. Thus lower plasma P concentrations were found in pigs receiving 1,250 PU/g than previously predicted (Lei et al., 1992a). Moreover, a significant effect of dietary phytase on plasma Ca concentration was shown only in the current study. Three factors may have contributed to these differences: 1) initial BW of pigs in current study was lighter; 2) dietary

P and Ca concentrations were not exactly the same in these two studies; and 3) the current study was conducted in the winter whereas the other was conducted in the summer. Likewise, differences in plasma P concentrations of pigs receiving the same treatment in Exp. 2.1 and 2.2 of this study may also be attributed to the differences in initial BW, dietary P and Ca concentrations, and feeding regime. In addition, we found that plasma P concentration appeared to be the most sensitive and convenient measure of the effect of dietary phytase on phytate-P utilization in our previous study (Lei et al., 1992a). However, in this study plasma P concentration kept increasing linearly with dietary phytase activity beyond the point at which other major measures were maximized.

Dietary Ca utilization was also improved by dietary phytase supplements, as shown previously (Nasi, 1990, Lei et al., 1992a). Other elements such as Mg, Cu, Fe, and Zn are also bound with phytate in corn and soybean meal and bioavailabilities are greatly limited (Reddy et al., 1982). Therefore, utilization of these elements might be improved by supplemental dietary phytase as well (Nasi, 1990). However, no such effects of dietary phytase were shown consistently in this study. The biologically available concentrations of these elements provided by the regular mineral premix may have been too high for phytase to show any effect. Special control of dietary levels of these

elements may be necessary to demonstrate the effect of dietary phytase. Measures other than plasma concentrations of these elements also may be useful.

Implications

Results of this study indicated that inorganic P supplementation of corn-soybean meal diets for weanling pigs could be almost completely eliminated by adding 1,200 units of A. niger phytase activity per gram diet. Pigs receiving this level of dietary phytase activity make near maximum responses in daily gains, gain/feed, plasma alkaline phosphatase activity, and plasma Ca concentrations. These responses were approximately 90% of those of pigs fed the regular diet supplemented with inorganic P. These pigs also maintained plasma P concentrations in the normal range and utilized dietary P and Ca quite efficiently. Accordingly, inorganic P resources could be saved and P in swine manure reduced by at least 50%.

EXPERIMENTAL SERIES III
SIMULTANEOUS IMPROVEMENTS IN PHYTATE
PHOSPHORUS AND ZINC BIOAVAILABILITY
BY SUPPLEMENTAL DIETARY PHYTASE
(Submitted to J. Nutr.)

ABSTRACT

Two experiments were conducted to determine the effects of supplemental microbial phytase on utilization of dietary zinc and phytate phosphorus in weanling pigs. Experiment 3.1 was a 2 X 3 factorial arrangement of treatments with 24 pigs for 4 wk. Two levels of phytase (A. niger), 0 or 1350 units/g, and three levels of zinc, 0, 30, or 60 mg/kg as zinc sulfate were added to a corn-soybean meal basal diet. Weekly measures included growth performance, plasma alkaline phosphatase activity, and plasma mineral concentrations. In experiment 3.2, mineral balances were determined in 12 pigs fed the basal diet or the diet with added zinc (30 mg/kg) or phytase (1350 units/g). The results indicated that either supplemental phytase or zinc increased plasma alkaline phosphatase activity and plasma zinc concentrations, but only in the absence of the other. Supplemental phytase decreased plasma alkaline phosphatase activity in pigs supplemented with zinc. Supplemental phytase also significantly enhanced weight gain, feed intake, gain/feed, plasma concentrations of inorganic phosphorus and iron, and retention of phosphorus and calcium. Neither supplemental phytase nor zinc affected plasma concentrations of copper

and magnesium or zinc retention. Supplementing corn-soybean meal diets with microbial phytase at 1350 units/g feed appears to improve bioavailability of zinc as well as of phytate phosphorus in weanling pigs.

INDEXING KEY WORDS:

- phytase • phytate • pig
- phosphorus • zinc

Introduction

Over 50% of phosphorus in corn, soybean meal, and other plant seeds is in the form of phytate (myo-inositol phosphates) that is poorly available to pigs and other simple-stomached animals (Reddy et al. 1982). Consequently, provision of an inorganic phosphorus supplement in pig diets composed mainly of these ingredients is routine (NRC 1988). This supplement not only increases diet cost but may result in accumulation of excess phosphorus in cropland to which swine manure is applied (Cromwell 1991). Of significance in both human and animal nutrition is the formation of insoluble complexes of phytate with zinc at certain concentrations of calcium in the digestive tract, thereby greatly inhibiting zinc absorption (O'Dell and Savage 1960, Simpson and Wise 1990). Thus, typical corn-soybean meal diets for swine do not provide sufficient available zinc to meet the requirement (NRC 1988) even though these diets

contain a level of zinc that would be adequate for pigs consuming a casein-glucose diet (Shanklin et al. 1968). Indeed, removal of phytate from infant soy formulas increased zinc absorption from 16% to 47% in suckling rats and from 27% to 45% in monkeys (Lonnerdal et al. 1988).

Phytases, myo-inositol hexaphosphate phosphohydrolases (E.C. 3.1.3.8), catalyze the stepwise removal of inorganic orthophosphate from phytate and produce five classes of intermediate products, myo-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates (Gibson and Ullah, 1990). Supplements of microbial phytase (Jongbloed et al. 1992, Lei et al. 1992a, Nasi 1990, Nelson et al. 1971, Simons et al. 1990) or cereal phytase (Pointillart et al. 1987) in diets for pigs and poultry effectively improves phytate phosphorus utilization. In our earlier study (Lei et al. 1992b), we demonstrated that supplementing corn-soybean meal diets with A. niger phytase at 1200 phytase units (PU)/g of feed appeared to maximize utilization of phytate phosphorus and may obviate almost completely the need for inorganic phosphorus supplementation in weanling pigs. Since bioavailability of zinc in foods of plant origin is a function of phytate concentration (Bobilya et al. 1991, Ferguson et al. 1989, Lonnerdal et al. 1988), we hypothesized that enhanced dietary phytate phosphorus utilization by supplemental microbial phytase might also increase the bioavailability of zinc. Two experiments were

conducted with weanling pigs to determine whether phytase supplements would produce simultaneous improvements in the bioavailability of zinc as well as phytate phosphorus in a corn-soybean meal diet.

Materials and Methods

Phytase, Zinc, and Diets. The microbial phytase used in this study was produced by a genetically modified strain of A. niger (var. ficuum, 3-phytase). The enzyme product was kindly provided by Alko Ltd., Rajamaki, Finland and phytase activity was approximately 500,000 PU/g. Actual phytase activity was confirmed as previously described (Lei et al. 1992a) before the product was mixed with other ingredients in the preparation of the complete diet. One PU is defined as the amount of enzyme that liberates 1 nmol of inorganic P from sodium phytate per minute at pH 5 and 37 °C. The basal diet (BD) was a fortified corn-soybean meal diet without supplemental inorganic phosphorus or zinc (**Table 3.1**). The analyzed concentrations of different minerals in the experimental diets are also listed in **Table 3.1**. Zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, ACS grade, Columbus Chemical Industries, Columbus, WI) was used as the supplemental zinc source.

TABLE 3.1
Composition and nutrient values of the basal diet¹

Item		Concentration
<u>Ingredient</u>		<u>g/kg</u>
Corn (ground, shelled)		773.4
Soybean meal (44% CP)		200.0
Calcium carbonate (38% Ca)		10.0
L-Lysine HCl		2.6
Salt (NaCl)		3.5
Vitamin-trace mineral premix ²		5.0
Vitamin E-Se premix ³		5.0
Antibiotic premix ⁴		.5
<u>Analyzed zinc concentration (as fed)</u>		<u>mg/kg</u>
Diet	Phytase PU/g	Total zinc mg/kg
Experiment 1 ⁵		
1	0	27
2	0	50
3	0	86
4	1350	25
5	1350	47
6	1350	94
Experiment 2 ⁶		
Basal	0	30
+ Zinc	0	55
+ Phytase	1350	32

¹ The basal diet provided all nutrients at recommended concentrations (NRC, 1988) with the exception of phosphorus, calcium, and zinc.

² Vitamin-trace mineral premix (g/kg): vitamin A acetate (30,000 IU/g), 22; vitamin D₃ (3000 IU/g), 44; menadione sodium bisulfite, 0.44; riboflavin, 0.66; niacin, 3.52; d-pantothenic acid, 2.64; choline chloride, 36.56; vitamin B₁₂ (trituated), 3.96; MnO, 11.45; KIO₃, 0.18; CuSO₄·5H₂O, 7.93; FeSO₄·H₂O, 35.68; butylated hydroxytoluene, 9.91.

³ Vitamin E-Se premix: α -tocopheryl acetate (500 IU/g), 0.15 g/kg; Na₂SeO₃, 44 mg/kg.

⁴ Supplied 55 mg of chlortetracycline per kilogram diet.

⁵ The basal diet was analyzed to contain per kilogram: phosphorus, 2.9 g, calcium, 4.6 g, magnesium, 1.6 g, zinc, 27 mg, copper, 10 mg, and iron, 108 mg.

⁶ The basal diet was analyzed to contain per kilogram: phosphorus, 3.0 g, calcium, 4.7 g, zinc, 30 mg, and copper, 15 mg.

Animals and Treatments. All pigs used in the two experiments were weanling crossbreds (Landrace-Yorkshire-Hampshire). Experimental housing was maintained at 22-25 °C, with a 12 h light:dark cycle. In experiment 3.1, twenty four pigs (4-wk old, $7.38 \pm .37$ kg body weight) were reared in 12 stainless steel metabolism pens (two pigs per pen) with slotted floors. A 2 X 3 factorial arrangement of treatments with 2 levels of supplemental phytase, 0, and 1350 PU/g, and 3 levels of supplemental zinc, 0, 30, and 60 mg/kg in the BD was conducted for four wk. Pigs were given ad libitum access to feed and water. All pigs were fed the low phosphorus and zinc BD for 10 d to deplete partially the phosphorus and zinc reserves before the formal trial was conducted.

In experiment 3.2, twelve pigs (3-wk old, $6.00 \pm .62$ kg body weight) were allotted equally into groups receiving the BD, the BD plus zinc, 30 mg/kg (+ Zinc), or the BD plus phytase activity, 1350 PU/g (+ Phytase). Pigs were housed in individual stainless steel metabolism cages and fed the BD for two wk to deplete the phosphorus and zinc reserves somewhat before the actual experimental period. Pigs were then fed their designated diets and given ad libitum access to distilled water for 20 d.

Sample Collection and Measurements. In experiment 3.1, individual pig weights and pen feed consumption were measured weekly. Blood samples of all pigs were taken weekly from the anterior vena cava for assay of plasma inorganic

phosphorus, calcium, magnesium, copper, iron, and zinc concentrations, and plasma alkaline phosphatase (AP, E.C.3.1.3.1) activity. In experiment 3.2, phosphorus, calcium, and zinc balance trials were conducted as previously described (Lei et al. 1992a). Total collections of feces and urine from individual pigs were made during the last 4 d of the trial. Dietary intake and fecal and urinary mineral excretion rates for the balance period were calculated as the average per kilogram body weight per day for each animal (Kimmel et al. 1992). Blood samples were taken from each pig at d 0, d 10, and at d 20 for assay of plasma phosphorus, calcium, and zinc concentrations, and plasma AP activity. Body weights also were recorded at each bleeding.

Assays. Concentrations of phosphorus in feed, feces, urine, and blood plasma were determined by a colorimetric method (Gomori 1942), and concentrations of calcium and zinc in these samples plus other elements in plasma and feed were determined by flame atomic absorption spectrophotometry (Model IL 951, Instrumentation Laboratory, Inc., Wilmington, MA). Plasma AP activity was determined on the day that blood samples were drawn by the method outlined by Sigma Chemical (1987).

This study was approved by the All-University Committee on Animal Use and Care of Michigan State University.

Statistics. Data from experiment 3.1 were analyzed as a

split-plot model with factorial arrangement of treatments and time repeated measurements (Gill, 1986). Pen was considered the experimental unit. Balance of phosphorus, calcium, and zinc in experiment 3.2 were analyzed as a randomized complete block model with 3 treatments. Plasma measures in experiment 3.2 were analyzed as a split-plot model with repeated measurements (d 10 and d 20). The Bonferroni t -test was used for conditional comparisons of treatment means. Standard errors of mean differences, instead of standard errors of single means, for all measures were listed as recommended by Gill (1986). Interactions were considered to have modest importance if $P < 0.20$, but primary comparisons were declared significant only if $P < .05$, unless indicated otherwise.

Results

Experiment 3.1

Main Effects. Statistical analyses of the main effects of supplemental phytase and zinc and their interactions on various measures are presented in **Table 3.2**. Supplemental phytase and zinc interacted to affect plasma AP activity ($P < 0.005$), plasma zinc concentrations ($P < 0.01$), and daily feed intake ($P < 0.09$). In addition, supplemental phytase

TABLE 3.2
Significances and standard errors of mean differences of main effects of dietary phytase and zinc, and their interactions on various measures¹

Measures	Significance P <			Standard error of mean difference ²			df ⁴
	Phy ³	Zn	Phy*Zn	Phy	Zn	Phy*Zn	
<u>Growth perforamces</u>							
Weight gain	0.0006	0.83	0.44	32	40	56	16
Feed intake	0.0001	0.58	0.09	53	64	91	24
Gain/feed	0.05	0.89	0.96	38	46	66	19
<u>Plasma alkaline phosphatase activity</u>							
	0.06	0.002	0.005	13.7	16.8	23.7	15
<u>Plasma mineral concentrations</u>							
Phosphorus	0.0001	0.07	0.67	0.09	0.11	0.15	23
Calcium	0.05	0.21	0.87	0.12	0.14	0.20	24
Zinc	0.0005	0.0008	0.01	1.25	1.54	2.17	23
Copper	0.45	0.75	0.33	1.23	1.52	2.14	15
Iron	0.03	0.97	0.67	4.59	5.63	6.85	22
Magnesium	0.22	0.22	0.35	0.08	0.10	0.13	20

¹ Data were analyzed as split-plot model with 2 X 3 factorial arrangement of treatments and time repeated measurements.

² Units for standard error of mean differences of various measures are the same as described in the following tables.

³ Phytase.

⁴ Calculated degrees of freedom of error.

affected all other measures ($P < 0.05$) except plasma copper and magnesium concentrations. However, supplemental zinc tended to affect only plasma phosphorus concentrations ($P < 0.07$) beside its interactive effects with supplemental phytase on plasma AP activity, plasma zinc concentrations, and feed intake. Time affected ($P < 0.01$) all the response measures taken. Strong interactions of time and phytase ($P < 0.004$) were observed on weight gain, feed intake, plasma AP activity, and plasma concentrations of phosphorus and zinc. Interactions of time and zinc were found on plasma zinc concentrations ($P < 0.03$) and plasma AP activity ($P < 0.0003$). Interactions of time both with phytase and zinc appeared for gain/feed ($P < 0.004$), weight gain ($P < 0.1$), plasma AP activity ($P < 0.08$), and plasma concentrations of zinc ($P < 0.2$).

Growth Performance. Effects of supplemental phytase and zinc on daily feed intake, weight gain, and gain/feed are presented in Table 3.3. Supplemental phytase tended to increase daily feed intake, but the increase varied with time and dietary zinc concentrations. In wk 4, supplemental phytase increased ($P < 0.05$) daily feed intake at all three levels of dietary zinc. However, in wk 1 and wk 3, supplemental phytase increased daily feed intake only in pigs supplemented with zinc at 30 mg/kg. Phytase produced no significant increase in feed intake in wk 2. Supplemental

TABLE 3.3
Daily feed intake, weight gain, and gain/feed of pigs receiving different dietary levels of supplemental phytase activity and zinc¹

Phytase, PU ² /g	0			1350		
Zinc, mg/kg	0	30	60	0	30	60
<u>Feed intake, g/d</u>						
Wk 1	550	466	539	672	698	557
Wk 2	729	878	729	885	929	904
Wk 3	846	807	878	1034	1138	1023
Wk 4	927	774	968	1341	1365	1216
Overall ³	763	731	778	983	1032	925
<u>Weight gain, g/d</u>						
Wk 1	343	268	273	399	453	438
Wk 2	321	414	397	524	562	456
Wk 3	416	326	412	537	544	484
Wk 4	411	369	424	667	606	589
Overall ³	373	344	376	532	541	492
<u>Gain/feed, g/kg</u>						
Wk 1	625	575	501	588	644	786
Wk 3	491	405	469	521	477	473
Wk 4	443	475	441	499	443	486
Overall ³	488	470	484	543	524	532

¹ Data were analyzed as split-plot model with 2 X 3 factorial arrangement of treatments and time repeated measurements. Significances and standard error of mean differences of treatment factors are listed in Table 2. Values are means of four pigs.

² Phytase unit (see text for definition).

³ Comparisons of overall means should not be taken because of interactions of treatments with time on the three measures.

phytase greatly improved weight gain ($P < 0.05$) in all 4 wk of the study, independent of dietary zinc concentrations. Supplemental phytase also improved gain/feed ($P < 0.05$) during the first 2 wk of the study. In contrast, supplemental zinc alone apparently did not affect weight gain or gain/feed.

Plasma Alkaline Phosphatase Activity. Effects of supplemental phytase and zinc on plasma AP activity are presented in **Table 3.4**. Pigs fed the BD had the lowest plasma AP activity. Supplementing the BD with zinc at either 30 or 60 mg/kg increased plasma AP activity ($P < 0.05$) in all 4 wk of the study, but the increase was shown only in pigs receiving no dietary phytase. The three groups of pigs supplemented with phytase had similar plasma AP activity. Unlike supplemental zinc, supplemental phytase did influence plasma AP activity at all levels of dietary zinc. Without supplemental zinc, phytase increased ($P < 0.1$) plasma AP activity in the first 3 wk. With supplemental zinc, phytase decreased ($P < 0.05$) plasma AP activity in the last 2 wk.

Plasma Concentrations of Minerals. Effects of supplemental phytase and zinc on different element concentrations in plasma are presented in **Tables 3.4** and **3.5**. Pigs fed the BD had the lowest plasma zinc concentrations. Supplementing the BD with either phytase or zinc increased ($P < 0.05$) plasma zinc concentrations in all 4 wk of the

TABLE 3.4

Plasma alkaline phosphatase activity and plasma zinc, phosphorus, and calcium concentrations in pigs receiving different dietary levels of supplemental phytase activity and zinc¹

Phytase, PU^2/g	0			1350		
Zinc, mg/kg	0	30	60	0	30	60
<u>Plasma alkaline phosphatase, U^3/L</u>						
Wk 0	132	164	138	147	121	149
Wk 1	99	194	185	155	157	175
Wk 2	68	201	142	127	151	154
Wk 3	66	257	190	129	148	140
Wk 4	103	286	228	119	134	143
<u>Plasma zinc, $\mu\text{mol/L}$</u>						
Wk 0	9.2	9.9	8.9	8.5	7.7	8.5
Wk 1	5.9	12.9	15.7	14.3	16.3	14.8
Wk 2	4.3	12.5	13.5	13.7	15.9	16.8
Wk 3	3.5	14.8	14.5	14.5	16.6	19.7
Wk 4	3.5	10.8	13.7	13.1	16.3	13.5
<u>Plasma phosphorus, mmol/L</u>						
Wk 0	1.7	1.6	1.6	1.5	1.7	1.5
Wk 1	1.4	1.6	1.4	2.0	2.2	1.7
Wk 2	1.0	1.1	1.1	2.0	2.0	2.1
Wk 3	1.0	1.0	0.8	1.9	2.0	2.0
Wk 4	0.8	0.7	0.7	2.0	2.1	1.7
<u>Plasma calcium, mmol/L</u>						
Wk 0	2.9	2.8	2.8	2.7	2.7	3.0
Wk 1	3.0	2.9	3.1	2.6	3.0	2.9
Wk 2	2.9	3.0	3.1	2.8	2.7	2.8
Wk 3	2.7	2.9	2.6	2.8	2.7	2.8
Wk 4	2.4	2.6	2.7	2.3	2.3	2.7

(Continued on next page)

(Continued, TABLE 3.4)

¹ Data were analyzed as split-plot model with 2 X 3 factorial arrangement of treatments and time repeated measurements. Significances and standard error of mean differences of treatment factors are listed in Table 2. Values are means of four pigs.

² Phytase unit (see text for definition).

³ Sigma unit (Sigma Procedure No. 425, 1987).

TABLE 3.5
Plasma iron, copper, and magnesium concentrations in pigs
receiving different dietary levels of supplemental phytase
activity and zinc¹

Phytase, PU^2/g	0			1350		
Zinc, mg/kg	0	30	60	0	30	60
<u>Plasma iron, $\mu\text{mol/L}$</u>						
Wk 0	36.4	39.8	42.1	49.6	46.1	41.3
Wk 1	42.0	35.2	36.8	40.5	46.8	38.9
Wk 2	33.4	34.6	43.9	43.9	48.2	41.6
Wk 3	28.2	37.1	32.7	51.1	37.3	43.0
Wk 4	39.3	46.3	39.6	53.8	49.5	51.6
<u>Plasma copper, $\mu\text{mol/L}$</u>						
Wk 0	22.5	21.3	21.7	22.5	21.1	23.0
Wk 1	20.8	21.3	21.3	21.9	21.2	19.2
Wk 2	20.5	20.0	19.5	25.3	19.7	23.1
Wk 3	19.5	21.3	21.1	23.4	20.3	20.9
Wk 4	16.6	19.1	22.5	18.3	19.5	20.5
<u>Plasma magnesium, mmol/L</u>						
Wk 0	1.5	1.4	1.4	1.4	1.3	1.4
Wk 1	1.3	1.3	1.2	1.5	1.5	1.3
Wk 2	1.4	1.1	1.1	1.4	1.3	1.3
Wk 3	1.5	1.3	1.1	1.3	1.4	1.3
Wk 4	1.2	1.0	1.1	1.0	1.1	1.2

¹ Data were analyzed as split-plot model with 2 X 3 factorial arrangement of treatments and time repeated measurements. Significances and standard error of mean differences of treatment factors are listed in Table 2. Values are means of four pigs.

² Phytase unit (see text for definition).

study. However, the increases resulting from supplemental phytase appeared only in the absence of supplemental zinc and vice versa. Plasma zinc concentrations in the three groups of pigs receiving phytase were similar. Higher supplemental zinc (60 mg/kg) did not increase plasma zinc concentrations further at either dietary phytase level. Pigs fed phytase maintained normal plasma phosphorus concentrations, as shown previously (Lei et al. 1992b), through the entire study. Meanwhile, pigs receiving no dietary phytase had plasma phosphorus concentrations that gradually decreased to less than half of those in pigs fed phytase ($P < 0.05$) by the end of the study. Dietary phytase also increased plasma iron concentrations ($P < 0.05$) in wk 3 and wk 4 and decreased plasma calcium concentrations ($P < 0.05$) in wk 2. The only effect of dietary zinc alone was on plasma phosphorus concentrations in wk 2. Pigs had higher plasma phosphorus concentrations ($P < 0.05$) when supplemented with zinc at 30 mg/kg rather than at 60 mg/kg. However, neither supplemental phytase nor zinc consistently affected plasma concentrations of copper or magnesium.

Experiment 3.2

Mineral Balance. Balances of phosphorus, calcium, and zinc in pigs fed the BD, the BD plus zinc, or the BD plus phytase are presented in **Table 3.6**. With essentially the same

TABLE 3.6

Balance of phosphorus, calcium, and zinc in pigs fed the basal diet or basal diet supplemented with zinc sulfate or microbial phytase¹

Item	Basal	+ Zinc ²	+ Phytase ³	SED ⁴	P < ⁵
<u>Phosphorus, mmol/kg·d</u>					
Intake	3.29	3.33	3.42	0.31	0.91
Fecal ⁶	2.46 ^b	2.56 ^b	1.04 ^a	0.18	0.0001
Urinary	0.01	0.02	0.03	0.01	0.49
Retained	0.82 ^a	0.75 ^a	2.35 ^b	0.23	0.0001
% of intake	24.92 ^a	22.52 ^a	69.59 ^b	4.69	0.0001
<u>Calcium, mmol/kg·d</u>					
Intake	3.86	4.22	3.98	0.38	0.65
Fecal	2.31 ^b	2.63 ^b	1.25 ^a	0.29	0.002
Urinary	1.05	0.78	0.55	0.27	0.23
Retained	0.50 ^a	0.81 ^a	2.18 ^b	0.18	0.0001
% of intake	12.95 ^a	19.19 ^a	54.77 ^b	4.67	0.0001
<u>Zinc, μmol/kg·d</u>					
Intake	15.17 ^a	29.13 ^b	17.41 ^a	2.32	0.0004
Fecal	20.26	31.72	22.69	5.47	0.14
Urinary	1.89	1.57	1.98	0.62	0.79
Retained	- 6.98	- 4.16	- 7.26	4.86	0.79
% of intake	-46.01	-14.28	-41.70	29.91	0.48

¹ Data were analyzed as random complete block model with three treatments. Values are means of four pigs.

² Supplemented with 30 mg zinc as ZnSO₄·7H₂O per kilogram basal diet.

³ Supplemented with A. niger phytase at 1350 units/g of basal diet.

⁴ Standard error of differences between any two treatment means.

⁵ Significance of main effects.

⁶ Means within a row lacking a common superscript letter differ (P < .05).

dietary intake, pigs fed the BD plus phytase retained more than twice as much phosphorus and calcium ($P < 0.05$) daily than the other two groups. As expected, daily fecal excretions of phosphorus and calcium were markedly reduced in pigs supplemented with phytase. Urinary excretions of phosphorus were lowest in pigs fed the BD followed by pigs supplemented with zinc and phytase. Urinary excretions of calcium were in the opposite order. However, no significant difference between treatments was found in urinary excretions of these two elements. Fecal and urinary excretions and retentions of phosphorus and calcium in pigs fed the BD were not different from pigs fed the BD plus zinc. All three groups of pigs were in negative zinc balance. Due to the higher dietary zinc intake ($P < 0.05$), pigs supplemented with zinc had less negative balance and greater fecal zinc excretions than the other two groups of pigs. Urinary zinc excretions were similar in the three treatment groups. In all, neither excretion via feces or urine nor retention of zinc was significantly different among the treatment groups.

Plasma Concentrations of Minerals and Alkaline Phosphatase Activity. Effects of supplemental phytase and zinc on plasma phosphorus, calcium, and zinc concentrations, and plasma AP activity are presented in **Table 3.7**. Pigs supplemented with phytase maintained normal plasma phosphorus concentrations

TABLE 3.7

Plasma inorganic phosphorus, calcium, and zinc concentrations and plasma alkaline phosphatase activity of pigs fed the basal diet or basal diet supplemented with zinc sulfate or microbial phytase in experiment 3.2^{1,2}

Time	Basal	+ Zinc ³	+ Phytase ⁴
<u>Plasma inorganic phosphorus, mmol/L</u>			
D 0	1.5	1.6	1.7
D 10 ⁵	1.5 ^{ab}	1.4 ^a	1.8 ^b
D 20	1.4 ^a	1.2 ^a	2.3 ^b
(SED ⁶ = .13, df of error = 8)			
<u>Plasma calcium, mmol/L</u>			
D 0	2.5	2.4	2.4
D 10	2.8 ^b	2.6 ^{ab}	2.4 ^a
D 20	2.9 ^b	2.7 ^{ab}	2.3 ^a
(SED = .10, df of error = 9)			
<u>Plasma zinc, μmol/L</u>			
D 0	9.7	9.5	10.3
D 10	10.2	11.9	10.8
D 20	8.9 ^a	11.9 ^{ab}	12.5 ^b
(SED = 1.18, df of error = 8)			
<u>Plasma alkaline phosphatase, U⁷/L</u>			
D 0	145	121	135
D 10	161	177	178
D 20	127	102	101
(SED = 27.1, df of error = 10)			

¹ Data were analyzed as split-plot model with three treatments and time repeated measurements. Values are means of four pigs.

² Significance of main effects of treatment on: plasma phosphorus ($P < 0.0006$), plasma calcium ($P < 0.002$), plasma zinc ($P < 0.1$), and plasma alkaline phosphatase ($P < 0.97$).

³ Supplemented with 30 mg zinc as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ per kilogram basal diet.

(Continued on next page)

(Continued, TABLE 3.7)

⁴ Supplemented with A. niger phytase at 1350 units/g of basal diet.

⁵ Means within a row lacking a common superscript letter differ ($P < 0.05$).

⁶ Standard error of differences between any two treatment means at a given time.

⁷ Sigma unit (Sigma Procedure No. 425, 1987).

which were higher ($P < 0.05$) than those in pigs fed the BD or the BD plus zinc. Plasma calcium concentrations in pigs receiving phytase were lower ($P < 0.05$) than in pigs fed the BD. Plasma calcium concentrations in pigs fed the BD or the BD plus zinc were not significantly different. Plasma zinc concentrations in pigs fed the BD were lower than those of pigs supplemented with phytase ($P < 0.05$) and zinc ($P < 0.1$) at the end of the experiment. The latter two groups had similar plasma zinc concentrations. Unlike the case in experiment 3.1, plasma AP activity was not affected by dietary treatments.

Weight Gain. All pigs were in good health and gaining weight through the entire experiment. The overall daily gains in pigs fed the BD, the BD plus zinc, and the BD plus phytase were 125, 123, and 151 g, respectively.

Discussion

Typical corn-soybean meal diets for pigs contain approximately 3 g of total phosphorus/kg, of which 60%, 10%, and 6% is as myo-inositol hexakis, pentakis, and tetrakisphosphate, respectively (Jongbloed et al. 1992, Lei et al. 1992a and b, Simons et al. 1990). Phytase activity in the stomach and small intestine, either from the dietary ingredients and/or mucous cells, is negligible (Jongbloed et al. 1992). Substantial dietary phytate generally passes to

the large intestine intact, where microflora may break it down, but phosphorus absorption is limited (Jongbloed 1987). Therefore, dietary phytate phosphorus is mainly excreted in the feces. As shown in previous studies (Jongbloed et al. 1992, Lei et al. 1992b, Nasi 1990, Simons et al. 1990) as well as in this study, no more than a quarter of the total phosphorus in these diets is retained by pigs. As expected, pigs fed such diets develop phosphorus deficiency as indicated by low feed intake, poor growth rate and efficiency, hypophosphatemia, hypophosphaturia, hypercalcemia, hypercalciuria, and elevated plasma AP activity (Lei et al. 1992a and b, Pointillart et al. 1987). Pigs receiving no supplemental phytase in this study showed nearly all of these signs except elevated plasma AP activity, which was probably limited by the low available zinc in the BD. Supplements of inorganic phosphorus in these corn-soybean meal diets overcome phosphorus deficiency and support normal performance (Lei et al. 1992b). Nevertheless, pigs supplemented with inorganic phosphorus still excrete the same, or a greater, amount of fecal phosphorus (Lei et al. 1992b). Thus, supplemental inorganic phosphorus does not resolve the problem of excess phosphorus in manure originating from the poor digestibility of phytate phosphorus in the foresegments of the gastrointestinal tract of pigs. Alternatively, supplementing corn-soybean meal diets with sufficient microbial phytase activity, such as

1200 PU/g in our previous study (Lei et al. 1992b) and 1350 PU/g in this study, appears to release sufficient phosphorus from phytate to meet the requirement of pigs. Of equal or greater importance, fecal phosphorus excretion is reduced by more than 50%. In addition, dietary calcium utilization is also improved. Therefore, our studies, together with those of others (Jongbloed et al. 1992, Nasi 1990, Simons et al. 1990), indicate that judicious use of microbial phytase in swine diets may not only greatly reduce the need for supplements of inorganic phosphorus, a nonrenewable resource and the third largest expense in the diet, but also alleviate excess manure phosphorus pollution which is a severe problem facing the swine industry (Cromwell 1991). Effects of supplemental zinc on plasma phosphorus concentrations were observed only in wk 2 of experiment 3.1. Basically, the positive effects of supplemental phytase on phytate phosphorus utilization in this study appeared to be independent of dietary zinc concentration. However, zinc deficiency has been shown to modulate biochemical responses to dietary phosphorus and calcium in rats (Kimmel et al. 1992).

Zinc in foods of plant origin is less bioavailable than that of animal origin because of the adverse effect of phytate on zinc utilization (Bobilya et al. 1991, Lonnerdal et al. 1988). Removal or reduction of phytate in food generally improves zinc bioavailability (Lonnerdal et al.

1988, Reddy et al. 1982). However, attempts to enhance zinc utilization in vivo by supplementing the diet with microbial phytase have not been previously reported. In this study, we demonstrated that supplements of A. niger phytase, just as supplements of zinc, in a corn-soybean meal BD greatly increased plasma zinc concentrations in both trials. We attribute this increase to the effect of supplemental phytase on phytate degradation and to zinc release. Supplemental phytase did not affect plasma zinc concentrations when highly available zinc was provided in the same diets.

Improvement of zinc utilization by phytase was also illustrated by the responses of plasma AP activity with different treatments. Alkaline phosphatase, a zinc containing enzyme, plays a key role in phosphorus metabolism. Activity of AP in plasma generally increases with phosphorus deficiency (Boyd et al. 1983, Miller et al. 1964) and decreases with zinc deficiency (Miller et al. 1968, Shanklin et al. 1968). Pigs receiving no supplemental phytase in experiment 1 were subjected to suboptimal available phosphorus. Thus, elevated plasma AP activity in these pigs would be expected. However, increased plasma AP activity occurred only in pigs supplemented with zinc. In contrast, plasma AP activity in pigs unsupplemented with zinc decreased. Plasma AP activity in pigs supplemented with phytase was normal (Miller et al. 1968) and was unaffected

by supplemental zinc. Therefore, supplementing microbial phytase at 1350 PU/g appeared to release not only sufficient phytate phosphorus but also sufficient zinc from the BD for pigs to maintain normal plasma AP activity. In contrast, phosphorus deprivation in pigs unsupplemented with phytase resulted in increases in plasma AP activity when highly available zinc (i. e. zinc sulfate) was provided. Yet, the increase was apparently limited in pigs fed the BD in which all zinc was from plant origin. We are uncertain why plasma AP activity failed to respond to supplemental dietary phytase or zinc in experiment 3.2.

Criteria for evaluating zinc bioavailability are not well established because it is still difficult to relate zinc deficiency signs and the biochemical roles of zinc in the body, particularly at the molecular level (Miller et al. 1991). Baker (1991) pointed out that plasma zinc concentration and AP activity were often misleading criteria of zinc bioavailability. He suggested that the best measures were probably body weight gain and bone zinc accumulation which indeed have been shown to be reliable in recent studies (Hunt and Johnson 1992, Wedekind et al. 1992). In our study, supplemental phytase significantly improved growth rate and efficiency. The improvement may have resulted from enhanced utilizations of dietary phosphorus and zinc by phytase. Supplemental zinc alone was not shown to promote performance directly in this study. The

phosphorus deficiency in pigs unsupplemented with phytase may have precluded the possible growth-promotion effect of supplemental zinc. The relatively low available dietary phosphorus and high growth rate in pigs supplemented with phytase may essentially have prevented supplemental zinc from further improving growth or feed intake. A linear increase in serum zinc concentration, instead of weight gain, was demonstrated by Miller et al. (1981) in weanling pigs supplemented with zinc from zinc oxide or metallic zinc dust at 25 and 50 mg/kg of feed. Responses of plasma zinc concentration to dietary zinc intake was found to agree with those of zinc retention, femur zinc concentration, and weight gain in neonatal pigs fed different sources of zinc (Bobilya et al. 1991). Earlier, serum alkaline phosphatase activity was successfully used in our laboratory as a criterion of zinc requirement of pigs (Miller et al. 1968, Shanklin et al. 1968). Currently, plasma alkaline phosphatase activity has been proposed as a useful indicator for monitoring zinc deficiency in humans (Ishikawa et al., 1981). Most importantly, responses of plasma AP activity and plasma zinc concentration to supplemental phytase and zinc in this study were very consistent. Thus, we consider that the changes of these two measures represented the improvement of dietary zinc bioavailability by supplemental phytase.

Zinc balance is also used to determine zinc requirement

and bioavailability (Bobilya et al. 1991, Shanklin et al. 1968). Nasi (1990) demonstrated no improvement in zinc retention associated with dietary phytase supplement in pigs. Likewise, pigs supplemented with phytase at 1350 PU/g in the BD in this study were in negative zinc balance as were pigs fed only the BD. It is difficult to explain how supplemental phytase increased plasma zinc concentrations without enhancing dietary zinc utilization, at least absorption. Moreover, even pigs supplemented with zinc at 30 mg/kg diet were still in negative zinc balance. In fact, total zinc concentration in the zinc-supplemented diet (55 mg/kg) was below the requirement (80 mg/kg, NRC 1988). However, pigs supplemented with zinc at 30 mg/kg had plasma zinc concentrations and plasma AP activities similar to those of pigs supplemented with zinc at 60 mg/kg. Besides the suboptimal intake of zinc, endogenous zinc excretion (Bobilya et al. 1991, Newland et al. 1958) may have contributed to the negative zinc balance. Ziegler et al. (1987) reported that half of the total fecal zinc in infancy originated from endogenous zinc excretion. Differences in phosphorus status between pigs supplemented with and without phytase also may have complicated zinc balance. Tao and Hurley (1975) found that low-calcium intakes resulted in release of skeletal zinc during bone resorption. Likewise, the low-phosphorus intake of pigs unsupplemented with phytase in this study may also have initiated bone

resorption, or at least reduced bone formation, and thus freed some zinc. The relative excess of zinc may have decreased dietary zinc absorption and(or) increased endogenous zinc excretion. In contrast, high levels of dietary phosphorus have been shown to increase fecal zinc excretion (Greger and Snedeker, 1980). The relatively higher available phosphorus intake of pigs supplemented with phytase, as compared to those that were unsupplemented, may have increased fecal zinc excretion somewhat, and thus, offset or minimized the expected positive effect of phytase on zinc absorption. Overall, it is not unusual to observe a negative balance of trace elements tested over a short period of time (Mertz 1987). Intrinsic and(or) extrinsic labeling of zinc in food may permit better estimates of the true bioavailability of zinc (Fairweather-Tait et al. 1991).

Besides zinc, phytate also chelates with other cations (Reddy, 1982). Although supplemental phytase appeared to increase only plasma iron concentrations, appropriate experimental designs may demonstrate improvement in bioavailability of other mineral elements by supplemental phytase. In conclusion, supplements of microbial phytase at 1350 PU/g diet appear to release sufficient zinc and phosphorus from a corn-soybean meal diet for pigs to maintain normal plasma phosphorus and zinc concentrations and normal growth and feed utilization without supplements of either inorganic phosphorus or zinc. Whether the released

zinc is sufficient to maintain normal zinc balance and bone zinc deposition in pigs deserves further study.

Implications

Supplementing corn-soybean meal diets with sufficient microbial phytase activity appears to not only increase bioavailability of phytate phosphorus, but also to improve zinc utilization as measured by plasma zinc concentrations, plasma alkaline phosphatase activity, and growth performance.

EXPERIMENTAL SERIES IV
INTERACTIONS OF PHYTASE, VITAMIN D, AND
CALCIUM ON PHYTATE PHOSPHORUS UTILIZATION
(Submitted to J. Anim. Sci.)

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ABSTRACT

The objective of this study was to determine the effect of dietary Ca and vitamin D on efficacy of microbial phytase (A. niger) in improving phytate-P utilization. A 2 X 2 X 2 factorial arrangement of treatments was conducted with two dietary levels of phytase (unit/g), 750 (suboptimal) and 1200 (optimal); of vitamin D (IU/kg), 660 (normal) and 6600 (high); and of Ca (%), .4 (low) and .8 (normal). Sixty-four weanling pigs (4-wk-old, $8.04 \pm .50$ kg BW) were allotted to 16 pens and fed the corn-soybean meal diets (no inorganic P added) with variable levels of phytase, vitamin D, and Ca. Individual pig weights and pen feed consumption were measured at d 10, d 20, and d 30. Blood samples were taken from each individual pig at the same time to assay plasma inorganic P and Ca concentration and alkaline phosphatase (AP) activity. The results indicated a strong ($P < .05$) adverse effect of normal dietary Ca on all the response measures. The depressive effect of normal Ca on performance was greater ($P < .05$) at normal vitamin D level or at optimal phytase level than at the other level of these two factors. The elevation in plasma AP activity by normal

dietary Ca was greater ($P < .05$) at the suboptimal than at the optimal phytase level. The decrease in plasma inorganic P and increase in plasma Ca by normal dietary Ca were overwhelming, but the magnitude of the changes still varied with levels of vitamin D and phytase. Overall, the best response of measures occurred when optimal phytase level was combined with levels of low Ca and normal vitamin D. In conclusion, normal level of Ca in the low-P, corn-soybean meal diets greatly reduced the efficacy of supplemental phytase. Raising vitamin D in the diets partially offset this adverse effect, but did not produce further improvement when Ca level was low.

Key Words: Pigs, Phytase, Vitamin D, Calcium, Phytate, Phosphorus, Plasma Alkaline Phosphatase

Introduction

Supplementing swine diets with microbial phytase (A. niger) greatly improves phytate-P bioavailability and decreases P excretion in the manure (Nasi, 1990; Simons et al., 1990; Cromwell, 1991; Jongbloed et al., 1992; Leunissen and Young, 1992). In a series of earlier studies, we demonstrated that supplemental phytase at 1200 phytase units (PU)/g of corn-soybean meal diets maximized phytate-P utilization and essentially obviated the need for inorganic P addition in weanling pigs (Lei et al., 1992b). However,

only a single dietary level of Ca and vitamin D, two key nutrients which strongly interact with P (Littledike and Goff, 1987) and affect phytate-P utilization (Wise, 1983), was used in all these studies. High levels of Ca in the diets of rats (Taylor and Coleman, 1979; Nahapetian and Young, 1980) and poultry (Edwards and Veltman, 1983; Ballam et al., 1985; Sheideler and Sell, 1987) consistently decreased the availability of phytate-P. Likewise, vitamin D deficiency was found to aggravate the disturbances of P and Ca metabolism resulting from high dietary phytate-P (Pointillart et al., 1985). In contrast, dietary supplements of cholecalciferol improved phytate-P bioavailability to pigs considerably (Fontaine et al., 1985). Recently, Mohammed et al. (1991) reported that the adverse effects of a high-phytate, low inorganic P diet on chicks were overcome by the independent but synergistic effects of lowering Ca and simultaneously raising cholecalciferol in the diets. Therefore, we proposed that dietary Ca and vitamin D would affect the efficacy of supplemental phytase by interacting with phytate-P. This experiment was conducted to determine the effect of dietary concentrations of Ca and vitamin D on phytase improvement of phytate-P utilization by weanling pigs.

Materials and Methods

Experimental Design. This study was arranged as a 2 X 2 X 2 factorial in randomized complete blocks. Because of repeated measurements over time, the entire design is a split-block. The three treatment factors included dietary phytase, Ca, and vitamin D. The two dietary levels of phytase (PU/g) were set at 750 (suboptimal) and 1200 (optimal), of Ca (%) at .4 (low) and .8 (normal), and of vitamin D (IU/kg) at 660 (normal) and 6600 (high).

Phytase and Diets. The microbial phytase (A. niger, Alko Ltd., Rajamaki, Finland), the confirmation of the actual activity of enzyme product, and the process of phytase incorporation into diets were the same as described previously (Lei et al., 1992a). A phytase unit (PU) is defined as the amount of enzyme that liberates 1 nmol of inorganic P from sodium phytate per minute at pH 5 and 37 °C. Calcium carbonate (Calcium Products, Inc., Swayzee, IN) and vitamin D₃ (Carl Akey, Lewisburg, OH) were used to achieve the dietary levels of these two nutrients. The basal diet (BD) was a fortified corn-soybean meal diet without supplemental inorganic P. The composition and the analyzed concentrations of P and Ca in the basal diet and the experimental diets are presented in Table 4.1.

Animals. Sixty-four weanling crossbreds (Landrace-Yorkshire-Hampshire, 4-wk-old) were grouped into heavy (8.91

$\pm .61$ kg BW) and light ($7.17 \pm .36$ kg BW) blocks based on body weight. The 32 pigs within each block were allotted into 8 pens of four pigs each. Housing, management, P-depletion procedures, and the experimental periods were the same as in previous studies (Lei et al., 1992a, b).

Sample Collection and Measurements. Individual pig weights and pen feed consumption were measured at d 10, d 20, and d 30. Blood samples were taken at the same frequency from all pigs for assay of plasma inorganic P and Ca concentrations and plasma alkaline phosphatase (AP) activity (Lei et al., 1992a).

Statistics. Data were analyzed as a split-block model with factorial treatments and time repeated measurements (Gill, 1986). Pen was considered the experimental unit. As recommended by Gill (1986), standard errors of differences of means, instead of standard errors of single means, are presented to overcome possible correlations induced by repeated measurement. The Bonferroni t -test was used for conditional comparisons of treatment means. Interactions were considered to have modest importance if $P < .30$, but primary comparisons were declared significant only for $P < .05$, unless indicated otherwise.

Correlations between various response measures were developed by the GLM procedure of SAS (1988).

Table 4.1. Composition of the basal and experimental diets.

Phytase, PU ^a /g	750						1200					
	660			6600			660			6600		
Vitamin D, IU/kg	Basal			4	8	4	8	4	8	4	8	
Calcium, g/kg	Basal			4	8	4	8	4	8	4	8	
<u>Ingredient, g/kg</u>												
Corn	775.1	773.6	762.9	771.6	760.9	772.7	762.0	770.7	760.0	772.7	760.0	
Soybean meal (44% CP)	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	
L-Lysine HCl	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	
Salt (NaCl)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	
Vitamin-trace mineral ^b	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
Vitamin E-Se ^c	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
Antibiotic ^d	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	
Phytase ^e	--	1.5	1.5	1.5	1.5	2.4	2.4	2.4	2.4	2.4	2.4	
CaCO ₃ ^f	8.3	8.3	19.0	8.3	19.0	8.3	19.0	8.3	19.0	8.3	19.0	
Vitamin D ^g	--	--	--	1.5	1.5	--	--	1.5	1.5	--	--	
<u>Analyzed, as fed</u>												
P, g/kg	3.2	3.2	2.9	3.2	3.1	3.4	3.1	3.1	3.1	3.1	2.9	
Ca, g/kg	4.9	4.8	5.5	5.2	5.4	8.8	9.0	9.5	9.5	9.5	9.5	

^a Phytase unit, see definition in context.

(Continued on Next Page)

(Continued, Table 1)

- ^b Supplied the following amounts per kilogram diet: vitamin A, 3,307 IU; vitamin D₃, 660 IU; menadione, 5.5 mg; riboflavin, 3.3 mg; niacin, 18.4 mg; d-pantothenic acid, 13.4 mg; choline, 110 mg; vitamin B₁₂, 20 µg; Zn, 75 mg; Fe, 58 mg; Mn, 33 mg; Cu, 10 mg; I, .20 mg.
- ^c Supplied 17 IU of vitamin E and .3 mg of Se per kilogram diet.
- ^d Supplied 55 mg of chlortetracycline per kilogram diet.
- ^e Aspergillus niger phytase (Alko Ltd., Rajamaki, Finland) supplied activity 500,000 PU/g product.
- ^f Contained 38% Ca.
- ^g Contained vitamin D 3,000 IU/g.

Results

Main Effects and Interactions of Treatments. The significances of main effects and their interactions of dietary phytase, vitamin D, and Ca and of block are summarized in Table 4.2. Dietary Ca significantly affected all the response measures whereas vitamin D alone did not affect any of these measures. Dietary phytase showed an effect only on plasma Ca concentration ($P < .06$). Marginal interaction ($P < .12$) of dietary phytase, vitamin D, and Ca was observed on ADFI and plasma Ca concentrations. The same type interaction but less significant ($P < .25$) was also shown on ADG and plasma AP activity. Dietary phytase and Ca appeared to interact on gain/feed ($P < .20$), plasma AP activity ($P < .25$), and plasma Ca concentration ($P < .29$). Similarly, dietary vitamin D seemed to interact with dietary Ca on all the response measures except plasma AP activity. In addition, body weight (block) had significant effect on all the measures but gain/feed or plasma AP activity. The standard errors of difference of two means for the main effects and the interactions, and the calculated degrees of freedom of error for each response criterion (Gill, 1986) are presented in Table 4.3.

Table 4.2. Probabilities of type I errors for test of significance of main effects and interactions on response measures

Factor	Performance			Plasma		
	ADG	ADFI	G/F	P	Ca	AP
<u>Main effect</u>						
Phytase (Phy)	.68	.95	.31	.85	.06	.56
Vitamin D (D)	.92	.46	.54	.52	.17	.43
Calcium (Ca)	.007	.04	.0005	.0002	.0001	.06
Block	.04	.006	.71	.05	.001	.22
<u>Interactions</u>						
Phy * D	.86	.97	.61	.41	.79	.77
Phy * Ca	.88	.73	.20	.53	.29	.25
D * Ca	.27	.34	.24	.35	.30	.74
Phy * D * Ca	.25	.12	.83	.57	.12	.24

Table 4.3. Standard errors of mean differences and degrees of freedom for various measures

Measure	Main effect	Two way interaction	Three way interaction	Error df
Daily gain, g	34.8	49.2	69.6	10.2
Feed intake, g/d	55.9	79.0	111.7	10.7
Gain/feed, g/kg	26.5	37.0	53.0	23.8
Plasma P, mg/dL	.22	.31	.44	12.9
Plasma Ca, mg/dL	.23	.32	.45	21.9
Plasma AP, U/dL	.92	1.30	1.84	14.2

Daily Gain, Daily Feed Intake and Gain/Feed. The effects of dietary phytase, vitamin D, and Ca on ADG, ADFI, and gain/feed are presented in Table 4.4. Phytase at 750 or 1200 PU/g of the corn-soybean basal diets without added inorganic P supported the pig growth at normal rate and efficiency (NRC, 1988) when dietary Ca level was low (.4%). In contrast, the effect of the same amount of phytase on ADG, ADFI, and gain/feed was markedly reduced or limited at the normal dietary Ca (.8%). Overall, pigs receiving the normal level of dietary Ca had much lower ADG than those receiving the low level of dietary Ca at d 10 ($P < .05$), d 20 ($P < .01$), and d 30 ($P < .01$). This type of decrease in ADG was much greater at the normal vitamin D level ($P < .02$) than at the high vitamin D level, and was even intensified at the optimal phytase level. At the combined optimal level

Table 4.4. Daily gain, feed intake, and gain/feed of pigs receiving different levels of supplemental microbial phytase, vitamin D, and calcium in the diets.

Phytase, PU ^a /g	750				1200			
	660		6600		660		6600	
Calcium, g/kg	4	8	4	8	4	8	4	8

Daily gain, g

d 10	252	166	250	161	237	121	188	167
d 20	374	303	431	287	477	237	397	358
d 30	497	307	440	314	573	303	488	408

Feed intake, g/d

d 10	486	428	582	408	466	322	386	439
d 20	828	729	905	736	922	637	790	865
d 30	1058	882	1120	890	1192	840	1093	1062

Gain/feed, g/kg

d 10	505	387	426	397	502	354	484	370
d 20	434	416	479	395	529	377	503	411
d 30	467	348	394	353	480	367	446	385

^a Phytase unit (1 nmol inorganic P released from sodium phytate per minute at 37 °C).

of phytase and normal level of vitamin D, pigs receiving the normal Ca level grew less than half the rate ($P < .05$) as those receiving the low Ca level. However, at otherwise combined levels of phytase and vitamin D, the differences in ADG between the two levels of Ca were not significant. Similarly, ADFI was greatly suppressed at the normal dietary Ca level, and the largest decline occurred at the combined levels of optimal phytase and normal vitamin D. But, the decrease was not significant until d 30. Poor feed efficiency resulted from feeding the pigs with normal dietary Ca just after d 10 ($P < .01$). The lowering effect of normal dietary Ca on gain/feed was significant at the optimal but not the suboptimal level of phytase. Likewise, the same effect was significant at the normal but not the high level of vitamin D. Unlike in the case of ADG or ADFI, this decreasing effect did not vary with the three way interaction. Compared to the suboptimal phytase level, optimal phytase level tended to improve ADG, ADFI, and gain/feed. Such improvement appeared to be more visible when levels of vitamin D and Ca in the diets were either both low or both high, though differences on the response measures were never significant. In addition, the heavy pigs ingested more feed ($P < .05$) and thus grew faster ($P < .05$) than the light pigs. But, there was no difference ($P > .05$) in gain/feed between these two groups.

Plasma Inorganic P and Ca Concentrations and Alkaline Phosphatase Activity. Effects of dietary phytase, vitamin D, and Ca on plasma P and Ca concentrations and AP activity are presented in Table 4.5. As shown previously (Lei et al., 1992b), phytase at 750 or 1200 PU/g of the basal diets resulted in a fairly normal plasma P status in pigs fed the low level of dietary Ca. However, at the normal level of dietary Ca, plasma P concentrations were decreased whereas plasma Ca concentrations and AP activities were elevated. Plasma P concentrations were lower ($P < .01$) at the normal level of Ca than at the low level Ca through the entire study. This difference tended to be attenuated at the optimal level of phytase and at the high level of vitamin D. However, these interactions, either two way or three way, were weak ($P > .35$). As expected, plasma Ca concentrations in pigs receiving the normal level of dietary Ca were higher ($P < .01$) than those of pigs receiving the low level of dietary Ca. Though these differences were overwhelming across all levels of phytase and vitamin D, their magnitude varied considerably because of a marginal three way interaction of phytase, vitamin D, and Ca ($P < .12$). When optimal phytase level was combined with normal vitamin D level, the differences in plasma Ca concentrations between pigs fed the

Table 4.5. Plasma concentrations of inorganic P and Ca, and plasma alkaline phosphatase activity of pigs receiving different levels of supplemental microbial phytase, vitamin D, and calcium in the diets.

Phytase, PU ^a /g		750				1200			
Vitamin D, IU/kg		660		6600		660		6600	
Calcium, g/kg		4	8	4	8	4	8	4	8
<u>Plasma inorganic P, mg/dL</u>									
d 0		5.2	4.9	5.0	5.1	4.8	5.2	4.8	4.6
d 10		5.8	4.0	5.8	4.5	5.7	4.4	5.6	4.0
d 20		6.4	4.4	6.5	4.8	6.9	4.3	6.7	4.5
d 30		5.8	3.4	5.7	4.3	6.0	3.7	6.0	4.1
<u>Plasma Ca, mg/dL</u>									
d 0		8.7	10.0	9.4	9.3	9.1	9.1	9.9	9.2
d 10		12.0	14.2	13.0	14.4	11.9	13.1	11.2	13.8
d 20		12.3	15.0	12.5	15.1	12.9	14.8	12.6	15.7
d 30		12.9	15.7	12.9	16.0	12.8	14.7	13.1	15.1
<u>Plasma alkaline phosphatase, U/dL</u>									
d 0		17.7	17.4	20.7	15.8	16.2	16.3	16.4	16.2
d 10		11.9	15.1	12.8	12.7	12.7	11.5	11.7	12.0
d 20		11.2	14.1	11.1	15.1	13.0	12.2	10.1	14.7
d 30		9.4	13.5	9.5	11.5	11.9	12.5	9.4	10.6

^a Phytase unit (1 nmol inorganic P released from sodium phytate per minute at 37 °C).

normal and low Ca levels were appreciably smaller than those shown at other occasions. Besides, dietary phytase alone was shown to reduce plasma Ca concentrations ($P < .05$) at d 10 and d 30. High level of vitamin D in the diets tended to enhance plasma Ca concentrations. Plasma AP activity was elevated in the pigs receiving normal level of Ca compared with that of pigs receiving the low level of Ca, but differences were significant only at the suboptimal level of phytase and at d 20 and d 30. Optimal phytase activity in the diets appeared to minimize these differences to a nonsignificant scale. Dietary levels of vitamin D had little effect on plasma AP activity. In addition, heavy pigs had higher ($P < .05$) plasma concentrations of P and Ca than the light pigs. But plasma AP activity was not different between these two groups of pigs.

Correlations among Various Response Measures. A strong positive correlation ($P < .001$) was found between ADG and ADFI at d 10 ($r = .92$), d 20 ($r = .89$), and d 30 ($r = .90$). Moderate positive correlations ($.5 < r < .8$, $P < .05$) were observed among ADG, ADFI, gain/feed, and plasma P concentrations. Plasma P and Ca concentrations were negatively correlated at d 10 ($r = -.59$, $P < .02$), d 20 ($r = -.71$, $P < .002$), and d 30 ($r = -.71$, $P < .002$). Likewise, plasma P concentrations and AP activity were also inversely correlated at d 20 ($r = -.52$, $P < .04$) and d 30 ($r = -.53$, $P < .03$). Plasma Ca concentrations and AP activity were

positively correlated at d 20 ($r = .60$, $P < .01$).

Discussion

Dietary P, phytate, Ca, and vitamin D are closely related in metabolism in the animal body (Wise, 1983; Pointillart et al., 1984, 1985, 1987). But recent studies on effect of microbial phytase on utilization of phytate-P by pigs during starting (Lei et al., 1992 a, b; Leunissen and Young, 1992) and growing-finishing phases (Nasi, 1990; Simons et al., 1990; Cromwell, 1991; Jongbloed et al., 1992) were almost exclusively conducted with a single level of Ca or vitamin D. In most cases, dietary Ca level was relatively low. In the present study, the efficacy of phytase on improving phytate-P availability was greatly reduced at the normal level of dietary Ca compared to that at the low level of dietary Ca. When all pigs were fed the corn-soybean meal diets without added inorganic P, supplementing phytase at either 750 or 1200 PU/g feed supported only pigs receiving low Ca to perform at a normal rate of gain (NRC, 1988) and to maintain normal plasma P status, as shown previously (Lei et al., 1992b). In contrast, the same amount of phytase activity in the diets with normal Ca concentration (.8%) failed to produce the same improvements, as indicated by appreciably lower ADG, ADFI, and gain/feed and unfavorable changes in plasma inorganic P and Ca concentrations and AP

activity. Calcium, the major divalent cation in the diets for many species, can progressively precipitate all the phytate by forming extremely insoluble Ca-phytate complex in the intestine (Wise, 1983; Nelson and Kirky, 1987). Consequently, phytate-P, as well as Ca itself, is largely unavailable to digestion (Wise, 1983). It has been conclusively demonstrated that high levels of Ca in the diets of rats (Taylor and Coleman, 1979; Nahapetian and Young, 1980) and of poultry (Edwards and Veltman, 1983; Ballam et al., 1985; Sheideler and Sell, 1987) decreased the availability of phytate-P considerably. Lowering dietary Ca from 1% to .5% enhanced phytate-P digestibility by 15% in chicks (Mohammed et al., 1991). However, the data on the effects of dietary Ca on phytate-P utilization by pigs were scarce (Pointillart et al., 1989). Jongbloed (1987) reported that absorption of P in diets without supplemental inorganic P was inversely related to dietary Ca up to .65%. Meanwhile, retention of dietary P was improved by the increase in dietary Ca. Pointillart et al. (1989) found that elevating dietary Ca from .9% to 1.4% in the diets containing .5% P (all from plant) intensified the P deficiency secondary to high phytate feeding. But they failed to observe a detrimental effect of high dietary Ca on phytate-P absorption or retention and suggested that the absence of decreased P absorption might result from the appropriate dietary vitamin D level (1,000 IU/kg).

The effect of dietary Ca on performance and plasma P status varied considerably with dietary vitamin D level, though vitamin D alone had no significant effects on any of the response measures. The high level of vitamin D in the diets appeared to reduce the adverse effects of normal dietary Ca on ADG, ADFI, gain/feed, and plasma inorganic P concentrations. This type of interaction between vitamin D and Ca was in agreement with that observed by Mohammed et al. (1991). They found that introduction of a high level of cholecalciferol (10 fold higher than normal) to the chick diets led to a marked increase in circulating levels of $1,25-(OH)_2D_3$. As a result, availability of Ca and phytate were significantly increased, and the hypophosphatemia associated with the low P intake was alleviated. Likewise, absorption or retention of P in a diet for pigs with .6% P (80% Phytate-P) and .6% Ca was virtually doubled when the diet was supplemented with cholecalciferol at 1000 IU/kg (Fontaine et al., 1985). However, the formation of unavailable Ca-phytate, suggesting by a simultaneous increase in fecal Ca and P excretion ($r > .92$, $P < .05$) with time, took place in vitamin D-depleted pigs (Pointillart et al., 1985). As vitamin D supplementation was shown to have no effect on phytase or AP activity in intestinal mucosa of pigs (Fontaine et al., 1985), it may oppose the depressive effect of Ca in phytate-P availability by activating Ca absorption and thus preventing, or at least reducing, Ca-

phytate formation (Pointillart et al., 1989). On the other hand, increasing dietary Ca from .5% to 1.0% did not influence $1,25-(\text{OH})_2\text{D}_3$ levels in plasma of chicks fed low-P diets (Mohammed et al., 1991).

Phytase level also influenced the action of dietary Ca. Optimal level of phytase in the diets appeared to magnify the differences in performance measures between the two levels of dietary Ca. But optimal level of phytase tended to minimize the same type of differences in plasma AP activity, and to a lesser extent in plasma inorganic P or Ca concentrations. Based on the relationship between dietary phytase activity and plasma measures (Lei et al., 1992a, b) and the correlations among these measures shown in this study, we would expect higher levels of phytase to free more P from phytate and then to reduce the resultant elevation in plasma AP activity. Superficially, it may look like to be contradictory that optimal phytase alleviated the adverse effect of dietary Ca on plasma P status, but aggravated the detrimental effect of dietary Ca on performance. However, if we assume that the effect of normal dietary Ca on phytate was certain, the differences in performance between the two levels of Ca then would mainly depend on the effect of phytase. Just as previously shown (Lei et al., 1992b), phytase at 1200 PU/g feed resulted in greater improvement in performance at the low dietary Ca than phytase at 750 PU/g feed. On the other hand, the differences in performance

measures between the two levels of phytase at normal dietary Ca were relatively small. Thus, the differences between the two levels of dietary Ca became significant at the optimal level but not at the suboptimal level of phytase. As phytase was provided in the diets, no severe P deficiency developed. Only plasma P concentrations were significantly related to performance measures with modest coefficient. Besides, the differences in most measures between the two levels of phytase were relatively smaller than those observed previously (Lei et al., 1992b). Some interactions between phytase and Ca or vitamin D may have somewhat confounded the effect of phytase.

Among the eight combinations of the three dietary treatments, optimal phytase combined with normal vitamin D and low Ca seemed to produce the best overall response. Simultaneous lowering of Ca and elevation of vitamin D in the diets did not cause additive benefits compared to singly reducing dietary Ca. This was different from what was observed by Mohammed et al. (1991). They found that simultaneous lowering of dietary Ca and elevation of vitamin D in the low-P diets for chicks additively improved phytate-P utilization. However, when microbial phytase was incorporated into the diets in this study, dietary Ca rather than vitamin D became more crucial.

Implications

Supplementing corn-soybean meal diets with microbial phytase greatly improves phytate phosphorus utilization and thus essentially obviates the need for inorganic phosphorus supplementation. However, this can only be achieved at moderately low levels of dietary calcium. Supplying calcium at a dietary level normally recommended or used results in an appreciable decrease in phytase efficacy. Introducing high levels of vitamin D in the diets may partially offset this adverse effect of Ca, but does not further improve phytate phosphorus utilization in low Ca diets with microbial phytase.

GENERAL DISCUSSION

GENERAL DISCUSSIONS

Progress in this Research

The results of the four consecutive studies on supplemental A. niger phytase in diets for weanling pigs lead to the followings conclusions:

1. Supplementing the enzyme up to 750 PU/g of a low-P, corn-soybean meal basal diet (BD) increased P retention by 50% and decreased fecal P excretion by 42%. Raising the enzyme activity to 1,200 or 1,350 PU/g of the BD resulted in relatively greater improvements in these two measures.

2. Plasma inorganic P concentrations, ADG, and ADFI increased linearly with dietary phytase activity from 0 to 750 PU/g of the BD.

3. Responses of ADG, ADFI, and plasma alkaline phosphatase activity maximized at approximately 1,200 PU/g of the BD. However, plasma inorganic P and Ca concentrations kept linearly increasing and decreasing, respectively, up to 1,350 PU/g of the BD.

4. One thousand units of phytase activity supported the retention of 1.1 mg P from the BD and were equivalent in effect to .91 mg P from mono-dibasic calcium phosphate. Pigs

receiving the BD with phytase at 1,200 PU/g retained virtually the same amount of P as those receiving the inorganic P supplemented control diets.

5. Plasma concentrations of inorganic P and Ca and daily urinary excretions of P and Ca were moderately correlated. High concentrations of plasma inorganic P in pigs supplemented with inorganic P in their diets led to increased daily urinary excretions of P.

6. Supplementing this enzyme at 1,350 PU/g of a corn-soybean meal diet without added inorganic forms of P and Zn appeared to release sufficient Zn and phytate-P from the diet to maintain normal status of plasma inorganic P and Zn and normal rate and efficiency of gain.

7. Utilization of dietary Ca was also improved by supplemental phytase. However, different levels of phytase did not influence plasma concentrations of Mg, Cu, Fe, and Zn when these elements were provided at the recommended levels in the diets.

8. All improvements in the various measures by adding phytase to the diets as described above were achieved at reduced dietary Ca levels. Normal Ca in the diets greatly suppressed the efficacy of phytase. Raising vitamin D level in the diets partially offset this adverse effect. It appears that the optimal Ca needed for maximum efficacy of supplemental dietary phytase is different from the metabolic Ca requirement.

Part of the above conclusions was also supported by the results of other researchers (Nasi, 1990; Simons et al., 1990; Cromwell, 1991; Jongbloed et al., 1992; Leunissen and Young, 1992). However, their approaches were quite different from that used in this research. Researchers in Europe (Nasi, 1990; Simons et al., 1990; Jongbloed et al., 1992) studied the effects of A. niger phytase on phytate-P and total P digestion and(or) retention. But, they did not have an inorganic P supplemented diet as a positive control. In addition, only a limited number of pigs were tested, and effect of phytase on performance, plasma inorganic P status or bone traits was not determined or reported. Researchers at the University of Kentucky (Cromwell, 1991) compared the effects of two levels of phytase (500 and 1,000 PU/g) on performance and bone strength. Inorganic P supplemented diets were used as a positive control. However, plasma inorganic P status and P balance were not measured. Leunissen and Young (1992) of the University of Guelph compared the effect of 500 and 1,000 PU/g feed with that of regular inorganic P supplementation on all the four major response measures taken by the above two groups. However, their basal diet was not simply composed of corn and soybean meal and small portion of inorganic P was supplemented. Again, only a limited number of pigs were tested at a fairly short period (3 wk). In comparison, four major experiments were conducted consecutively with a total of 258 pigs in

this research. A series of 7 graded levels of phytase activity was compared. Both negative control (the BD) and positive control (inorganic P supplements) were used. Measurements included performance, plasma mineral concentrations and alkaline phosphatase activity, and mineral balance. Systematic data were generated from fairly large samples and thereby were very consistent. Furthermore, the simultaneous improvement in Zn and phytate-P utilization by phytase were demonstrated. Interactions of dietary phytase, vitamin D, and Ca were also determined.

Applications of this Research

The followings implications may be inferred from the above conclusions:

1. Supplementing A. niger phytase at 1,200 PU/g of corn-soybean meal diets essentially obviates the need for inorganic P supplementation. Thus, diet cost would be decreased and large amounts of inorganic P resources would be saved.
2. Replacing inorganic P by phytase in the diets would eliminate, at least, alleviate the P pollution originating from the excess P in the swine manure in areas of intensive animal production.
3. Supplementing phytase may reduce the Zn requirement of pigs. This enzyme may also be used to improve

bioavailability of Zn in infant soy formula and to treat other foods of plant origin consumed by humans.

4. Dietary Ca level should be reduced to permit full efficacy of supplemental phytase.

In addition, Cromwell (1991) suggested that supplementing phytase at 1,000 PU/g of corn-soybean meal diets could replace all the inorganic P supplementation in finishing pigs. Jongbloed and Kemme (1990) demonstrated that proper pelleting feeds with phytase did not reduce the enzyme activity. If all these results were applied in swine production, major impact in nutrition and environment could be expected.

Limitations of this Research

The major concern associated with the interpretation and application of this research results was a possible carry-over effect. Low-P feeding of weanling pigs may be detrimental to their later development and(or) performance, particularly to breeding herds. The lack of testing phytase on bone traits amplify this concern. In all, no economical source of phytase is available. Supplementing phytase at the optimal level shown in this research would be more expensive than the routine addition of different sources of inorganic P. Therefore, future use of phytase in swine production mainly depends on the success of continuous seeking high

producers of phytase and(or) improving the enzyme production by biotechnology. Optimizing dietary conditions may maximize phytase efficacy and then reduce the required level of the enzyme and facilitate the economy of phytase usage.

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