SUPPLEMENTING MICROBIAL PHYTASE TO DIETS FOR SWINE AND POULTRY

by

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ABSTRACT

This study was conducted to determine the efficacy of Natuphos® phytase for improving the bioavailabilities of phytate P and other nutrients (CP, AA, Ca, and Zn) bound to phytate in the diets for pigs, broilers, and turkey poults, and to evaluate P and Zn equivalency values of phytase. In a 5-wk study with young pigs (BW = 7.5 kg; n = 96) fed a soybean meal-based semi-purified diet (SP), performance, P absorption, and bone characteristics were improved, as graded levels of phytase (0, 350, 700, 1,050, and 1,400 U/kg of diet) were added to the low P diets (.05 and .16% available P, aP). In comparison with the .32% aP diet, fecal P excretion decreased 25 to 50% by adding phytase. The replacement of 1 g P as defluorinated phosphate (DFP) would require about 676 U of Natuphos® phytase. Effect of phytase on Ca and N absorption were variable. Three experiments were conducted with 1,856 broilers (d 1 to 21) fed corn-soybean meal-based diets (CS, Exp. 1 and 2) or SP, (Exp. 2 and 3). A 3 x 7 factorial arrangement of treatments was used in Exp. 1 with .20, .27, and .34% nonphytate P (nP) and 0, 200, 400, 600, 800, 1,000, and 1,200 U phytase/kg of diet. Four graded levels of phytase (0, 350, 700, and 1,050 U/kg of diet) and nP (.27, .36, .45, and .54%) were used in Exp. 2 and 3

with .27% nP as a basal diet. Adding phytase consistently increased BW gain, feed intake, toe ash percentage, and apparent retention of P and Ca. The magnitude of the responses was greater with the lower nP than with the higher nP (Exp. 1). In comparison with the .45% nP diet, P excretion of broilers decreased 25 to 60% by addition of phytase. The average of P equivalency values of phytase from these three experiments indicates that the release of 1 g P as DFP requires 920 and 830 U of Natuphos® phytase for broilers fed SP and CS, respectively. Apparent N retention was increased with addition of phytase in broilers fed CS (only measured in Exp. 3). In a study with turkey poults (n = 480, d 1 to 29) fed CS, a 2 x 2 x 2 factorial arrangement of treatments was used with .45 and .60% nP, 22.5 and 28.0% CP, and 0 and 750 U of phytase/kg of diet. Microbial phytase enhanced apparent and true ileal digestibility of N and AA, apparent retention of N and P, performance and toe ash content. The major improvement of phytase on N and AA digestibility ranged from 1 to 4 percentage units was obtained at .45% nP with 28.0% CP or .60% nP with 22.5% CP. The effect of phytase on the utilization of Zn was determined in broilers (d 1 to 21, n = 384) fed a corn-soy isolate diet (20 ppm Zn). Supplemental phytase (150, 300, 450, and 600 U/kg of diet) improved Zn utilization based on measurements of Zn content in toe, tibia, and liver, apparent Zn rentention and performance. The results indicate that about 1 mg of Zn was released per 100 U of Natuphos® phytase over the range of phytase added. It was observed in pigs that the stomach was the site of highest added phytase activity. In summary, microbial phytase is effective in improving the utilization of P, Ca, Zn, N, and AA in the diets for pigs, broilers, and turkey poults.

Key Words: Pig, Broiler, Turkey, Phytase, Phosphorus, Zinc, Amino acids

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Chapter I

Introduction

Environmental protection is one of the most important issues in modern society. Modern commercial swine and poultry production supplies high quality and economical protein food to us. However, if not properly managed, the large amount of manure produced could lead to environmental pollution (Cromwell and Coffey, 1991). Of the components present in the manure, P, N, and trace minerals (probably Cu and Zn) are of greatest concern.

The possible solutions to the problem of environmental pollution from the manure could involve one or more of the following: limit swine and poultry production, recycle the manure, and decrease the excretion of P, N, and trace minerals. Swine and poultry production is being restricted in some countries and will be restricted in other countries if solutions to the problem of manure disposal are not developed and implemented.

One of the possible approaches to decreasing excretion of P from animals is to develop ways to improve the utilization of phytate P in plant ingredients. It could be done by transgenic plants, feed processing, and feeding phytase from plants and microorganisms. Currently, feeding microbial phytase appears the most practical and reasonable approach, certainly in the near future, to improve phytate P utilization.

Swine and poultry are able to utilize only 10 to 35% of the P present in plant ingredients (NRC, 1988; 1994). In several types of plant seeds used as feed ingredients, 60 to 80% of the P is present in the form of phytic acid, but swine and poultry lack the enzyme phytase to act on phytate. As a result, inorganic P is used to meet the P requirements for these animals. Consequently, both phytate P and inorganic P are excreted to the environment from manure.

Phytic acid has the potential to form a wide variety of insoluble salts with cations. Calcium, Mg, Zn, Cu, Co, Mn, and Fe are among the minerals that can be involved (Morris 1986). Zinc and Cu have the strongest binding affinity (Vohra et al., 1965). This binding potentially renders the minerals unavailable for intestinal absorption. Phytate can also bind with protein and the phytate-protein complexes may reduce the utilization of the protein and amino acids (AA) (Anderson, 1985).

Phytase is the enzyme known to release the orthophosphate group from the phytate molecule (Gibson and Ullah, 1990). The enzyme occurs widely in microorganisms, plants, and certain animal tissues. However, the activity of phytase in the body and in the diets of swine and poultry is very limited. Recently, a microbial phytase from a genetically engineered *Aspergillus* was used to improve the availability of the phytate P in corn-soybean meal diets (Simons et al., 1990). Limited information also suggests that microbial phytase may improve the utilization of Ca, Mg, Zn, Fe, Cu, and CP and AA. Little is known about the equivalency values of microbial phytase for inorganic P and Zn in swine and poultry diets. Little is also known about the active sites of microbial phytase in gastrointestinal tract (GIT) of animals.

The objectives of this study were 1) to determine the effectiveness of Natuphos® phytase for improving P availability in diets for swine and poultry, and in reducing P excretion with a range of dietary P and phytase levels; 2) to generate response equations for use in nutrition-production models and to determine the P equivalency values of microbial phytase in the diets for swine and poultry; 3) to measure the efficacy of microbial phytase on N and amino acid digestibility in turkey poults; 4) to evaluate the effects of phytase on Zn utilization in broilers and to calculate Zn equivalency values of phytase; 5) to determine the active sites of supplemental microbial phytase in GIT of young pigs.

Chapter II

Literature Review

Introduction

Many things in the world can be divided into two aspects. One is the good and the other is the bad. Under certain conditions, the bad can be changed into the good and vise versa. Phosphorus possesses the good as a necessary element in life processes. On the other hand, P also possesses the bad as a pollutant from animal manure in the environment. Science and technology can make it possible to take advantage of the good aspect while reducing the bad aspect. An example is to supplement the diets for swine and poultry with microbial phytase.

Phosphorus

Metabolic Functions of Phosphorus.

Phosphorus is a well known essential element. It plays versatile functions as phosphate (PO_4^{-3}) or as phosphate compounds which are summarized in Figure 2-1. About 80% of P is found as hydroxyapatite in the hard tissues (bones and teeth) of the animal body. Phosphate esters and anhydrides dominate the living world (Westheimer, 1987). The genetic materials, DNA and RNA, are phosphodiesters. The principal reservoirs of biochemical energy, ATP and creatine phosphate, are phosphates. Most of the coenzymes (CoA, FAD, FMN, NAD, NADP) are esters of phosphoric or pyrophosphoric acid. Many intermediary metabolites such as glucose-6-phosphate (G-6-P), phosphoenolpyruvate (PEP), acyl carrier protein (ACP) are phosphate esters. Phospholipid (PL) maintaining membrane integrity is also a phosphate compound (Stryer, 1987). Phosphate compounds cAMP and inositol triphosphate (IP_3) play a role of a second messenger in cell metabolism (Guyton, 1991; Swenson and Reece, 1993).

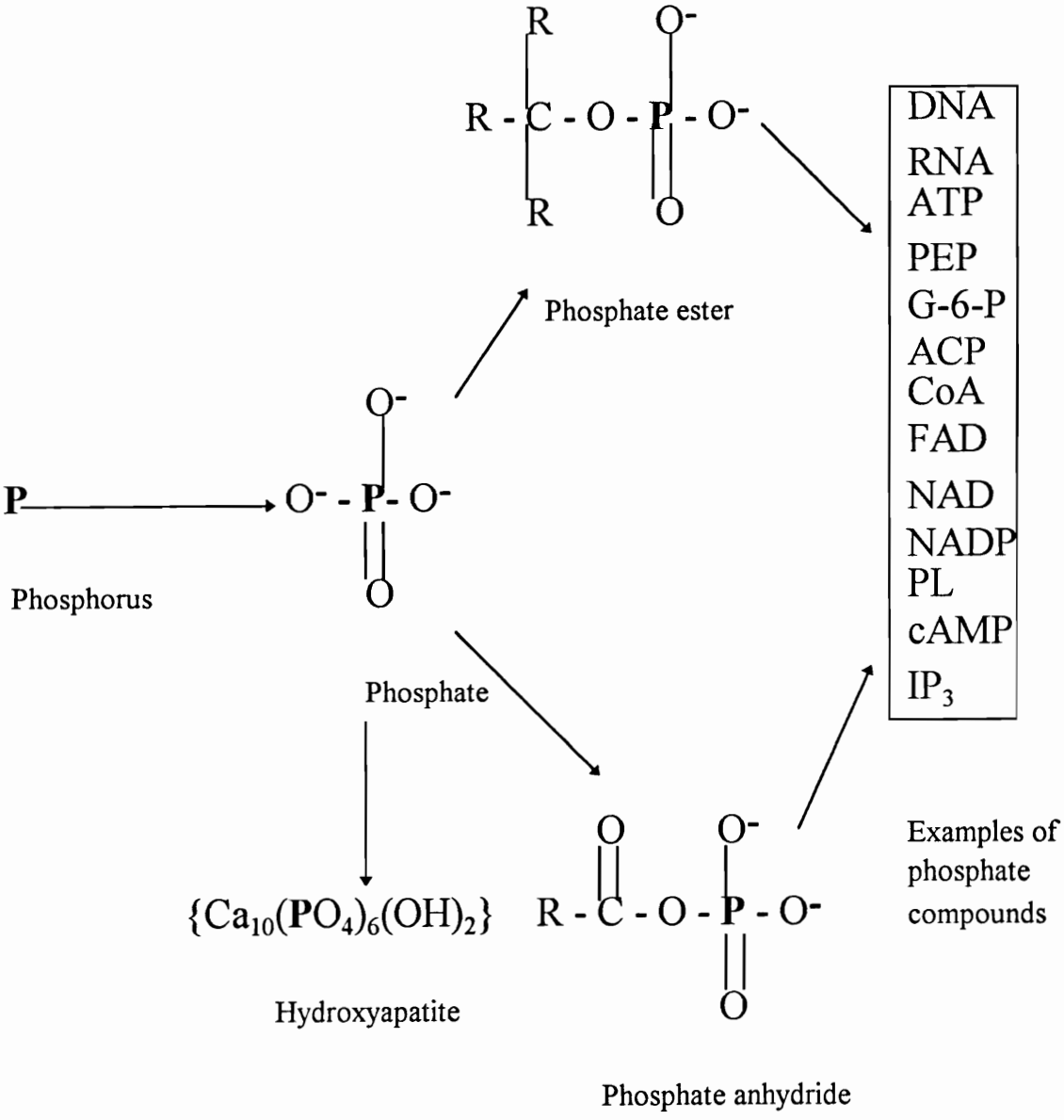

Figure 2-1. Metabolic functions of phosphate compounds

Phosphate can also maintain osmotic pressure and acid-base balance with other compounds in animal body. Phosphate is also involved in the control of appetite and in the efficiency of feed utilization (Underwood, 1981).

Response Criteria for Assessing Phosphorus Nutrition.

A number of response criteria have been used for assessing P nutrition in young swine and poultry. The criteria mainly include performance, characteristics of bones (Combs et al., 1991a, b; Cromwell et al., 1993; Denbow et al., 1995) or blood (Lei et al., 1993a; Perney et al., 1994), and apparent P digestibility or retention (Dellaert et al., 1990; Simons et al., 1990; Veum et al., 1994).

Performance

Most of the feeding trials in swine and poultry nutrition measured performance: gain, feed intake, and gain:feed to evaluate P requirement or availability. Peo (1991) reviewed a number of studies in swine conducted from 1962 to 1986 and concluded that performance of young pigs has good sensitivity. Performance of young poultry has been extensively used to determine the nutrient requirements (NRC, 1994).

Bone Characteristics

It is widely accepted that the dietary level of P necessary for maximal bone mineralization is higher than that necessary for maximal growth rate (NRC, 1988). Bone characteristics are generally more responsive over a wide range of dietary P levels than growth rate and gain:feed (Kornegay, 1985; Peo, 1991). However, comparison of data in the literature is often difficult because of a lack of standardization of the bone and the bone criteria. Researchers have used several bones, such as femur, humerus, rib, mandible, metacarpal, metartarsal, tooth, turbinate of pigs (Crenshaw et al., 1981a,b; Koch et al., 1984; Cromwell et al., 1993), tibia and toe of poultry (Edwards, 1993;

Denbow et al., 1995; Ravindran et al., 1995) to determine various characteristics, such as dimensional, compositional, and mechanical traits.

Generally, the dimensional characteristics (weight, length, and outside diameter) of bones of pigs are not very responsive to dietary P change, whereas the thickness of the wall of the bones is responsive, with the effect being less for P level above NRC recommendations. In compositional characteristics, bone ash has been used widely in assessing bone mineralization because of its ease of determination. A positive relationship between bone ash (dried fat-free) and dietary P levels has been reported for baby pigs (Rutledge et al., 1961; Miller et al., 1962, 1964), for weanling pigs (Combs et al., 1962; Hoppe et al., 1990), for growing-finishing pigs (Cromwell et al., 1970; Pond et al., 1975; Mahan et al., 1980), and for broilers (Simons et al., 1990; Schoner et al., 1993). The P and Ca content of bone expressed as a percentage of bone ash is relatively constant over a wide range of dietary P levels. Phosphorus and Ca content of bone ash would not be sensitive to changes in P intake. But, P and Ca content of dry bones or dry fat-free bones are as sensitive as bone ash, but would be much more labor intensive than bone ash.

The mechanical characteristics determined (Miller et al., 1962, 1964; Baker and Haugh, 1979; Arthur et al., 1983) include maximal load or breaking strength, maximal bending moment (force and length), maximal stress (force per unit of bone area), stiffness (slope of the straight-line portion of the force-deformation curve), modulus of elasticity (Young's modulus of elasticity), and flexural modulus (resistance of bone to bending). In general, breaking load and bending moment have been used often because of their simplicity of determination and high sensitivity over a wide range of P levels (Kornegay, 1985; Peo, 1991; Wilson, 1991). Stress, modulus of elasticity, and flexural modulus are more difficult to calculate because they require the determination of moment of inertia, which varies according to the shape of the bone.

Blood Characteristics

Blood characteristics used include serum or plasma P and Ca concentrations and alkaline phosphatase (AP) activity. The P concentration of whole blood in pigs ranges from 350 to 450 mg/L (Peo, 1991). Plasma inorganic P concentration in pigs is about 70 to 100 mg/L (Miller et al., 1964; Miller and Ullrey, 1987; Friendship and Henry, 1992), while in broilers it is 30 to 70 mg/L (Perney et al., 1993). Serum P concentration is known to be linearly increased and serum Ca and AP activity are linearly decreased with increasing dietary P levels (Kornegay and Thomas, 1981; Lei et al., 1993a,b; Perney et al., 1993). Serum P and Ca were evaluated by Peo (1991) as a low sensitive indicator. But the results of recent studies in pigs (Lei et al., 1993a,b) and in broilers (Perney et al., 1993) showed serum P and Ca to be highly responsive to dietary P levels. Boyd et al. (1983) and Peo (1991) concluded that AP activity was a sensitive criteria. But in the evaluation of Dellaert et al. (1990), the sensitivity of AP activity was very low.

Apparent Phosphorus Digestibility

The determination of apparent P digestibility in pigs was often conducted in balance trials (Atteh and Leeson, 1983; Kornegay and Kite, 1983; Grandhi, 1984). But it was concluded by Peo (1991) as "almost meaningless for evaluating the biological value of a P supplement." However, in the comparison of different techniques to assess the bioavailability of feed phosphates in pig feeding, Dellaert et al. (1990) reported that the apparent P digestibility was the most accurate criteria to determine the bioavailability of P, even better than bone characteristics. The method has been widely used in recent studies (Simons et al., 1990; Jongbloed et al., 1992; Mroz et al., 1994; Veum et al., 1994). The apparent P retention of poultry has also been widely measured (Simons et al., 1990; Edwards et al., 1993; Schoner et al., 1993). Other criteria that have been used include

hair P content of pigs, and P solubility in diet or feces (Kornegay et al., 1981; Coffey et al., 1994).

Factors Affecting Phosphorus Absorption.

The site of P absorption is located in the proximal end of the duodenum where P is absorbed in the orthophosphate (PO_4^{-3}) form (Bartter, 1964; Iving, 1964). However, whether P is absorbed by a passive or active system, or both, has not been elucidated (Peo, 1991; McDowell, 1992). The amount of P absorbed depends upon several factors, such as P sources and levels, P requirement and P nutritional status of animals, Ca level, Ca to P ratio, vitamin D, and other minerals, pH in GIT of animals and dietary fiber. Generally, inorganic P sources have higher absorption than organic P sources. Most of P in plant ingredients exists in the organic form as phytate, which can not be directly absorbed by swine and poultry. Higher dietary P decreases P absorption. Animals with P deficiency have high P absorption. High levels of Ca or a wider Ca to P ratio decreases P absorption (Jongbloed, 1987). But normal dietary levels of Ca enhance P absorption (Fox et al., 1978). Vitamin D and K increase P absorption, but high levels of Mg, Al, and Fe decrease P absorption (McDowell, 1992). The absorption of P is facilitated by a low pH (Swenson and Reece, 1993). Dietary fiber, which promotes the passage of digesta in GIT, may decrease P absorption.

Pollution of Phosphorus from Animal Manure.

Adequate amounts of available P (aP) are required for optimum growth, reproduction, and bone development in swine and poultry. Since much of the P in the diets for swine and poultry is nutritionally unavailable, inorganic phosphates are used to meet the P requirement. Consequently, large amounts of P are excreted in the manure. This leads to P pollution in the environment (Cromwell and Coffey, 1991). Isermann (1990) estimated for agriculture in Germany that 73% of the P input was derived from

mineral fertilizers, 26% from feedstuffs including minerals feeds, and 1% from sewage sludge. Only 35% of the P input leaves the agricultural sector in the form of marketed animals and plant products. The remainder of the P (65% of the input) mainly accumulates in the soil, and reaches surface waters due to soil erosion, surface run-off and drainage. It has been estimated that in the U.S., swine and poultry annually produce approximately 700,000 tons of P (Sweeten, 1992). This creates potential for substantial environmental impact of P contamination of surface run-off and ground water. Consideration must be given to dealing with this serious situation.

Phytate

Separate Lines of Phytate Research Interest.

The evolution of research on phytate has followed two separate lines of interest, one involving inositol phosphates as second messengers controlling Ca flux within cells (Irvine, 1988), the other involving the nutritional aspects of phytic acid (Reddy et al., 1982; Gibson and Ullah, 1990).

Inositol Phosphate as Second Messengers

It has been reported (Swenson and Reece, 1993) that in the Ca and phosphatidylinositol system in the cells for muscle contraction, the activated phosphodiesterase hydrolyzes a membrane phospholipid into two second messengers, inositol triphosphate (IP_3) and diacylglycerol. The IP_3 acts to release Ca into the cytosol. The increased Ca binds to calmodulin, which then activates myosin light chain kinase. The phosphorylated myosin light chain forms a cross-bridge that binds actin to form actomysin.

Nutritional Concerns of Phytate

In the nutritional aspect of phytate, human nutritionists are more concerned with the effects of phytate on trace mineral utilization. Human nutritionists today advocate that people take more vegetable or fiber food in order to reduce cancer (especially the cancers

in GIT) incidence. However, when people eat more vegetable food, they consume more phytates, too. The phytates in food decrease the utilization of minerals or trace minerals because of the chelating activity of phytate. Animal nutritionists are more concerned with the utilization of dietary P and the nutrients bound to phytate, and P pollution from animal manure.

Chemical Structure.

Phytic acid was discovered by Turting in 1855 (Reddy et al., 1982). However, the structure of phytic acid was a subject of controversy. It has now been accepted that phytic acid is a myo-inositol 1, 2, 3, 4, 5, 6 hexa, dihydrogen phosphate (IUPAC-IUB, 1968) with an empirical formula of $C_6H_{18}O_{24}P_6$ (Reddy, et al., 1982; Gibson and Ullah, 1990). At neutral pH, phosphate groups have either one or two negatively charged oxygen atoms (Erdman, 1979). It is apparent that various cations could strongly chelate between two phosphate groups or weakly with one phosphate group (Figure 2-2).

Phytate Binds Other Minerals

Phytic acid forms a variety of insoluble salts with di- and trivalent cations at neutral pH (Vohra et al., 1965; Oberleas, 1973). Calcium, Mg, Zn, Cu, Fe, Co, Mn, and Cr are among the minerals that can be involved (Maga, 1982; Reddy, et al., 1982; Morris, 1986). Zinc and Cu have the strongest binding affinity (Maddaiah et al., 1964; Vohra et al., 1965). The binding potentially renders the minerals unavailable for intestinal absorption.

Phytate Binds Proteins

Phytate has the potential of binding with protein at low and neutral pH (Cosgrove, 1980; Anderson, 1985; Thompson, 1986). The phytate-protein complexes may reduce the utilization of proteins and AA. In general, at low pH, the proteins are positively charged and can form insoluble complexes with the negatively charged phytate because of strong

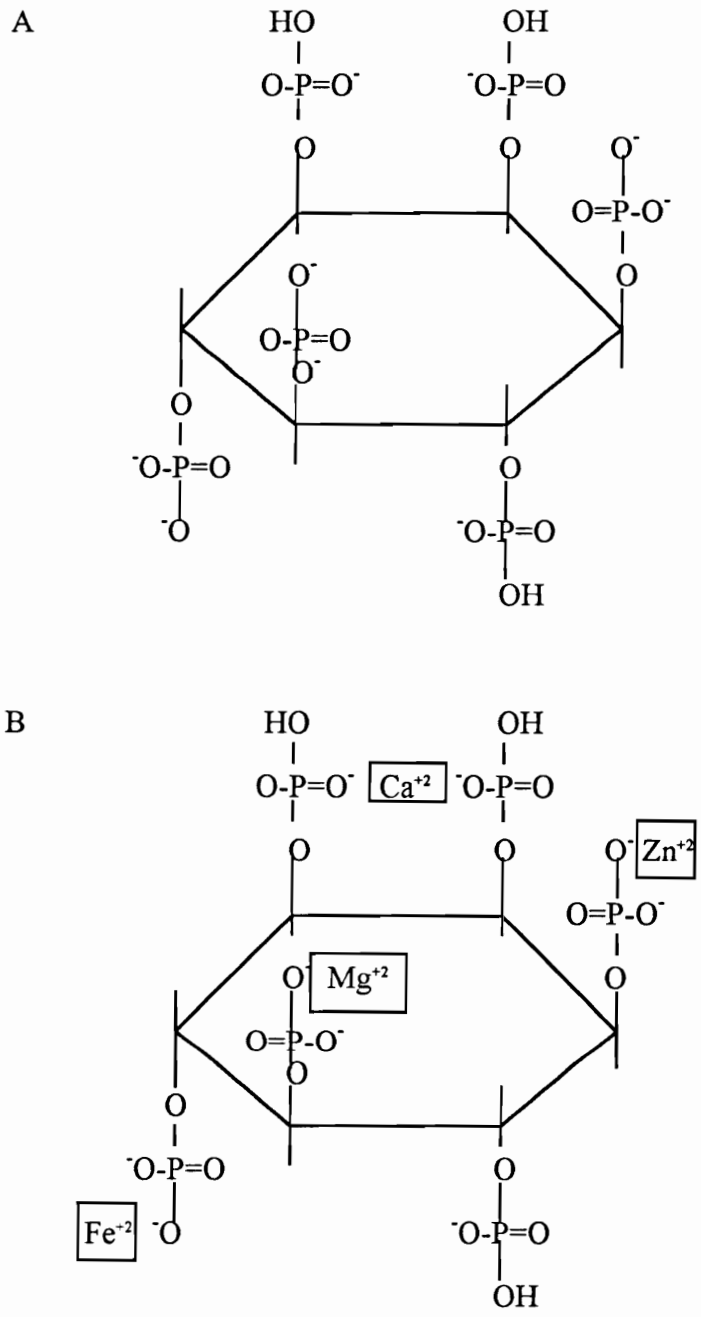

Figure 2-2. Chemical structure of phytic acid (A) and phytic acid chelate (B) at neutral pH (from Erdman, 1979)

electrostatic interactions (Cheryan, 1980; Reddy et al., 1982). The positive charges of the proteins could be the terminal alpha amino group, epsilon amino group of lysine, guanido group of arginine, and histidine residues (Barre and van Huot, 1965a, b). When the pH is raised, the proteins bind with phytate mediated by multivalent cations such as Ca, Mg, and Zn (Figure 2-3). The common binding site for the ternary complex appears to be the ionized carboxyl groups and the unprotonated imidazole group of histidine. The complexing of phytate with proteins may change protein structure, which in turn may lead to decreased solubility, digestibility, and functionality. Phytate may also form complexes with proteases, such as trypsin and pepsin (Camus et al., 1976; Singh and Krikorian, 1982) in the GIT. The complexes may decrease the activity of digestive enzymes, which decreases the digestibility of dietary proteins.

Contents in Plant Ingredients.

Phytate P constitutes the major portion of total P (tP) in various types of plant ingredients (Nelson et al., 1968; Kirby and Nelson, 1988; Eeckhout and De Paepe, 1994). In general, the proportion of phytate P varies from 60 to 75% of the tP (Table 2-1). Phytate in cereals is not distributed uniformly in the various morphological parts of the kernel, but is associated with specific components in the seeds (Oberleas, 1973). Almost 90% of phytate in corn is located in the germ portion of the kernel (O'Dell, 1969; O'Dell et al., 1972). Phytate in wheat and rice is concentrated in the aleurone layer of the kernel and in the bran. More than 80% of phytate in rice is present in the outer bran (O' Dell et al., 1972; De Boland et al., 1975). The endosperm of wheat and rice is almost devoid of phytate. Phytate in oilseeds which contain little or no endosperm is distributed throughout the kernel located within subcellular inclusions known as protein bodies or globoids (Erdman, 1979). However, phytates in soybean are unique in that, although associated with globoids, they appear to have no specific site of localization.

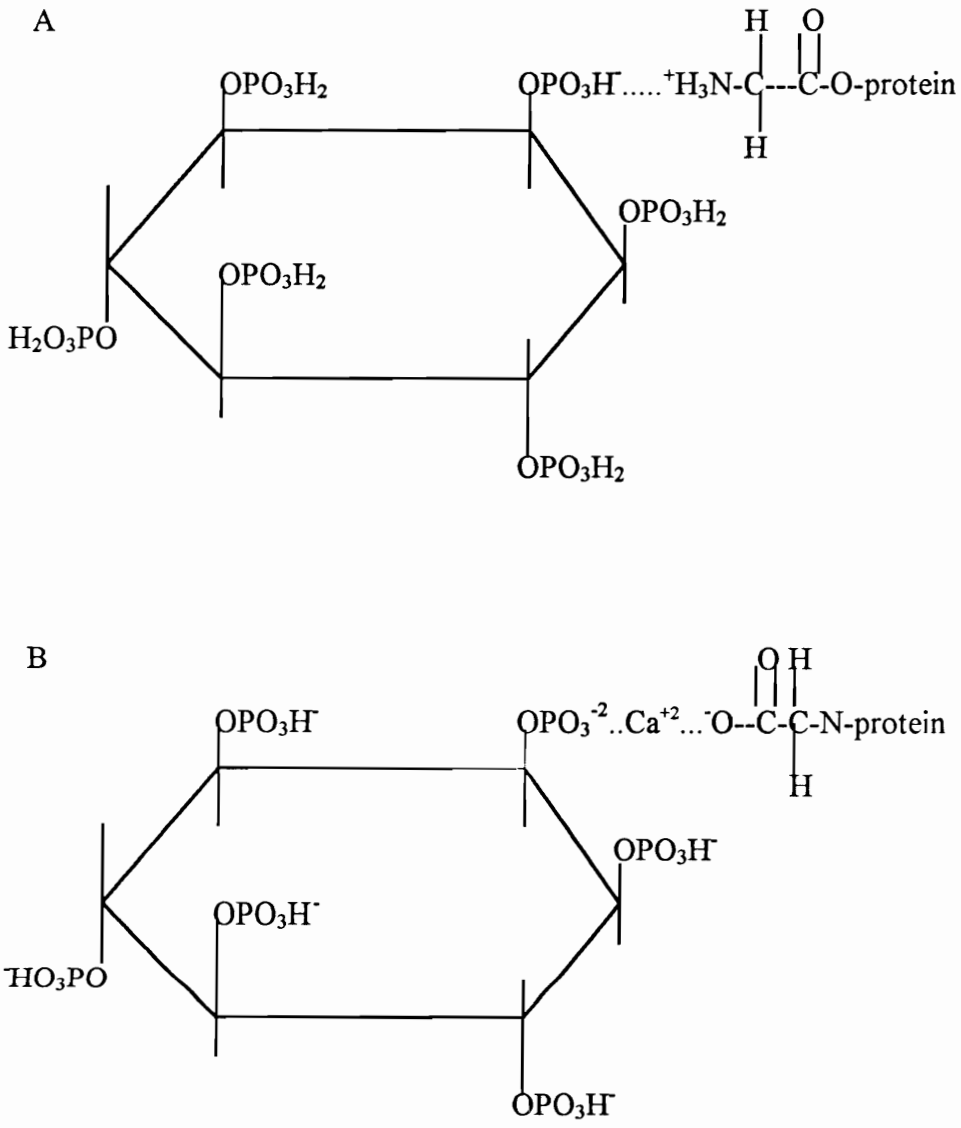

Figure 2-3. Phytic acid-protein complexes at low pH (A) and neutral pH (B) (from Anderson, 1985)

Table 2-1. Phytate phosphorus contents and phytase activity in some plant ingredients

Ingredients	Phytate phosphorus %[a]		Phytate phosphorus as % of tP[a]		Phytase activity units/kg[b]
Cereals and By-products					
Barley	.21	(.19-.24)[c]	58	(55-62)[c]	400
Corn	.20	(.19-.29)	68	(61-76)	15
Oats	.28	(.16-.35)	69	(48-78)	40
Rice	.27	(.25-.28)	77	(74-81)	20
Sorghum	.22	(.19-.29)	68	(61-76)	25
Triticale	.30	(.14-.53)	66	(30-90)	1500
Wheat	.24	(.19-.29)	68	(61-78)	700
Wheat bran	.88	(.60-1.27)	76	(68-93)	1200
Oilseed Meals					
Canola meal	.65	(.46-.78)	58	(36-70)	15
Peanut meal	.35	(.32-.43)	58	(47-69)	3
Soybean meal	.37	(.28-.40)	57	(46-61)	40
Sunflower meal	.44	(.32-.51)	77	(73-80)	60

[a]Data from Nelson et al. (1968), Reddy et al. (1982), Kirby and Nelson (1988), Pointillart et al., 1993; Eeckhout and De Paepe (1994), and Ravindran et al. (1994).
[b]Data from Pointillart et al. (1987, 1991; 1993), and Eeckhout and De Paepe (1994).
[c]Data in parentheses refer to ranges reported in the literature.

Phytate contents of feed are widely variable, depending on the stage of maturity, cultivar, climatic factors, soil factors, moisture, location, the year of growing, and degree of processing. (Reddy, et al., 1982).

Phytate and Phosphorus Availability.

Phosphorus in phytate is efficiently absorbed by ruminants because rumen microorganisms produce sufficient phytase to hydrolyze the phytate P. In swine and poultry, however, there is a negative correlation between phytate contents and P availability in plant ingredients (Table 2-1 and 2-2). Similar estimates of P availability in

Table 2-2. Bioavailability of phosphorus in plant ingredients for swine and poultry

Ingredients	Bioavailability of P for swine, %[a,b]	Bioavailability of P for poultry, %[c]
Cereals		
Barley	31	36
Corn	12	28
Oats	23	33
Triticale	46	33
Wheat	50	31
Oilseed Meals		
Canola meal	21	26
Peanut meal	12	21
Soybean meal, dehulled	25	35
Soybean meal, 44% protein	35	40
Sunflower meal	3	15

[a]Adopted from Cromwell (1992).
[b]Relative to the availability of P in monosodium phosphate (given 100%).
[c]Adopted from NRC (1994).

cereals fed to pigs were obtained by bone breaking strength (Cromwell 1992) or digestibility trials (Jongbloed, 1987; Jongbloed and Kemme, 1990; Jongbloed et al., 1991). The availability of P in cereal grains is quite variable, ranged from 12 to 50%. Phosphorus availability in corn is very low because of its high percentage of phytate. The availability of P in triticale and wheat fed pigs is considerably higher and in barley it is intermediate in availability. This higher availability is attributed to the presence of naturally-occurring phytase in the seed coat (Table 2-1). Phosphorus availability in cereal by-products is variable. Wheat bran has high P availability because of the natural phytase. Phosphorus availability in soybean meal is lower, which is similar to that in canola meal. Phosphorus availability in sunflower meal and peanut meal is very low because of the high content or

percentage of phytate. Therefore, the low P availability of plant feedstuffs for swine and poultry is because of a lack of dietary and endogenous phytase to release the P in phytate.

Methods Used to Improve the Utilization of Phytate.

Feed Processing

Several processing methods have been used to improve the utilization of phytate P in plant ingredients, including autoclaving, autolysis, cooking, fermentation, germination, milling, and soaking (Reddy, et al., 1982). De Boland et al. (1975) found that autoclaving inositol hexaphosphate and isolated soy protein for two hours resulted in reduction of phytate and release of P. Kumar et al. (1978) reported that cooking decreased both water- and acid-soluble phytate P in green gram, cowpea and chickpea. Germination eliminates considerable amounts of phytate from the plant seeds or grains (Reddy et al., 1982). Milling followed by germ separation can remove 89% of the phytate in corn, in which phytate concentrates in germ (O'Dell et al., 1972). However, many of the methods are initiated and mainly used in human nutrition and food processing. Currently, most of them are not economical in the feed industry for animals. Some methods, such as autoclaving (Takemasa and Hijikuro, 1991) and soaking (Nasi and Helander, 1994), are being tested in animal experiments.

Feeding Phytase

A feeding approach to improve the utilization of phytate is to replace corn in part by wheat, triticale or rye bran. Newton et al. (1983) found that dietary P absorption was increased by using 10 or 20% wheat bran substitutes for corn in the diet for growing pigs. Schwartz et al. (1986) found improved absorption and retention of other minerals in humans consuming wheat bran. In the studies of Pointillart et al. (1984, 1987, 1991) with growing pigs, the utilization of phytate P in the diets containing wheat, triticale or rye bran was significantly greater than that in the corn-soybean meal diets. Nelson (1976) reported

that substituting 50% of corn with wheat in the diets for chicks and laying hens improved phytate hydrolysis. Nahashon et al. (1994) reported that a phytase containing material and its carrier, condensed cane molasses solubles, improved P retention in laying hens.

An important feeding method to improve the utilization of phytate is to supplement microbial phytase in the diets for swine and poultry. Nelson et al. (1968) were the first to show that microbial phytase has the potential to hydrolyze the phytate P in plant-derived ingredients. This was confirmed in subsequent studies (Rojas and Scott, 1969; Nelson et al., 1971). But the low supply and high cost of producing phytase limited its commercial exploitation in the past. Recently, recombinant DNA technology was used for the production of microbial phytase from genetically engineered *Aspergilus* strains (Simons et al., 1990). This has made microbial phytase available as a feed additive.

Phytase

Phytase, myo-inositol hexaphosphate phosphohydrolase, is an acid phosphatase that catalyzes the stepwise removal of phosphate from phytate as well as from a variety of natural and synthetically phosphorylated substrates (Gibson and Ullah, 1990). Two classes of phytase (3-phytase and 6-phytase) are recognized by the International Union of Biochemistry (IUPAC-IUB, 1975). The 3-phytase (EC 3.1.3.8.) initially hydrolyzes the ester bond at the 3-position of phytate; whereas, 6-phytase (EC 3.1.3.26.) first removes orthophosphate from the 6-position of phytate (Nayini and Markakis, 1986). Typically, 3-phytase is more common in microorganisms (Cosgrove, 1970), while 6-phytase is present in plant tissues, especially in plant seeds.

Sources of Phytases

Phytase in Microorganisms. Phytases have a wide occurrence in microorganisms, plants, and certain animal tissues. The phytase activity of microorganisms has been studied extensively. Fungi, especially *Aspergilli*, yeast, and bacteria exhibit phytase

activity. Phytase activity also occurs in rumen and soil microorganisms and in *mycorrhizal fungi* (Yamamoto et al., 1972; Irving and Cosgrove, 1974; Nayini and Markakis, 1984). *Aspergilli* are rich sources of microbial phytase (Shieh and Ware,1968).

Phytase in Plants. Plant seeds such as rice, wheat, barley, corn, rye, soybean, and other leguminous and oil seeds have a wide range of phytase activity. An increase in phytase activity concomitant with a decrease in phytate accompanies germination (Gibson and Ullah, 1988). Among the commonly used grain and legumes, wheat (Lim and Tate, 1973), triticale (Pointillart et al., 1987), and rye (Pointillart, 1991) are relatively rich in phytase activity. Their grain and by-products are used as sources of dietary phytases to improve the availability of phytate P (Pointillart et al., 1987; Pointillart, 1991).

Phytase in Animal Tissues. Phytases have been detected in some animal tissues. McCollum and Hart (1908) were the first to report phytase in the liver and blood of calves. Other researchers measured phytase activity in the small intestinal mucosa of calf, chicken and man (Bitar and Reinhold, 1972), rabbits, guinea pigs and hamsters (Cooper and Gowing, 1983), rats (Moore and Veum, 1983; Yang et. al., 1991a,b), and pigs (Pointillart et al., 1987). Mucosal production of phytase is induced by low dietary P in chicks (McCuaig et al., 1972) but not in rats (Moore and Veum, 1983). A high phytate diet induced an increase (1.8 vs. 3.6 *m*U/mg protein) in rat intestinal phytase activity (Yang et al., 1991b). The phytase activity in animal tissues is generally considered to be negligible for monogastric animals to use phytate in the diets.

Commercial Microbial Phytase

With the development of modern biotechnology, microbial phytase from *Aspergilli* has been made available as a commercial product. Natuphos® (5,000 U/g), produced by BASF (Ludwigshafen, Germany), is now being marketed in Austria, Brazil, Bulgaria, Canada, Finland, Germany, Netherlands, Norway, Switzerland, and Taiwan

(Dunn, 1994; Schwartz, 1994). There are two other phytase products: Finase® (500 U/g), produced by ALKO Ltd. (Rajamarki, Finland), and Allzyme® (50 U/g), produced by Alltech (Nicholasville, Kentucky, USA).

Characteristics of Microbial Phytases

Assay. Phytases from different sources have been purified and characterized. Phytase activity is assayed by measuring the inorganic phosphate liberated from phytic acid (phytate). One unit of microbial phytase activity is equal to the amount of enzyme that liberates 1 μmol of orthophosphate from 5.1 mM phytic acid (sodium phytate) in 1 min under a standard condition at 37 to 40 ^{o}C and pH 5.0 to 5.5 (Simons et al., 1990; Jongbloed et al., 1992). The activity also can be determined by separating the unhydrolized phytate by ion exchange (Harland and Oberleas, 1977) and by assaying the electrical conductivity of the reaction mixture (Collatz and Bailey, 1921).

Physical Characteristics. As estimated by their migrations in a calibrated gel filtration column, the molecular weight of the enzyme from *Aspergillus* was approximately 90 kDa (Ullah, 1988a, b). When analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the purified protein ran as two broadly diffused bands at 85 and 100 kDa from *Aspergillus* (Gibson and Ullah, 1988). Phytase from *Aspergillus* consists of 594 amino acid residues, of which, 104 amino acid residues are Asp and Glu and 30 from Lys and Arg. Thus, phytase is an acidic phosphatase (Ullah, 1988b; Gibson and Ullah, 1988).

The isoelectric point (pI) of 4.5 for *Aspergillus* phytase was deduced from chromatofocusing. The molecular forms of phytase from *Aspergillus* stained positive for carbohydrate with periodic acid-Sciff's reagent (PAS) after SDS-PAGE (Gibson and Ullah, 1988).

Enzymatic Characteristics. ***Optimum pH.*** The optimum pH of microbial phytase from *Aspergillus* showed two peaks, the highest activity was observed at pH 5.0 to 5.5 and the second highest activity was at pH 2.5 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). Shieh et al. (1969), and Irving and Cosgrove (1974) observed that the enzyme was 40% less active at pH 2.5 than at pH 5.0; whereas Simons et al. (1990) reported that the enzyme was 50% less active at pH 5.5 than at pH 2.5. The enzyme retained most of its activity at pH 6.0, but no activity was observed at pH 7.0 and above.

Optimum Temperature. *Aspergillus* phytase showed its highest activity at 58 ^{o}C (Gibson and Ullah, 1988). There was a sluggish range from 20 to 30 ^{o}C before the peak and the activity fell rapidly at 65 ^{o}C after the peak. However, the enzyme held for 10 min. at 68 ^{o}C and subsequent assayed at 58 ^{o}C retained 40% of the original activity.

Substrate Specificity. Microbial phytases displayed a preference for phytic acid with a μM Km, but a variety of phosphoesters were accommodated as substrates such as p-nitrophenylphosphate, phenylphosphate, ATP, and Di-H pyrophosphate (Irving and Cosgrove, 1974; Ullah, 1988b; Gibson and Ullah, 1990).

Activators and Inhibitors. The data available indicate that phytases are activated by several divalent cations. *Aspergillus* phytase was slightly stimulated by Co^{2+} and Mn^{2+} (Ullah, 1988b). Sodium fluoride and inorganic orthophosphate were effective inhibitors of *Aspergillus* phytase (Irving and Cosgrove, 1974; Gibson and Ullah, 1988). Orthophosphate was a noncompetitive inhibitor (Gibson and Ullah, 1990). Sodium

fluoride inhibited the reaction of *Aspergillus* phytase at pH 2.5 but not at pH 5.0. The cations Cu^{2+}, Cu^{3+}, Zn^{2+} and Fe^{3+} were inhibitory to *Aspergillus* phytase (Scheuermann et al., 1988a,b). Calcium can act both as an activator (Scheuermann et al., 1988a,b) and inhibitor (Schulz and Osluge, 1972). At high Ca contents (> 7 g/kg) and pH 6, there is a formation of Ca-phytate which precipitates and thus can not be attacked by phytase (Lantzsch, 1989).

Moisture and Time. It was reported that the minimum water content for phytase action must be about 25% (Hoppe, 1992). The water content of 80% (Bos, 1990) or 97% (Simons et al., 1990) was used in some *in vitro* studies. The time for assay of phytase activity was established as 1 h. The time used *in vitro* studies ranges from 1 to 24 h.

Mechanism of Action

The amino acid residues in the active site of microbial phytase could be Lys, Arg, and His because the phytase binds with phytate which possesses negative charges. Phytase is a hydrolase. Microbial phytase from *Aspergillus* is a 3-phytase (Nayni and Markakis, 1986). Firstly, the phytase removes the 3-position phosphate from the phytate, myo-inositol hexaphosphate (IP_6) and forms a myo-inositol 1, 2, 4, 5, 6-pentaphosphate (IP_5) and orthophosphate (PO_4^{-3}). i.e.

$$IP_6 + H_2O \xrightarrow{\text{phytase (pH, temperature)}} IP_5 + PO_4^{-3}$$

Under adequate conditions, phosphate in the hexakisphosphate is hydrolyzed and released. Then, phytase proceeds in a stepwise manner, producing five classes of intermediate products (myo-inositol penta-, tetra-, tri-, bis-, and monophosphates) of variable stereochemistry.

Efficacy of Phytase *in vitro*

Incubation of phytate with microorganism cultures resulted in hydrolysis of phytate because the cultures possessed phytase activity. Raun et al. (1956) observed the presence of inorganic P after a washed suspension of rumen microorganisms was incubated in an artificial rumen system with Ca-phytate. The hydrolysis of phytate was found in fermented peanut press cake (Fardiaz and Markakis (1981), in fermented corn with natural lactic acid (Lopez et al., 1983), and in fermented pearl millet (Kheturpaul and Chauhan, 1990). Microbial phytase from *Aspergillus* was used to incubate the specific plant seeds. The limited data indicate that microbial phytase could release phytate P from soybean meal (Nelson et al., 1968; Han, 1989; Simons et al., 1990), corn (Simons et al., 1990), cotton seed meal (Han and Wilfred, 1988), and rapeseed meal (Zyla and Koreleski, 1993). The amount of P released by phytase was influenced by the amount of added phytase, amount of phytate present in the feed, the temperature, moisture, time, and pH of the reaction (Han and Wilfred, 1988; Simons et al., 1990).

It is possible to make low phytate diets for animals by incubating the plant seeds with crude culture products containing phytase or with purified phytase products. The P in the treated grains were more efficiently used by animals than that in untreated grains, but the process was uneconomical. Han (1989) estimated that feeding phytase treated feed would be 17 times more expensive than adding inorganic P. In the study of Zyla et al. (1993), commercial phytase was used in an *in vitro* procedure for prediction of P release from poultry feeds. Increasing levels of phytase (0, 250, 500, and 1,000 U/kg of diet) resulted in a linear release of P from feed samples. The amount of P released from feed samples *in vitro* was correlated with weight gains of turkeys fed diets containing phytase for 1 wk. In addition to orthophosphate liberation, an increase in amount of

protein in a solid state fermentation of canola meal by *Aspergillus* phytase was also observed (Nair and Duvnjak, 1990; Nair et al., 1991).

Effectiveness of Supplemental Microbial Phytase

A few studies have been conducted for evaluating the effectiveness of supplemental microbial phytase in swine (weaning and growing-finishing) and poultry (broilers, turkey poults, and laying hens). The diets were mostly based on corn-soybean meal, which contains minimal plant phytase activity.

Improving Performance.

Adding microbial phytase at levels from 100 to 1,500 U/kg of diet to low P diets of pigs increased average daily gain (ADG) by 10 to 20%, and increased average daily feed intake (ADFI) by 2 to 24% (Simons et al., 1990; Beer and Jongbloed, 1992; Jongbloed et al., 1992). Adding similar amounts of phytase to the low P diets of broilers increased BW gain and feed intake by 10 to 80% (Saylor et al., 1991; Schoner et al., 1991; Vogt, 1992). It turns out that the improved gains due to phytase were mediated primarily via an increased feed intake. The effects of adding phytase on gain:feed were conflicting in the literature. Some showed increasing responses of 6 to 20%, but some showed no improvement. Reducing P in diets of layers decreased their performance, but adding phytase to the low P diets resulted in recovery of egg production, egg mass, and feed efficiency (Peter, 1992; Simons et al., 1992).

Improving Bioavailability of Phytate Phosphorus.

Adding phytase to the low P diets of pigs increased apparent P digestibility by 18 to 30%, and phytic acid ileal digestibility by 50 to 75% (Simons et al., 1990; Beer and Jongbloed, 1992; Jongbloed et al., 1992). Supplemental phytase in the diets of pigs also increased plasma P concentration, decreased plasma alkaline phosphatase activity (Lei et al., 1993a, b, c), increased bone breaking strength (Cromwell et al., 1993; Ketaert et al.,

1993), and increased P and Ca retention (Hoppe et al., 1992; Lei et al., 1993a,b; Mroz et al., 1994). Adding phytase to the low P diets of broilers and turkey poults increased livability, tibia breaking strength (Denbow et al., 1995; Ravindran et al., 1995), toe ash content, and P retention (Schoner and Hoppe, 1992). However, the responses from phytase addition manifested a wide range of variability at various phytase doses and with different measurements. The quantitative characterization for the responses are very limited in the literature.

Replacing Inorganic Phosphorus.

Since phytase improves the availability of phytate P, it can reduce the need for inorganic P in diets for swine and poultry. It is important to know the replacement values of inorganic P by microbial phytase or P equivalency values of microbial phytase. However, the data about the equivalency values are very limited. In some of the evaluations, the response of added phytase was compared with the response of a positive control group (assuming P requirement was met). Lei et al. (1993a) estimated the equivalency value of phytase by a P balance trial in pigs with a basal diet (B) containing .33% P, B + 1,200 U phytase/kg of diet, and B + .20 % P diet from monodibasic Ca phosphate (MCP). Based on the difference in daily P intake and retention of the pigs, 1,000 U of phytase was equivalent to .91 g inorganic P. However, it may have been that the one level of phytase was too high to get an efficient response. Simons et al. (1992) compared the growth response of broilers fed 250, 500, or 750 U of phytase/kg of diet with the response from the P control group. They found that 500 U of phytase was equivalent to 1.0 g P from MCP. However, a deficiency of the study was that only one level of P was used for the comparison, whereas the P utilization could be influenced by many factors such as dietary Ca level or Ca to P ratio.

It is reasonable for the calculation of P equivalency value to compare the responses of graded levels of inorganic P with the responses of graded levels of phytase. Some response data from graded levels of P or phytase are available. But most of them have not been used to develop the equivalency value. Using some of the data, Hoppe and Schwarz (1993) calculated the equivalency values of inorganic P from MCP for pigs. By assuming a linear relationship between phytase activity and increase in digestible P, 430 U of phytase was equal to 1 g P. It is noteworthy that the response of phytase on P digestibility may not be linear and the response to phytase of other criteria may be different from P digestibility.

Decreasing Phosphorus Excretion.

Phytase addition in low P diets of pigs led to the highest decreases in P excretion of 70% (Simons et al., 1990), 55% (Beers and Jongbloed, 1992), 58% (Lantzsch and Wjst, 1992) and 48% (Paullauf et al., 1992). The average reduction in P excretion of pigs from the literature is about 56%. Supplemental phytase to the diets of broilers resulted in a decrease of P excretion by 50% (Simons et al., 1990; Schoner et al., 1992, 1993).

Potential to Improve the Utilization of Other Nutrients.

Microbial phytase may improve the utilization of other minerals (Ca, Mg, Zn, Cu, and Fe), proteins, and AA bound to phytate. Lei et al. (1993c) found that the bioavailability of Zn for pigs was improved when phytase was added to a low P and Zn corn-soybean meal diet with 0, 30, and 60 ppm of Zn. In the studies of Pallauf et al. (1992) and Nasi and Helander (1994), adding microbial phytase to the diets of young pigs significantly improved apparent absorption of Zn, Mg, Fe, and Cu. Thiel et al. (1993) found that femur Zn content of the chicks fed the basal diet (30 ppm Zn) with addition of 700 U phytase/kg of diet was equal to that fed a diet containing 39 ppm of Zn without added phytase. In the study of Biehl et al. (1995), adding phytase (1,200 U/kg of diet) to

the low Zn diet (13 ppm) increased growth rate and tibia Zn content of broilers. However, in the study of Roberson and Edwards (1994), adding phytase (750 U/kg of diet) did not improve Zn retention in broilers. Most studies with phytase have not been conducted to evaluate the effect of phytase on trace minerals. The possible reason is due to the inadequate facilities to prevent contamination by trace minerals, and that the diets used are probably rich in trace minerals added.

Ketaren et al. (1993) found that addition of phytase increased protein deposition and retention of pigs, but had no effect on CP digestibility. Mroz et al. (1994) reported that supplemental microbial phytase improved the apparent digestibility of protein and some AA in pigs. Phytase also improved N absorption in laying hens (Van der Klis and Versteegh, 1991). The limited data in evaluation of phytase on digestibility of AA in swine and poultry may be due to the expense of amino acid analysis.

Factors Affecting the Effectiveness of Phytase

According to the principles of enzyme reaction, an enzyme (E) combines with a specific substrate (S) to form a specific intermediate complex (E-S), which proceed to form a product (P) or to dissociate into E and S (Stryer, 1987). The factors affecting the effectiveness of phytase on the different parts of phytase action are summarized in Figure 2-4.

Factors Related to Substrate and Enzyme

Phytate and Phytase Contents

The reaction favors forming the products when the amount of substrate or enzyme activity in the reaction was increased (Figure 2-4). The results from feeding experiments indicate that the response measurements of phytase were increased with the increase of added phytase in the diets (Simons et al., 1990; Cromwell et al., 1993; Lei et al., 1993a,b). The responses from the same amount of added phytase in diets with high phytate were

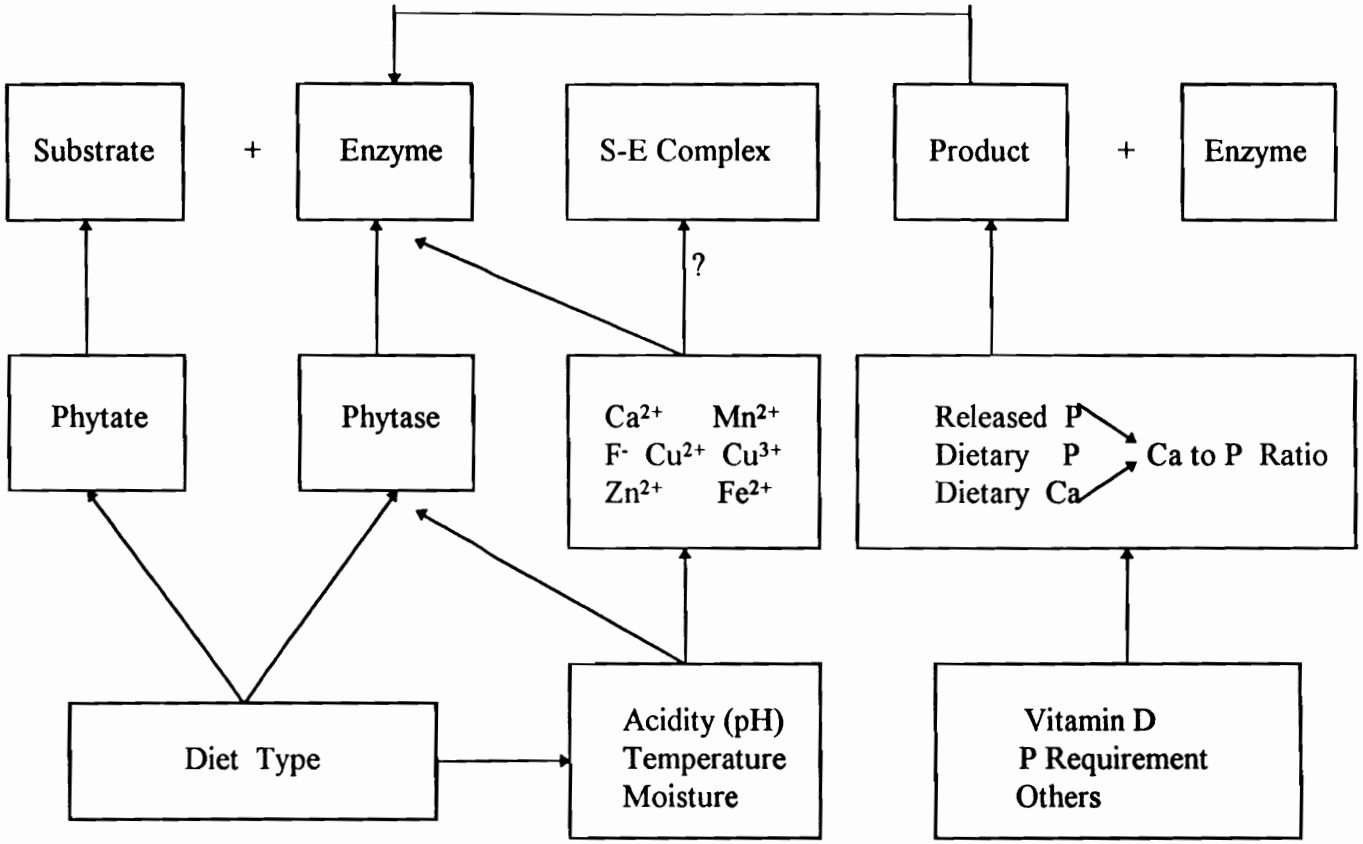

Figure 2-4. Factors of affecting the effectiveness of phytase

greater than those in diets with low phytate (Cromwell et al., 1993). The concentration of phytate and phytase is affected by diet type. Corn-soybean meal diets (CS) have higher phytate content than soybean meal based semi-purified diets (SP). Thus, the effect of phytase on phytate P could be higher in CS than that in SP. The responses of adding microbial phytase to diets containing wheat or triticale were greater than that of adding phytase to the corn-soybean meal diet because of the natural phytase activity in wheat or triticale (Schoner et al., 1991).

Dietary Acidity (pH)

Acids have been added to diets for pigs, especially for young pigs (Giesting et al., 1991; Risley et al., 1992; Easter, 1993), which decreased dietary pH. Mineral supplements also change the acidity of the diets (Patience et al., 1987). Dietary acidity may change the pH in the GIT and in turn influences the activity of supplemental phytase.

Pelleting Temperature

Pelleting has been employed in the feed industry for reduction of *Salmonella*, dust, and wastage of feed, and for improvement of feed efficiency and digestibility (Van Schoubroek et al., 1971). The temperature used for pelleting influences the activity of phytase. Jongbloed and Kemme (1990) reported that pelleting diets containing wheat, wheat middlings or barley at 80 °C decreased the P and Ca absorbability by 10 percentage units or more. In the study of Simons et al. (1990) on the stability of added phytase, samples steam heated to 50 °C before pressing and reaching 81 °C after pelleting retained 94% of initial phytase activity. In samples preheated to 65 °C, 83 and 46% of the activity remained after pelleting when the temperature reached 84 and 87 °C, respectively. Newman (1991) reported that partially purified phytase from *Aspergillus niger* lost about 16% of its activity upon exposure to 90 °C for 30 min.

Moisture and Others

Moisture is one of the necessary conditions for phytase action. Liquid feeding is one feeding system for pigs in some countries. Adding phytase in liquid feeds may have more benefits than in dry feeds because it increases the time of phytase exposure to the phytate at the adequate moisture. However, no data exist about the effect of liquid feeding on phytase activity.

The activators and inhibitors from other cations on phytase have been reported (Gibson and Ullah, 1990). But their action sites on substrate, enzyme, or complex in the reaction are unclear. Little is known about the effects of these factors on phytase under feeding typical conditions in animals.

Factors Related to Product of Reaction.

Dietary Phosphorus

In enzymatic reactions, products can feedback to inhibit the activity of the enzyme. The major product of phytase action on phytate is orthophosphate, which is the chemical compound added supplemental inorganic P. Thus, dietary levels of available P (aP) or nonphytate P (nP) could influence the effectiveness of phytase. It was observed that the responses to phytase addition were greatest at the lowest nP diets from the experiments with factorial arrangement of aP and phytase (Denbow et al., 1995; Ravindran et al., 1995). Adding phytase to broiler diets with higher than recommended nP levels resulted in no phytase responses (Swick and Ivey, 1991; Perney et al., 1993).

Dietary Calcium

It is well known that an excess of dietary Ca decreases P utilization (NRC, 1988, 1994). Dietary levels of Ca may be an important factor that determines the extent of phytate hydrolysis (Wise, 1983; Ballam et al., 1984) through formation of insoluble Ca phytate (Nelson, 1967). Studies *in vitro* have shown that high Ca was an inhibitor in

phytase action. Jongbloed et al. (1993) investigated three levels of dietary Ca (.4, .6, and .8%) using a basal diet (tapioca, corn, hominy feed, barley, soybean meal, and sunflower meal) containing .43% P. Apparent P absorption decreased as the level of Ca increased, and increased as the level of phytase increased. However, the magnitude of the increase due to phytase was reduced as dietary Ca increased.

Generally, the effectiveness of phytase should be best at marginally deficient levels of Ca and P because P and Ca released by phytase plus the available P and Ca present in the diet will meet the requirement of P and Ca for animals. Since phytase increases Ca availability, however, the adequate level of Ca may be altered into a high level of Ca after phytase is added. The high level of Ca resulted by phytase may decrease P utilization and then decrease the effectiveness of phytase. Lei et al. (1994) reported that the ability of phytase to improve phytate P availability in pigs was greatly reduced at a normal dietary level (.92%) of Ca (Ca:tP was 3.0:1). In the study of Schoner and Hoppe (1992) with broilers fed P deficient diets (.35%), the Ca level had no effect on P utilization with supplementation of inorganic P. But the P utilization in the treatment of phytase addition was decreased by an increased dietary Ca level (.60, .75, and .90%). However, Huyghebaert et al. (1991) found that the improvement of P utilization from phytase addition at 250 and 500 U/kg of diet was unaffected by increasing the Ca content (.75 and 1.0%).

Dietary Calcium to Phosphorus Ratio

The dietary Ca to P ratio is important for animals to efficiently utilize both minerals and other nutrients. Since the levels of both Ca and P could be excessive, adequate or low, Ca to P ratio is complicated for discussion. It is well known that wide Ca to P ratio decreases P utilization (NRC, 1988; 1994). The participation of phytase and different efficacy of phytase on Ca and P utilization make consideration of the Ca to P ratio more

complicated. The basic question is what level of Ca in a Ca to P ratio should be: excessive, adequate or low and how low. In some studies of phytase (Simons et al., 1992; Cromwell et al., 1993; Lei et al., 1994), Ca levels were kept constant and Ca to P ratios varied among different treatments. In some studies of phytase (Denbow et al., 1995; Ravindran et al., 1995), Ca to P ratios were kept constant (2:1), but Ca levels varied. In other studies of phytase, both Ca and Ca to P ratios were manipulated. Thus, it is difficult to interpret the available data.

The important issue in phytase studies is to know how much more P is released from phytate by phytase and at what Ca to P ratio. Otherwise, it is not reasonable to draw a conclusion about the effects of Ca to P ratios on the effectiveness of phytase. Schoner et al. (1993) evaluated the effect of Ca to P ratio on phytase efficacy with a complete 3 x 5 factorial design. The Ca to P ratios were 1.71 (6.0:3.5), 2.14 (7.5:3.5), and 2.57 (9.0:3.5) and phytase levels were from 125 to 1,500 U/kg of diet. I calculated that the P released by phytase at the levels of 250, 500, 750, and 1,000 U/kg of diet were .41, .69, .88, and 1.01 g at 1.71 ratio; .37, .58, .73, and .82 g at 2.14 ratio; and .41, .64, .80, and .90 g at 2.57 ratio, respectively. Phytase released more P from phytate at the narrow Ca to P ratio than those at the wide ratio. The other important issue in phytase study is to use the Ca to aP ratio instead of Ca to tP ratio. In Ca to tP ratio, it is unclear if the aP in diets meet or is close to the requirement. It is also necessary to know how much aP is present in the diets and how much P is released from phytate by phytase.

Dietary Vitamin D

In the enzyme reaction, the reaction favors forming product when the product is removed quickly. Vitamin D enhances the enterocytes of the small intestine to transport P into the plasma compartment (Deluca et al., 1989). Vitamin D regulates Ca absorption in intestines of animals through its receptor in nuclei of mucosal cell, where its action turns

on the genes expressing Ca-binding protein that acts as the receptor in the active absorption of Ca (Wasserman et al., 1992). Thus, improving absorption of P and Ca by vitamin D could increase the effectiveness of phytase. Vitamin D also could improve the absorption of phytate by stimulating the hydrolysis of phytate (Mohammed et al., 1991).

In the study of Roberson and Edward (1994) with broilers, phytate P retention was increased by adding phytase (600 U/kg diet) or 1, 25$(OH)_2$-D_3 (5 μg/kg diet), and was increased additively when both were fed. Biehl et al. (1995) reported that total bone ash of broilers increased 56% from the addition of .10% P to the P deficient diet (.10% aP), 64% from 1,200 U of phytase/kg of diet, 60% from 10 μg/kg diet di-OH D_3, and 98% from the combination of phytase and di-OH D_3. Lei et al. (1994) found that increasing vitamin D levels in the pig diet partially offset the adverse effect of normal level of dietary Ca on phytase efficacy.

Phosphorus Requirement and Other Factors.

Different animal species have different P requirements (NRC, 1988, 1994). The higher P requirement is met by supplementing more inorganic P. However, when the inorganic P levels in diets are high, the effectiveness of phytase may be decreased. Other factors affecting P absorption (discussed before) also may influence the effectiveness of supplemental phytase. However, data on these points are not currently available.

Summary

Phosphorus is a nutritionally essential element. Response criteria including performance, characteristics of bones and blood, apparent P digestibility or retention have been used for assessing P nutrition in swine and poultry. Modern intensive animal production leads to P pollution from swine and poultry manure because of the phytate P in diets and added inorganic P to meet the P requirement. Phytate is a storage form of P in plant seeds and acts as a second messenger in animals. Most of the P in plant ingredients

is in phytate which is unavailable for swine and poultry. Several methods have been used to improve the availability of phytate P. Supplementation of feed with microbial phytase seems to be an effective approach. Microbial phytase is obtained from a genetically engineered *Aspergillus* strain, and its characteristics including optimal pH and temperature, activators and inhibitors have been identified. Microbial phytase is effective in the low P diets of swine and poultry for improving P bioavailability and for decreasing P excretion. Phytase also may improve the utilization both of other minerals and of protein and amino acids bound to phytate. The effectiveness of phytase could be affected by several factors, such as dietary phytate content, amount of added phytase, dietary levels of Ca, P or Ca to P ratio, and vitamin D.

Chapter III

Effectiveness of Natuphos® Phytase in Improving the Bioavailabilities of Phosphorus and Other Nutrients in Soybean Meal-based Semi-purified Diets for Young Pigs

ABSTRACT: Crossbred young pigs (n = 96, BW = 7.5 kg) were used in a 5-wk trial to determine the effectiveness of supplemental Natuphos® phytase in improving the bioavailabilities of P and other nutrients in a semi-purified diet with soybean meal as the only P source in the basal diet. Two available P (aP) levels (.05 and .16%) and five phytase levels (0, 350, 700, 1,050, and 1,400 U/kg of diet) were used in a 2 x 5 factorial arrangement of treatments. The Ca:total P was maintained at 2:1 in the 10 treatments. In addition to the 10 diets, two extra diets (Ca:total P = 1.46:1) were formulated to supply the recommended level of aP (.32%) with 0 and 1,400 U of phytase/kg of diet. Graded levels of phytase resulted in linear increases of ADG ($P < .05$), ADFI ($P < .001$ at .16% aP only) and gain:feed ($P < .05$). Effects of adding phytase to the .32% aP diet were only observed in wk 1 to 2 with increases of only ADG ($P < .01$) and gain:feed ($P < .05$). Apparent digestibility (or absorption) coefficients (ADC) of DM, P, Ca, and N were estimated, using chromic oxide as an indicator during wk 4 and 5. When phytase and P were added to low P diet, ADC of P was increased ($P < .001$), but only small and variable changes in ADC of DM, Ca, and N were observed. Fecal P excretion (g/d) decreased as microbial phytase was added ($P < .001$) and increased with added P ($P < .001$). In comparison to .32% aP diet, fecal P excretion was decreased 25 to 50% by addition of phytase. The addition of phytase to the .32% aP diet further improved ($P < .01$) ADC of P (54.5 vs 61.8%) and decreased ($P < .001$) fecal P excretion (1.62 vs 1.38 g/d). Characteristics of fourth metacarpals and tenth ribs were consistently improved by both

increasing dietary phytase and P. Based on an assessment of R^2 values from the second order translog equations, ADG, ADFI, P apparent absorption, and bone ash percentage and shear force were sensitive indicators. Phosphorus equivalency of microbial phytase was calculated using response equations for ADG and P apparent absorption. The average function for the release of P (Y, g/kg) by microbial phytase (X, U/kg diet) was developed with aP levels of .05 and .16%: $Y = 1.546 - 1.504e^{-.0015X}$. The replacement of 1 g of P as defluorinated phosphate would require about 676 U of microbial phytase. This represents 77% of released P from phytate.

Key Words: Pig, Phosphorus, Phytase, Soybean meal, Bioavailability

Introduction

Soybean meal is the main protein supplement fed to pigs in the U.S. About 60% of P in soybean meal is in the form of phytate (Nelson et al., 1968; Reddy et al., 1982), which is poorly available to pigs and leads to the large amount of P present in pig manure (Cromwell and Coffey, 1991). Phytase (EC 3.1.3.8.) releases orthophosphate groups from the phytate molecule (Gibson and Ullah, 1990). But phytase activity in the gastrointestinal tract of pigs and in soybean meal are limited (Pointillart et al., 1984, 1987; Hoppe et al., 1993). Recently, a microbial phytase from *Aspergillus* has been used to improve the availability of phytate P in corn-soybean meal diets (Simons et al., 1990; Cromwell et al., 1993b; Lei et al., 1993a; Pallauf et al., 1994). There is also some evidence that microbial phytase will enhance the utilization of DM, Ca, and CP in pigs (Ketaren et al., 1993; Mroz et al., 1994; Pallauf et al., 1994). Little is known about P equivalency values of microbial phytase.

The purpose of this study was to evaluate the effectiveness of graded levels of Natuphos® phytase for improving the absorption and utilization of P, Ca, and N, and apparent digestibility of DM in young pigs fed soybean meal-based semi-purified diets

containing two levels of available P (aP). Also, response equations were generated for the evaluation of sensitive indicators and the calculation of P equivalency values of microbial phytase using the performance, apparent absorption, and bone measurements.

Materials and Methods

Animals and Management

A total of 96 crossbred pigs (equal number of barrows and gilts) were used. The pigs were weaned between 28 and 35 d of age and were given a 7-d adjustment before dietary treatments were started. During the adjustment period they were fed a diet containing 22% CP for 4 d (Maximum Wean 10-15, Southern States Cooperative, Richmond, VA 23261) and then a 20% CP corn-soybean meal diet containing 10% dried whey for the remaining 3 d. After the adjustment period, pigs were weighed (average BW = 7.5 ± .2 kg) and randomly placed on treatments from outcome groups based on gender and weight. Littermates were balanced across treatments as much as possible. The pigs (one barrow and one gilt) were housed in double deck nursery pens (.6 m x .9 m) with expanded metal floors and a baffle between decks. Each pen was equipped with a nipple waterer and a stainless steel feeder.

The study was conducted in two similar, environmentally controlled rooms with 24 pens in each room. Room temperatures were initially set at 29 °C and were lowered about 2 °C per week after the second week. A continuous lighting regime and recommended air ventilation rates were maintained. Pigs had free access to water and diets, but due to the poor flowability, the diets were agitated six times per day to ensure that pigs were receiving near ad libitum intake. The care and treatment of pigs followed published guidelines (Consortium, 1988).

Treatments and Basal Diet

A completely randomized design in a 2 x 5 factorial arrangement of treatments was employed to evaluate the response of weanling pigs to five levels of phytase and two levels of aP. Dietary aP levels were formulated to have .05 and .16% aP [or .22 and .32% total P (tP), respectively], and each level of aP was supplemented with 0, 350, 700, 1,050, and 1,400 U phytase/kg of diet (assayed phytase activity of diet samples was 20, 320, 610, 900, and 1,320 U/kg of diet). A unit (U) is defined as the quantity of enzyme which liberates 1 μmol of inorganic P per min from 5.1 mM sodium phytate at pH 5.5 and 37 °C (Engelen et al., 1994). These P levels were formulated below the current NRC (1988) recommendations to ensure maximum response to phytase additions. In addition to the ten diets described above, two additional diets were formulated to supply the recommended level of P (.32% aP or .48% tP); one diet was fed without phytase and one was fed with 1,400 U of phytase/kg of diet. The diet without the addition of phytase served as the positive control. Each of the 12 dietary treatments was fed to four replicate pens of two pigs each.

The basal diets were semi-purified diets and contained only soybean meal as the protein and organic P source (Table 3-1). Soybean meal supplied all the P contained in the basal diet and the estimated aP content was .05%. Corn starch, dextrose, and soybean oil were used as energy sources. The desired levels of aP in the other basal diets were achieved by the addition of defluorinated phosphate (DFP, from Fine CDP, Southern Bag Corp., Valdosta, GA 31803). The Ca:tP was maintained at 2:1 in all basal diets except the .32% aP diets where a ratio 1.46:1 was used. Limestone and DFP were added to the diets at the expense of corn starch. Since phytate was supplied only from the soybean meal, the dietary content of phytate P, .13%, was similar in all diets, based on NRC (1988). Chromic oxide was included at a level of .10% in diets as an indigestible indicator

Table 3-1. Percentage composition and calculated analysis of the basal diets

Item	Available P, %		
	.05[a]	.16[a]	.32[b]
Ingredients	----------%----------		
Soybean meal (48.5% CP)	33.65	33.65	33.65
Corn starch[c]	29.86	29.21	28.66
Dextrose[d]	25.25	25.25	25.25
Soybean oil	6.00	6.00	6.00
Cellulose[e]	3.00	3.00	3.00
Ground limestone[f]	.93	.93	.54
Defluorinated phosphate[g]	---	.65	1.59
Vitamin premix[h]	.50	.50	.50
Trace mineral premix[i]	.20	.20	.20
Salt	.40	.40	.40
Selenium premix[j]	.05	.05	.05
Chromic oxide	.10	.10	.10
Methionine	.06	.06	.06
Calculated analysis, %			
Crude protein	16.32	16.32	16.32
Lysine	1.05	1.05	1.05
Methionine & Cysteine	.53	.53	.53
Ca	.43	.64	.70
Total P	.22	.32	.48
Available P	.05	.16	.32

[a]Zero, 350, 700, 1,050 and 1,400 U phytase per kilogram of diet were added. Phytase (Natuphos®-5000 U/g) was supplied by BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234.
[b]Zero and 1,400 U phytase per kilogram of diet were added.
[c]Food grade, National Starch and Chemical Co., Bridgewater, NJ 08807.
[d]Clintose, ADM Corn Processing, Clinton, Iowa 52732.
[e]Purified cellulose, Grade BH200 International Filler Corp., New York 14120.
[f]Limestone Dust Corp., Bluefield, VA 24605.
[g]Fine CDP, Southern Bag Corp., Valdosta, GA 31803.
[h]Supplied per kilogram of diet: retinyl acetate, 3027 μg; cholecalciferol, 22 μg; dl-α-tocopherol acetate, 22 mg; riboflavin, 8.8 mg; niacin, 44 mg; choline, 880 mg; d-pantothenic acid, 44 mg; d-biotin, .88 mg;cyanocobalamin, 44 μg; Vitamin K (as menadione dimethylprimidinol bisulfite), 4.4 mg.
[i]Supplied per kilogram of diet: Zn, 300 mg; Fe, 350 mg; Mn, 120 mg; Cu, 35 mg; I, 4 mg.
[j]Supplied .3 mg selenium per kilogram of diet.

for digestibility measurements. To obtain a homogeneous distribution of the indicator in the diet, chromic oxide was first mixed in a small mixer with corn starch at a ratio of 1:3

(w/w) and this mixture then was ground in a laboratory mill to pass through 1 mm sieve (Dellaert et al., 1990).

Sampling and Analysis

During the 5-wk test, pigs were weighed individually and pen feed intakes were recorded at weekly intervals. Grab fecal samples were collected from each pen during wk 4 and 5. During each of these weeks, feces were collected twice daily (morning and evening) on 3 alternate d. Collections from each of the 3 d within a week were pooled and frozen at -20 °C in airtight plastic bags for subsequent analysis. After thawing, fecal samples were dried in an oven at 70 °C. The dried samples, along with representative samples of diets, were ground to pass through a 1 mm sieve. Dry matter was determined according to standard AOAC (1990) methods. After a wet nitric-perchloric acid digestion of samples, total P concentrations were assayed photometrically (AOAC, 1990), and Ca and Cr contents were determined with an atomic absorption spectrophotometer. Nitrogen content was determined using the Kjeldahl method. The apparent absorption of Ca, P, and N over the total tract was calculated (Dellaert et al., 1990).

At the end of wk 5, all barrows (one per replicate pen - four per treatment) were slaughtered for collection of bone samples. The front foot and the tenth rib on the left and right sides were removed and frozen in airtight plastic bags. The foot samples were later thawed, extraneous tissue was removed and the fourth metacarpal was retained. The rib samples were also defleshed. The width of the bones at the narrow and wide dimensions of the bone shaft were measured. Bones were then refrozen in airtight plastic bags for shear force and shear energy determination as described by Combs et al. (1991b).

The shear force of the fourth metacarpal and the tenth rib were determined using an Instron Universal Testing Machine (Model 1123, Instron Corp., Canton, MA 02021). Bones were thawed in airtight plastic bags immediately prior to testing to prevent

desiccation. After the shear failure test, wall thickness was measured using dial calipers. Shear stress values were calculated according to the formula of Combs et al. (1991b) for metacarpals and Wilson (1991) for ribs. After the shear test, the bones were oven-dried at 100 °C for 24 h and ashed in a muffle furnace at 600 °C for 24 h. Bone ash was expressed as a percentage of the dry weight.

Statistical Analysis and Calculation of Phosphorus Equivalency Values

The data were analyzed by the GLM procedures of SAS (1990). Performance and absorption data were analyzed using the pen as the experimental unit, while bone measurements were analyzed using individual pig values as the experimental unit. Linear and quadratic effects of supplemental phytase within each aP level and within dietary aP levels without added phytase were tested using orthogonal polynomials. A comparison between the positive control diets with and without phytase was made using nonorthogonal contrasts. Second order translog functions were derived for the 2 x 5 factorial with the following model:

$$\text{LnY} = \alpha_0 + \alpha_1 D_1 + \alpha_2 \text{LnX} + \alpha_3(\text{LnX})^2 + \alpha_4 D_1 \text{LnX}$$

Where, Y = response measurements; X = phytase added (U/kg of diet); D_1 = aP when aP = .05% in the diet, D_1 = 0 and when aP = .16%, D_1 = 1. The most sensitive indicators were determined by examining the R^2 values of the second order translog equations from all of the measurements.

Nonlinear or linear functions which best fit the data were derived using treatment means for phytase levels at each aP level, and for three aP levels without phytase added. The nonlinear regression model used was $Y = a(1 - be^{-kX})$ and the linear regression model was $Y = a + bX$, where, Y = response measurements; X = aP (%) or phytase added (U/kg of diet). The functions generated from all the measurements with higher r^2, except bone shear stress, apparent digestibility of DM, Ca and N with lower r^2, and P excretion with

opposite response trend between aP levels without added phytase and phytase levels, were used to calculate P equivalency values of phytase. The equation for aP and equation for added phytase at each of the two levels of aP were set equal. For example, the equation for ADG at .05% was as follows:

$$256.5 + 487.0Y = 446.9(1 - .335e^{-.0003X})$$

$$Y = .3910 - .3074e^{-.0003X}$$

where, Y = aP (%); X = phytase added (U/kg of diet).

The resulting equations were used to calculate the equivalent aP (%) at 250, 500, 750, and 1,000 U of phytase/kg of diet in order to compare the data with those from others. Values for ADG and apparent absorption of P were used to determine the amount of P released and the released P was expressed as a percentage of phytate P. The amount of P released per 100 U of phytase was also calculated. The average function for amount of P released (Y, g/kg), released P as a percentage of phytate P (Y, %), and amount of P released per 100 U of phytase (Y, g) by microbial phytase (X, U/kg of diet) was developed for aP levels of .05 and .16%.

Results

Performance

During the 5-wk trial, ADG ($P < .01$), ADFI ($P < .10$) and gain:feed ($P < .01$) of weanling pigs were linearly increased by increasing amounts of dietary aP (Table 3-2). Graded levels of phytase linearly increased ADG ($P < .05$) and gain:feed ($P < .001$) of pigs fed .05% aP diets, whereas pigs fed .16% aP diets responded with linear increases in ADG ($P < .001$), ADFI ($P < .001$), and gain:feed ($P < .05$). The ADFI response to added phytase was much greater for pigs fed .16% aP diets than for those fed .05% aP, resulting in an aP x phytase interaction ($P < .10$). No interactions were observed for any other

Table 3-2. Performance, apparent digestibility of dry matter, and absorption of phosphorus, calcium and nitrogen, and fecal P excretion of young pigs fed soybean meal-based, semi-purified diets containing varying amounts of phosphorus and supplemental phytase[a]

Diets	1	2	3	4	5	6	7	8	9	10	11	12	
Available P, %			.05					.16				.32	
Added phytase (U/kg diet)	0	350	700	1,050	1,400	0	350	700	1,050	1,400	0	1,400	SEM
Avg daily gain, g	297	315	325	342	351	308	367	365	400	406	423	446	17
Avg daily feed, g	630	631	624	654	670	613	724	703	757	764	739	754	33
Gain:feed, g/kg	471	499	521	523	524	502	507	519	528	531	572	592	14
DM digestibility, %	91.9	91.6	90.9	91.3	90.9	90.5	90.8	91.8	91.1	90.6	90.2	90.4	.26
P absorption, %	35.4	38.8	41.7	44.1	42.3	41.2	47.4	53.3	52.2	50.0	54.5	61.8	1.64
Ca absorption, %	69.6	67.9	67.3	71.2	68.0	69.6	69.8	75.9	73.5	69.4	73.6	74.1	2.24
N absorption, %	90.3	90.0	89.1	88.9	88.8	88.7	88.8	89.9	88.8	87.5	87.5	87.5	.49
P excretion, g/d	.90	.85	.80	.81	.85	1.15	1.22	1.04	1.16	1.22	1.62	1.38	.03

Probability

	PY[b]	aP	PY*aP	Lin	Lin 1	Lin 2	Quad	Quad 1	Quad 2	Con 1[c]	Con 2[d]	Con 3[e]
Avg daily gain, g	.001	.001	.497	.001	.021	.001	.312	.723	.269	.357	.004	.070
Avg daily feed, g	.003	.001	.053	.001	.327	.001	.372	.920	.160	.689	.060	.133
Gain:feed, g/kg	.004	.114	.733	.001	.001	.032	.357	.312	.800	.228	.002	.265
DM digestibility, %	.201	.032	.001	.151	.007	.454	.091	.359	.001	.671	.001	.107
P absorption, %	.001	.001	.578	.001	.001	.001	.001	.091	.001	.002	.001	.121
Ca absorption, %	.406	.058	.390	.725	.972	.644	.208	.863	.053	.869	.276	.523
N absorption, %	.040	.030	.097	.005	.012	.124	.161	.502	.009	.937	.001	.734
P excretion, g/d	.001	.001	.003	.688	.181	.437	.003	.047	.022	.001	.001	.082

[a]Performance comes from four pens (two pigs per pen) per treatment mean; Average initial weight, 7.53 kg. Digestibilit and absorption coefficients come from eight pens (two pigs per pen for weeks 4 and 5) per treatment mean.
[b]PY = added phytase, U/kg diet.
[c]Con 1 = contrast for diet 11 vs 12.
[d]Con 2 = contrast for diet 1, 6, and 11, linear.
[e]Con 3 = contrast for diet 1, 6, and 11, quadratic.

measurements. Response curve equations for ADG, ADFI, and gain:feed during wk 1 to 5 were developed (Table 3-3 and 3-4).

Phytase (1,400 U/kg diet) was added to the diets containing .32% aP to evaluate any possible "extra-P" effects of the enzyme. The results revealed that such an effect was seen only during wk 1 to 2 of the trial; pigs fed the positive control diet with added phytase grew faster ($P < .01$, 335 vs 270 g) and were more efficient in feed utilization ($P < .05$, 663 vs 588 g/kg) than pigs fed positive control diet without phytase. However, during wk 3 to 5, there was no response of phytase on performance; over the 5-wk trial, performance means were only numerically larger for pigs fed the .32% aP with added phytase.

Apparent Digestibility and Absorption Coefficients

Increasing the amount of aP without adding phytase linearly decreased ($P < .001$) the digestibility of dry matter and absorption of N (Table 3-2). The absorption of P was linearly increased ($P < .001$) as the dietary level of aP increased, but there was no effect on Ca absorption. An aP x phytase interaction ($P < .001$) was observed for DM digestibility. At .05% aP, DM coefficients linearly decreased ($P < .01$) as the level of phytase increased, but at .16% aP, DM coefficients increased ($P < .10$) from 0 to 1,050 U of phytase/kg of diet and then decreased at 1,400 U of phytase/kg of diet. Addition of graded levels of phytase increased absorption of P ($P < .001$); whereas, an effect of phytase on Ca absorption was only observed at .16% aP ($P < .05$). An aP x phytase interaction ($P < .10$) for N absorption revealed a linear decrease ($P < .05$) at .05% aP as the level of phytase increased, but there was a quadratic increase ($P < .01$) at .16% aP. Adding 1,400 U of phytase/kg of diet to the diets containing .32% aP increased ($P < .01$) the absorption of P, but did not change the absorption of Ca and N, and the digestibility of DM. Adjusting absorption and digestibility coefficients for P, Ca, and N, as suggested by

Dellaert et al. (1990), resulted in only small changes and responses were unchanged. Response curve equations of DM, P, Ca, and N were developed (Tables 3-3 and 3-4).

Table 3-3. Second order translog functions for performance, apparent digestibility coefficients, fecal P excretion, and bone characteristics of young pigs fed soybean meal-based semi-purified diets containing varying amounts of phosphorus and supplemental phytase

	Coefficients of equations[a]						
Item	α_0	α_1	α_2	α_3	α_4	P-value	R^2
Performance, 1-5 wk							
ADG, g	5.500	.0914	.0099	.0054	.0080	.001	.63
ADFI, kg	-.5949	.0557	.0027	.0030	.0118	.001	.52
Gain/feed, g/kg	6.094	.0357	.0072	.0023	-.0038	.011	.33
Apparent Digestibility Coefficients, %							
Dry matter	4.524	-.0096	-.0003	-.0002	.0013	.042	.12
Phosphorus	3.486	.1750	-.0055	.0044	.0037	.001	.46
Calcium	4.207	.0251	-.0021	.0007	.0039	.388	.05
Nitrogen	4.524	-.0129	.0007	-.0009	.0011	.040	.12
Excretion of P, g/d	-.1600	.2870	-.0085	.0005	.0079	.001	.69
Bone Characteristics[b]							
Fourth metacarpal							
Shear force	.8613	.2653	.0218	.0056	.0018	.001	.52
Shear stress	.9842	.2007	.0211	.0094	.0022	.015	.23
Shear energy	.1585	.2914	.0234	.0046	.0001	.001	.41
Ash, %	3.036	.1228	.0097	.0037	.0038	.001	.71
Tenth rib							
Shear force	-1.630	.4294	.0394	.0102	.0097	.001	.75
Shear stress	.1861	.2682	.0236	.0055	-.0071	.003	.30
Shear energy	-1.346	.5246	.0308	.0062	.0026	.001	.58
Ash,%	3.368	.1904	.0173	.0031	-.0021	.001	.81

[a]Model: $LnY = \alpha_0 + \alpha_1 D_1 + \alpha_2 LnX + \alpha_3 (LnX)^2 + \alpha_4 D_1 LnX$.
D_1 = available P (aP), %. When aP = .05%, $D_1 = 0$; when aP = .16%, $D_1 = 1$.
X = added phytase, U/kg of diet.
[b]Units are N, N/cm^2 and N-mm, respectively for shear force, stress and energy.

Table 3-4. Nonlinear or linear functions for performance, apparent digestibility coefficients and bone characteristics of young pigs fed soybean meal-based semi-purified diets containing varying amounts of phosphorus and supplemental phytase

Item	Equation[a]	P-value	r^2
	.05% aP		
Performance, 1-5 wk			
ADG, g	$Y = 446.9(1 - .335e^{-.0003X})$		.99
ADFI, kg	$Y = .621 + .00003X$	.074	.71
Gain:Feed, g/kg	$Y = 529.2(1 - .108e^{-.0022X})$		.99
Apparent digestibility coefficients of P, %			
	$Y = 43.86(1 - .198e^{-.0019X})$		.93
Bone characteristics[b]			
Fourth metacarpal			
Shear force	$Y = .571 + .00006X$	.006	.94
Shear stress	$Y = .624 + .000043X$	.613	.10
Shear energy	$Y = 1.848(1 - .2355e^{-.0015X})$		.58
Ash, %	$Y = 25.33 + .0011X$	.136	.58
Tenth rib			
Shear force	$Y = .487(1 - .290e^{-.0012X})$		.60
Shear stress	$Y = 1.63 + .0003X$	.124	.60
Shear energy	$Y = .490(1 - .325e^{-.0011X})$		.98
Ash, %	$Y = 39.65(1 - .158e^{-.0015X})$		.87
	.16% aP		
Performance, 1-5 wk			
ADG, g	$Y = 416.1(1 - .2540e^{-.0016X})$		.93
ADFI, kg	$Y = .758(1 - .186e^{-.0027X})$		.88
Gain:Feed, g/kg	$Y = 605.9(1 - .173e^{-.0003X})$		.98
Apparent digestibility coefficients of P, %			
	$Y = 51.85(1 - .209e^{-.0036X})$		.88
Bone characteristics[b]			
Fourth metacarpal			
Shear force	$Y = .743 + .00007X$	.033	.82
Shear stress	$Y = .824(1 - .109e^{-.0036X})$		.91
Shear energy	$Y = 2.206(1 - .1536e^{-.0028X})$		.60
Ash, %	$Y = 28.23 + .0027X$	.004	.96
Tenth rib			
Shear force	$Y = .703(1 - .346e^{-.0022X})$		.91
Shear stress	$Y = 2.030 + .0003X$	.032	.83
Shear energy	$Y = .773(1 - .255e^{-.0024X})$		.55
Ash, %	$Y = 46.66(1 - .146e^{-.0014X})$		.96

[a]X = Added phytase, U/kg of diet.
[b]Units are N, N/cm^2 and N-mm, respectively for shear force, stress and energy.

Fecal P excretion was linearly increased ($P < .001$) when the amount of aP (without phytase) was increased. Addition of graded levels of phytase decreased fecal P excretion ($P < .01$) up to about 1,050 U of phytase/kg of diet in the .05% aP diets and up to about 700 U of phytase/kg of diet in the .16% aP diets. Supplementing 1,400 U of phytase/kg of diet to the .32% aP diet decreased ($P < .001$) fecal P excretion.

Bone Characteristics

Increasing levels of aP linearly increased shear force ($P < .001$), shear stress ($P < .10$), shear energy ($P < .05$) and percentage of ash ($P < .001$) in metacarpals and tenth ribs (Tables 3-5). Adding graded levels of phytase linearly increased shear force ($P < .05$), shear energy ($P < .10$), and ash percentage ($P < .001$) of metacarpals; whereas, shear stress was not consistently affected by phytase addition. Addition of graded levels of phytase linearly increased ($P < .01$) all the characteristics of tenth ribs with the quadratic effect significant for shear force ($P < .01$) and ash percentage ($P < .01$). Supplementation of 1,400 U of phytase/kg of diet to the diet containing .32% aP improved shear force ($P < .01$), shear stress ($P < .05$) and ash percentage ($P < .001$) of metacarpals, and shear energy ($P < .001$) of the ribs. Response curve equations were developed (Tables 3-3 and 3-4).

Sensitive Indicators

The second order translog equations from the measurements of ADG, ADFI showed R^2 values .63 and .52 ($P < .001$); whereas, the equation of gain:feed had a low R^2 (.33; Table 3-3). The equations for P absorption had an R^2 of .46 ($P < .001$); and the equations for DM coefficients, Ca and N absorption had low R^2 (.12, .05, and .12, respectively). The equation for fecal P excretion had an R^2 of .69 ($P < .001$). For measurements of bone characteristics, the equations for shear force and ash percentage

Table 3-5. Metacarpal and rib characteristics of young pigs fed soybean meal-based, semi-purified diets containing varying amounts of phosphorus and supplemental phytase[a]

Diets	1	2	3	4	5	6	7	8	9	10	11	12	
Available P, %			.05					.16			.32		
Added phytase (U/kg diet)	0	350	700	1,050	1,400	0	350	700	1,050	1,400	0	1,400	SEM
Meta. shear force	.58	.58	.61	.64	.66	.73	.78	.78	.84	.83	1.03	1.23	.04
Meta. shear stress	.69	.63	.55	.65	.76	.74	.79	.84	.81	.82	.82	.98	.07
Meta. shear energy	1.46	1.43	1.80	1.88	1.68	1.87	2.09	2.08	2.37	2.09	3.21	3.48	.18
Meta. ash, %	25.8	25.3	25.5	27.0	26.9	28.2	29.4	29.7	31.4	31.9	33.9	37.3	.84
Rib shear force	.35	.38	.41	.51	.43	.46	.57	.65	.73	.66	.99	1.06	.04
Rib shear stress	1.67	1.61	1.88	2.17	1.94	1.99	2.10	2.28	2.39	2.32	2.72	2.43	.20
Rib shear energy	.33	.39	.40	.44	.46	.59	.73	.64	.84	.74	1.07	1.51	.06
Rib ash, %	33.3	36.5	36.3	39.5	38.5	39.9	42.4	43.7	45.8	45.3	50.1	52.0	.61

	Probability											
	PY[b]	aP	PY*aP	Lin	Lin 1	Lin 2	Quad	Quad 1	Quad 2	Con 1[c]	Con 2[d]	Con 3[e]
Meta. shear force	.188	.001	.892	.017	.120	.060	.895	.581	.444	.002	.001	.015
Meta. shear stress	.433	.001	.369	.350	.586	.431	.164	.020	.690	.047	.087	.170
Meta. shear energy	.041	.001	.727	.076	.077	.430	.095	.580	.085	.917	.054	.753
Meta. ssh, %	.001	.001	.130	.001	.014	.001	.436	.198	.865	.001	.001	.014
Rib shear force	.001	.001	.135	.001	.003	.001	.002	.203	.002	.975	.001	.001
Rib shear stress	.001	.034	.695	.004	.068	.028	.415	.369	.793	.440	.006	.108
Rib shear energy	.001	.001	.312	.001	.004	.001	.075	.757	.035	.001	.001	.077
Rib ash, %	.001	.001	.617	.001	.001	.001	.003	.068	.022	.212	.001	.048

[a]Four pigs per treatment Mean. Units are N, N/cm^2, and N-mm, respectively for shear force, stress, and energy.
[b]PY = added phytase, U/kg diet.
[c]Con 1 = contrast for diet 11 vs 12.
[d]Con 2 = contrast for diet 1, 6, and 11, linear.
[e]Con 3 = contrast for diet 1, 6, and 11, quadratic.

showed R^2 values ranged from .52 to .81; whereas the equation of shear stress had a low R^2 (< .30) and the R^2 values for shear energy were intermediate. Measurements of ADG, ADFI, P apparent absorption coefficient (%), fecal P excretion (g/d), shear force and ash percentage of fourth metacarpals and tenth ribs were determined to be sensitive indicators for evaluating the effects of phytase for improving the availability of phytate P in soybean meal for pigs.

Phosphorus Equivalency Values of Phytase

Phosphorus equivalency values of phytase were calculated using response equations for graded levels of aP and phytase for all the measurements except bone stress, apparent digestibility and absorption of DM, Ca, and N, and P excretion (Table 3-6). However, only P equivalency values for ADG and apparent absorption of P were used for calculating released P. These two measurements were chosen because of their sensitivity and economic importance, and relative ease of determination. Adding phytase at 250, 500, 750, and 1,000 U/kg of diet could replace .52, .83, 1.08, and 1.21 g of inorganic P as DFP in the diet. The released P from phytate P was 40, 64, 83, and 93%, respectively. The total amount of P released increased as the phytase level increased, but the amount of P released per 100 U of phytase decreased. The replacement of 1 g of P would require about 777 U of microbial phytase when the .05% aP level was fed; about 623 U of phytase would be required when the .16% aP level was fed. About 676 U of phytase would be required to release 1 g of P when the two aP levels are combined.

Table 3-6. Phosphorus equivalency values of microbial phytase for young pigs fed soybean meal-based semi-purified diets containing varying amounts of phosphorus and supplemental phytase

Available P, %	.05				.16						
Added phytase (U/kg diet)	250	500	750	1,000	250	500	750	1,000	Equation[a,b]	P-value	R^2
Equivalent of aP, %											
Avg daily gain	.105	.130	.150	.165	.185	.230	.265	.285	Y = 256.5 + 487.0X	.001	.89
Avg daily feed intake	.100	.120	.135	.155	.235	.320	.360	.380	Y = .584 + .433X	.046	.74
Gain:feed	.125	.165	.190	.200	.160	.175	.195	.210	Y = 449.1 + 379.2X	.001	.98
Absorption of P	.105	.130	.150	.160	.230	.260	.285	.290	Y = 31.0 + 71.6X	.001	.99
Metacarpal shear force	.060	.070	.080	.090	.170	.180	.190	.200	Y = .486 + 1.66X	.045	.99
Metacarpal shear energy	.060	.075	.085	.090	.135	.150	.155	.160	Y = 1.156 + 6.47X	.123	.99
Metacarpal ash percentage	.050	.060	.070	.075	.165	.195	.215	.245	Y = 23.94 + 30.3X	.082	.98
Tenth rib shear force	.085	.095	.105	.110	.160	.185	.200	.205	Y = .174 + 2.429X	.162	.94
Tenth rib shear energy	.070	.080	.090	.095	.180	.195	.205	.210	Y = .174 + 2.77X	.041	.99
Tenth rib ash percentage	.085	.110	.125	.135	.200	.225	.245	.255	Y = 30.1 + 62.35X	.010	.99
Mean equivalent of aP, %[c]	.105	.130	.150	.163	.208	.245	.275	.288			
Released P, %[d]	.055	.080	.100	.113	.048	.085	.115	.128			
% of phytate P[e,f]	42.3	61.5	76.9	86.9	36.9	65.4	88.5	98.5			
Mean of the two aP levels											
Release P, %[g]					.052	.083	.108	.121			
% of phytate P[h]					39.6	63.5	82.7	92.7			
Released P (%)/100 U of phytase[i]					.021	.017	.014	.012			

[a]Equations for phytase, see Table 3-4.
[b]Y = response; X = available P, %.
[c]Mean equivalent of aP comes from the data of average daily gain and absorption of P.
[d]Equivalency equation $Y = .3910 - .3074e^{-.0003X}$ at .05% aP; $Y = .3277 - .2170e^{-.0016X}$ at .16% aP.
[e]Phytate P (60% total P) in this diet is .13%.
[f]Equivalency equation $Y = .1796 - .1213e^{-.0019X}$ at .05% aP; $Y = .2912 - .1514e^{-.0036X}$ at .16% aP.
[g]$Y = 1.546 - 1.504e^{-.0015X}$. Y = released P (g/kg) and X = phytase activity (U/kg of diet).
[h]$Y = 118.0 - 115.2e^{-.0016X}$. Y = % of phytate P released and X = phytase activity (U/kg of diet).
[i]$LnY = -4.60 + 1.31LnX - 138(LnX)^2$. Y = released P (g)/100 U of phytase and X = phytase activity (U/kg of diet).

Discussion

Effectiveness of Natuphos® Phytase

The results of this experiment indicate that Natuphos® phytase is effective in improving P availability from phytate P in soybean meal for young pigs. Adding 1,050 U of phytase to the basal diet increased ADG by 30% (Table 3-2), ADFI by 24%, and gain:feed by 11% at .05% aP, increased P apparent absorption by 23%, deceased fecal P excretion by 10% at .05% aP (at 700 U phytase of .16% aP diet), and increased bone shear force by 40% (Table 3-3). These results agree with other findings in pigs (Jongbloed et al., 1992; Cromwell et al., 1993a,b; Lei et al., 1993a,b). Phytase improved the apparent absorption of phytate bound P in soybean meal, and the increased P absorbed was well utilized, as evidenced by improved bone mineralization.

Maximum Responses

The maximum apparent absorption of P appears to occur at phytase levels of 700 to 1,050 U/kg of diet in the soybean meal-based semi-purified diet with .05 or .16% aP (Figure 3-1). The addition of 1,400 U of phytase/kg of diet produced a lower response than the 1,050 U of phytase/kg of diet. The response curves for ADG, shear force, and ash percentage of metacarpal and tenth rib (Figure 3-1, Tables 3-2 and 3-5) also indicated that the maximum response levels of phytase were at the range of 700 to 1,000 U/kg of diet. The results of Lei et al. (1993b) indicated that the response to added Finase® phytase appeared to reach a plateau at approximately 1,200 U/kg of diet in weanling pigs fed corn-soybean meal diets with .05% aP. Veum et al. (1994) demonstrated that the maximum response of added Natuphos® phytase ranged between 800 to 1,200 U/kg of diet for

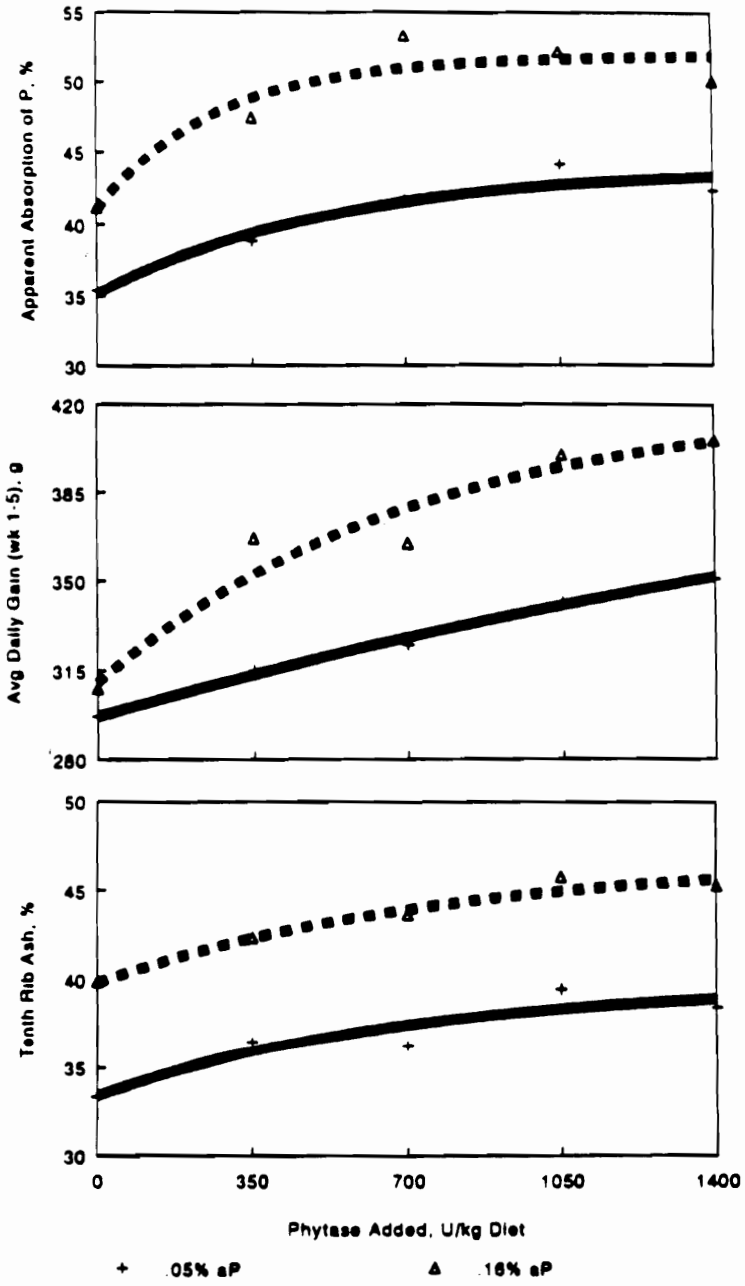

Figure 3-1. Response curves of young pigs fed soybean meal based, semi-purified diets containing two levels of phosphorus and five levels of phytase. Top: P absorption; Middle: average daily gain; Bottom: tenth rib ash percentage. For the equations see Table 3-4.

growing pigs fed a canola-grain sorghum diet with .06% aP (.44% tP). It appears that the maximum response of added phytase is below or around 1,000 U/kg of diet when included in low P diets of pigs.

In comparison to the .32% aP diet (positive control), the maximum performance (combining ADG, ADFI, and gain:feed) of pigs with phytase addition to .05 or .16% aP diets was 88.4 or 97.4% of the DFP response. Similarly, the maximum P absorption of pigs with phytase addition were 80.9 and 97.8% of the positive control diet. The maximum responses of bone (combining metacarpal and tenth rib) shear force, stress, energy, and ash percentage of pigs fed .05 or .16% aP diets with added phytase compared with the positive control were 57.8 or 77.7%, 86.3 or 95.2%, 50.8 or 76.2%, and 79.3 or 92.7%, respectively. All the responses from phytase addition at .05% aP diets were lower than those at .16% aP diets. A partial explanation is that the Ca to aP ratio in .05% aP diets was wider than that in .16% aP diets (8.6 vs 4.0), though both of them had the same Ca to tP ratio (Table 3-1). The wider Ca to aP ratio may impair the utilization of P (Qian *et al.*, 1995). The addition of phytase will also improve the availability of Ca, probably through the release of Ca from the phytate complex, thus the effect of a widening Ca:tP may be worsened. Another possible reason is that the amounts of available Ca and P (.43 % Ca and .13% aP) in .05% aP diets after phytase addition were still deficient; whereas, the amounts of available Ca and P (.64% Ca and .24% aP) in .16% aP diets after phytase addition were close to the level of the NRC (1988) recommendations.

Phosphorus Excretion

Adding phytase to the low aP diet linearly or quadratically decreased fecal P excretion, whereas adding inorganic P increased fecal P excretion (Table 3-2). In comparison to the diet of .32% aP (NRC recommended level), fecal P excretion decreased 25 to 50% by addition of phytase to the low aP diets. The results presented here agree with other findings in pigs fed corn-soybean meal diets (Jongbloed et al., 1992; Cromwell et al., 1993a; Lei et al., 1993b). Obviously, adding phytase to pig diets provides an important means of environmental protection, because P can be considered an environmental pollutant which contaminates surface soil and water (Cromwell and Coffey, 1991). It is estimated that in the US swine annually produce approximately 460,000 tons of P as a waste product (Sweeten, 1992). A 30% reduction in P excretion would mean about 138,000 tons less P excreted by swine annually in the US.

Phosphorus Equivalency Values of Phytase

Using the P equivalency function shown in Table 3-6 ($Y = 1.546 - 1.504e^{-0015X}$), the replacement of 1 g of inorganic P as DFP would require about 676 U of phytase. The P equivalency values are close to those calculated from published data using the same type of diets, or in corn-soybean meal diets for pigs (Yi et al., 1994). Hoppe et al. (1993) reported 1 g P from monocalcium phosphate (MCP) was equivalent to 380 U of phytase when based on P retention and 403 U of phytase when based on phalanx crude ash, when pigs were fed a corn-oat-soybean meal-based diet. In a review paper, Hoppe and Schwarz (1993) concluded that for diets based mainly on corn-soybean meal, 500 U of phytase was equivalent to 1 g P from MCP (= .8 g digestible P).

Percentage of Phosphorus Released from Phytate

Using the P equivalency equations generated in this study and based on additions of 250, 500, 750, and 1,000 U of phytase/kg of diet, 40, 64, 83, and 93% of the phytate P in soybean meal was released. Cromwell et al. (1993b) observed that adding 250, 500, and 1,000 U of phytase/kg of diet would release 14, 22, and 43% of the phytate P in a soybean meal-based semi-purified diet fed to growing pigs in terms of the measurements of bone shear force. Hoppe et al. (1993), in a study using pigs fed a grain-soybean meal diet, reported that adding 250, 500, and 1,000 U of phytase/kg of diet released 15, 42, and 57% of the phytate P, based on bone ash content, and P and Ca retention. Our results are also supported by the observation of Jongbloed et al. (1992) who reported that the ileal digestibility of phytic acid was increased from 9 to 59% by addition of 1,500 U of phytase/kg of diet to a corn-soybean meal diet fed to growing pigs.

Improvement of "Extra-phosphorus"

The results of this experiment indicate that adding phytase (1,400 U/kg of diet) to the diets containing near the recommended level of P (.32% aP and .48% tP) further improved several response measurements; the apparent absorption of P was increased 13% ($P < .002$), metacarpal shear force was increased 19%, and ash percentage was increased 10% ($P < .001$). Similar results were also found in young pigs fed corn-soybean meal basal diets (Kornegay and Qian, 1994) or canola-grain sorghum basal diets (Veum et al., 1994). Other supporting evidence is that as response equations for the data of ADG and ADFI from five levels of phytase (0, 350, 700, 1,050, and 1,400 U/kg diet) and three levels of aP without phytase (.05, .16, and .32%) were used to calculate P equivalency

values of phytase, the P released from phytate in soybean meal approached 100% (Table 3-6). The extra beneficial effects could have come from the improved utilization of other nutrients. Therefore, phytase not only improves P utilization, but may also improve the utilization of other nutrients such as CP, AA, other minerals and trace minerals bound to phytate. Supporting evidence has also been provided in the studies of Pallauf et al. (1992, 1994), Lei et al. (1993c), and Mroz et al. (1994).

Effects of Phytase on Calcium and Nitrogen

Effects of P and phytase additions resulted in only small and variable changes in the apparent digestibility of DM, and absorption of Ca and N in this study. Phytic acid exists as phytin in plants (Cosgrove, 1980). Because of its anionic phosphate groups, phytate possesses the ability to bind with cationic minerals and cationic groups of protein (Reddy et al., 1982; Morris, 1986; Champagne et al., 1990). Ketaren et al. (1993) found that addition of phytase increased growth rate, protein deposition, protein retention and energy retention, but had no effect on the apparent digestibility of DM and CP. Mroz et al.(1994) reported that addition of phytase enhanced the apparent digestibilities of DM, OM, Ca, CP, and AA. Clearly, further research is needed to determine the effects of adding phytase on the utilization of CP, amino acids, other minerals and trace minerals bound to phytic acid in the diets of pigs and poultry.

Sensitive Indicators

Results in this experiments indicate that the measurements of ADG, ADFI, P apparent digestibility coefficient (%), fecal P excretion (g/d), shear force and ash percentage of metacarpal and tenth rib are relatively sensitive indicators. Average daily

gain and ADFI, especially ADG, of young pigs were also found to be sensitive indicators in the studies of Cromwell et al. (1993a,b) and Lei et al. (1993a,b) with addition of graded levels of phytase. The apparent absorption coefficient of P was considered the best indicator to determine the nutritional values of feed phosphates in the study of Dellaert et al. (1991). Apparent absorption of P measured with .10% chromic oxide in the diets of presented experiment support their observation.

Bone indicators are often used to evaluate P and Ca status in pigs (Koch and Mahan, 1985; NRC, 1988; Combs et al., 1991a,b). Generally, metacarpal, metatarsal and femur of pigs were used. In this experiment, the measurements from shear force and ash percentage of tenth rib were sensitive. Shear energy is the energy required to deform a bone to the point of fracture, which represents the area (Newton-mm) under the force-deformation curve up to the point of fracture. Bone shear energy was sensitive, but not as sensitive as shear force and ash percentage. In this study, both metacarpal and tenth rib shear stress showed low sensitivity. As both shear force and area increase, shear stress tends to remain constant. Similar responses were observed by Kornegay and Qian (1994).

Implications

Natuphos® phytase was effective in improving P bioavailability from phytate in soybean meal for pigs and provided a means of decreasing P in pig manure. The P equivalency values of microbial phytase for 1 g P as DFP was about 676 U when the data for .05 and .16% aP were averaged. This represents 40, 64, 83, and 93% of P released from phytate when 250, 500, 750, and 1,000 U of phytase/kg of diet were added. As the phytase level increased, the total amount of P released increased, but the amount of P

released per 100 U of phytase decreased. Adding phytase to the highest level of aP (.32%) further improved P availability and decreased fecal P excretion. Phytase may improve the utilization of Ca, CP, and DM in pigs. The measurements of ADG, ADFI, P apparent absorption, bone ash percentage, and shear force are sensitive indicators.

Chapter IV

Response of Broilers to Graded Levels of Natuphos® Phytase Added to Corn-Soybean Meal-Based Diets Containing Three Levels of Nonphytate Phosphorus

ABSTRACT: Male day-old broilers (n = 920) were fed 0, 200, 400, 600, 800, 1,000, and 1,200 U of phytase/kg of diet in combination with .20, .27 or .34% nonphytate P (nP) in a 21-d trial. Total P (tP) levels were .44, .51 or .58%, respectively. In addition to these 21 diets, a positive control P diet supplied .45% nP (.69% tP). A Ca to tP ratio of 2:1 was maintained except a 1.45:1 in the control diet. Phytase additions linearly increased ($P <$ .01) BW gain, feed intake, toe ash percentage, and apparent retention (% of intake) or total amount (g/bird) of retained Ca and P, and linearly decreased ($P <$ .01) P excretion (g/kg of DM intake) at each level of nP, with the magnitude of the response inversely related to the level of nP. Above normal mortality was observed only in the .20% nP diet without phytase. Adding nP linearly increased ($P <$ .01) BW gain, feed intake, toe ash percentage, Ca retention (% of intake and g/bird), total amount (g/bird) of P retained, and P excretion, and linearly decreased ($P <$ .01) apparent retention (%) of P. Based on an assessment for the R^2 values of the second order translog equations, BW gain, feed intake, toe ash percentage, appparent retention (% of intake) of Ca and P, total amount of retention (g/bird) of DM, Ca, and P were sensitive indicators. Derived linear or nonlinear response equations for BW gain and toe ash percentage at the two lower nP (.20 and .27%) levels were used to calculate P equivalency values of microbial phytase. Results show that 939 U of microbial phytase is equivalent to 1 g of P from defluorinated phosphate in broiler starter diets using corn-soybean meal as the source of phytate P. The

amount of P released per 100 U of phytase decreased as the total amount of phytase increased.

Key Words: Phytase, Broilers, Phosphorus equivalency

Introduction

It has been documented that microbial phytase is effective in releasing a significant portion of the P bound in phytate present in corn and soybean meal and making it available to broilers (Nelson et al., 1968; Simons et al., 1990; Schoner et al., 1991). Phosphorus excretion of broilers can be reduced when supplemental phytase is included in the diets (Schoner et al., 1990; Simons et al., 1990). The amount of P excreted would be related to the nonphytate and total P (tP) fed and to the level of supplemental phytase. Body weight gain, feed intake and toe ash percentage of poultry fed soybean meal-based semi-purified diets were sensitive indicators for assessing the efficacy of phytase for broilers and turkey poults fed soybean meal-based semi-purified diets (Denbow et al., 1995; Ravindran et al., 1995), and broilers and turkey poults fed corn-soybean meal diets (Potter, 1988; Potter et al., 1995). These measurements may be sensitive for broilers fed corn-soybean meal diets.

Apparent retention (%) or total amount of retention (g/bird) of P and Ca of broilers may also be sensitive indicators for evaluating P availability (Schoner et al. 1993). Only limited information is available about P equivalency values of phytase in broilers fed corn-soybean meal diets. Schoner et al. (1991) reported that 700 U of microbial phytase was equivalent to 1 g P as monocalcium phosphate (MCP) when based on P retention of broilers fed a corn-soybean meal diet for 14 d; a value of 762 U of phytase was obtained when the calculation was based on crude ash in the body. Schoner et al. (1993), using a similar diet, reported that 570 U of phytase was equivalent to 1 g P as MCP based on BW gain at 14 d and 1,050 U of phytase was equal to 1 g P when based on P retention. At 40

d, 850 U of phytase was equal to 1 g P for both measurements. In each of the above studies (Schoner et al., 1991, 1993), only one level of P was used in each of the trials.

The purpose of this study was: (1) to study the response of broilers to graded levels of microbial phytase added to corn-soybean meal-based diets containing three levels of nonphytate P, (2) to evaluate several measurements for their usefulness in predicting the responses to P and phytase additions, and (3) to determine P equivalency values of phytase.

Materials and Methods

Birds and Treatments

Peterson x Arbor Acres male broiler chicks (n = 920) were used in a 3 x 7 factorial arrangement of treatments with four replicate pens (10 birds/pen) to evaluate the response of broilers to seven levels of phytase (Natuphos®; BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07823-1234) in combination with three levels of nonphytate P (nP). Dietary P levels were formulated at .20, .27, and .34% nP (or .44, .51, and .58% tP), respectively, and each level of P was supplemented with 0, 200, 400, 600, 800, 1,000, and 1,200 U of phytase/kg of diet. A U of phytase activity is defined as the quantity of enzyme which liberates 1 μmol of inorganic P per min. from 5.1 mM sodium phytate at pH 5.5 and 37 °C (Engelen et al., 1994). These dietary P levels were formulated below the current NRC (1994) recommendations to ensure maximum responses with phytase additions. In addition to the 21 diets described above, a positive control diet was formulated to supply the recommended level of .45% nP or .69% total P.

Diets and Management

Composition of the diets is shown in Table 4-1. Since the nP level of .15% supplied by the corn and soybean meal was thought to be inadequate, inorganic P (.05%) was added to increase the nP level to .20%; this level of P without phytase, however, was

Table 4-1. Percentage composition and calculated analysis of the basal diets

Item	Nonphytate phosphorus, %			
	.20[a]	.27[a]	.34[a]	.45[b]
Ingredients	----------%----------			
Ground yellow corn	57.81	57.39	56.98	57.29
Soybean meal (48.5% CP)	37.10	37.10	37.10	37.10
Stabilized fat	2.00	2.00	2.00	2.00
Ground limestone	1.79	1.82	1.84	0.92
Defluorinated phosphate[c]	.30	.69	1.08	1.69
Vitamin premix[d]	.20	.20	.20	.20
Trace mineral premix[e]	.20	.20	.20	.20
Salt	.40	.40	.40	.40
DL-methionine	.20	.20	.20	.20
Calculated analysis, %				
Crude protein	23.07	23.04	23.00	23.03
Lysine	1.32	1.32	1.32	1.32
Methionine & Cysteine	.93	.93	.93	.93
Ca	.88	1.02	1.16	1.00
Total P	.44	.51	.58	.69
Nonphytate P	.20	.27	.34	.45

[a]Zero, 200, 400, 600, 800, 1,000 and 1,200 U phytase per kilogram of diet were added to each of the basal diets. Phytase (Natuphos® - 5,000 U/g) was supplied by BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234. Based on NRC (1984), the calculated phytate P in the diets was .24%.

[b]Positive control diet; no phytase was added.

[c]Fine CDP, Southern Bag Corp., Bluefield, VA 24605.

[d]Supplied per kilogram of diet: retinyl acetate, 908 μg; cholecalciferol, 66 μg; dl-α-topherol acetate, 26 mg; menadione sodium bisulfite complex, .75 mg; riboflavin, 7.5 mg; d-calcium pantothenate, 9.7 mg; niacin, 26.4 mg; cyanocobalamin, .011 mg; choline chloride, 1,012 mg; d-biotin, .31 mg; folic aicd, 3.1; thiamin•HCl, 8 mg; pyridoxine HCl, 3.1 mg; ethoxyquin, 50 mg; and virginiamycin, 2.9 mg.

[e]Supplied per kilogram of diet: manganese, 88 mg; zinc, 95 mg; iron, 100 mg; copper, 12.5 mg; iodine, 4 mg; and selenium, .6 mg.

expected to result in some mortality (Potter et al., 1995). The desired levels of nP in the basal diets were achieved by adding varying levels of defluorinated phosphate (DFP). The Ca to tP ratio was maintained at 2:1 in all diets, except the positive control diet that had a ratio of 1.45:1. Limestone and DFP were added to the diets at the expense of corn. Since the phytate was supplied from only the corn and soybean meal, the dietary percentage of phytate P (.24%) was similar in all diets based on NRC (1984). The birds were housed in electrically heated, raised wire-floored starting batteries in an environmentally controlled room. The treatments were assigned randomly to 4 pens of 10 chicks each except for the positive control diet, which had 8 pens of 10 chicks each. The diets were fed in mash form from 1 to 21 d of age. Birds had *ad libitum* access to feed and water at all times. The care and treatment of broilers followed published guidelines (Consortium 1988). Body weights and feed intake were recorded on a pen basis at weekly intervals. Mortality were recorded daily.

Sampling and Analysis

During the third week (d 18 to 20), the total excreta from each pen were collected. Feed intake and production of excreta were measured quantitatively per pen over 3 consecutive d. Excreta from each pen were stored in plastic bags at -20 °C. After thawing, excreta were dried in an oven at 70 °C and weighed. Excreta, along with diet samples, were ground to pass a 1 mm sieve. Dry matter was determined according to AOAC (1990) procedures. Following a nitric-perchloric acid wet digestion, P concentrations were determined photometrically and Ca contents were determined with an atomic absorption spectrophotometer. Apparent retention (% of intake or g/bird) of P and Ca during the 3 d were calculated for each dietary treatment.

On d 21, all surviving chicks were killed. Toe samples were obtained by severing the middle toe through the joint between the 2nd and 3rd tarsal bones from the distal end.

The left middle toes of all chicks within a pen were pooled, and the right middle toes from the same chicks were pooled, yielding two samples of toes per pen. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 600 °C for 4 h (Potter, 1988). Toe ash was expressed as a percentage of dry weight.

Statistical Analysis and Calculation of Phosphorus Equivalency Values

Data were analyzed by the GLM procedures of SAS (1990) with pen means as the experimental unit except toe ash percentage which included foot (left and right toes). Linear and quadratic effects of added phytase within each P level and comparisons between dietary P levels were tested using nonorthogonal contrast polynomials. Second order translog equations were derived for the 3 x 7 factorial arrangement of treatments with the model:

$$LnY = \alpha_0 + \alpha_1 LnX_1 + \alpha_2 LnX_2 + \alpha_3 (LnX_1)^2 + \alpha_4 (LnX_2)^2 + \alpha_5 LnX_1 LnX_2;$$

where Y = response measurements; X_1 = nP (%); and X_2 = phytase added (U/kg of diet). Nonlinear or linear functions which best fit the data were also derived for the seven phytase levels at each P level and for the four P levels (.20, .27, .34, and .45% nP) without addition of phytase, with the nonlinear model $Y = a(1 - be^{-kX})$ and the linear model $Y = a + bX$. Where, Y = response measurements; and X = nP (%) or phytase added (U/kg diet). Nonlinear and linear equations for nP (no added phytase) and for phytase at the three levels of nP for all measurements were examined for high r^2. The R^2 values of the second order translog equations were also examined. Because of high R^2 values and economic importance or ease of obtaining, BW gain and toe ash percentage were used to generate the P equivalency equations. The equations for nP and for added phytase at each of the two lower levels of nP (.20 and .27%) were set equal. For example, the equation for BW gain at .20% nP was as follows:

$$625.3(1 - 3.09e^{-10.3Y}) = 557.1(1 - .312e^{-.0036X})$$

$$Y = -.0971Ln(.0353 - .09\ e^{-.0036X})$$

where, Y = nP (%); X = phytase added (U/kg of diet).

The resulting equations were used to calculate the equivalent nP (%) at 250, 500, 750, and 1,000 U of phytase/kg of diet in order to compare the data with results of other studies.

Results

Performance and Toe Ash Percentage

Body weight gains were increased ($P < .001$) by dietary P and phytase additions (Table 4-2), but a P level x phytase level interaction ($P < .001$) was observed. Gains were improved by phytase additions at all P levels; however, the magnitude of the response was greatest at lower rates of phytase addition for the lower nP levels (Figure 4-1A). At .20% nP level, gains improved ($P < .001$) up to 1,000 U of phytase/kg of diet and then reached a plateau. The response appeared to reach a plateau at 800 U/kg of diet for broilers fed diets containing .27% nP ($P < .001$). On the .34% nP diet, the response appeared to reach a plateau at 600 U/kg of diet levels ($P < .01$).

Total feed intake followed a similar pattern to that of average BW gains (Table 4-2). The addition of both nP and phytase increased feed intake, with the greatest response for phytase at the lowest nP (.20%) level. Gain:feed (g/kg) was linearly improved by increasing dietary nP level ($P < .05$). The addition of phytase improved gain:feed only at .20% nP ($P < .01$). The R^2 values of all response equations of gain:feed were low. Dietary additions of nP ($P < .001$) and phytase ($P < .001$) linearly increased toe ash percentage (Table 4-3). The response to phytase was observed at all levels of nP, although the magnitude of the response seemed to decrease as level of nP increased (Figure 4-1B).

Table 4-2. Body weight gain, feed intake, and gain:feed of broilers fed corn-soybean meal-based diets containing varying amounts of nonphytate phosphorus and supplemental phytase from 1 to 21 days of age[a]

Phytase added	Nonphytate P, %			
(U/kg diet)	.20	.27	.34	.45
BW gain, g/bird[b]				
0	380[c]	511[d]	562[e]	613
200	487	544	581	
400	508	556	580	
600	524	564	595	
800	548	587	598	
1,000	560	585	594	
1,200	557	595	612	
Total feed intake, g/bird[b]				
0	652[c]	835[d]	906[f]	963
200	810	873	915	
400	819	876	903	
600	876	924	943	
800	905	929	946	
1,000	860	918	933	
1,200	872	935	965	
Gain:feed, g/kgg				
0	584[h]	613	620	636
200	604	624	644	
400	622	634	643	
600	599	610	631	
800	606	632	633	
1,000	652	638	637	
1,200	642	637	636	

[a]Four pens of 10 chicks each per mean, except .45% nP which had 8 pens of 10 chicks each). The root MSE were 26, 38, and 30, respectively for BW gain, total feed intake and gain:feed. The pooled SEM for a single treatment mean would equal MSE/$\sqrt{n}$.

[b]Phosphorus main effect (linear, $P < .001$; quadratic, $P < .02$); P x phytase interaction ($P < 001$).

[c]Phytase effect (linear, $P < .001$; quadratic, $P < .001$).

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

[e]Phytase effect (linear, $P < .005$).

[f]Phytase effect (linear, $P < .015$).

[g]Phosphorus main effect (linear, $P < .03$).

[h]Phytase effect (linear, $P < .003$).

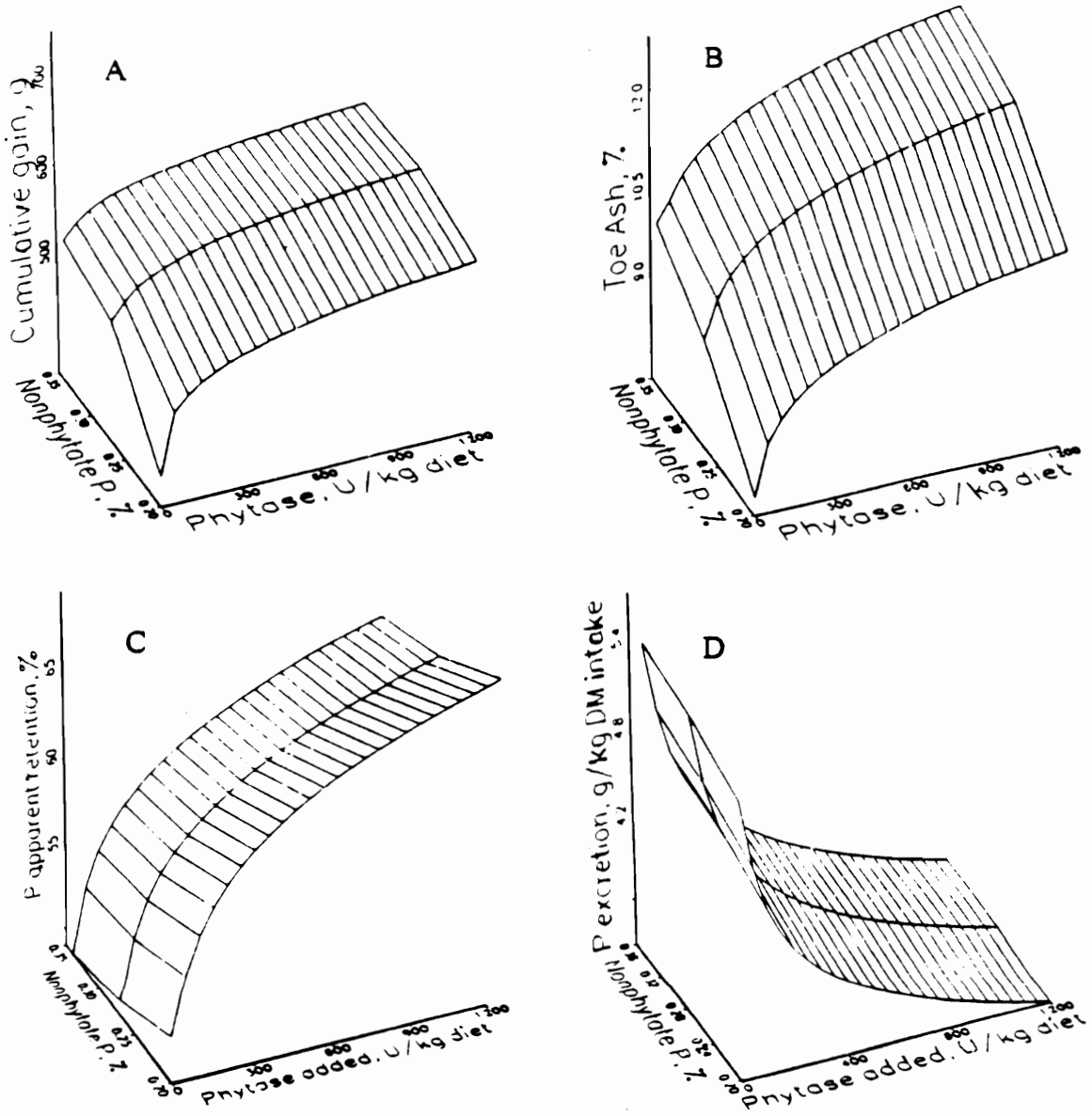

Figure 4-1. Influence of dietary nonphytate P and phytase additions on body weight gain (A), toe ash percentage (B), P retention (C), and P excretion (D) of broilers fed a corn-soybean meal-based diet. For the equations see Table 4-6.

Table 4-3. Ash percentage of dried toes from broilers fed corn-soybean meal-based diets containing varying amounts of nonphytate phosphorus and supplemental phytase from 1 to 21 days of age[a]

Phytase added (U/kg diet)	Nonphytate P, %[b]			
	.20[c]	.27[c]	.34[c]	.45
0	8.2	10.0	11.3	13.3
200	8.7	10.5	11.9	
400	9.3	11.1	11.9	
600	9.8	11.5	11.8	
800	9.9	10.9	12.3	
1,000	10.5	11.6	12.2	
1,200	11.1	12.6	12.9	

[a]Four pens of 10 chicks each per mean, except .45% nP which had eight pens of 10 chicks each. The root MSE was .6 and the pooled SEM for a single mean would equal MSE/$\sqrt{n}$.
[b]Phosphorus major effect (linear, $P < .001$; quadratic, $P < .05$).
[c]Phytase effect (linear, $P < .001$).

Apparent Utilization of Dry Matter and Retention of Phosphorus and Calcium

Utilization (% of intake and g/bird) of DM, retention (% of intake and g/bird) of Ca and P, and excretion (g/kg DM intake) of P during the d 18 to 20 are shown in Tables 4-4 and 4-5. Increasing dietary nP (%) level linearly increased ($P < .01$) utilization (% of intake and g/bird) of DM, retention (% of intake and g/bird) of Ca and P, and excretion of P (g/kg DM intake). Increasing nP (%) also linearly increased the total amount (g/bird)of P retained ($P < .01$), but linearly decreased apparent retention (% of intake) of

Table 4-4. Dry matter utilization and calcium retention of broilers fed corn-soybean meal-based diets containing varying amounts of nonphytate phosphorus and supplemental phytase from 18 to 20 days of age[a]

Phytase added (U/kg diet)	Nonphytate P, %							
	.20		.27		.34		.45	
Dry matter utilization[b]	%	g/bird	%	g/bird	%	g/bird	%	g/bird
0	74.0[c]	83.0[c]	75.8[d]	116.5[d]	75.5[e]	131.8[e]	77.5	142.4
200	75.4	111.0	75.2	123.2	76.8	138.1		
400	76.3	116.0	77.5	129.8	76.9	127.9		
600	76.4	117.5	77.1	134.0	77.2	139.2		
800	76.5	127.8	77.9	134.1	77.9	136.4		
1,000	77.2	125.0	77.7	133.7	77.5	138.6		
1,200	76.2	128.1	77.1	139.4	78.1	144.5		
Calcium retention[f]								
0	52.3[g]	.626	52.3[h]	1.007	54.1[i]	1.312	63.6	1.628
200	51.0	.810	56.6	1.248	55.7	1.376		
400	50.5	.756	55.8	1.147	57.4	1.381		
600	53.5	.863	58.5	1.215	54.2	1.289		
800	53.0	.918	57.8	1.268	57.5	1.491		
1,000	57.1	.996	61.5	1.334	55.0	1.253		
1,200	58.7	1.027	64.0	1.581	62.6	1.643		

[a]Four pens of 10 chicks each per mean, except 0.45% nP which had eight pens of 10 chicks each. The root MSE was 1.6 and 8.7, 3.0 and 0.098, respectively for DM utilization and calcium retention (% of intake and g/bird). The pooled SEM for a single treatment mean was MSE/$\sqrt{n}$.

[b]Phosphorus main effect (linear, $P < .01$).

[c]Phytase effect (linear, $P < .01$; quadratic, $P < .07$)

[d]Phytase effect (linear, $P < .03$).

[e]Phytase effect (linear, $P < .04$).

[f]Phosphorus main effect (linear, $P < .001$; quadratic, $P < .001$).

[g]Phytase effect (linear, $P < .001$; quadratic, $P < .02$ for % of intake).

[h]Phytase effect (linear, $P < .001$; quadratic, $P < .03$ for % of intake; $P < .07$ for g/bird).

[i]Phytase effect (linear, $P < .005$; quadratic, $P < .03$).

Table 4-5. Phosphorus retention and excretion of broilers fed corn-soybean meal-based diets containing varying amounts of nonphytate phosphorus and supplemental phytase from 18 to 20 days of age[a]

Phytase added (U/kg diet)	Nonphytate P, %							
	.20		.27		.34		.45	
Phosphorus retention[b]	%	g/bird	%	g/bird	%	g/bird	%	g/bird
0	58.7[c]	.348[c]	54.7[c]	.494[c]	52.1[c]	.566[c]	54.9	.888
200	60.6	.506	60.6	.619	56.9	.660		
400	59.7	.482	60.8	.606	58.8	.639		
600	61.8	.497	63.4	.653	58.4	.672		
800	62.3	.524	64.0	.677	63.4	.774		
1,000	66.0	.559	64.8	.567	63.2	.787		
1,200	67.2	.599	70.6	.872	63.4	.794		
Phosphorus excretion[d], g/kg DM intake								
0	2.19[c]		2.66[c]		2.98[c]		3.75	
200	2.23		2.46		2.83			
400	2.12		2.37		2.69			
600	2.00		2.17		2.68			
800	2.01		2.18		2.56			
1,000	1.78		2.12		2.55			
1,200	1.74		2.01		2.48			

[a]Four pens of 10 chicks each per mean, except .45% nP which had eight pens of 10 chicks each. The root MSE were 3.4, .06, and .36, respectively for P retention (% of intake and g/bird) and excretion. The SEM for a single treatment was MSE/$\sqrt{n}$.

[b]Phosphorus main effect (linear, $P < .002$; quadratic, $P < .05$).

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

[d]Phosphorus main effect (linear, $P < .001$).

P ($P < .01$). Addition of graded levels of phytase linearly increased utilization of DM ($P < .05$) and retention of Ca ($P < .01$), and P ($P < .001$, Figure 4-1C). The amount of P excreted was linearly decreased ($P < .001$) as phytase was added. In comparison to the positive control diet (.45% nP), P excretion was reduced by 25 to 54% with addition of 200 to 1,200 U of phytase/kg of diet at various nP (%) levels. The magnitude of the response (Figure 4-1D) was greatest for the lower nP levels (41 to 54% at .20% nP; 34 to 46% at .27% nP; 25 to 34% at .34% nP).

Response Equations and Phosphorus Equivalency Values

Second order translog equations of performance, toe ash percentage, apparent utilization of DM, retention of P and Ca, and P excretion were generated (Table 4-6). All the measurements had high R^2, except gain:feed and apparent utilization (% of intake) of DM, which had low R^2. Linear or nonlinear response equations were also generated with four nP levels (.20, .27, .34, and .45%) without added phytase and with seven phytase levels at each of three nP levels (.20, .27, and .34%) for all the measurements (Table 4-7). The equations for all the measurements with high R^2 except apparent retention (% of intake) of P were used to calculate P equivalency values of phytase (Table 4-8). The apparent retention of P had an opposite response trend between nP levels without phytase addition (decreasing) and phytase levels (increasing) at the two lower nP levels. When BW gain and toe ash percentage are weighted equally and averaged across the two lower nP levels for development of a P equivalency equation, 939 U of microbial phytase was equivalent to 1 g of P from DFP. The percentage of P released from phytate P was 16, 27, 36, and 44%, respectively for 250, 500, 750 and 1,000 U of phytase/kg of diet. The P released (%) per 100 U of phytase was decreased as the total amount of phytase increased.

Table 4-6. Second order translog equations of performance, toe ash percentage, apparent utilization of dry matter, and retention of calcium and phosphorus and phosphorus excretion of broilers fed corn-soybean meal diets

Item	Coefficients of second order translog equations[a]							
	α_0	α_1	α_2	α_3	α_4	α_5	P-value	R^2
Performance								
Weight gain, g	6.228	-.3138	-.0408	-.2927	.0032	-.0412	.001	.84
Feed intake, g	6.697	-.3064	-.0361	-.2584	.0024	-.0352	.001	.76
Gain:feed, g/kg	6.438	-.0074	-.0047	-.0344	.0009	-.0060	.028	.15
Toe ash, %	2.114	-.5039	-.0114	-.3761	.0055	-.0178	.001	.77
Apparent utilization or retention, %								
Dry matter	4.362	.0555	.0003	.0054	.0008	-.0014	.001	.29
Calcium	4.080	.2661	-.0063	.0341	.0050	-.0096	.001	.46
Phosphorus	3.891	.1342	.0143	.0821	.0049	-.0023	.001	.62
Apparent utilization or retention, g/bird								
Dry matter	4.158	-1.216	-.0431	-.6493	.0042	-.0445	.001	.78
Calcium	-.7211	-1.785	-.0431	-1.096	.0089	-.0478	.001	.85
Phosphorus	-.0505	.5024	-.0267	-.1323	.0082	-.0404	.001	.79
Excretion of P (g/kg DM intake)	2.743	1.673	-.0132	.4088	-.0087	.0012	.001	.88

[a]Model: $LnY = \alpha_0 + \alpha_1 LnX_1 + \alpha_2 LnX_2 + \alpha_3 (LnX_1)^2 + \alpha_4 (LnX_2)^2 + \alpha_5 LnX_1 LnX_2$; where, X_1 = nonphytate P, %; X_2 = phytase added, U/kg diet.

Table 4-7. Nonlinear or linear equations of performance, toe ash content, apparent utilization of dry matter, and retention of calcium and phosphorus, and phosphorus excretion[a].

Item	.20% nP		.27% nP		.34% nP	
	Equation	r^2	Equation	r^2	Equation	r^2
Performance						
Weight gain, g	$Y = 557.1(1 - .312e^{-.0036X})$	.98	$Y = 609.0(1-.157e^{-.0015X})$	.98	$Y = 622.7(1-.094e^{-.0011X})$	.88
Feed intake, g	$Y = 877.9(1 - .255e^{-.0051X})$	.94	$Y = 945.5(1-.117e^{-.0019X})$	.92	$Y = 902.8 + .046X$	.73
Toe ash, %	$Y = 8.28 + .0023X$	.99	$Y = 10.10 + .0018X$	.82	$Y = 11.42 + .0010X$	.82
Apparent utilization or retention, %						
Dry matter	$Y = 76.7(1 - .036e^{-.0041X})$	.90	$Y = 77.8(1-.031e^{-.0022X})$	.62	$Y = 78.0(1-.031e^{-.0023X})$	.91
Calcium	$Y = 50.1 + .006X$	.72	$Y = 53.0 + .008X$	.90	$Y = 54.0 + .004X$	.40
Phosphorus	$Y = 58.1 + .007X$	.89	$Y = 56.3 + .011X$	.89	$Y = 53.9 + .009X$	.87
Apparent utilization or retention, g/bird						
Dry matter	$Y = 96.84 + .031X$	.73	$Y = 139.5 (1-.167e^{-.0020X})$	.96	$Y = 131.6 + .0085X$	.46
Calcium	$Y = .671 + .0003X$	.91	$Y = 1.041 + .0004X$	.77	$Y = 1.30 + .0002X$	.24
Phosphorus	$Y = .585(1 - .365e^{-.0022X})$	.82	$Y = .526 + .0002X$	.51	$Y = .584 + .0002X$	.90
P excretion, g/kg of DM intake	$Y = 2.263 - .0004X$	.91	$Y = 2.586 - .0005X$	.93	$Y = 2.917 - .0004X$	.94

[a]Y = response measurements; X = added phytase (U/kg of diet).

Table 4-8. Calculated phosphorus equivalency values of phytase[a]

nP, %	.20				.27					
Phytase, U/kg diet	250	500	750	1,000	250	500	750	1,000	Equation	r^2
Equivalent of nP, %										
BW gain	.255	.290	.310	.310	.310	.340	.360	.380	$Y = 625.3(1 - 3.09e^{-10.3X})$	.99
Feed intake	.265	.290	.300	.310	.305	.330	.355	.370	$Y = 972.6(1 - 3.44e^{-11.7X})$	.99
Toe ash percentage	.225	.250	.270	.300	.300	.320	.345	.370	$Y = 21.3(1 - .908e^{-1.97X})$	.99
DM apparent utilization, %	.340	.385	.405	.415	.375	.425	.455	.470	$Y = 71.8 + 12.34X$	.87
Ca apparent retention, %	.230	.265	.295	.330	.305	.355	.395	.445	$Y = 41.2 + 45.82X$	.82
DM retention, g/bird	.240	.260	.285	.315	.305	.335	.355	.370	$Y = 146.9(1 - 3.55e^{10.5X})$	.99
Ca retention, g/bird	.220	.235	.250	.260	.290	.315	.340	.360	$Y = 2.22(1 - 1.60e^{-3.99X})$	.99
P retention, g/bird	.260	.285	.300	.310	.315	.340	.370	.390	$Y = -.0888 + 2.10X$	.97
Mean of equivalent of nP, %[b]	.240	.270	.290	.305	.305	.330	.353	.375		
Released P, %	.040	.070	.090	.105	.035	.060	.083	.105		
Percentage of phytate P[c]	16.7	29.2	37.5	43.8	14.6	25.0	34.6	43.8		
Means of the two nP levels										
Released P (%)[d]					.038	.065	.087	.105		
Percentage of phytate P[e]					15.6	27.1	36.0	43.8		
Released P (%)/100 U of phytase[f]					.015	.013	.012	.011		

[a]Equations for phytase, see Table 4-7.

[b]Only BW gain and toe ash percent used for calculation of equivalent nP %.

[c]Phytate P in this diet is .24% (See Table 4-1).

[d]$Y = 1.849 - 1.799e^{-.0008X}$ $r^2 = .99$; Y = released P (g/kg) and X = phytase activity (U/kg diet).

[e]$Y = 88.17 - 84.9e^{-.0007X}$, $r^2 = .99$; Y = phytate P released (%) and X = phytase activity (U/kg diet).

[f]$LnY = -1.978 + .202LnX - .034(LnX)^2$, $r^2 = .99$; Y = released P (g)/100 U phytase and X = phytase activity (U/kg diet).

Mortality

During the 21-d experiment, 14, 0, and 4 of the broilers died from diets containing .20, .27, and .34% nP levels. Seven of the 14 birds that died were from the .20% nP diet without added phytase; the number of deaths declined to normal levels with phytase addition of 200 U/kg diet or more. In the broilers fed .27 and .34% nP diet, mortality was normal and not influenced by phytase addition. There were no deaths in broilers fed .45% nP.

Discussion

Performance

The addition of Natuphos® phytase to the corn-soybean meal diets improved all the measurements, especially at the lower nP (%) levels when fed to broilers during a 21-d test. These results indicate that microbial phytase is effective in improving P availability. The maximum growth responses of broilers to phytase level was reduced with increasing levels of dietary nP (%). The maximum growth responses appeared to occur at 1,000, 800, and 600 U of phytase/kg diet, respectively for .20, .27, and 0.34% nP.

The improved growth responses to the addition of phytase were primarily mediated by increased feed intake. The improvements of phytase on gain:feed were observed only at the .20% nP level. These results are in agreement with previous findings (Schoner, et al. 1991; Vogt, 1992; Denbow et al., 1995). However, the responses for ash percentage of toes and the retention (% of intake and g/bird) of P and Ca of these 1 to 21 d-old broilers to the supplemental phytase levels did not reach a plateau. It appears that the supplemental phytase may have potential benefits for later growth of broilers, which is supported by the findings of Schoner et al. (1993) who fed broilers 40 d. They reported the P equivalency values of phytase at 14 d as 1 g P as MCP = 570 U of phytase for BW

gain and 1 g P = 1,050 U of phytase for P retention. At 40 d, 850 U of phytase was equal to 1 g P for both measurements.

Phosphorus Excretion

Phosphorus excretion (g/kg DM intake) was linearly decreased with increasing amount of phytase in this experiment (Table 4-5). The magnitude of reduced P excretion was greater at .20 and .27% nP than that at .34% nP (Figure 4-1). This result indicates that microbial phytase provides a means of reducing P pollution in poultry manure. In comparison to the positive control level of nP (.45%), P excretion was reduced by 25 to 54% with addition of 200 to 1,200 U of phytase. In terms of the model of Schoner et al. (1990): one manure unit corresponded to 350 broilers with a P discharge of 55 kg P_2O_5 per year, the number of broilers per manure unit could increase from 350 to 435 or 525 for the same amount of P excretion. This data confirmed the finding of Yi et al. (1994).

Effects of Phytase on Calcium

The results presented here indicate that supplemental phytase improved Ca availability with an increase in apparent retention (% of intake) and total of Ca retained (g/bird). These findings are supported by other observations (Schoner et al., 1991, 1993; Yi et al. 1994). In a broiler study designed to measure the effect of phytase on Ca availability, Schoner et al. (1994) reported that 500 U of microbial phytase was equivalent to .35 g Ca as measured by BW gain and .56 g Ca as measured by phalanx ash. Phytic acid can form insoluble salts with Ca^{++} (Oberleas, 1973; Morris, 1986), potentially rendering Ca unavailable for intestinal absorption. Phytase has the ability to release Ca^{++} from these insoluble salts and makes Ca available for absorption in broilers.

Sensitive Indicators

The results in this experiment demonstrate that the measurements of BW gain, feed intake, toe ash percentage, apparent retention (% of intake) of Ca and P, total amount of

utilization or retention (g/bird) of DM, Ca, and P, and P excretion are sensitive indicators of evaluating P availability and phytase efficacy in broiler diets. Body weight gain and toe ash percentage were found to be sensitive measurements to evaluate P availability in diets of poultry (Simons et al. 1990; Schoner et al. 1993). Several tibia, metatarsal, and toe measurements, as well as BW gain were examined in broilers fed deficient to adequate levels of P from seven P sources and a dicalcium phosphate dihydrate standard for 3 wks (Ravindran et al., 1995b). They found that BW gain and toe ash percentage were equally or more sensitive for assessment of P availability than tibia ash, and that other measurements including tibia specific gravity, tibia shear force, toe shear force and metatarsal shear force were of limited value. Birds are sensitive to dietary P because of their characteristic low P storage and fast growth. Retention (% of intake and g/bird) directly reflects the absorption and utilization of dietary P in the body of broilers. Thus, these measurements are useful in predicting the responses to supplemental P and phytase. Measurement of the total amount of P retained (g/bird) appears even better than those of apparent retention (% of intake) of P. This was also observed in the study of Schoner et al. (1993). However, because of their economic importance, ease of determination and sensitivity, BW gain and toe ash percentage may be the measurements of choice in many situations.

Phosphorus Equivalency Values of Phytase

The results of this experiment indicate that about 938 U of phytase were required to replace 1 g of inorganic P as DFP based on equally weighted BW gain and toe ash percentage for equivalent P values averaged across .20 and .27% nP (Table 4-8). This value was obtained using the nonlinear equation, $Y\ (g\ P) = 1.849 - 1.799e^{-.0008X\ (U\ of\ phytase)}$. The amount of P released per 100 U of phytase decreased as the total amount of phytase was increased. Yi et al. (1994) reported P equivalency values of 1,598 U of phytase in

Exp. 1 and 922 U in Exp. 2 for broilers fed a similar soybean meal-based diet at .27% nP (.45% tP), but they reported a lower P equivalency (766 U of phytase) for broilers fed a corn-soybean meal diet at .27% nP. Denbow et al. (1995) reported that the values of released P were higher at the higher phytase levels for .20% nP (.38% tP) compared with .27% nP (.45% tP). The P equivalency values of phytase for 1 g of P were 609 U and 1, 133 U, respectively, for .20 and .27% nP. The P equivalency values for the average of the two P levels was 822 U of phytase.

Schoner et al. (1991) reported that 700 U of phytase were equivalent to 1 g P as MCP when P retention data from broilers fed a corn-soybean meal diet for 14 d was used in the calculation; a value of 762 U of phytase was obtained when the calculation was based on crude ash in the total body. The diet used by Schoner et al. (1991) contained .60% Ca and .45% tP that included .10% as MCP. Schoner et al. (1993) used a corn-soybean meal basal diet (.60% of Ca and .35% of tP) without added inorganic P. They reported the P equivalency values of phytase at 14 d as 1 g P as MCP = 570 U of phytase for BW gain and 1 g P = 1,050 U of phytase for P retention. At 40 d, 850 U of phytase was equal to 1 g P for both measurements. Simons and Versteegh (1992) suggested, for broilers during the first 14 d, that 250 U of phytase were equivalent to .5 g P as MCP. This interpretation of their data suggests that the response per 250 U of phytase decreased as the amount of phytase was increased from 500 to 750 U/kg of diet; inorganic P was reduced in the diet as the amount of phytase increased. The difference in P equivalency values may be due to diet type, response indicators, and inorganic P level and source used.

In the calculation of P equivalency values in this experiment, all the data for birds fed .34% nP diet were omitted because of the inconsistency of response measurements (Table 4-7). The R^2 values for the equations of Ca retention (% of intake and g/bird) were low. Since .34% nP was close to the optimum level of P (NRC recommended .45%

nP), phytase released P at .34% nP was less than those at .20 and .27% nP level. This indicates that the responses of phytase in corn and soybean meal were influenced by the dietary nP level. This is supported by the findings from the *in vitro* study of Irving and Cosgrove (1974). They found that inorganic orthophosphate was an inhibitor of *Aspergillus ficuum* phytase.

The wide Ca to tP ratio (2:1) used in this experiment probably reduced the overall response to phytase and inorganic P. Schoner et al. (1993) reported that feeding high levels of Ca with a constant low level of tP (.35%) reduced the increase in BW gain, feed intake, P and Ca retention resulting from added phytase. Their Ca totP ratios were 1.71:1, 2.14:1, and 2.57:1 from the lowest to the highest level of Ca. Similar negative effects of widening the Ca to tP ratio on these response measurements were observed for the lower levels of inorganic P, but not for the higher levels. In our laboratory, Qian et al. (1995) found negative effects on response measurements (BW gain, feed intake, gain:feed, toe ash percentage, P and Ca retention) of widening the Ca to tP ratio (1.1:1 to 2:1) at each phytase (0, 300, 600 or 900 U/kg of diet) and P (.27 and .36% nP) level. The magnitude of the effect of widening the Ca to tP ratio was much larger at the lower P level. For example, widening the Ca to tP ratio from 1.4:1 to 2:1 decreased phytase efficiency by 7.4% for the .27% nP diets and 4.9% for the .34% nP diets.

Implications

Natuphos® phytase was effective for improving P availability in corn-soybean meal diets fed to broilers with the magnitude of the response inversely related to the level of dietary nP. About 939 U of phytase was equivalent to 1 g of P from defluorinated phosphate in broiler diets. The amount of P released per 100 U of phytase decreased as the total amount of phytase increased. In comparison to the .45% nP diet, P excretion

could be reduced by 25 to 54% with addition of 200 to 1,200 U of phytase at various nP (%) level. Phytase also improved Ca utilization in broilers.

Chapter V

Improving Phytate P Availability in Corn and Soybean Meal for Broilers Using Natuphos® Phytase and Calculation of P equivalency Values of Phytase

ABSTRACT: Two experiments were conducted to determine the effectiveness of Natuphos® phytase for improving P availability of soybean meal-based semi-purified diets (SP, Exp. 1 and 2) or corn-soybean meal-based diets (CS, Exp. 2) fed to broilers (1 to 21 d, n = 936). Phosphorus equivalency values of phytase were calculated. The basal diet was formulated to contain .27% nonphytate P (nP); which contained .45% total P (tP) in SP with .17% P as defluorinated phosphate (DFP) and .51% tP in CS with .12% P as DFP. Both basal diets were supplemented with DFP to provide .36, .45, and .54% nP or with 350, 700, and 1,050 U of phytase/kg of diets. Supplementing DFP and phytase linearly increased BW gain ($P < .001$), feed intake ($P < .001$), and ash percentage of dried toes ($P < .01$). Phytase addition increased apparent retention (%) of P ($P < .05$), Ca ($P < .01$ in Exp. 2), N ($P < .10$ in Exp. 2 for CS), and increased apparent digestibility of DM ($P < .05$), and linearly decreased ($P < .01$) P excretion. In comparison to the .45% nP diet, P excretion was reduced 42 to 51% by addition of phytase. The addition of DFP linearly decreased apparent retention (%) of P ($P < .05$) and Ca ($P < .01$ in Exp. 2), and increased P excretion ($P < .01$). Nonlinear or linear response equations for the effects of P and phytase levels, generated for the measurements of BW gain and toe ash percentage, were used to calculate the P equivalency values. Phytase levels of 250, 500, 750, and 1,000 U/kg of diet released 12, 25, 37, and 50% P from phytate in SP (average of Exp. 1 and 2) and 23, 33, 43, and 51% P from phytate P in CS, respectively. The average of released P values for SP diets in Exp. 1 and 2 gave a P equivalency value of 1 g P = 1,116 U of

phytase. The P equivalency value for CS diets fed only in Exp. 2 was 766 U of phytase = 1 g P as DFP.

Key Words: Phosphorus, Phytase, Broiler, Equivalency values

Introduction

Corn and soybean meal are the major feed sources in poultry diets in the US. More than 60% of P in corn and soybean meal is in the form of phytate (Nelson et al., 1968; Reddy et al., 1982). This phytate P has low availability (NRC, 1994), which leads to the use of inorganic P sources to meet the P requirements of poultry. Therefore, large amounts of P are present in poultry manure (Cromwell and Coffey, 1991). Phytase (EC 3.1.3.8.) is the enzyme known to release the orthophosphate group from the phytate molecule (Gibson and Ullah, 1990). But the activity of endogenous phytase and that naturally occuring in the diets of poultry is very limited (Davies and Motzok, 1972; Pointillart et al., 1984). Recently, a microbial phytase from *Aspergillus* was used to improve the availability of the phytate P of corn-soybean meal diets (Simons et al., 1990; Schoner et al., 1991). Data using pigs indicated that microbial phytase might influence the utilization of DM (Jongbloed et al., 1992; Ketaren et al., 1993), and other nutrients such as Ca and CP (Lei et al., 1994; Mroz et al., 1994; Yi et al., 1994). Little is known about the P equivalency values of microbial phytase in poultry diets fed graded levels of phytase.

The present experiments were conducted to determine the effectiveness of Natuphos® phytase for improving P, Ca, and N apparent retention and P excretion in broilers fed soybean meal-based semi-purified diets or corn-soybean meal-based diets. Equations were generated to calculate P equivalency values of phytase using each of these basal diets.

Materials and Methods

Birds, Diets, and Treatments

Peterson x Arbor Acres male broiler chicks (n = 936) were used in two 21-d experiments to investigate the effectiveness of supplemental Natuphos® phytase (BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07823-1234) and added inorganic P on performance, toe ash percentage, and P, Ca, and N utilization and P excretion of broilers fed soybean meal-based semi-purified diets (SP, Exp. 1 and 2) or corn-soybean meal-based diets (CS, Exp. 2). Eight treatments were designed for each diet type (Table 5-1). The basal diets were formulated to contain .27% nonphytate P (nP). The SP basal diet contained .45% total P (tP) that included .17% P as defluorinated phosphate (DFP) and the CS basal diet contained .51% tP that contained .12% P as DFP. This level of nP was selected based on the results of previous broiler phytase trials (Chapter IV and Denbow et al., 1995), and the need to maintain the dietary nP below the current NRC (1994) recommendations to ensure a strong response to phytase additions but minimal mortality from P deficiency. Each basal diet was supplemented with DFP to provide .36, .45, and .54% nP or with 350, 700, and 1,050 units (U) of phytase/kg of diet. Also, 1,050 U of phytase/kg of diet was added to .54% nP diets with each diet type. The assayed P and Ca contents and phytase activity are given in Table 5-1. The Ca to tP ratio was maintained at 2:1 in all diets. Limestone and DFP were added to the diets at the expense of corn starch (Table 5-2). A unit of phytase activity is defined as the quantity of enzyme that liberates 1 μmol of inorganic P per min from 5.1 mM sodium phytate at pH 5.5 and 37 °C (Engelen et al., 1994). Since phytate was supplied only from the soybean meal in SP, the dietary percentage of phytate P (.18%) was similar in Diets 1 to 8. The dietary percentage of phytate P (.24%) in CS was also similar in Diets 9 to 16, based on NRC (1984).

Table 5-1. Dietary treatments and assayed P and Ca contents and phytase activity in Exp. 1 and 2

Diet[a]	nP %	Total P, %		Total Ca, %		Phytase, U/kg diet		Diet type[b]
		Calculated	Assayed	Calculated	Assayed	Added	Assayed	
1	.27	.45	.48	.90	.90	0	<50	SP[c]
2	.36	.54	.54	1.08	1.18	0	<50	SP
3	.45	.63	.72	1.26	1.28	0	<50	SP
4	.54	.72	.80	1.44	1.36	0	<50	SP
5	.27	.45	.54	.90	.84	350	313	SP
6	.27	.45	.54	.90	.90	700	610	SP
7	.27	.45	.56	.90	.82	1,050	973	SP
8	.54	.72	.81	1.44	1.36	1,050	946	SP
9	.27	.51	.55	1.02	.89	0	<50	CS[c]
10	.36	.60	.59	1.20	.92	0	<50	CS
11	.45	.69	.69	1.36	1.13	0	<50	CS
12	.54	.78	.78	1.53	1.27	0	<50	CS
13	.27	.51	.54	1.02	.96	350	331	CS
14	.27	.51	.54	1.02	.96	700	658	CS
15	.27	.51	.60	1.02	.96	1,050	987	CS
16	.54	.78	.78	1.53	1.28	1,050	935	CS

[a]Only diets 1 to 8 used in Exp. 1.
[b]SP = soybean meal-based semi-purified diets, CS = corn-soybean meal-based diets.
[c]Phytate content was calculated to be .18% in SP and .24% in CS based on NRC (1984).

Feeding and Management

Broilers, obtained from a commercial hatchery, were allotted randomly on the day hatched to 36 pens (10 chicks per pen) in Exp. 1, and 72 pens (8 chicks per pen) in Exp. 2. They were housed in electrically heated, raised wire-floored starting batteries in an environmentally controlled room. Within each diet type (SP vs CS), the basal diet (Diet 1 or 9) was assigned randomly to eight pens, whereas, the other seven treatments were assigned to four pens. Diets were fed in a mash form for 21 d. Birds had *ad libitum*

Table 5-2. Percentage composition of diets[a]

	Semi-purified diets				Corn-soybean meal diets			
nP, %	.27[b]	.36	.45	.54[c]	.27[b]	.36	.45	.54[c]
Ingredients	----------%----------							
Corn (8.8% CP)	---	---	---	---	54.99	54.56	54.00	53.44
Soybean meal (48.5% CP)	47.50	47.50	47.50	47.50	37.50	37.40	37.44	37.48
Corn starch[d]	30.03	29.48	28.92	28.36	---	---	---	---
Dextrose[e]	10.00	10.00	10.00	10.00	---	---	---	---
Soybean oil	6.00	6.00	6.00	6.00	4.00	4.00	4.00	4.00
Cellulose[f]	3.00	3.00	3.00	3.00	---	---	---	---
Ground limestone[g]	1.30	1.32	1.35	1.38	1.82	1.84	1.86	1.88
Defluorinated phosphate[h]	.92	1.45	1.98	2.51	.69	1.20	1.70	2.20
Vitamin premix[i]	.40	.40	.40	.40	.20	.20	.20	.20
Trace mineral premix[j]	.20	.20	.20	.20	.20	.20	.20	.20
Salt	.40	.40	.40	.40	.40	.40	.40	.40
DL-methionine	.21	.21	.21	.21	.20	.20	.20	.20

[a]All diets were formulated to contain 23.0% crude protein, and .93% methionine plus cystine. Semi-purified diets contained 1.51% lysine and corn-soybean meal diets contained 1.32% lysine.

[b]Zero, 350, 700 and 1,050 U of phytase per kilogram of diet were added. Phytase (Natuphos®-5,000 U/g) was supplied by BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07823-1234.

[c]Zero and 1,050 U of phytase per kilogram of diet was added.

[d]Food grade, National Starch and Chemical Co., Bridgewater, NJ 08807.

[e]Clintose, ADM Corn Processing, Clinton, IA 52732.

[f]Purified cellulose, Grade BH200, International Filler Corp., NY 14120.

[g]Limestone Dust Corp., Bluefield, VA 24605.

[h]Fine CDP, Southern Bag Corp., Valdosta, GA 31083.

[i]Supplied per kilogram of diet in SP diets: retinyl acetate, 1816 µg; cholecalciferol, 132 µg; dl-α-tocopheryl acetate, 53 mg; menadione sodium bisulfite, 1.5 mg; riboflavin, 15 mg; d-calcium pantothenate acid, 19.4; niacin, 52.8; cyanocobalamin, 22 µg; choline chloride, 2,025 mg; biotin, .62 mg; folic acid, 6.2 mg; thiamin•HCl, 16 mg; pyridoxine•HCl, 6.2 mg; ethoxyquin, 100 mg; virginiamycin, 5.8 mg. One-half of these amounts were supplied in the corn-soybean meal diets.

[j]Supplied per kilogram of diet: manganese, 88 mg; zinc, 95 mg; iron, 100 mg; copper, 12.5 mg; iodine, 4 mg; selenium, .6 mg.

access to feed and water. The care and treatment of broilers followed published guidelines, (Consortium, 1988). Body weights and feed intake were recorded on a pen basis at weekly intervals. Mortality was recorded daily.

Sampling and Analysis

During the third week (d 18 to 20), a total collection of excreta from each pen was carried out. Feed intake and production of excreta were measured per pen over the 3 consecutive d. Excreta from each pen were stored in plastic bags at -20 °C. After thawing, excreta were dried in an oven at 70 °C and weighed. Excreta, along with diet samples, were ground to pass through a 1 mm sieve. Dry matter was determined according to AOAC (1990) procedures. Following a nitric-perchloric acid wet digestion, P concentrations were determined colorimetrically (AOAC, 1990). Calcium contents were determined with an atomic absorption spectrophotometer. Nitrogen concentrations in Exp. 2 were determined by the Kjeldahl method (AOAC, 1990). Apparent retention (percentage of intake) of P, Ca, and N were calculated for each dietary treatment.

On d 21, all surviving chicks were sacrificed. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left middle toes of all chicks within a pen were pooled, and the right middle toes from the same chicks were pooled, yielding two samples of toes per pen. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 600 °C for 4 h.

Statistical Analysis and Calculation of Phosphorus Equivalency Values

Data were analyzed by the GLM procedures of SAS (1990) with pen means as the experimental unit except toe ash percentage which included foot (left and right toes). Linear and quadratic effects of added DFP and supplemental phytase were tested using orthogonal polynomials. Comparison of main effects between SP and CS in Exp. 2 were also made by orthogonal contrast. Comparisons between the diet containing .54% nP and diet containing .54% nP plus supplemental phytase were made using non-orthogonal contrasts.

Nonlinear and linear functions that best fit the data were derived for phytase levels and for nP levels for SP and CS with the nonlinear model: $Y = a(1 - be^{-kX})$ and linear model: $Y = a + bX$. Where, Y = the response measurements; X = nP(%) or phytase added (U/kg of diet). The nonlinear or linear response equations with higher r^2 value for added nP and the equation of phytase levels were set equal and solved. For example, the equation for toe ash percentage was as follow:

$$14.15(1 - .857e^{-5.98Y}) = 11.76 + .00077X$$

$$Y = -.1672\text{Ln}(.1971 - .0000635X)$$

The resulting equations were used to calculate the equivalent P (%) at 250, 500, 750, and 1,000 U of phytase/kg of diet in order to compare the data with those from others. These values were used to determine the amount of P released and mean of BW gain and toe ash percentage were used to calculate the P equivalency values of phytase for each diet type.

Results

Experiment 1

Performance and Toe Ash Percentage

Increasing the level of nP, in the form of DFP, linearly increased ($P < .001$) BW gains, feed intake, and ash percentage of toes of broilers (Table 5-3). Body weight gain and feed intake reached a plateau at .45% nP, but ash percentage of toes continued to increase to .54% nP. Gain:feed was unaffected by increasing the level of nP. Adding

Table 5-3. Effects of defluorinated phosphate or phytase supplementation of a soybean meal-based semi-purified diet on performance and toe ash of broiler chicks (d 1 to 21, Exp. 1)[a]

Diet	Nonphytate P %	Added phytase U/kg	BW gain g	Feed intake g	Gain per feed g/kg	Toe ash %
1. Basal (B)	.27	---	520	791	658	11.6
2. B + DFP	.36	---	624	947	659	12.8
3. B + DFP	.45	---	646	998	648	13.3
4. B + DFP	.54	---	636[b]	977[b]	651	13.7[c]
5. B + phytase	.27	350	597	924	646	12.1
6. B + phytase	.27	700	606	925	655	12.3
7. B + phytase	.27	1,050	620[d]	956[d]	649	12.8[e]
8. B + DFP + phytase	.54	1,050	673[f]	1,056[f]	651	13.8
MSE[g]			28	46	30	.5

[a]Four pens (10 birds per pen) per treatment mean, except diet 1 which had eight pens per treatment mean. DFP = defluorinated phosphate.
[b]Phosphorus effects (linear, $P < .001$; quadratic, $P < .01$ or .001).
[c]Phosphorus effects (linear, $P < .001$; quadratic, $P < .07$).
[d]Phytase effects (linear, $P < .001$; quadratic, $P < .10$).
[e]Phytase effect (linear, $P < .01$).
[f]Contrast (diet 4 vs 8; $P < .08$).
[g]MSE = the root square mean error and pooled SEM = MSE/$\sqrt{n}$, where n = 8 (diet 1) or 4 (all others) pens.

phytase to the .27% nP diet linearly increased ($P < .001$) BW gains, feed intake, and ash percentage of toes. Gain:feed was not influenced by supplemental phytase. Addition of phytase to diets containing above NRC (1994) recommended levels of nP (.54%) increased ($P < .10$) BW gains and feed intake. However, gain:feed and ash percentage of toes were unaffected by adding phytase.

Utilization or Retention of Phosphorus, Calcium, Dry Matter and Phosphorus Excretion

Increasing the level of nP linearly decreased ($P < .05$) the retention (%) of P and linearly increased ($P < .001$) P excretion (Table 5-4). The numerical value of DM digestibility was lower for broilers fed .27% nP diets than those fed the diets with addition of DFP or phytase. Retained Ca showed decreasing trend ($P = .12$) with increased levels of DFP. The reduction in P and Ca retention and the increase in P excretion were more evident when the dietary level of nP was increased from .45 to .54% nP.

Adding tiered levels of phytase increased ($P < .10$) P retention and linearly reduced ($P < .001$) P excretion. The major increase in P retention or decrease in P excretion occurred with 350 U of phytase/kg of diet. Additions of phytase to the basal diet with .27% nP resulted in an 18 to 25% reduction in the P excreted. When compared with the .45% nP (NRC, 1994, recommendation) diet, adding phytase decreased P excretion by 42 to 47%. Calcium retention was only numerically increased with added phytase to 700 U/kg of diet. The digestibility of DM was increased from 0 to 350 U of phytase/kg of diet and then slightly decreased for 700 and 1,050 U of phytase/kg of diet (quadratic, $P < .05$). Addition of phytase to the .54% nP diet had no effects on P retention or excretion, but increased DM digestibility ($P < .05$) and decreased Ca retention ($P < .10$). Mortality was within normal ranges during the trial, and difference in mortality was not observed between dietary treatments.

Table 5-4. Effects of defluorinated phosphate or phytase supplementation of a soybean meal-based semi-purified diet on the apparent digestibility of DM, retention of P and Ca, and the excretion of P of broiler chicks (Exp. 1)[a]

Diet	Nonphytate P %	Added phytase U/kg	DM %	P %	Ca %	P excretion g/kg DM intake
1. Basal (B)	.27	---	72.8	58.5	41.2	2.21
2. B + DFP	.36	---	75.1	57.9	41.2	2.65
3. B + DFP	.45	---	75.2	57.2	38.6	3.12
4. B + DFP	.54	---	74.6	47.1[b]	31.0	4.39[c]
5. B + phytase	.27	350	77.6	68.1	42.8	1.82
6. B + phytase	.27	700	76.4	64.4	46.1	1.66
7. B + phytase	.27	1,050	75.9[d]	64.4[e]	39.6	1.72[f]
8. B + DFP + phytase	.54	1,050	78.2[g]	50.3	22.6[h]	4.05
MSE[i]			2.2	5.6	7.7	.38

[a]Based on total collection of excreta from day 18 to 20; four pens (10 birds per pen) per treatment mean, except diet 1 which had eight pens (ten birds per pen) per treatment mean. DFP = defluorinated phosphate.
[b]Phosphorus effect (linear, $P < .02$).
[c]Phosphorus effects (linear, $P < .001$; quadratic, $P < .06$).
[d]Phytase effects (quadratic, $P < .04$).
[e]Phytase effects (linear, $P < .08$; quadratic, $P < .02$).
[f]Phytase effects (linear, $P < .001$; quadratic, $P < .03$).
[g]Contrast (diet 4 vs 8, $P < .03$).
[h]Contrast (diet 4 vs 8, $P < .1$).
[i]MSE = the root square mean error and pooled SEM = MSE/$\sqrt{n}$, where n = 8 (diet 1) or 4 (all others) pens.

Phosphorus Equivalency Values of Phytase

Phosphorus equivalency values of phytase were calculated using equations for BW gain and toe ash percentage (Table 5-5) because of their economic importance and ease of determination. Phytase levels of 250, 500, 750, and 1,000 U of phytase/kg of diet were used for these calculations. When BW gain and toe ash percentage values were weighted

Table 5-5. Calculated phosphorus equivalency values of phytase and nonlinear or linear equations for available P and phytase levels (Exp. 1)

	Phytase, U/kg diet			
	250	500	750	1,000
Equivalent of nP, %				
BW gain, g[a]	.305	.325	.330	.335
Toe ash, %[b]	.285	.305	.325	.335
Mean of equivalent of nP, %[c]	.295	.315	.328	.335
Released P, %[d]	.025	.045	.048	.065
Percentage of phytate P[e,f]	14	25	32	36
Released P (%)/100 U of phytase[g]	.010	.009	.008	.007

[a]Equation for nP was $Y = 652.8(1 - 40.7e^{-19.6X})$, $r^2 = .94$,
and for phytase was $Y = 617.1(1 - .156e^{-.0042X})$, $r^2 = .99$.
Where Y = BW gain (g); X = nP (%) or phytase (U/kg of diet).
[b]Equation for nP was $Y = 14.15(1-.857e^{-5.98X})$, $r^2 = .99$,
and for phytase was $Y = 11.76+.00077X$, $r^2 = .99$.
Where Y = toe ash percentage; X = nP (%) or phytase (U/kg diet).
[c]Body weight gain and toe ash percentage weighted equally for these means.
[d]$Y = .15 + .000532X$, $r^2 = .95$;
where Y = released P (g) and X = phytase activity (U/kg of diet).
[e]Phytate P in soybean meal (.18%) makes up 60% of the total P.
[f]$Y = 42.6(1 - 1.097e-.002X)$, $r^2 = .99$;
where Y = phytate P released (%) and X = phytase activity (U/kg of diet).
[g]$LnY = -6.96 + 1.721LnX - .1589(LnX)^2$, $r^2 = .99$;
where Y = released P (g)/100 U of phytase and X = phytase activity (U/kg of diet).

equally, 1,598 U of microbial phytase was equivalent to 1 g of P as DFP. It was estimated that 14, 25, 32, and 36% of the phytate P in soybean meal would be released by the addition of 250, 500, 750, and 1,000 U of phytase/kg of diet. The response per 100 U of phytase decreased by .003% from 250 to 1,000 U of phytase/kg of diet.

Experiment 2

The main effects of diet type are shown in Table 5-6. Broilers fed SP ate less ($P < .01$), but were more efficient ($P < .001$) and grew faster ($P < .001$) than broilers fed CS. Toe ash percentage, and apparent retention of P and Ca were higher ($P < .001$) for broilers fed SP than for those fed CS. Retention of N was higher ($P < .001$) for CS than for SP, but DM digestibility was numerically higher ($P = .32$) for broilers fed SP than for CS. Phosphorus excretion was higher ($P < .001$) for broilers fed CS than those fed SP.

Table 5-6. Average performance, toe ash percentage from 1 to 21 d, and apparent digestibility of DM, and retention of P, N and Ca, and P excretion of broilers fed soybean meal-based semi-purified diets and corn-soybean meal-based diets (d 18 to 20, Exp. 2)[a]

Item	SP[b]	CS[b]	SEM	P-value
Performance				
BW gain, g	659	633	4.7	.001
Feed intake, g	919	953	8.7	.008
Gain:feed, g/kg	717	665	5.4	.001
Toe ash, %	11.6	11.2	.4	.001
Apparent digestibility or retention, %				
DM	75.3	74.8	.2	.32
P	53.9	47.5	.5	.001
N	65.0	68.5	.8	.001
Ca	42.5	27.5	.6	.001
Phosphorus excretion (g/kg DM intake)	2.9	3.3	.1	.001

[a]SP = soybean meal-based semi-purified diets; CS = corn-soybean meal-based diets.
[b]Thirty-six pens (eight birds per pen) per diet type mean.

Performance and Toe Ash Percentage

Similar significant increases in BW gain and feed intake were observed for increasing levels of nP and phytase supplemented into the two types of diets (Table 5-7). Body weight gain and feed intake reached a plateau at .45% nP and at 700 U of phytase/kg of diet with .27% nP for broilers fed SP. However, BW gain and feed intake continued to increase to .54% nP for broilers fed CS, but tended to reach a plateau at 700 U of phytase/kg of diet with .27% nP. The addition of phytase (1,050 U/kg of diet) to the .54% nP diet improved gain:feed in SP ($P < .10$), but BW gain and feed intake were not affected. Adding DFP and/or phytase linearly increased toe ash percentage for SP ($P < .001$) and for CS ($P < .001$ and $P < .10$, respectively for nP and phytase). Phytase added to the .54% nP diet increased toe ash percentage of broilers fed CS ($P < .01$), but not of broilers fed the SP.

Apparent Retention of Phosphorus, Calcium, Nitrogen, and Phosphorus Excretion

Apparent digestibility of DM ($P < .02$), and retention (%) of P ($P < .001$) and Ca ($P < .001$) linearly decreased as the level of nP increased in SP (Table 5-8); however, only apparent retention of P ($P < .001$) and Ca ($P < .001$) were linearly decreased as the level of nP increased in CS. Adding phytase linearly increased the apparent digestibility of DM ($P < .05$ for SP, $P < .01$ for CS), and retention of P ($P < .01$ for SP and CS), N ($P < .01$ for CS), and Ca ($P < .001$ for SP and CS). The addition of phytase to the .54% nP diet decreased the apparent retention of Ca in SP ($P < .001$), and increased apparent retention of Ca in CS ($P < .05$). Phosphorus excretion linearly increased ($P < .001$) as the level of DFP was increased; but linearly decreased ($P < .01$) as phytase was added to the .27% nP diet. Compared to the .45% nP diet, P excretion was reduced 30 to 60% by adding phytase. The addition of 1,050 U of phytase/kg of diet to the .54% nP diet increased ($P < .05$) P excretion.

Table 5-7. Effects of defluorinated phosphate or phytase supplementation of soybean meal-based semi-purified and corn-soybean meal-based diet on performance and toe ash percentage of broiler chicks (d 1 to 21, Exp. 2)[a]

Diet	Nonphytate P %	Added phytase U/kg	BW gain g	Feed intake g	Gain per feed g/kg	Toe ash %
Soybean meal-based semi-purified diets						
1. Basal (B)	.27	---	601	852	706	10.4
2. B + DFP	.36	---	655	924	710	11.7
3. B + DFP	.45	---	692	989	701	12.4
4. B + DFP	.54	---	693[b]	967[c]	718	12.7[d]
5. B + phytase	.27	350	607	882	689	10.9
6. B + phytase	.27	700	668	912	732	11.2
7. B + phytase	.27	1,050	661[e]	905[f]	731[g]	12.3[h]
8. B +DFP + phytase	.54	1,050	693	923	751[i]	12.8
Corn-soybean meal-based diets						
9. Basal (B)	.27	---	537	802	666	9.6
10. B + DFP	.36	---	610	919	666	11.3
11. B + DFP	.45	---	662	978	678	12.3
12. B + DFP	.54	---	683[b]	1,026[c]	666	12.6[d]
13. B + phytase	.27	350	586	880	665	10.9
14. B + phytase	.27	700	645	1,005	644	10.6
15. B + phytase	.27	1,050	657[e]	960[f]	686	11.0[h]
16. B +DFP + phytase	.54	1,050	681	1,048	650	13.4[j]
MSE[k]			28	51	31	.5

[a]Four pens (eight birds per pen) per treatment mean, except diet 1 and 9 which had eight pens per treatment mean. DFP = defluorinated phosphate.
[b]Phosphorus effects (linear, $P < .001$; quadratic, $P < .05$).
[c]Phosphorus effects (linear, $P < .002$; quadratic, $P < .03$ for SP).
[d]Phosphorus effects (linear, $P < .001$; quadratic, $P < .001$ for SP).
[e]Phytase effects (linear, $P < .001$ for SP; $P < .001$ for CS).
[f]Phytase effect (linear, $P < .02$ for SP; $P < .001$ for CS; quadratic, $P < .05$ for CS).
[g]Phytase effects (linear, $P < .01$ for SP).
[h]Phytase effects (linear, $P < .001$ for SP; $P < .06$ for CS).
[i]Contrast (diet 4 vs 8, $P < .07$).
[j]Contrast (diet 12 vs 16, $P < .007$).
[k]MSE = the root square mean error and pooled SEM = MSE/$\sqrt{n}$, where n = 8 (diet 1 or 9) or 4 (all others) pens.

Table 5-8. Effects of defluorinated phosphate or phytase supplementation of soybean meal-based semi-purified and corn-soybean meal-based diets on digestibility of DM, retention of P, Ca, and N, and the excretion of P in broiler chicks (d 18 to 20, Exp. 2)[a]

Diet	nP %	phytase U/kg	DM %	P %	N %	Ca %	P Exc. g/kg
Soybean meal-based semi-purified diets							
1. Basal (B)	.27	---	75.7	56.5	65.7	45.0	2.09
2. B + DFP	.36	---	76.2	47.9	68.8	47.0	2.82
3. B + DFP	.45	---	73.5	41.5	63.6	34.6	4.21
4. B + DFP	.54	---	74.4[b]	45.0[c]	63.4	37.1[d]	4.62[e]
5. B + phytase	.27	350	75.1	62.9	60.4	46.3	2.01
6. B + phytase	.27	700	76.6	63.8	67.7	48.6	1.96
7. B + phytase	.27	1,050	76.9[f]	70.0[g]	63.8	55.0[g]	1.68[g]
8. B + DFP + phytase	.54	1,050	73.6	40.6	63.9	23.7[h]	4.93[i]
Corn-soybean meal-based diets							
9. Basal (B)	.27	---	74.1	49.9	65.3	26.8	2.76
10. B + DFP	.36	---	74.5	44.3	69.4	19.5	3.29
11. B + DFP	.45	---	74.6	45.6	69.1	24.0	3.75
12. B + DFP	.54	---	74.8	33.3[j]	70.0	15.1[d]	4.67[e]
13. B + phytase	.27	350	75.0	51.5	66.6	33.1	2.62
14. B + phytase	.27	700	75.6	56.1	69.2	35.9	2.37
15. B + phytase	.27	1,050	76.3[g]	62.8[g]	71.2[k]	43.1[g]	2.23[g]
16. B +DFP + phytase	.54	1,050	74.5	34.1	70.9	20.9[l]	5.14[h]
MSE[m]			1.2	5.4	4.1	8.8	.19

[a]Based on total collection of excreta from day 18 to 20; four pens (eight birds per pen) per treatment mean, except diets 1 and 9 that had eight pens per treatment mean. DFP = defluorinated phosphate.

[b]Phosphorus effect (linear, $P < .02$).

[c]Phosphorus effect (linear, $P < .001$; quadratic, $P < .001$).

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

[e]Phosphorus effect (linear, $P < .001$, quadratic, $P < .06$).

[f]Phytase effect (linear, $P < .03$).

[g]Phytase effect (linear, $P < .005$).

[h]Contrast (diet 4 vs 8; $P < .001$).

[i]Contrast (diet 4 vs 8; $P < .05$).

[j]Phosphorus effect (linear, $P < .001$; quadratic, $P < .02$).

[k]Phytase effect ($P < .01$).

[l]Contrast (diet 12 vs 16, $P < .02$).

[m]MSE = the root square mean error and pooled SEM = MSE/$\sqrt{n}$, where n = 8 (diet 1 or 9) or 4 (all others) pens.

Phosphorus Equivalency Values of Phytase

Nonlinear and linear response equations for SP and CS with increasing levels of DFP or supplemental phytase were developed using treatment means (Table 5-9). The P equivalency value of phytase for 1 g P was calculated using the equation derived from the average for BW gain and toe ash percentage for each diet type (Table 5-10). For SP, 922 U of microbial phytase was equivalent to 1 g of P as DFP, and for CS, 766 U of phytase was equivalent to 1 g of P. Based on the addition of 250 to 1,000 U of phytase/kg of diet, 11 to 52% and 24 to 59% of phytate P was released for SP and CS diets, respectively. Phytase appears to release more phytate P in CS than in SP. The response per 100 U of phytase generally decreased as the total amount of phytase added was increased in CS, but lightly increased in SP. Mortality was within normal ranges (3%) during the trial, and differences in mortality were not observed between the various dietary treatments.

Discussion

The results obtained from these 21-d studies with d-old Peterson x Arbor Acres male broiler chicks indicate that Natuphos® phytase is effective in improving phytate P availability in corn and soybean meal for broilers. Supplemental phytase of 350, 700, or 1,050 U/kg of diet to SP or CS increased BW gain 11 to 22%, feed intake 6 to 25%, toe ash percentage 4 to 18%, and P retention 3 to 25%. The responses agree with other findings (Simons et al., 1990; Schoner et al., 1991; Vogt, 1992). The BW gains of broilers with addition of phytase (700 to 1,050 U/kg of diet in SP, 1,050 U/kg of diet in CS) to the .27% nP basal diet were very close to those of .45% nP diet (NRC recommendation). However, improved BW gains were mainly related to the increased feed intake.

Table 5-9. Nonlinear or linear response equations for the various measurements of broilers (1 to 21 days) fed soybean meal-based semi-purified or corn-soybean meal-based diets with increased levels of nonphytate phosphorus and supplemental phytase (Exp. 2)

Item	Nonphytate phosphorus[a]	r^2	Phytase added[b]	r^2
Soybean meal-based semi-purified diets				
BW gain, g	$Y = 706.2(1 - 1.70e^{-8.99X})$	.99	$Y = 775.8(1 - .232e^{-.0005X})$	.80
Feed intake, g	$Y = 986.3(1 - 2.53e^{-10.8X})$	.93	$Y = 915.3(1 - .0693e^{-.0023X})$	.93
Gain:feed, g/kg	$Y = 837.6(1 - .167e^{-.1965X})$	.22	$Y = 812.1(1 - .1377e^{-.0001X})$	.54
Toe ash, %	$Y = 13.0(1 - 1.69e^{-7.93X})$	.99	Y = 10.3 + .0016X	.92
Apparent retention, %				
DM	Y = 77.9 - 7.43X	.49	Y = 75.3 + .0015X	.62
P	Y = 66.1 - 45.5X	.68	Y = 57.1 + .012X	.94
N	Y = 70.8 - 13.5X	.39	Y = 64.1 + .0005X	.01
Ca	Y = 57.1 - 40.0X	.60	Y = 43.8 + .009X	.89
P excretion, g/kg DM intake	Y = .616 + 9.99X	.96	Y = 2.12 - .0004X	.86
Corn-soybean meal-based diets				
BW gain, g	$Y = 724.2(1 - 1.25e^{-5.80X})$	.99	$Y = 720.5(1 - .258e^{-.0011X})$	.98
Feed intake, g	$Y = 1096(1 - 1.037e^{-5.12X})$	.99	$Y = 998.6(1 - .1919e^{-.0022X})$	.84
Gain:feed, g/kg	$Y = 691.6(1 - .0421e^{-.5783X})$	.06	$Y = 686.0(1 - .0334e^{-.0007X})$	.08
Toe ash, %	$Y = 13.0(1 - 2.44e^{-8.19X})$	.99	$Y = 10.8(1 - .115e^{-1.01X})$	.92
Apparent retention, %				
DM	Y = 73.6 + 2.19X	.93	Y = 74.2 + .002X	.99
P	Y = 65.1 - 53.7X	.78	Y = 48.6 + .012X	.94
N	$Y = 69.6(1 - 619.5e^{-35.0X})$	.90	Y = 65.0 + .006X	.99
Ca	Y = 35.2 - 34.2X	.60	Y = 27.0 + .015X	.98
P excretion, g/kg DM intake	Y = .826 + 6.89X	.98	Y = 2.77 - .0005X	.98

[a]Y = response and X = nonphytate P (%).
[b]Y = response and X = phytase activity (U/kg diet).

Table 5-10. Calculated phosphorus equivalency values of phytase (Exp. 2)[a]

Item	Phytase, U/kg diet			
	250	500	750	1,000
	Semi-purified diets			
Equivalent of nP, %				
BW gain[a]	.285	.315	.345	.380
Toe ash percentage[a]	.290	.310	.345	.385
Mean of equivalent of nP, %[b]	.288	.313	.345	.383
Released P, %[c]	.018	.043	.075	.113
Percentage of phytate P[d,e]	10	24	42	63
Released P (%)/100 U of phytase[f]	.007	.009	.010	.011
	Corn-soybean meal diets			
Equivalent of nP, %				
BW gain[a]	.315	.365	.410	.450
Toe ash percentage[a]	.330	.335	.335	.335
Mean of equivalent of nP, %[b]	.323	.350	.373	.393
Released P, %[c]	.053	.080	.103	.123
Percentage of phytate P[d,e]	22	33	43	51
Released P (%)/100 U of phytase[f]	.021	.016	.014	.012

[a]Equations for calculation, see Table 5-9.
[b]Body weight gain and toe ash percentage weighted equally.
[c]$Y = -.17 + .001268X$ $r^2 = .99$ for SP; $Y = 2.451 - 2.233\ e^{-.0006X}$, $r^2 = .99$ for CS; Y = released P (g) and X = phytase activity (U/kg of diet).
[d]Phytate P in soybean meal (.18%); in corn-soybean meal (.24%) (see Table 5-1).
[e]$Y = -9.5 + .071X$, $r^2 = .99$ for SP; $Y = 101.0 - 92.19e^{-.0006X}$, $r^2 = .99$ for CS; Y = phytate P released (%) and X = phytase activity (U/kg of diet).
[f]$LnY = -6.02 + .8389LnX - .0416(LnX)^2$, $r^2 = .99$ for SP;
$LnY = -.769 + .057LnX - .036(LnX)^2$, $r^2 = .99$ for CS.
Y = released P (g)/100 U of phytase and X = phytase activity (U/kg of diet).

Retention of Phosphorus, Calcium, and Nitrogen and Utilization of Dry Matter

The retention of P (percentage of intake) was increased as the addition of phytase increased and DFP was increased, which agrees with previous findings of Schoner et al,

(1991, 1993) when broilers were fed a P deficient corn-soybean meal diet. Schoner et al, (1991) fed a basal diet with .6% Ca and .45% tP (.1% P from monocalcium phosphate, MCP); whereas, Schoner et al. (1993) fed a basal diet with three Ca levels (.60, .75, or .90%) with .35% tP (all P from corn and soybean meal). Total body P was measured by Schoner et al. (1991) and amount of P retained was measured by Schoner et al. (1993).

Improvements in the utilization of Ca, N (not measured for Exp. 1), and DM by supplemental phytase were found in these experiments. These results are supported by other findings (Yi et al., 1994a). Schoner et al. (1991, 1993) also reported improved Ca retention of broilers given supplemental phytase. Phytic acid exists as phytin in plants (Cosgrove, 1980). Because of its chelating ability, phytate may reduce the availability of essential minerals (Reddy et al., 1982; Morris, 1986). Phytate also has the ability to bind protein and to form a ternary complex, protein-cation-phytic acid (Cheryan, 1980; Wise, 1983; Champagne et al., 1990), which may reduce protein utilization (Thompson and Serraino, 1986). The results presented here provided evidence to support this hypothesis.

The response of broilers to phytase supplementation of diets containing above-recommended levels of nP (.54%) was not consistent. Addition of 1,050 U of phytase/kg of diet to the .54% nP diet increased BW gain and feed intake, but did not affect gain:feed in Exp. 1. Supplemental phytase increased gain:feed in SP, but did not change BW gain and feed intake in Exp. 2. The unexpected depression observed in Ca apparent retention in SP containing .54% nP is noteworthy. It is believed that high levels of P inhibit Ca absorption by the formation of insoluble Ca-P complexes in the intestinal tract (Guyton, 1986). However, the addition of 1,050 U of phytase/kg of diet to CS containing .54% nP increased Ca retention and toe ash percentage. It turns out that the response may be related to the diet type because the birds fed SP had better utilization of nutrients than those fed CS (see late discussion).

Phosphorus Excretion

In comparison to the NRC recommended nP level of .45%, addition of 350, 700, or 1,050 U of phytase/kg of diet decreased P excretion by 47, 50, and 52% in SP and by 30, 37, and 41% in CS, respectively. Schoner et al. (1990) calculated that one manure unit corresponded to 350 broilers with a P discharge of 55 kg P_2O_5 per year. The data presented here indicate that adding phytase to broiler diets could decrease P discharge from 55 to 33 kg of P_2O_5 per year, and increase the number of broilers per manure unit from 350 to 490 for the same amount of P excretion. Adding phytase to the diets for poultry provides a means of environmental protection, because P is considered an environmental pollutant, which contaminates surface soil and water. It is estimated that approximately 250,000 tons of P are produced annually as a waste product by poultry in the US (Cromwell and Coffey, 1991). A 30% reduction in P excretion would mean 75,000 tons less P excreted by poultry annually in the US.

Phosphorus Equivalency Values of Phytase

Results of these experiments demonstrated that the average P equivalency value of phytase for SP is (averaged for Exp. 1 and 2) 1,100 U = 1 g P and 766 U = 1 g P for CS, when BW gain and toe ash percentage values are weighted equally and averaged. Denbow et al. (1995) reported that 1 g of P as DFP could be released with 1,133 U of phytase when broilers were fed SP diets containing .27% nP: a lower phytase value, 609 U = 1 g P, was reported for broilers fed SP diets containing .20% nP. A value of 700 U of phytase was suggested by Schoner et al. (1991) to be equivalent to 1 g P from MCP, when P contained in the carcass was used in the calculation; a value of 762 U of phytase was obtained when the calculation was based on crude ash in the total body. Schoner et al. (1993) reported the P equivalency value of phytase at 14 d as 1 g P as MCP = 570 U of

phytase for BW gain and 1 g P = 1,050 U of phytase for P retention. At 40 d, 850 U of phytase was equal to 1 g P for both measurements.

Different measurements could result in various P equivalency values. In these studies, BW gain, feed intake, toe ash percentage, and P excretion were the most sensitive indicators. These measurements consistently showed higher r^2 in the nonlinear or linear response equations for graded levels of nP and added phytase in both diet types (Table 5-9). However, P excretion demonstrated an opposite trend between added DFP and phytase. Feed intake was similar response pattern with BW gain. Thus, BW gain and toe ash percentage were used to calculate P equivalency values of phytase in these studies. Again, BW gain of birds would be an efficient indicator from the standpoint of being nondestructive and easy to measure. Toe ash percentage would require the least amount of laboratory labor.

Equivalency values and percentage of P released from phytate indicate that the addition of phytase to CS may release more phytate P than when added to SP. This may be due to the fact that CS has a higher concentration of phytate than SP (.24 vs .18%), and the amount of P released by phytase is correlated with the amount of phytate in the diets. The other reason for the difference is that the comparison was made by the responses of graded levels of nP with the responses of graded levels of phytase in each diet type, and the DFP responses in SP were higher than those in CS (Table 5-6).

Implications

The results further confirmed the effectiveness of Natuphos® phytase for improving P availability in corn and soybean meal fed to broilers and for reducing P excretion in broilers at practical dietary P level. The average of released P values for soybean meal-based semi-purified diets in Exp. 1 and 2 gave a P equivalency value of 1 g P = 1,116 U of phytase; the P equivalency value for corn-soybean meal-based diets in Exp.

2 was 766 U of phytase = 1 g P as defluorinated phosphate. Phytase improved Ca and N retention in broilers.

Chapter VI

Effect of Microbial Phytase on Nitrogen and Amino Acid Digestibility and Nitrogen Retention of Turkey Poults Fed Corn-soybean Meal Diets

ABSTRACT: The effect of microbial phytase on N and amino acid (AA) digestibility and N retention was investigated in a 29-d trial using 480 British United Turkey female poults fed corn-soybean meal diets. A 2 x 2 x 2 factorial arrangement of treatments was used with .45 and .60% nonphytate P (nP), 22.5 and 28.0% crude protein (CP), and 0 and 750 U of microbial phytase/kg of diet. At .45% nP, adding phytase to either 22.5 or 28.0% CP diets increased BW gain ($P < .01$), percentage ($P < .01$) and weight ($P < .10$) of toe ash; at .60% nP, the magnitude of the effect of phytase was less ($P > .10$) than observed for .45% nP and inconsistent. Apparent and true ileal digestibility of N and AA was estimated by using chromic oxide as an indicator at d 24. At .45% nP, adding phytase to 22.5% CP diets resulted in improved trend in the apparent and true ileal digestibility of N and AA, except cysteine or methionine; adding phytase to 28.0% CP diets increased the digestibility of N and most of AA ($P < .001$ to .10). At .60% nP, adding phytase to 22.5% CP diets increased the apparent and true ileal digestibility of N and all the AA ($P < .001$ to .10), but did not change digestibilities at 28.0% CP diets. Adding phytase also increased ($P < .001$ to .10) apparent ileal digestibility of DM and P at .45% nP for both CP diets, but only 22.5% CP diets at .60% nP. The total excreta was collected at d 27 to 29. Adding phytase to .45% nP diets increased apparent utilization of DM ($P < .01$ to .10) and N ($P < .05$ to .10) at both CP levels; retention of P was only increased ($P < .10$) at 22.5% CP. At .60% nP, adding phytase increased DM utilization ($P < .05$) and N retention ($P < .10$) only at 22.5% CP; P retention was not affected. In summary,

microbial phytase enhanced growth performance, toe ash, ileal N and AA digestibility, and apparent N and P retention.

Key Words: Turkey, Phytase, Nitrogen, Amino acid, Phosphorus, Ileal digestibility

Introduction

Phytate has the potential of binding with protein at low and neutral pH (Cosgrove, 1980; Anderson, 1985; Thompson, 1986). The phytate-protein complexes may reduce the utilization of the proteins and amino acids (AA). Phytate may also form complexes with proteases, such as trypsin and pepsin (Camus et al., 1976; Singh and Krikorian, 1982) in the gastrointestinal tract. The complexes may decrease the activity of digestive enzymes, then decrease the digestibility of dietary proteins.

Phytase (E.C.3.1.3.8.), myo-inositol hexaphosphate phosphohydrolase, is the enzyme that releases P from phytate (Gibson and Ullah, 1990). It has been shown that supplemental microbial phytase improved dietary phytate P bioavailability in birds (Simons et al., 1990; Yi et al., 1994b; Denbow et al, 1995). Phytase may also improve the utilization of protein and AA which could be bound to phytate. Ketaren et al. (1993) found that addition of phytase to the diet of young pigs increased protein deposition and retention of pigs, but had no effect on CP digestibility. Mroz et al. (1994) reported that supplemental microbial phytase improved the apparent digestibility of protein and some AA in pigs. In the studies of Yi et al. (1994a,b) and Kornegay and Qian (1994), the apparent N absorption of pigs and N retention of broilers were improved, when phytase was added to the diet. Phytase also improved N absorption in laying hens (Van der Klis and Versteegh, 1991).

The objective of this experiment was to investigate the effect of microbial phytase on protein and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets.

Materials and Methods

Birds, Treatments, and Diets

British United Turkey poults (n = 480) were used in a bioassay feeding trial to investigate the effect of microbial phytase on the utilization of dietary protein and AA. A 2 x 2 x 2 factorial arrangement of treatments was used with two levels of nonphytate P (.45 and .60% nP), two levels of crude protein (22.5 and 28.0% CP), and two levels of phytase (0 and 750 U/kg of diet). Dicalcium phosphate and limestone were added to the diet at the expense of corn starch to maintain the desired P and Ca levels. A Ca to nP ratio of 2:1 was maintained in all the diets as recommended by NRC (1994). The 22.5% CP level [80% of NRC (1994) recommendation] was obtained by substituting corn starch for corn and soybean meal; the ratio of corn and soybean meal was maintained constant across diets (Table 6-1). Microbial phytase was Natuphos® provided by BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234. The percentage composition of the basal diets is shown in Table 6-1 and the assayed ingredients are shown in Table 6-2.

Feeding and Management

Turkey poults were randomly allotted on the day of hatched to 48 pens (10 birds/pen). They were housed in electrically heated, wire-floored starting batteries in an environmentally controlled room. The eight treatments were randomly assigned to 48 pens (six pens per treatment). The diets were fed in a mash form. Birds had *ad libitum* access to feed and water through 21 d. The care and treatment of birds followed published guidelines (Consortium, 1988).

Body weight and feed intake of the birds were recorded on a pen basis at d 6, 13, and 20. At the end (d 29) of the experiment, BW of the remaining birds was recorded on a pen basis. Mortality was recorded daily.

Table 6-1. Percentage composition of diets[a]

Nonphytate P, %	.45		.60	
Crude protein, %	22.50	28.00	22.50	28.00
Ingredients	----------%----------			
Corn (8.5% CP)	30.04	37.55	30.04	37.55
Corn starch[b]	18.74	1.17	17.63	---
Soybean meal (48.5% CP)	40.94	51.20	40.96	51.20
Soybean oil	6.00	6.00	6.00	6.00
Ground limestone	1.05	1.03	1.35	1.35
Dicalcium phosphate[c]	1.81	1.65	2.62	2.50
Vitamin premix[d]	.60	.60	.60	.60
Trace mineral premix[e]	.20	.20	.20	.20
Salt	.40	.40	.40	.40
DL-methionine	.20	.20	.20	.20
Calculated nutrients				
CP	22.62	28.22	22.62	28.22
Methionine + cysteine	.88	1.05	.88	1.05
Lysine	1.28	1.61	1.28	1.61
Ca	.90	.90	1.20	1.20
Total P	.67	.73	.82	.89
Nonphytate P (nP)	.45	.45	.60	.60
Ca:nP	2.00	2.00	2.00	2.00

[a]Zero and 750 U phytase per kilogram of diet were added to each of the basal diets. Phytase (Natuphos® -5,000 U/g) was supplied by BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234. The diets for the digestibility test contained .05% of Cr_2O_3 at the expense of corn starch.

[b]Food grade, National Starch and Chemical Co., Bridgewater, NJ 08807.

[c]DYNAFOS®, Pitman-Moore, Inc. Mundelein, Illinois 60060.

[d]Supplied per kilogram of diet: 7,920 IU of vitamin A, 7,920 ICU of vitamin D3, 79 IU of vitamin E, 2.2 mg of vitamin K, 22 mg of riboflavin, 29 mg of calcium pantothenate, 99 mg of niacin, .033 mg of vitamin B12, 3,036 mg of choline chloride, .92 mg of biotin, 9.2 mg of folic acid, 22 mg of thiamin•HCl, 9.2 mg of pyridoxine•HCl, 150 mg of ethoxyquin and 8.7 mg of virginiamycin.

[e]Supplied per kilogram of diet: 88 mg of manganese, 95 mg of zinc, 100 mg of iron, 12.5 mg of copper, 4 mg of iodine and .6 mg of selenium.

Table 6-2. Assayed ingredients of the diets[a]

Nonphytate P, %	.45		.60	
Crude protein, %	22.50	28.00	22.50	28.00
Ingredients	----------	----------	%----------	----------
Nitrogen	3.78	4.74	4.05	4.56
Methionine (Met)	.50	.60	.52	.63
Cystine (Cys)	.38	.47	.40	.47
Lysine (Lys)	1.34	1.70	1.47	1.70
Threonine (Thr)	.86	1.09	.94	1.10
Arginine (Arg)	1.63	2.06	1.77	2.06
Histidine (His)	.63	.79	.68	.80
Isoleucine (Ile)	1.01	1.27	1.11	1.30
Leucine (Leu)	1.90	2.35	2.04	2.38
Phenylalanine (Phe)	1.16	1.47	1.27	1.49
Valine (Val)	1.13	1.43	1.22	1.44
Proline (Pro)	1.16	1.45	1.27	1.48
Aspartic acid (Asp)	2.44	3.10	2.67	3.12
Serine (Ser)	.95	1.24	1.03	1.24
Glutamic acid (Glu)	3.91	4.97	4.26	5.00
Glycine (Gly)	.95	1.20	1.04	1.21
Alanine (Ala)	1.06	1.34	1.16	1.35
Tyrosine (Tyr)	.75	.99	.83	1.01
Total amino acid (TAA)	21.80	27.64	23.77	27.89
Ca	.96	.96	1.20	1.20
Total P (tP)	.72	.77	.83	.92
Ca : tP	1.33	1.25	1.45	1.30

[a]The N contents were analyzed by standard methods (AOAC, 1990). The Ca contents were analyzed with an atomic absorption spectrophotometer. The P contents were analyzed with colorimeter (AOAC, 1990). The amino acids contents were hydrolyzed with 6 N HCl and analyzed by HPLC (Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MI 65211).

Sampling With Indicator Method for Digestibility

On d 21, the birds were transferred into developer batteries and fed twice a day during the next 2 d on the same diets while being trained to eat all their feed in 2 h (Mitaru et al., 1985). The birds were fed in the morning at 0900 and allow 2 h to eat (0900-1100), and then again in the afternoon at 1500 (1500-1700). At each feeding, the birds received 25 g of diet per kg BW (modified from Ford and Hewitt, 1979). After the second day, six pens of birds per treatment were randomly divided into two groups (three pens per group within a treatment). One group of three pens was fed the same diet as previously fed (Table 6-1) and the other group was fed a protein-free diet (Table 6-3), each for 1 d followed by a 24 h fast. After they were fasted, the two groups from each treatment was fed their respective test diet shown in Table 6-1 or protein-free diet shown in Table 6-3 with the addition of .05% of Cr_2O_3 at the expense of corn starch. Five of the birds per pen

Table 6-3. Protein-free diets[a]

Nonphytate P, %	.45	.60
Ingredients	----------%----------	
Corn starch[b]	46.38	45.24
Dextrose[c]	40.00	40.00
Soybean oil	6.00	6.00
Cellulose[d]	3.00	3.00
Limestone	.99	1.32
Dicalcium phosphate[b]	2.43	3.24
Vitamin premix[b]	.60	.60
Trace mineral premix[b]	.20	.20
Salt	.40	.40

[a]The diets for the digestibility test contained .05% Cr_2O_3 added at the expense of corn starch.
[b]See Table 6-1.
[c]Clintose, ADM Corn Processing, Clinton, Iowa 52732.
[d]Purified cellulose, Grade BH200 (International Filler Corp., New York 14120).

were weighed and killed 3 h post cibum by cervical dislocation. The ileum was dissected within 5 min after killing. The ileum was defined as extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-cecal junction (Payne et al., 1968). Ileal contents were removed and pooled for the five birds in a pen; these composited samples were lyophilized. The ileal contents from the three replicate pens of birds fed protein-free diets were further pooled to make one composite sample per treatment for determination of endogenous N and AA.

Sampling and Measurement of Toes

Toe samples of the killed birds were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left middle toes of the five birds within a pen were pooled, and the right middle toes from the same birds were pooled, yielding two samples of toes per pen. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 600 °C for 4 h.

Sampling With Total Collection Method

The remaining birds (five or less if any mortality) were used for a total collection of excreta to evaluate the apparent utilization of DM and retention of N, P, and Ca. The birds were given *ad libitum* access to the respective diets as used in the previous feeding trial. A total excreta of the birds was collected during d 27 to 29. Feed intakes were recorded starting 24 h before the collection time and ending 24 h before the end of collection. The excreta were dried at 60 °C and ground to pass through 1 mm sieve.

Laboratory Analysis

The samples were analyzed for moisture and N content in diets, ileal contents, and excreta by standard methods (AOAC, 1990). Chromium and Ca in the diets, ileal, and excreta (only Ca) samples were analyzed with an atomic absorption spectrophotometer following a nitric-perchloric acid wet digestion. Phosphorus in the diets and ileal samples

was analyzed colorimetrically (AOAC, 1990). Amino acids in the diets and ileal contents were hydrolyzed with 6 N HCl and analyzed by HPLC (Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MI 65211). Apparent ileal digestibility values of N, individual AA, DM, P, and Ca, and true ileal digestibility values of N, individual AA were calculated as described by Varnish and Carpenter (1975).

Statistical Analysis

The data of performance, toe ash percentage and weight, digestibility of N, AA, P, Ca, and DM, and apparent utilization or retention of DM, N, P, and Ca of the birds were subjected to analysis of variance by the GLM procedures of SAS (1991). The model included nP, CP, and phytase, and two way and three way interactions. Comparisons between the diets with and without added phytase at each CP level of the two nP (%) levels were made using nonorthogonal contrast.

Results

Performance and Toe Ash

The performance and toe ash contents of turkey poults with the various dietary treatments are shown in Table 6-4. The main effect of microbial phytase (750 U/kg diet) indicates increased BW gain ($P < .001$), feed intake ($P < .05$), and toe ash contents ($P < .05$); but no influence on gain:feed. Reducing the dietary CP (22.5 vs 28.0%) decreased the performance and toe ash weight of the birds but did not influence toe ash percentage. Reducing dietary nP (.45 vs .60%) did not influence the performance of the birds during the first 20 d; but decreased the BW gain from d 21 to 29, and from d 1 to 29 ($P < .01$). Reducing dietary nP decreased toe ash percentage ($P < .001$), but did not influence the toe ash weight. The interactive effects of phytase and nP on toe ash contents ($P < .05$), and BW gain from d 1 to 29 ($P < .01$) were observed, which indicates that the effect of phytase on toe ash contents and BW gain was dependent on dietary nP level. The

Table 6-4. Performance and toe ash of turkey poults fed corn-soybean meal diets with two nonphytate phosphorus (nP) levels, two crude protein (CP) levels and two phytase (PY) levels

Diet	nP	CP	PY	D 1 to 20[a]			D 24 toe ash[b]		D 1 to 29 gain[b]
				Gain	Feed	Gain:feed			
	%	%	U/kg	g	g	g/kg	%	mg	g
1	.45	22.5	0	428	522	822	12.3	74.5	821
2	.45	22.5	750	488	604	807	13.2	85.7	952
3	.45	28.0	0	520	597	885	11.8	91.8	988
4	.45	28.0	750	578	606	960	13.2	98.9	1,125
5	.60	22.5	0	441	555	796	13.5	77.3	879
6	.60	22.5	750	471	554	853	13.0	80.4	931
7	.60	28.0	0	532	616	865	13.2	102	1,099
8	.60	28.0	750	583	661	885	13.7	98.5	1,144
Pooled SEM				16	22	33	.28	2.6	23
						Main Effects			
PY (U/kg)		0		480	572	842	12.7	86.5	947
		750		530	606	876	13.3	90.8	1,038
CP (%)		22.5		457	559	820	12.9	79.5	896
		28.0		553	620	899	13.0	97.9	1,089
nP (%)		.45		503	582	869	12.6	87.7	975
		.60		507	596	850	13.3	89.7	1,013
				----------Probability----------					
PY				.001	.040	.155	.001	.027	.001
CP				.001	.001	.002	.967	.001	.001
nP				.750	.387	.425	.001	.315	.013
PY * CP				.683	.665	.569	.045	.151	.979
PY * nP				.412	.464	.853	.002	.016	.011
CP * nP				.628	.162	.223	.141	.100	.149
PY * CP * nP				.627	.063	.178	.513	.711	.840
Contrast diet 1 vs 2				.011	.011	.737	.010	.004	.001
Contrast diet 3 vs 4				.014	.763	.115	.001	.081	.001
Contrast diet 5 vs 6				.181	.993	.228	.224	.435	.114
Contrast diet 7 vs 8				.030	.154	.668	.144	.312	.173

[a]Six replicate pens (10 birds per pen) per treatment.
[b]Six replicate pens (five birds per pen) per treatment.

interactive effect of phytase and CP on toe ash percentage ($P < .05$) was observed, which indicates that the effect of phytase on toe ash percentage was dependent on dietary CP level. An interactive effect of CP and nP on toe ash weight ($P < .10$) was observed. The three-way interaction of phytase, CP, and nP on feed intake was observed ($P < .10$). This indicates that the effect of phytase on feed intake was dependent on dietary CP and nP levels.

At .45% nP, adding phytase to the 22.5% CP diets increased BW gain and feed intake of the birds during the first 20 d ($P < .01$), and BW gain from d 1 to 29 ($P < .001$), toe ash contents ($P < .01$); whereas, addition of phytase to the 28.0% CP diets increased BW gain ($P < .01$) and toe ash contents ($P < .001$ to .10), but did not influence feed intake. Gain:feed was not influenced by phytase at either CP level. At .60% nP, adding phytase to the 22.5% diets only showed increased pattern of BW gain, gain:feed, and toe ash weight of the birds, but did not influence feed intake and toe ash percentage; whereas, addition of phytase to the 28.0% CP diets only resulted in increased BW gain during the first 20 days ($P < .05$).

Apparent Ileal Digestibility of Nitrogen and Essential Amino Acids

Apparent ileal digestibility coefficients (AID) of N and essential amino acids (EAA) are shown in Table 6-5. Main effects indicate 1) that the addition of phytase increased the AID of N and all of the EAA ($P < .001$ to .01), 2) that reducing the dietary CP level increased the AID of N, Cys, and Thr ($P < .001$ to .05) with the AID of the other EAA, except Met, being only showed improved trend, and 3) that reducing dietary nP level increased ($P < .01$ to .10) the AID of N and all the AA, except Cys. A three-way interaction of phytase, CP, and nP on the AID of N and all the AA, except Met, was observed ($P < .05$ to .10). This confirmed that the effects of phytase on the AID of N and AA were dependent on the dietary CP and nP levels.

Table 6-5. Apparent ileal digestibility of dietary nitrogen and essential amino acids in turkey poults fed corn-soybean meal diets with two nonphytate phosphorus (nP) levels, two crude protein (CP) levels and two phytase (PY) levels[a]

Diet	nP	CP	PY	N	Met	Cys	Lys	Thr	Arg	His	Ile	Leu	Phe	Val
	%	%	U/kg	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
1	.45	22.5	0	90.8	95.2	86.1	93.8	89.2	94.4	92.3	91.5	91.1	91.6	90.8
2	.45	22.5	750	91.8	95.8	88.1	94.5	90.2	95.0	93.1	92.2	92.0	92.4	91.6
3	.45	28.0	0	89.6	95.0	82.5	93.1	88.1	93.8	91.5	90.5	90.5	91.0	90.1
4	.45	28.0	750	91.1	95.5	85.6	94.4	89.3	94.9	92.8	92.4	92.0	92.6	91.9
5	.60	22.5	0	88.8	94.1	84.7	92.4	86.4	92.8	90.5	89.4	89.1	89.6	88.8
6	.60	22.5	750	91.5	95.6	87.9	94.4	89.5	94.8	92.7	92.1	91.9	92.3	91.4
7	.60	28.0	0	89.1	94.8	83.1	92.9	87.1	93.4	91.1	90.1	89.8	90.4	89.7
8	.60	28.0	750	89.1	95.1	83.9	92.9	87.4	93.5	91.0	90.0	90.0	90.4	89.4
Pooled SEM				.54	.32	.63	.40	.57	.47	.48	.58	.58	.59	.55
				Main Effects										
PY (U/kg)		0		89.6	94.8	84.1	93.0	87.7	93.6	91.3	90.4	90.1	90.7	89.9
		750		90.9	95.5	86.4	94.0	89.1	94.6	92.4	91.7	91.5	91.9	91.1
CP (%)		22.5		90.8	95.2	86.7	93.8	88.8	94.3	92.1	91.3	91.0	91.5	90.7
		28.0		89.6	95.1	83.8	93.3	87.9	93.9	91.6	90.8	90.6	91.1	90.3
nP (%)		.45		90.8	95.4	85.6	94.0	89.2	94.5	92.4	91.7	91.4	91.9	91.1
		.60		89.7	94.9	84.9	93.1	87.6	93.6	91.3	90.4	90.2	90.7	89.8
				----------	----------	----------	----------	Probability	----------	----------	----------	----------	----------	----------
PY				.005	.007	.001	.005	.004	.011	.008	.007	.007	.009	.007
CP				.020	.851	.001	.130	.050	.312	.153	.235	.307	.371	.320
nP				.008	.067	.157	.013	.002	.016	.006	.008	.013	.013	.006
PY * CP				.176	.154	.513	.264	.131	.293	.211	.340	.258	.283	.267
PY * nP				.880	.363	.537	.946	.484	.727	.914	.980	.741	.916	.875
CP * nP				.844	.465	.760	.933	.731	.989	.960	.704	.797	.741	.667
PY * CP * nP				.052	.242	.074	.047	.097	.092	.054	.027	.074	.056	.031
Contrast diet 1 vs 2				.237	.235	.038	.274	.218	.368	.288	.407	.311	.341	.322
Contrast diet 3 vs 4				.074	.355	.003	.049	.165	.122	.078	.035	.093	.074	.039
Contrast diet 5 vs 6				.004	.004	.003	.004	.002	.008	.005	.005	.005	.007	.006
Contrast diet 7 vs 8				.970	.511	.342	.965	.694	.850	.925	.876	.837	.983	.776

[a]Each value comes from three replicate pens of five birds/pen; PY = phytase added, U/kg of diet.

At .45% nP, adding phytase to the 22.5% CP diets only increased the AID of Cys ($P < .05$) with only improved trend in the AID of N and other AA. Adding phytase to the 28.0% CP diets increased the AID of N, Cys, Lys, His, Ile, Leu, Phe, and Val ($P < .01$ to .10) with only improved trend in the AID of Met, Thr, and Arg. At .60% nP, adding phytase to the 22.5% CP diets increased the AID of N and all the other AA ($P < .01$), while addition of phytase to the 28.0% CP diets did not change the AA digestibility.

Apparent Ileal Digestibility of Non-essential and Total Amino Acids

Apparent ileal digestibility coefficients (AID) of non-essential and total amino acids (NEAA, TAA) are shown in Table 6-6. Main effects indicate 1) that the addition of phytase increased AID of NEAA and TAA ($P < .001$ to .01), 2) that reducing the dietary CP level increased the AID of Pro, Asp, Gly, Ala and TAA ($P < .05$ to .10) with only improved trend in the AID of the other NEAA; 3) reducing dietary nP increased ($P < .01$ to .05) the AID of TAA and all the NEAA. A three-way interaction of phytase, CP and nP on the AID of TAA and all the NEAA, except Ser, was observed ($P < .05$ to .10). This confirmed that the effects of phytase on the AID of AA were dependent on the dietary CP and nP levels.

At .45% nP, adding phytase to the 22.5% CP diets numerically increased the AID of NEAA and TAA, while adding phytase to the 28.0% CP diets only increased the AID of Pro, Gly, and TAA ($P < .10$). At .60% nP, adding phytase to the 22.5% CP diets increased the AID of all NEAA and TAA ($P < .01$ to .10), while addition of phytase to the 28.0% CP diets did not change the AA digestibility.

Apparent Ileal Digestibility of Dry Matter, Phosphorus, and Calcium

Apparent ileal digestibility coefficients (AID) of DM, P and Ca are shown in Table 6-6. Main effects indicate 1) that the addition of phytase increased the AID of DM and P

Table 6-6. Apparent ileal digestibility of dietary non-essential and total amino acids, and dry matter, phosphorus and calcium in turkey poults fed corn-soybean meal diets with two nonphytate phosphorus (nP) levels, two crude protein (CP) levels and two phytase (PY) levels[a]

Diet	nP	CP	PY	Pro	Asp	Ser	Glu	Gly	Ala	Tyr	TAA	DM	P	Ca
	%	%	U/kg	%----------										
1	.45	22.5	0	91.3	90.8	91.0	93.0	89.9	91.6	92.9	91.7	72.4	53.3	56.1
2	.45	22.5	750	92.1	91.7	92.2	93.8	91.0	92.4	93.6	92.5	78.7	63.2	56.4
3	.45	28.0	0	89.9	89.5	90.5	92.3	88.8	90.7	92.6	90.7	73.4	52.4	55.6
4	.45	28.0	750	91.3	91.2	91.2	93.6	90.4	92.0	93.8	92.1	76.9	62.0	56.2
5	.60	22.5	0	88.9	88.5	88.9	91.1	87.7	89.6	91.2	89.7	72.6	48.3	52.1
6	.60	22.5	750	91.9	91.3	91.3	93.6	90.6	92.2	93.6	92.2	77.9	55.9	54.9
7	.60	28.0	0	89.3	88.9	88.7	91.8	88.2	90.1	92.0	90.2	73.7	52.6	52.4
8	.60	28.0	750	89.4	88.9	90.2	91.9	88.1	90.3	92.1	90.2	74.6	54.0	48.9
Pooled SEM				.55	.62	.86	.58	.58	.55	.51	.56	1.3	1.4	1.6
				Main Effects										
PY (U/kg)		0		89.9	89.4	89.8	92.0	88.7	90.5	92.2	90.6	73.2	51.7	54.1
		750		91.2	90.8	91.2	93.2	90.0	91.7	93.3	91.8	77.0	58.8	54.1
CP (%)		22.5		91.0	90.6	90.9	92.9	89.8	91.4	92.8	91.8	75.6	55.2	54.9
		28.0		90.0	89.6	90.1	92.4	88.9	90.8	92.6	90.6	74.6	55.3	53.3
nP (%)		.45		91.1	90.8	91.2	93.2	90.2	91.7	93.2	91.6	75.5	57.7	56.1
		.60		89.9	89.4	89.8	92.1	88.7	90.6	92.2	90.8	74.7	52.7	51.9
				----------Probability----------										
PY				.004	.008	.036	.013	.005	.007	.009	.008	.001	.001	.977
CP				.018	.051	.263	.244	.043	.113	.564	.093	.402	.915	.402
nP				.007	.006	.030	.020	.006	.012	.015	.008	.466	.001	.048
PY * CP				.160	.241	.597	.284	.146	.263	.252	.236	.065	.123	.430
PY * nP				.596	.875	.443	.750	.934	.653	.681	.823	.325	.020	.825
CP * nP				.949	.834	.946	.976	.852	.906	.764	.911	.690	.262	.512
PY * CP * nP				.040	.054	.838	.103	.052	.096	.079	.071	.671	.167	.375
Contrast diet 1 vs 2				.322	.531	.366	.358	.199	.321	.352	.300	.003	.001	.938
Contrast diet 3 vs 4				.091	.403	.557	.136	.074	.122	.119	.095	.087	.004	.856
Contrast diet 5 vs 6				.002	.047	.074	.009	.003	.006	.006	.006	.015	.002	.441
Contrast diet 7 vs 8				.931	.549	.256	.874	.915	.753	.853	.948	.595	.493	.395

[a]Each value comes from three replicate pens of five birds/pen; PY = phytase added, U/kg of diet; TAA = the total amino acids, including the essential amino acids in Table 6-5.

($P < .001$), but did not affect the AID of Ca, 2) that reducing dietary CP did not influence the AID of DM, P, and Ca, and 3) that reducing dietary nP increased the AID of P ($P < .01$) and Ca ($P < .05$), but did not affect the AID of DM. An interactive effect of phytase and CP on the AID of DM was observed ($P < .10$), which indicates that the effect of phytase on the AID of DM was dependent on dietary CP level. An interactive effect of phytase and nP on the AID of P was observed ($P < .05$), which indicate that the effect of phytase was dependent on dietary nP level.

At .45% nP, adding phytase to the 22.5 or 28.0% CP diets increased the AID of DM ($P < .01$ for 22.5% CP; $P < .10$ for 28.0% CP), and P ($P < .01$). At .60% nP, adding phytase to the 22.5% CP diets increased ($P < .05$) the AID of DM and P, but did not influence the AID of DM and P with 28.0% CP diets. At the two nP levels, adding phytase to either CP level did not influence Ca digestibility.

True Ileal Digestibility of Nitrogen and Essential Amino Acids

True ileal digestibility coefficients (TID) of N and EAA are shown in Table 6-7. Main effects indicate 1) that the addition of microbial phytase increased TID of EAA ($P < .001$ to .01), 2) that reducing the dietary CP increased the TID of EAA ($P < .001$ to .10), and 3) that reducing the dietary nP also increased the TID of EAA ($P < .01$ to .05) except Cys and Met. The interactive effects of phytase and CP on TID of EAA, except Cys and Arg, were observed ($P < .05$ to .1). The three-way interaction of phytase, CP, and nP on the TID of EAA was also observed ($P < .01$ to .10). This confirmed that the effects of phytase on TID of EAA were dependent on dietary CP and nP levels.

At .45% nP, adding phytase to the 22.5% CP diets increased the TID of Met ($P < .10$), while the TID of other EAA was only showed improved trend. Addition of phytase to the 28.0% CP diets increased ($P < .001$ to .10) the TID of all the EAA except Met. At

Table 6-7. True ileal digestibility of dietary nitrogen and essential amino acids in turkey poults fed corn-soybean meal diets with two nonphytate phosphorus (nP) levels, two crude protein (CP) levels and two phytase (PY) levels[a]

Diet	nP	CP	PY	N	Met	Cys	Lys	Thr	Arg	His	Ile	Leu	Phe	Val
	%	%	U/kg	----------					%----------					
1	.45	22.5	0	92.1	96.2	89.2	94.9	91.9	95.1	93.8	92.7	92.3	92.6	92.3
2	.45	22.5	750	92.9	96.9	90.0	95.6	92.7	95.8	94.2	93.4	93.1	93.4	93.1
3	.45	28.0	0	90.3	95.8	85.0	93.8	90.0	94.3	92.7	91.5	91.4	91.8	91.3
4	.45	28.0	750	92.1	96.2	88.2	95.1	91.5	95.4	93.9	93.3	92.8	93.3	93.0
5	.60	22.5	0	89.9	95.0	87.9	93.3	88.9	93.4	91.6	90.3	90.0	90.4	90.3
6	.60	22.5	750	93.4	97.2	91.4	95.8	93.2	95.9	94.6	93.8	93.4	93.7	93.4
7	.60	28.0	0	90.2	95.6	86.1	93.7	89.4	94.0	92.3	91.2	90.8	91.4	91.2
8	.60	28.0	750	90.1	95.9	86.4	93.6	89.5	94.0	91.9	90.9	90.8	91.0	90.6
Pooled SEM				.53	.29	.53	.37	.48	.45	.43	.54	.54	.56	.50
							Main Effects							
PY (U/kg)		0		90.6	95.7	87.0	93.9	90.1	94.2	92.6	91.4	91.1	91.6	91.3
		750		92.1	96.5	89.0	95.0	91.7	95.3	93.6	92.8	92.5	92.9	92.5
CP (%)		22.5		92.1	96.3	89.6	94.9	91.7	95.0	93.5	92.5	92.2	92.5	92.3
		28.0		90.7	95.9	86.4	94.1	90.1	94.4	92.7	91.7	91.4	91.9	91.5
nP (%)		.45		91.9	96.3	88.1	94.9	91.5	95.2	93.7	92.7	92.4	92.8	92.4
		.60		90.9	95.9	87.9	94.1	90.2	94.3	92.6	91.5	91.3	91.7	91.4
				----------				Probability						
PY				.002	.001	.001	.001	.001	.004	.005	.002	.003	.005	.003
CP				.002	.046	.001	.007	.001	.066	.016	.048	.081	.114	.046
nP				.023	.126	.600	.012	.002	.017	.003	.009	.012	.015	.010
PY * CP				.109	.015	.568	.075	.017	.126	.051	.087	.084	.088	.073
PY * nP				.620	.114	.900	.716	.153	.584	.422	.735	.466	.677	.978
CP * nP				.780	.698	.614	.875	.934	.949	.673	.719	.728	.617	.507
PY * CP * nP				.008	.091	.002	.009	.003	.034	.005	.007	.021	.014	.005
Contrast diet 1 vs 2				.319	.099	.298	.216	.234	.306	.518	.315	.314	.358	.281
Contrast diet 3 vs 4				.031	.427	.001	.031	.045	.097	.086	.035	.097	.076	.025
Contrast diet 5 vs 6				.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
Contrast diet 7 vs 8				.867	.502	.719	.799	.959	.996	.530	.656	.946	.656	.413

[a]Each value comes from three replicate pens of five birds/pen; PY = phytase added, U/kg of diet.

.60% nP, adding phytase to the 22.5% CP diet increased the TID of all the EAA ($P <$ .001) with no change in the TID of EAA when adding phytase to the 28.0% CP diets.

True Ileal Digestibility of Non-essential and Total Amino Acids

True ileal digestibility coefficients (TID) of NEAA and TAA are shown in Table 6-8. Main effects indicate 1) that the addition of microbial phytase increased TID of NEAA and TAA ($P <$ .001 to .05), 2) that reducing the dietary CP increased TID of TAA and NEAA ($P <$ .01 to .10) except Tyr, and 3) that reducing the dietary nP also increased the TID of TAA and NEAA ($P <$ .01 to .05). Interactive effects of phytase and CP on the TID of TAA and NEAA, except Ser and Glu, were observed ($P <$ .05 to .1), which indicates that the effect of phytase was dependent on dietary CP level. The three-way interaction of phytase, CP, and nP on the TID of TAA and NEAA, except Ser, were observed ($P <$ .01 to .05). This confirmed that the effects of phytase on the TID of TAA and the NEAA were depnedent on the dietary CP and nP levels.

At .45% nP, adding phytase to the 22.5% CP diets only showed increased trend in the TID of NEAA and TAA; the addition of phytase to the 28.0% CP diets increased the TID of Pro, Asp, Gly, Ala, and TAA ($P <$ .05 to .10), the others were only numerically increased. At .60% nP, adding phytase to the 22.5% CP diets increased the TID of all AA ($P <$.001 to .05), but did not change the TID with 28.0% CP diets.

Apparent Utilization of Dry Matter and Retention of Nitrogen, Phosphorus, and Calcium

Apparent utilization of DM and retention of N, P, and Ca are shown in Table 6-9. Main effects indicate 1) that the addition of microbial phytase increased the apparent utilization of DM ($P <$ .05) and retention of N ($P <$ .05) and P ($P <$ .05 for g/bird), 2) that reducing dietary CP increased ($P <$ .001) the percentage utilization of DM and retention of N, decreased ($P <$ .02) the total amount of utilized DM and retained N and P; but did not influence Ca retention, and 3) reducing dietary nP increased ($P <$ .05) the percentage

Table 6-8. True ileal digestibility of dietary non-essential and total amino acids in turkey poults fed corn-soybean meal diets with two nonphytate phosphorus (nP) levels, two crude protein (CP) levels and two phytase (PY) levels[a]

Diet	nP	CP	PY	Pro	Asp	Ser	Glu	Gly	Ala	Tyr	TAA
	%	%	U/kg	----------	----------	----------	----------	----------	----------	----------	----------
1	.45	22.5	0	92.7	91.9	93.3	93.9	91.6	92.9	93.9	93.1
2	.45	22.5	750	93.3	92.9	94.1	94.7	92.7	93.7	94.6	93.9
3	.45	28.0	0	90.9	90.5	92.0	92.9	90.2	91.8	93.5	91.8
4	.45	28.0	750	92.5	92.2	92.9	94.2	91.6	93.1	94.6	93.3
5	.60	22.5	0	89.9	89.5	90.7	91.8	89.2	91.0	92.1	90.9
6	.60	22.5	750	93.9	93.0	93.9	94.8	93.0	94.1	95.1	94.1
7	.60	28.0	0	90.5	90.0	90.4	92.5	89.8	91.3	93.0	91.5
8	.60	28.0	750	90.5	89.8	91.6	92.5	89.5	91.3	93.1	91.4
Pooled SEM				.50	.58	.81	.56	.52	.50	.48	.51
						Main Effects					
PY (U)	0			91.0	90.7	91.6	92.8	90.2	91.7	93.1	91.8
	750			92.6	91.8	93.1	94.0	91.7	93.1	94.3	93.2
CP (%)	22.5			92.5	91.8	93.0	93.8	91.7	92.9	93.9	93.0
	28.0			91.1	90.6	91.8	93.0	90.3	91.9	93.5	92.0
nP (%)	.45			92.3	91.8	93.1	93.9	91.5	92.9	94.2	93.0
	.60			91.2	90.6	91.7	92.9	90.4	91.9	93.3	92.0
				----------	----------	----------	Probability	----------	----------	----------	----------
PY				.001	.003	.018	.007	.001	.002	.003	.002
CP				.002	.010	.049	.078	.002	.010	.275	.015
nP				.007	.007	.025	.022	.007	.018	.025	.014
PY * CP				.056	.099	.399	.139	.021	.077	.087	.086
PY * nP				.236	.711	.250	.632	.509	.455	.372	.593
CP * nP				.848	.716	.993	.955	.784	.646	.516	.856
PY * CP * nP				.004	.018	.378	.047	.009	.023	.033	.017
Contrast diet 1 vs 2				.387	.275	.483	.315	.162	.254	.302	.269
Contrast diet 3 vs 4				.045	.060	.459	.122	.079	.088	.134	.063
Contrast diet 5 vs 6				.001	.001	.013	.002	.001	.001	.001	.001
Contrast diet 7 vs 8				.968	.802	.311	.969	.660	.960	.881	.882

[a]Each value comes from three replicate pens of five birds/pen; PY = phytase added, U/kg of diet; TAA = the total amino acids, including the essential amino acids in Table 6-7.

Table 6-9. Apparent utilization of dry matter and retention of nitrogen, phosphorus, and calcium of turkey poults fed corn-soybean meal diets with two nonphytate phosphorus (nP) levels, two crude protein (CP) levels and two phytase (PY) levels[a]

Diet	nP	CP	PY	DM		N		P		Ca	
	%	%	U/kg	%	g/bird	%	g/bird	%	g/bird	%	g/bird
1	.45	22.5	0	72.9	132	62.6	4.12	52.2	.679	47.0	.810
2	.45	22.5	750	76.1	153	66.1	4.75	57.6	.858	53.6	.967
3	.45	28.0	0	68.3	141	51.9	5.04	49.4	.790	46.6	.929
4	.45	28.0	750	71.3	160	58.5	5.86	50.1	.868	52.0	1.04
5	.60	22.5	0	71.8	146	58.5	4.30	48.1	.813	44.6	1.09
6	.60	22.5	750	75.9	149	67.8	5.04	49.4	.802	47.1	1.09
7	.60	28.0	0	71.0	160	57.6	5.74	45.9	.947	41.7	1.12
8	.60	28.0	750	69.9	165	54.7	5.77	45.9	.996	39.2	1.10
Pooled SEM				1.3	5	2.4	.25	2.0	.042	4.0	.088
						Main Effects					
PY (U/kg)		0		71.0	145	57.6	4.80	49.1	.809	45.0	.986
		750		73.3	157	61.8	5.35	50.7	.880	48.0	1.05
CP (%)		22.5		74.2	145	63.6	4.53	52.0	.789	48.1	.988
		28.0		70.1	157	55.8	5.60	47.8	.900	44.9	1.05
nP (%)		.45		72.1	146	59.8	4.94	52.5	.800	49.8	.937
		.60		72.1	155	59.6	5.21	47.3	.890	43.2	1.10
				----------Probability----------							
PY				.015	.002	.030	.005	.199	.021	.276	.331
CP				.001	.002	.001	.001	.007	.001	.247	.354
nP				.994	.023	.947	.151	.001	.005	.021	.015
PY * CP				.136	.904	.186	.492	.293	.761	.573	.799
PY * nP				.366	.034	.615	.330	.387	.105	.279	.245
CP * nP				.479	.341	.644	.811	.423	.086	.426	.536
PY * CP * nP				.169	.747	.035	.217	.562	.187	.742	.928
Contrast diet 1 vs 2				.075	.007	.333	.082	.065	.007	.236	.227
Contrast diet 3 vs 4				.099	.012	.072	.026	.798	.196	.337	.418
Contrast diet 5 vs 6				.027	.721	.010	.056	.658	.862	.655	.990
Contrast diet 7 vs 8				.528	.426	.401	.944	.982	.410	.665	.850

[a]Six replicate pens (five birds per pen) per treatment.

retention of P and Ca, but decreased ($P < .05$) the total amount of retained P and Ca. The interactive effects of phytase and nP on the total amount of utilized DM ($P < .05$) and P ($P < .10$) were observed, which indicates that the effects of phytase on DM utilization and P retention were dependent on dietary nP. The interactive effect of CP and nP on the total amount of retained P was observed ($P < .10$). The three-way interaction of phytase, CP, and nP on percentage N retained was also observed ($P < .05$). This confirmed that the effect of phytase on N retention was dependent on dietary CP and nP levels.

At .45% nP, adding phytase to the 22.5% CP diets resulted in an increased apparent utilization of DM ($P < .10$ or .01), N ($P < .10$ for g/bird), P ($P < .10$) and Ca (improved trend); addition of phytase to the 28.0% CP diets increased the apparent utilization of DM ($P < .10$ or .01), N ($P < .10$), P and Ca (improved trend). At .60% nP, adding phytase to the 22.5% CP diets increased the apparent utilization of DM ($P < .05$ for %) and N ($P < .10$), but did not influence P and Ca; addition of phytase to the 28.0% CP diets did not influence any of the measurements. Mortality of the birds was within normal ranges (3%) during the trial, and differences in mortality was not observed between the various treatments.

Discussion

Indicator Methods

This is the first report investigating the effect of microbial phytase on N and AA digestibility of turkey poults fed corn-soybean meal diets. It has been proven that evaluating microbial phytase efficacy up to the end of the small intestine is more accurate than evaluating it over the total tract, because the potential contributions of the hindgut microflora in degradation of phytate-protein complexes are excluded (Mroz et al., 1994). The indicator method with Cr_2O_3 (the analysis of ileal contents) has been proven more convenient and meaningful than conventional (the analysis of feces) methods (Varnish and

Carpenter, 1975). The indicator method was used in this experiment to assay the ileal N and AA digestibility of turkey poults. The average values (91.1 and 92.4%) and range (85 to 94% and 88 to 95%) of the apparent and true ileal digestibility of individual amino acids, except Met, obtained in this study are comparable to those obtained in turkeys or chicks fed corn and soybean meal (Parsons et al., 1981; Engster et al., 1985; Green et al., 1987). The higher apparent and true ileal digestibility of Met (95 and 96%) in this study are possibly due to the addition of .20% synthetic DL-Met to the diets, because free Met has high digestibility in poultry (NRC, 1994).

Effects of Phytase on Nitrogen and Amino Acid Digestibility

The results of this experiment indicate that the addition of 750 U of phytase/kg of diet to corn-soybean meal diets increases N and AA digestibility of turkey poults (Table 6-5 to 8). The results were demonstrated by the main effects of phytase and confirmed by the three-ways interactions of phytase, CP, and nP. However, the improvements of phytase on the digestibility were primarily seen at .45% nP with 28.0% CP diets and at .60% nP with 22.5% CP diets. The magnitude of the improvement in AID was 1 to 3 percentage units and the magnitude in TID was 1 to 4 percentage units. The improvements of phytase on the digestibility of N and AA at .45% nP with 22.5% CP diets were less than those above, and there was no effect of phytase at .60% nP with 28.0% CP diets.

It has been reported that phytate has the potential of binding with protein at low and neutral pH (Cosgrove, 1980; Anderson, 1985; Thompson, 1986). In general, at low pH, the proteins are positively charged and can form insoluble complexes with the negatively charged phytate because of strong electrostatic interactions (Cheryan, 1980, Reddy et al., 1982). The positive charges of the proteins could be the terminal alpha amino group, epsilon amino group of lysine, or guanidyl group of arginine, and histidine residues (Barre

and van Huot, 1965a, b). When the pH is raised, proteins bind with phytate mediated by multivalent cations such as Ca, Mg, or Zn as a bridge between negatively charged protein carboxyl groups and phytate (Anderson, 1985). The common binding site for the ternary complex appears to be the ionized carboxyl groups and the unprotonated imidazole group of histidine. The complexing of phytate with proteins can change the protein structure, which in turn decreases solubility, digestibility, and functionality of proteins, i.e. all the AA in the proteins. The association between phytate and protein begins in the seeds during ripening when phytate accumulates primarily in the protein-rich aleurone layer of monocotyledonous seeds and in the protein bodies of dicotyledonous seeds (O'Dell et al., 1972; Prattley et al., 1982). Therefore, when phytase cleaves the ester bond to release P from phytate, it also frees the binding groups of proteins bound to phytate, which makes the protein and AA available for absorption. In this experiment, it was confirmed that adding microbial phytase increased dietary phytate P digestibility at .45% nP with both CP levels and at .60% nP with 22.5% CP diets. When dietary P and CP requirements of poults were met, added phytase did not influence P absorption, or the digestibility of N and AA. In the study of Mroz et al. (1994) with pigs (45 to 110 kg), adding phytase (800 U/kg of diet) significantly increased the AID of Met and Arg only. This may be due to the fact that the P requirement of the pigs was met in the diet (.36% tP). The reasons for lower improvements of N and AA digestibility at .45% nP with 22.5% CP diets in this study were unkown. Further research is needed to clarify this phenomena.

Effects of Phytase on Retention of Nitrogen and Phosphorus

Consistently with the improved AID and TID of N and AA, adding phytase increased apparent retention or utilization of P, N and DM (Table 6-9). The increased P and N retention by phytase was also observed in previous studies in broilers (Yi et al., 1994b) and laying hens (Van der Klis and Versteegh, 1991). The results indicate that

adding phytase could reduce N excretion by more than 3 percentage units at 22.5% CP diets. Pollution of the environment with manure N is a major argument for improving N utilization and limiting N excretion by animals and poultry (Cromwell and Coffey, 1991; Sweeten, 1992). The use of microbial phytase in poultry diets would provide a means to reduce N excretion from poultry manure. In comparison with the data at .60% nP with 28.0% CP recommended by NRC (1994) as a positive control, reducing CP (22.5%) did not affect N excretion at .60% nP (41 vs 42%), but reduced N excretion by 12% at .45% nP (37 vs 42%). Adding phytase to the low CP diets decreased N excretion by 19 (34 vs 42%) and 24% (32 vs 42%). The potential of using phytase to increase N retention and reduce N excretion is further confirmed by growth response in this study. In comparison with the positive control, the BW gain of the birds during d 1 to 20 fed 22.5% CP (80% of recommended level) without phytase added at both nP levels gained 82 or 83% (428 or 441 g) of the control (532 g); however, the BW gain of the birds with phytase added increased to 89 or 92% (488 or 471 g) of the control (Table 6-4). Similar results were also found in BW gain of birds during d 1 to 29. It appears possible to reasonably reduce the dietary CP level without decreasing performance by adding microbial phytase.

It may be possible is to reduce dietary CP to 90% (25.2 vs 28.0%) without adverse effects on growth by adding phytase. Phytase (750 U/kg of diet) could improve the digestibility and utilization of N and AA, thus meeting the AA requirement of the turkey poults at a lower CP level. This would result in about a 10% decrease in N excretion. Another possibility is to reduce dietary CP to 80% (22.5 vs 28.0%) by adding phytase and synthetical AA, without adverse effects on growth. A comparison of AA requirements was made in Table 6-10. When dietary CP is reduced to 80% of the NRC level, the amount of Arg, Gly + Ser, His, Leu, Phe, Phe + Tyr, and Trp were still greater than the requirements. After addition of phytase, the deficit of available limiting AA was

Table 6-10. Comparison of CP and AA requirement (unit: %)

CP or AA	AA level		Difference	Available AA		Difference
	NRC[a]	.8NRC[b]		NRC[c]	.8NRC+PY[d]	
CP	28.00	22.80	-5.20	25.30	21.30	-4.00
Arg	1.60	1.70	+.10			
Gly + Ser	1.00	1.98	+.98			
His	.58	.65	+.07			
Ile	1.10	1.06	-.04	1.00	.99	-.01
Leu	1.90	1.94	+.04			
Lys	1.60	1.40	-.20	1.50	1.34	-.16
Met	.55	.51	-.04	.53	.50	-.03
Met + Cys	1.05	.90	-.15	.96	.86	-.10
Phe	1.00	1.22	+.22			
Phe + Tyr	1.80	2.00	+.20			
Thr	1.00	.90	-.10	.89	.84	-.05
Val	1.20	1.17	-.03	1.09	1.09	
Trp	.26	.35[e]	+.09			

[a]NRC,1994.
[b].8NRC = 80% of NRC level; mean of 22.5% CP diets at .45 and .60% nP.
[c]NRC requirements time the true ileal digestibility (%) at 28.0% CP of .60% nP in Table 6-7.
[d].8NRC + PY = 80% of NRC level; mean of 22.5% CP diets at .45 and .60% nP time the true ileal digestibility (%) at 22.5% CP of .60% nP in Table 6-7.
[e]Calculated from NRC (1994).

eliminated (Ile and Val) or decreased (Lys, Met + Cys, and Thr). Interestingly, a 10% deficit was remained for Lys (.16 vs 1.5%) and Met + Cys (.10 vs .96%), which is the same ratio as the deficit in growth response after adding phytase to the lower CP level. It appears that the growth response of birds fed 22.5% CP diets could be maintained at normal level by adding .16% Lys and .10% Met to the diets after adding phytase.

Implications

Supplemental phytase improved N and AA digestibility in the corn-soy bean meal diets fed to turkey poults. The improvements of phytase on the digestibility were primarily manifested at .45% nP with 28.0% CP diets and at .60% nP with 22.5% CP diets with the increased magnitude of 1 to 4 percentage units in AID and TID. Phytase also increased N and P retention, BW gain, feed intake, and toe ash contents of the birds.

Chapter VII

Supplemental Microbial Phytase Improves Zinc Utilization in Broilers

ABSTRACT: Day-old male broilers (n = 384) were used in a 21-d trial to investigate the effect of microbial phytase on the retention and utilization of Zn. A corn-soy isolate basal diet containing 20 ppm Zn was fed alone and supplemented with 5, 10, and 20 ppm Zn as $ZnSO_4 \cdot 7H_2O$ or with 150, 300, 450, and 600 U of phytase/kg of diet. Total excreta were collected during d 18 to 20. Toe, tibia, and liver samples were taken at the end of the experiment. Adding Zn and phytase to the low Zn basal diet linearly increased BW gain and feed intake of broilers ($P < .01$). The gain:feed was not changed by adding Zn but was decreased by adding phytase ($P < .01$). The amount of DM utilized was linearly increased by adding Zn and phytase ($P < .10$), but DM utilized as a percentage of intake was only increased by adding Zn ($P < .05$). The amount of Zn retained per bird was linearly increased by adding Zn and phytase ($P < .01$); however, Zn retained as a percentage of intake was linearly decreased by adding Zn but was linearly increased by adding phytase ($P < .10$). Ash percentage of toe and tibia were not affected by adding Zn but were linearly increased by adding phytase ($P < .10$); however, the amount of ash in toe or tibia was increased by Zn ($P < .05$) and phytase ($P < .01$ for toe). The concentration and amount of Zn in toe and tibia were linearly increased by adding Zn and phytase ($P < .001$). The concentration of Zn in liver was increased by adding Zn ($P < .10$). The amount of Zn retained in liver was linearly improved by adding Zn and phytase ($P < .05$). Nonlinear or linear response equations of the effects of Zn and phytase levels were generated and used to calculate the Zn equivalency values. The results indicate that about 1 mg of Zn was released per 100 U of phytase over the range of 150 to 600 U of phytase.

Key Words: Broiler, Phytase, Zinc, Utilization, Equivalency values

Introduction

Phytate has complexing potential to form a wide variety of insoluble salts with cations (Vohra et al., 1965; Oberleas, 1973; Morris, 1986). It has been reported that Zn has the strongest binding affinity with phytate (Maddaiah et al., 1964; Vohra et al., 1965; Reddy et al., 1982). Phytate has been shown to impair the bioavailability of Zn in humans (Anderson et al., 1983; Ferguson et al., 1989), rats (Lonnerdal et al., 1989), pigs (Bobilya et al, 1991), and chicks (Likuski and Forbes, 1964; Lease, 1966). Supplemental microbial phytase has been shown to be very effective for improving dietary phytate P bioavailability (Simons et al., 1990; Yi et al., 1994a,b; Denbow et al., 1995). A few studies also indicate that supplemental microbial phytase in the diets of young pigs improved the bioavailability and absorption or retention of Zn (Pallauf et al., 1992, 1994a,b; Lei et al., 1993). Thiel et al. (1993) found that Zn content of femur from chicks fed a diet containing 30 ppm Zn plus 700 U of phytase/kg of diet was equal to that of chicks fed a diet containing 39 ppm Zn without phytase. Adding phytase increased tibia Zn concentration but did not improve apparent Zn retention in broiler chicks (Roberson and Edward, 1994). The addition of 1,200 U of phytase/kg of diet to a glucose-soy concentrate diet (13 ppm of Zn) increased growth rate by 40% and total tibia Zn by more than 100% in chicks (Biehl et al., 1995).

The objective of this experiment was to investigate the influence of adding microbial phytase on Zn utilization in broilers fed a low Zn corn-soy isolate diet and to calculate Zn equivalency values of phytase.

Materials and Methods

Birds, Diets and Treatments

The effect of microbial phytase on the utilization of Zn was investigated using 384 Peterson x Arbor male broiler chicks fed corn-soy isolate diets. The basal diet met recommended (NRC, 1994) nutrient requirements except Zn (Table 7-1). The calculated

Table 7-1. Percentage composition of the basal diets[a]

Ingredients	%
Corn (8.5% CP)	54.40
Soy isolate (92% CP)[b]	20.00
Soybean oil	4.00
Corn starch[c]	17.67
Limestone[d]	.89
Defluorinated phosphate[e]	1.94
Vitamin premix[f]	.20
Trace mineral premix[g]	.20
Salt	.40
DL-methionine	.30
Calculated Ingredients	
CP	23.00
Lysine	1.28
Methionine + Cystine	.90
Ca	1.00
Total P	.65
Nonphytate P (nP)	.45
Ca:nP	2.22

[a]The assayed contents of Zn, total P, and Ca in the basal diet were 20 ppm, .61% and .93%, respectively. Based on NRC (1994), the calculated phytate P in the basal diet was .21%.

[b]Nonphytate P was assumed to make up 40% of total P in soy isolate, the same as reported for soy concentrate.

[c]Food grade, National Starch and Chemical Co., Bridgewater, NJ 08807.

[d]Limestone Dust Corp., Bluefield, VA 24605.

[e]Fine CDP, Southern Bag Corp., Valdosta, GA 31083.

[f]Supplied per kilogram of diet: retinyl acetate, 908 μg; cholecalciferol, 66 μg; dl-α-topherol acetate, 26 mg; menadione sodium bisulfite, .75 mg; riboflavin, 7.5 mg; d-calcium pantothenate, 9.7 mg; niacin, 26.4 mg; cyanocobalamin, .011 mg; choline chloride, 1,012 mg; d-biotin, .31 mg; folic acid, 3.1 mg; thiamin·HCl, 8 mg; pyridoxine·HCl, 3.1 mg; ethoxyquin, 50 mg; and virginiamycin, 2.9 mg.

[g]Supplied per kilogram of diet: manganese, 60 mg; iron, 80 mg; copper, 8 mg; iodine, .35 mg; and selenium, .15 mg.

and assayed Zn content of the basal diet was 20 ppm. The assayed Zn contents of corn, soy isolate, corn starch, and defluorinated phosphate (DFP) were 19, 38, 2.5, and 65 ppm, respectively. Eight treatments were as follows: three levels of Zn (5, 10 and 20 ppm Zn as $ZnSO_4$ $.7H_2O$, USP/FCC, from Fisher Chemical Fisher Scientific, Fair Lawn, NJ 07410) or four levels of Natuphos® phytase (150, 300, 450, and 600 U/kg of diet, from BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234.) were added to the low Zn basal diet. The basal diet without added Zn or phytase was fed as a control. Limestone and DFP were used to maintain the desired P and Ca levels. The Ca:nonphytate P was 2.22:1 in all diets as recommended (NRC, 1994). The assayed total P and Ca contents in the basal diets were .61 and .93%, respectively. Based on NRC (1994) data, the calculated phytate P in the basal diet was .21%.

Feeding and Management

Broilers were randomly allotted on the day of hatched to 48 pens (eight birds per pen). They were housed in electrically heated, stainless steel starting batteries (Alternative Design, Manufacturing & Supply, Inc., Siloam Spring, AR 72701) in an environmentally controlled room. The eight treatments were randomly assigned to 48 pens (six pens per treatment). The diets were fed in a mash form. Birds had *ad libitum* access to feed and mineral-free water from a reverse osmosis system (Rolp120-TF, Hydro, Research Triangle Park, NC 27709). Zinc concentration in the water was .4 pmm. The care and treatment of birds followed published guidelines (Consortium, 1988). Body weight and feed intake of the birds were recorded on a pen basis at weekly intervals during the 21-d experiment. Mortality was recorded daily.

Sampling and Analysis

The total excreta of the birds were collected during d 18 to 20. Feed intakes were recorded starting 24 h before the collection time and ending 24 h before the end of

collection. The excreta were kept in plastic bags and dried in a stainless steel oven at 60 °C and ground. All the birds were killed at the end of the experiment. Toe samples of the killed birds were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left middle toes of the birds within a pen were pooled, and the right middle toes from the same birds were pooled, yielding two samples of toes per pen. The composite samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 600 °C for 4 h. Three killed birds from each pen were randomly selected and samples of liver and left tibia were collected. After the soft tissues were removed, tibia samples were dried to a constant weight at 100 °C and then ashed in a muffle furnace at 600 °C for 4 h. The ash from toe and tibia was solubilized with nitric and perchloric acid (5:3, v/v) for measurement of Zn contents. Zinc concentrations in diets, excreta, liver, toe and tibia ash were analyzed with an atomic absorption spectrophotometer (AOAC, 1990) following nitric-perchloric acid wet digestion.

Statistical Analysis and Zinc Equivalency Values of Phytase

The data were subjected to analysis of variance by the GLM procedures of SAS (1991). The model included diet and replicate with linear and quadratic contrast for effects of supplemental Zn or microbial phytase. Nonlinear and linear functions which best fit the data were derived for four Zn levels and for five phytase levels with the nonlinear model: $Y = a(1 - be^{-kX})$ and linear model: $Y = a + bX$. Where Y = the response measurements; X = Zn (ppm) added or phytase added (U/kg of diet). The nonlinear or linear response equations for added Zn and added phytase levels with higher r^2 values were set equal and solved as shown in the example below for amount of Zn retained in toe:

$$159.7(1 - .5613e^{-.0573Y}) = 71.4 + .045X$$

$$Y = -17.45Ln(.9851 - .0005X)$$

where, Y = Zn added (ppm); X = phytase added (U/kg of diet)

The resulting equations were used to calculate the Zn equivalency values of microbial phytase at 150, 300, 450, and 600 U/kg of diet for the various measurements.

Results and Discussion

Performance and Apparent utilization of Dry Matter and Retention of Zinc

Adding graded levels of Zn to the low Zn basal diet linearly increased BW gain ($P < .01$) and feed intake, but did not influence gain:feed of broilers (Table 7-2). Addition of graded levels of phytase to the basal diet linearly increased BW gain ($P < .001$) and feed intake ($P < .001$), but linearly decreased gain:feed of broilers ($P < .01$). The improved BW gains by adding phytase were mainly related to the increased feed intake. In agreement, Schoner et al. (1991), Denbow et al. (1995), and Yi (Chapter IV and V) observed improvement in BW gain and feed intake, when phytase was added to the low P diets. However, the gain:feed was not affected by phytase in their studies. This may be due to the lower Zn in our diets. Even after the action of phytase, Zn in the diets may have not been adequate to meet the requirement of the birds.

The amount of DM utilized (Table 7-2) was linearly increased by adding Zn ($P < .001$) and phytase ($P < .10$), but DM utilized as a percentage of intake was only linearly increased by adding Zn ($P < .05$). The amount of Zn retained (mg/bird) was linearly improved by adding Zn ($P < .001$) and phytase ($P < .01$); however, Zn retained as a percentage of intake was numerically decreased by adding Zn but was linearly increased by adding phytase ($P < .10$). In agreement, Thiel and Weigand (1992) reported that the addition of 800 U of phytase/kg of diet to a diet containing 27 ppm Zn increased Zn retention and decreased Zn excretion. However, Roberson and Edward (1994) reported that Zn retention was not influenced by adding 600 U of phytase/kg of diet. This may be

Table 7-2. Effects of supplemental microbial phytase and zinc on performance (d 1 to 21), apparent utilization of DM and retention (d 18 to 20) of zinc of broilers[a]

Diet	Zn added ppm	Phytase added U/kg	BW gain g	Feed intake g	Gain: feed g/kg	DM utilization		Zn Retention	
						%	g/bird	%	mg/bird
1	0	0	396	530	747	83.7	75.9	37.9	6.89
2	5	0	428	600	714	83.2	87.2	35.8	9.39
3	10	0	437	605	721	83.7	87.6	35.4	11.14
4	20	0	443[b]	616[c]	722	84.7[d]	91.1[e]	34.2	13.34[e]
5	0	150	398	551	724	83.3	77.6	39.2	7.23
6	0	300	431	617	700	83.9	86.1	44.0	8.97
7	0	450	440	670	660	83.8	86.1	43.0	8.81
8	0	600	440[f]	630[g]	702[h]	83.5	85.6[i]	44.8[i]	9.09[j]
Pooled SEM			11	20	16	.40	3.5	2.96	.95

[a]Six pens (eight birds per pen) per treatment mean. The values of apparent retention were based on total collection of excreta from day 18 to 20.
[b]Zinc effect (linear, $P < .01$).
[c]Zinc effect (linear, $P < .01$).
[d]Zinc effect (linear and quadratic, $P < .05$).
[e]Zinc effect (linear, $P < .001$).
[f]Phytase effect (linear, $P < .001$).
[g]Phytase effect (linear, $P < .001$; quadratic, $P < .08$).
[h]Phytase effect (linear, $P < .01$; quadratic, $P < .03$).
[i]Phytase effect (linear, $P < .06$).
[j]Phytase effect (linear, $P < .01$).

related to the higher Zn contents (35 to 45 ppm) in their basal diets. The other studies with Zn and phytase (Thiel and Weigand, 1992; Roberson and Edward, 1994) did not give the Zn retention data.

Zinc Contents in Toe, Tibia, and Liver

Ash percentage of toes and tibias (Table 7-3) was not affected by Zn levels, but was linearly increased by adding phytase ($P < .05$ for toes; $P < .10$ for tibias); however, the amount of ash (mg/toe or tibia) was linearly increased by Zn ($P < .001$ for toes; $P <$

Table 7-3. Effects of supplemental microbial phytase and zinc on ash and zinc contents in toes and tibia of broilers (d 1 to 21)

Diet	Zn added ppm	Phytase added U/kg	Toe ash[a] DM basis %	Toe ash[a] DM basis mg/toe	Toe Zn[a] DM basis ppm	Toe Zn[a] DM basis μg/toe	Tibia ash[b] DM basis %	Tibia ash[b] DM basis mg/tibia	Tibia Zn[b] DM basis ppm	Tibia Zn[b] DM basis μg/tibia
1	0	0	12.5	15.3	71	8.8	36.5	570	125	195
2	5	0	12.7	18.0	88	12.5	36.9	611	146	247
3	10	0	12.7	17.7	113	15.6	36.8	633	208	356
4	20	0	12.7	18.8[c]	130[c]	19.3[c]	36.7	636[d]	291[e]	507[f]
5	0	150	13.1	16.6	82	10.3	37.4	578	122	189
6	0	300	12.9	17.5	83	10.7	37.4	590	137	216
7	0	450	13.6	19.0	84	11.7	37.4	609	150	237
8	0	600	13.2[g]	17.4[h]	104[i]	14.4[i]	37.8[j]	595	151[i]	230[i]
Pooled SEM			.21	.60	5.3	.8	.53	23	7.8	17

[a]Six pens (eight birds per pen) per treatment mean.
[b]Six pens (three birds per pen) per treatment mean.
[c]Zinc effect (linear, $P < .001$).
[d]Zinc effect (linear, $P < .04$).
[e]Zinc effect (linear, $P < .001$; quadratic, $P < .01$).
[f]Zinc effect (linear, $P < .001$; quadratic, $P < .04$).
[g]Phytase effect (linear, $P < .02$).
[h]Phytase effect (linear, $P < .01$; quadratic, $P < .04$).
[i]Phytase effect (linear, $P < .001$).
[j]Phytase effect (linear, $P < .09$).

.05 for tibias) and phytase ($P < .01$ for toes; numerical for tibias). The concentration (ppm, DM basis) and amount (μg/toe or tibia) of Zn in toes and tibias were linearly increased by adding Zn and phytase ($P < .001$). Adding 10 or 20 ppm Zn to the low Zn diets resulted in an increase of Zn content by 60 to more than 100%. In the study of Biehl et al. (1995), adding 10 ppm Zn to the Zn deficient diet (13 ppm) also increased total tibia

Zn by more than 100%. The magnitude of Zn content in toes and tibias indicates that bone Zn measurements are the most sensitive indicators among those examined in the present study for determining Zn utilization. This conclusion is also supported by the studies of Roberson and Edward (1994) with chicks. Adding phytase increased the ash percentage of toe and tibia because it affected P and Ca utilization which account for about 50% of ash (Qian et al., 1995, unpublished data). The amount of ash retained in toe or tibia in present study are good measurements to compare the effect of Zn and phytase. Both were linearly increased by adding Zn and phytase.

The concentration of Zn in the liver on a fresh or DM basis (Table 7-4) was linearly increased by adding Zn ($P < .05$ for fresh; $P < .10$ for DM) and phytase. The

Table 7-4. Effects of supplemental microbial phytase and zinc on the amount and concentration of Zn in liver of broilers (d 1 to 21)[a]

Diet	Zn added ppm	Phytase added U/kg	Zn, ppm fresh basis	Zn, ppm DM basis	Zn μg/bird
1	0	0	19.9	78.3	202
2	5	0	21.4	82.2	221
3	10	0	22.1	82.3	270
4	20	0	23.6[b]	88.5[c]	284[d]
5	0	150	21.6	82.1	210
6	0	300	20.9	83.5	206
7	0	450	21.8	84.4	222
8	0	600	21.4	82.7	244[e]
Pooled SEM			.81	2.87	16

[a]Six pens (three birds per pen) per treatment mean.
[b]Zinc effect (linear, $P < .02$).
[c]Zinc effect (linear, $P < .09$).
[d]Zinc effect (linear, $P < .001$).
[e]Phytase effect (linear, $P < .05$).

amount of Zn retained (μg/bird) in the liver was linearly increased by adding Zn ($P < .001$) and phytase ($P < .05$). The Zn concentration in liver was reported by Schell and Kornegay (1994) and Cheng et al. (1995) to be a sensitive measurement to evaluate the bioavailability of Zn sources in young pigs. The Zn concentration in liver of the birds in this study were increased by adding Zn and phytase, but the magnitude of the increase by Zn addition was smaller than the increase in the Zn concentration of bones.

Zinc Equivalency Values of Phytase

Nonlinear and linear response equations of birds fed graded levels of Zn or supplemental phytase were developed using treatment means of the various measurements (Table 7-5). The respective equations were solved and derived equation for each of the measurement was used to calculate Zn requirement values (Table 7-6). The addition of 150, 300, 450, and 600 U phytase/kg of diet to the low Zn corn-soy isolate diets for broilers released 1.4, 2.7, 3.9, and 5.1 mg of Zn respectively. The Zn equivalency equation, using averages without BW gain and Zn in liver, was Y = .20 + .0082X, where Y = Zn released (mg) and X = phytase (U/kg diet). The results indicate that about 1 mg of Zn is released per 100 U of phytase over the range of 150 to 600 U of phytase. The basal diet was calculated to contain .21% phytate (Table 7-1), and thus, 3.24 mmol (2100 mg / 648 MW) of phytate. If we assume that 1 molecule of phytate complexes with 1 molecule of Zn, then 211 mg of Zn (3.24 mmol x 65 MW) would be combined with phytate. Thus, 18 mg Zn (20 mg - 2 mg from defluorinated phosphate) in the basal diet (Table 7-1) has the potential to totally the complex with phytate. Therefore, the Zn equivalency values of phytase in this study is reasonable.

Our findings are generally supported by the results of Biehl et al. (1995) and Thiel et al. (1993). Biehl et al. (1995) reported that the total amount of tibia Zn of the chicks fed

Table 7-5. Nonlinear or linear response equations for the various measurements of broilers fed corn-soy isolate diets with increased levels of zinc and supplemental phytase[a]

Measurements	Equation	r^2
	Zinc	
Aapparent DM utilization, mg/bird	$Y = 90.2(1 - .1570e^{-.2621X})$	.97
Apparent Zn retention, mg/bird	$Y = 15.6(1 - .5585e^{-.0673X})$	.99
Toe ash, mg/toe	$Y = 18.4(1 - .1710e^{-.3631X})$	.93
Zn in toe (DM basis), ppm	$Y = 159.7(1 - .5613e^{-.0573X})$	.98
Total Zn in toe, μg	$Y = 24.31(1 - .6405e^{-.0570X})$	.99
Tibia ash, mg/tibia	$Y = 641.3(1 - .1128e^{-.1628X})$	.98
Zn in tibia (DM basis), ppm	$Y = 116.8 + 8.66X$	.98
Total Zn in tibia, μg	$Y = 185.8 + 16.1X$	.99
Total Zn in liver, μg	$Y = 314.9(1 - .3698e^{-.0711X})$	.93
Zn in liver (DM basis), ppm	$Y = 78.6 + .4797X$	.95
BW gain, g	$Y = 443.1(1 - .1064e^{-.2258X})$	.99
	Phytase	
Apparent DM utilization, mg/bird	$Y = 89.1(1 - .1579e^{-.0029X})$	.79
Apparent Zn retention, mg/bird	$Y = 9.85(1 - .3153e^{-.0026X})$	.86
Toe ash, mg/toe	$Y = 18.3(1 - .1712e^{-.0051X})$	.79
Zn in toe (DM basis), ppm	$Y = 71.41 + .045X$	.81
Total Zn in toe, μg	$Y = 8.652 + .0084X$	.92
Tibia ash, mg/tibia	$Y = 599.6(1 - .0520e^{-.0038X})$	.77
Zn in tibia (DM basis), ppm	$Y = 121.3 + .053X$	.87
Total Zn in tibia, μg	$Y = 189.8 + .079X$	.78
Total Zn in liver, μg	$Y = 197.7 + .0641X$	.81
Zn in liver (DM basis), ppm	$Y = 83.6(1 - .0637e^{-.0095X})$	.92
BW gain, g	$Y = 475.6(1-.1748e^{-.0016X})$	.91

[a]Y = response measurement; X = Zn added (ppm) or phytase added (U/kg of diet).

Table 7-6. Calculated zinc equivalency values (mg) of microbial phytase in broilers fed corn-soy isolate diets[a]

Item	Phytase, U/kg of diet			
	150	300	450	600
	----------mg----------			
Apparent DM utilization, g/bird	1.3	2.7	4.0	5.3
Apparent Zn retention, mg/bird	1.5	2.9	3.9	4.6
Toe ash retention, mg/toe	1.9	3.9	5.6	7.0
Zn in toe, ppm DM	1.6	3.2	4.8	6.6
Toe Zn retention, µg/toe	1.4	3.0	4.8	6.7
Tibia ash retention, mg/tibia	1.2	2.1	2.6	2.9
Zn in tibia, ppm DM	1.4	2.4	3.3	4.2
Tibia Zn retention, µg/tibia	1.0	1.7	2.5	3.2
Liver Zn retention, µg/bird	1.1	2.4	3.9	5.5
Zn in liver, ppm	7.8	9.8	10.3	10.4
BW gain	1.6	4.0	7.9	19.0
Mean (without gain and Zn in liver)	1.4	2.7	3.9	5.1
Mean from all the measurements	2.0	3.5	4.9	6.9
Mean (without BW gain)	2.0	3.4	4.6	5.6

[a]Equations for Zn and phytase see Table 7-5.

the basal diet (13 ppm Zn) with 1,200 U of phytase/kg of diet was equal to that of chicks fed the basal diet with 10 mg Zn/kg diet. Thiel et al. (1993) found that the Zn concentration of femur from chicks fed a diet containing 30 mg Zn/kg of diet plus 700 U of phytase/kg of diet was equal to that of chicks fed a diet containing 39 mg Zn/kg of diet without phytase.

The release of Zn was 2.0, 3.5, 4.9, and 6.9 mg, respectively for 150, 300, 450, and 600 U of phytase/kg of diet using the average calculated from all the measurements. The Zn equivalency equation was Y = .300 + .0107X, where Y = Zn released (mg) and X

= phytase (U/kg of diet). However, there was a very large increase of Zn equivalency values calculated from in BW gain from 300 to 600 U of phytase/kg of diet. Adding 600 U of phytase/kg of diet could release 19 mg Zn, which is about 100% of the Zn content (20 mg/kg diet). This value was extremely high compared to the others. This is because phytase not only improved Zn utilization, but also increased the utilization of other nutrients. Therefore, the BW gain values should be omitted from the average of the response measurements. When all BW gain values are removed, the release of Zn was 2.0, 3.4, 4.6, and 5.6 mg of Zn for 150, 300, 450, and 600 U of phytase/kg of diet, respectively. Using average without BW gain, the Zn equivalency equation was Y = .900 + .008X, where Y = Zn released (mg) and X = phytase (U/kg diet). Again, Zn equivalency values of phytase in the lower phytase levels (150 to 300 U/kg of diet) calculated from the measurement of Zn in liver were much higher than those from others. Although the response equation of phytase on the measurement of Zn in liver had a high r^2 (Table 7-5), the linear contrast was not significant (Table 7-4). Thus, the values calculated from Zn in liver should be also omitted from the average of the response measurements.

Implications

Natuphos® phytase is effective for improving Zn utilization in low Zn corn-soy isolate diets fed to broilers. About 1 mg of Zn was released per 100 U of phytase over the range of 150 to 600 U of phytase. Zinc contents in toe and tibia were the most sensitive indicators for evaluating Zn availability of broilers fed low Zn diets.

Chapter VIII

Sites of Phytase Activity in the Gastrointestinal Tract of Young Pigs

ABSTRACT: The sites of supplemental phytase activity in the gastrointestinal tracts (GIT) of young pigs fed added microbial phytase were determined in two tests. In Test 1, samples of diets and GIT (stomach, upper and lower small intestine) contents were taken from pigs that had been fed a soybean meal-based semi-purified diet containing two levels of available P (.05 and .16% aP), with or without added microbial phytase (1,050 U/kg of diet) for 5 wk. There was no detectable phytase activity in the diets and GIT contents of pigs fed the basal diets without added phytase, and phytase activity did not differ for diets or GIT contents between the two dietary P levels. For pigs fed diets with added phytase, phytase activity in the digesta from the stomach was higher ($P < .001$) than that from the upper small intestine (51 vs 31% of diet activity). No phytase activity was detectable in the digesta of the lower small intestine. In Test 2, samples of diets and GIT contents were taken from young pigs that had been fed a corn-soybean meal diet with added microbial phytase (750 U/kg of diet) containing one of three levels of citric acid (0, 1.5, and 3.0%). Adding citric acid at 3.0 ($P < .10$) and 1.5% (numerically) decreased phytase activity in the stomach digesta; but there was no difference between the 1.5 and 3.0% added citric acid. About 40% of the added phytase activity remained in the digesta of the stomach from pigs fed the diet without added citric acid; whereas, only 27% of the phytase activity remained in the stomach digesta from pigs fed the diet with added citric acid. Again, phytase activity in the digesta from the stomach was higher ($P < .05$) than that in digesta from the upper small intestine (16 and 10% of added activity, respectively for diets without and with citric acid). As in Test 1, no phytase activity was detectable in the lower small intestine digesta.

Key Words: Site, Phytase activity, Pigs

Introduction

Phytase (E.C.3.1.3.8.), myo-inositol hexaphosphate phosphohydrolase, is a special kind of acidic phosphatase (Gibson and Ullah, 1990). The optimal pH of the phytase from *Aspergillus ficuum* showed two response peaks: pH 5.0 to 5.5 and 2.5 (Shieh et al., 1969; Irving and Cosgrove, 1974; Simons et al., 1990). The variation of the pH in the gastrointestinal tract (GIT) of pigs may influence the active sites of added microbial phytase in the diet. Orthophosphate was reported as a noncompetitive inhibitor to phytase (Gibson and Ullah, 1990). However, it is unknown whether dietary available P influences the activity of phytase in GIT. Acidification of starter diets using organic acids has generally improved postweaning performance of young pigs (Risley et al., 1992; Easter, 1993). Mineral requirements are met by different sources of mineral supplements. The use of organic acid and mineral sources can change the acidity of the diets (Patience et al., 1987), and the diet acidity may change the pH of the digesta, which could influence phytase activity in the GIT. Little is known about the active sites of microbial phytase in the GIT of pigs. The objective of the present experiments was to determine the active sites of supplemental microbial phytase in the GIT of young pigs and effects of dietary available P and added citric acid on the sites.

Materials and Methods

Test 1

Samples were taken from pigs that had been fed soybean meal-based semi-purified diets containing two levels of available P (.05 and .16% aP), with or without 1,050 U of added microbial phytase/kg of diet (Yi et al., 1994a). The basal diet contained 33.65% soybean meal as the only protein and phytate source. Corn starch, dextrose and soybean oil were used as the energy sources. The estimated aP in the basal diet was .05%. The .16% aP level in the diet was achieved by the addition of defluorinated phosphate (Fine CDP, Southern Bag Corp., Valdosta, GA 31083). The Ca to total P ratio was maintained at 2:1 in all the diets. Defluorinated phosphate and limestone were added to the diets at the expense of corn starch. Since phytate was supplied only from the soybean meal, the dietary

content of phytate (.13%) was similar in all diets based on NRC (1988). Eight pigs per treatment were housed in groups of two (one barrow and one gilt) in nursery pens and fed for 5 wk.

Test 2

Samples were taken from pigs fed a corn-soybean meal diet with added microbial phytase (750 U/kg of diet) containing one of three levels (0, 1.5, and 3.0%) of citric acid (Radcliffe and Kornegay, 1994; unpublished data). The basal diet contained .39% total P or .115% aP and the Ca:P (aP plus expected release of P) was 2.5:1. All diets contained .24% phytate P based on NRC (1988). Eight pigs per treatment were housed in groups of two (one barrow and one gilt) in nursery pens and fed for 4 wk. The care and treatment of pigs in Test 1 and 2 followed published guidelines (Consortium, 1988).

Sampling

At the end of the experiments (Test 1 and 2), all barrows (one per replicate pen-four per treatment) were killed for collection of digesta samples. Immediately after slaughter, samples of GIT digesta were obtained from stomach, upper small intestine, and lower small intestine for determination of phytase activity in the different location. The following sections were isolated, ligated, and removed: stomach, upper small intestine (2 m section), and lower small section (2 m section). Samples of GIT contents were expelled into airtight plastic bags and frozen at -20 °C until they were lyophilized at -25 °C. The diet samples (500 g) were randomly taken from each treatment and mixed to determine phytase activity. The diet samples were ground in a laboratory mill to pass through a 1 mm sieve.

Analysis of pH in Stomach Digesta

Following the collection and mixing in Test 2, the pH of stomach digesta was determined within 5 min of collection as described by Risley et al. (1992), using the appropriate electrodes (Fisher Scientific AccupHast combination electrode #13-620-281 and Accoumet pH meter # 620, Fisher-Scientific, Pittsburgh, PA). Two grams of stomach

digesta were diluted in 18 mL of deionized water, mixed, and suspended on an electromagnetic mixer.

Phytase Source and Its Assay

Microbial phytase (Natuphos®) was provided by BASF Corp. (3000 Continental Drive North, Mount Olive, NJ 07828-1234). A unit of phytase is defined as the quantity of enzyme which liberates 1 μM of inorganic orthophosphate per min. from 5.1 mM sodium phytate at pH 5.5 and 37 °C (Engelen et al.,1994). Phytase activities of the diet and the digesta were measured by the method of Simons et al. (1990) and Engelen et al. (1994). The method is not very accurate for phytase activity less than 50 U.

Statistial Analysis

The data were analyzed by the GLM procedures of SAS (1990). The comparisons of phytase activities from diets and GIT contents between two levels of aP (Test 1), or among three levels of acid (Test 2) were made using nonorthogonal contrasts. The comparisons of phytase activities in digesta among different sections of the GIT at each level of aP (Test 1), or each level of acid (Test 2) were also made using nonorthogonal contrasts.

Results

Test 1

Phytase activities in diets and GIT contents of young pigs fed soybean meal-based semi-purified diets containing two levels of P and with or without added microbial phytase are shown in Table 9-1. Added phytase activity in diets and GIT contents were similar for the two dietary P levels ($P > .11$), suggesting that the level of dietary aP (.05 or .16%) within the range used in this test did not influence the activity of supplemental microbial phytase. There was no detectable phytase activity in the diets or in the GIT digesta of pigs fed the basal diet without added microbial phytase. This demonstrates that the phytase activity of dietary soybean meal, of bacterial flora in the GIT, and of intestinal mucosa was negligible.

Table 8-1. Phytase activity in diets and gastrointestinal tracts (GIT) of young pigs fed soybean meal-based semi-purified diets containing two levels of phosphorus and with or without microbial phytase addition

Diet	aP %	Phytase added[c] U/kg diet	Phytase assayed U/kg DM	Phytase in digesta (U/kg DM)[a, b]		
				Stomach	Upper SI	Lower SI
1	.05	0	0	< 50	<50	<50
2	.05	1,050	1,123	552 ± 71[d, x]	312 ± 55[d, y]	45 ± 35[d, z]
			(100%)	(48%)	(28%)	(4%)
3	.16	0	0	<50	<50	<50
4	.16	1,050	1,159	605 ± 94[d, x]	384 ± 54[d, y]	60 ± 36[d, z]
			(100%)	(52%)	(33%)	(5%)
Mean[d, e]		1,050	1,141	579 ± 42[x]	348 ± 27[y]	53 ± 18[z]
			(100%)	(51%)	(31%)	(5%)

[a]Mean ± SD, four samples per treatment mean.
[b]Number in parenthesis is the phytase activity in the GIT section expressed as a percentage of phytase activity in the diet.
[c]Phytase added is 1,167 U/kg DM.
[d]Comparisons between diet 2 and 4 were nonsignificant ($P > .11$).
[e]Mean ± SEM, eight samples per treatment mean from diet 2 and 4.
[x, y, z]Means within the same row with a different superscript differ ($P < .001$).

For pigs fed microbial phytase, phytase activity in the digesta from the stomach was higher ($P < .001$) than that from the upper small intestine, and the activity in the upper small intestine was higher ($P < .001$) than that from the lower small intestine. About 50% the phytase activity of the diets was detected in the digesta from the stomach of pigs fed the soybean meal-based semi-purified diets with added phytase, and only 30% of the phytase activity in the diet was observed in the digesta of the upper small intestine. Phytase

activity in the digesta of the lower small intestine of the pigs was negligible, which was about 5% of the added phytase activity and this amount was about the same as that in the diets without added phytase.

Test 2

The microbial phytase activity in the diets and GIT contents, and the pH of stomach digesta of young pigs fed corn-soybean meal diets with added phytase (750 U/kg of diet) containing one of the three levels of citric acid (0, 1.5, and 3.0%) are shown in Table 9-2. Adding citric acid at 3.0 ($P < .10$) and 1.5% (numerically) lowered phytase activity in stomach digesta, but there appeared to be no difference in phytase activity of the stomach digesta between the two levels of added citric acid ($P = .75$). About 40% of the added phytase activity was detected in the stomach digesta of pigs fed the basal diet without addition of organic acid; whereas, about 27% of added phytase activity was detected in stomach digesta of pigs fed diets with added citric acid. Phytase activity in the digesta from the stomach was higher than that from the upper small intestine or that from the lower small intestine ($P < .05$). Approximately 16% of added phytase activity for the basal diet and about 10% for the citric acid diets was present in the upper small intestine contents. Phytase activity in the lower small intestine contents was undetectable as in Test 1. Adding both levels of citric acid tended to reduce pH of stomach contents, but the difference was not significant ($P > .10$).

Discussion

Sites of Phytase Activity

The stomach was the site of greatest phytase activity in the pigs with activity varying from 40 to 50% of the added activity for diets (without added organic acids) followed by the upper small intestine (16 to 30% of the added phytase activity); negligible activity was observed in lower small intestine. Liebert et al. (1993) using chickens at 3 to 5 weeks of age reported that 25 to 50% of the added phytase activity was detected in the crop contents and that 10 to 25% of added phytase activity was detected in stomach when 500 or 1,000 U of phytase/kg of diet was added to the corn-soybean meal diets containing

Table 8-2. Phytase activity in the diets and gastrointestinal tracts (GIT), and pH in the stomach of young pigs fed corn-soybean meal diets with added microbial phytase and two levels of citric acid

Diet	Citric Acid %	Phytase added[c] U/kg diet	Phytase assayed U/kg DM	Phytase in digesta (U/kg DM)[a, b] Stomach	Upper SI	Lower SI	pH in Stomach
1	0	750	910 (100%)	370 ± 33[d, x] (41%)	148 ± 88[d, y] (16%)	48 ± 38[d, y] (5%)	3.84 ± .66[d]
2	1.5	750	957 (100%)	269 ± 149[d,e,x] (28%)	94 ± 68[d, y] (10%)	53 ± 44[d, y] (6%)	3.59 ± .86[d]
3	3.0	750	913 (100%)	250 ± 160[e, x] (27%)	102 ± 39[d, y] (11%)	24 ± 32[d, y] (3%)	3.63 ± .87[d]
Mean[f]		750	927 (100%)	296 ± 64[x] (32%)	114 ± 34[y] (12%)	42 ± 19[z] (5%)	3.69 ± .40

[a]Mean ± SD, four samples per treatment mean.
[b]Number in parenthesis is the phytase activity in the GIT section expressed as a percentage of phytase activity in the diet.
[c]Phytase added is 830 U/kg DM.
[d,e]Means within the same column with a different superscript differ ($P < .06$).
[f]Mean ± SEM, 12 samples per treatment mean.
[x, y, z]Means within the same row with a different superscript differ ($P < .05$).

.36, .43, .48, .54, and .64% P (.36% phytate P). No phytase activity was detected in the small intestine contents. Since no activity of phytase was detected in the small intestine of the chickens, they concluded that the main sites of phytase action were the crop and the stomach. The sites of phytase activity were also confirmed by concentrations of phytate P in the GIT. The high percentage of phytate P disappearance was also observed in crop and stomach contents. With addition of phytase, the contents of phytate P decreased 49, 74,

and 26%, respectively in the crop, the stomach, and the small intestine of the birds (Liebert et al., 1993). Gueguen et al. (1968) reported that plant phytase from activated wheat bran hydrolyzed phytate P mainly in the stomach of pigs. In a study with pigs, Lantzsch et al. (1992) found that because of dietary plant phytase, approximately 38% of phytate P from corn was absorbed in the stomach and proximal half of the small intestine, whereas up to 55% was absorbed by the end of the small intestine. Jongbloed et al. (1992), with growing and finishing pigs fitted with two simple T-cannulae located in the duodenum and in the terminal ileum, reported that 85% of added phytase activity (1,565 U/kg of diet) was detected in duodenal digesta of pigs fed a corn-soybean meal diet, and only 62% of added phytase activity was detected in pigs fed a soybean meal-tapioca-hominy feed-sunflower meal diet. No phytase activity was observed in the ileal digesta. Jongbloed et al. (1992) also reported that 60 to 74% of phytic acid could be hydrolyzed by the end of small intestine when 1,550 U of phytase/kg of diet was added.

The purified phytase protein from *Aspergillus ficuum* showed two broadly diffused bands at 85 and 100 kDa when analyzed by SDS-PAGE (Gibson and Ullah, 1990). The phytase from *Aspergillus ficuum* is an acidic phosphatase which consists of 594 amino acid residues, of which 104 amino acid residues are from Asp and Glu, and 30 from Lys and Arg. The isoelectric point of the phytase is 4.5 (Gibson and Ullah, 1990). The optimal pH of the phytase showed two response peaks, the highest activity was observed at pH 5.0 to 5.5 and the second highest activity was at pH 2.5 (Shieh et al., 1969; Irving and Consgrove, 1974; Simons et al., 1990). The pH (3.4 to 4.8) of stomach digesta of pigs is much lower than that (6.4 to 7.2) in the small intestine (Risley et al., 1992). Obviously, the phytase has greater activity within the pH of the stomach than the pH in the small intestine. The time for assay of phytase activity was established as 1 h (Simons et al., 1990; Engelen et al., 1994). It was reported that the half-life of the feed remaining in the stomach of pigs was about 1 h (Hoppe, 1992). The time of 1 h is comparatively long for the phytase reaction with phytate in feed ingredients.

Effects of Dietary Acidification

In Test 2, adding citric acid significantly (3.0%) or numerically (1.5%) decreased the phytase activity from the stomach digesta of pigs (Table 9-2). The results may be associated with the reduced pH in the diet by dietary acidification which might change the pH of the stomach digesta as evidenced with the numerical decrease in Test 2. The main effects of adding citric acid and decreasing the pH in the stomach were observed in the study of Radcliffe and Kornegay (1994, unpublished data). Phosphorus absorption was facilitated with a low pH (Swenson and Reece, 1993). Improved P absorption would promote phytase efficacy. Further research is needed in this field. Orthophosphate was reported as a noncompetitive inhibitor to phytase (Gibson and Ullah, 1990). However, the level of dietary aP (.05 or .16%) within the range used in Test 1 did not influence the activity of supplemental microbial phytase. Further research is needed to clarify this effect.

Implications

These results indicate that the stomach of young pigs is the site of highest activity of supplemented microbial phytase followed by the upper small intestine with negligible activity ocurring in the lower small intestine. There was no effect of the .05 and .16% available P on phytase activity. Adding citric acid may promote microbial phytase hydrolysis in stomach.

Chapter IX

General Discussion and Implications

Sensitive Indicators for Evaluating Phytase Efficacy

The sensitive indicators were determined by R^2 of second order translog models and r^2 of linear or nonlinear models for all the response equations in these studies. The results indicate that growth response, P apparent absorption (pigs) or retention (birds), and ash percentage of pig and bird bones are the most sensitive indicators to evaluate efficacy of phytase on the dietary P availability. Feed intake, P excretion of pigs and birds, shear force of tenth ribs and martacarpals of pigs are sensitive indicators. These findings are supported in a number of studies reviewed by Yi and Kornegay (1995). Zinc contents in toes and tibia are the most sensitive indicators to evaluate the efficacy of added phytase on Zn utilization in broilers. Apparent Zn retention and liver Zn contents, and body weight gain of birds fed low Zn diets are sensitive indicators.

Phosphorus and Zinc Equivalency Values of Phytase

The results of these studies confirmed other reports that Natuphos® phytase is very effective for improving the availability of phytate P in corn and soybean meal fed to young pigs, broilers, and turkey poults. The average of the P equivalency values of phytase from a number of measurements indicates that the release of 1 g P from phytate requires 676, 920, and 830 U of phytase for pigs fed soybean meal-based semi-purified diets (SP), and broilers fed SP, and corn-soybean meal diets (CS), respectively. The P equivalency values are close to those calculated from published data using the same type of diets (Yi et al., 1994; Yi and Kornegay, 1995). The results indicate that about 1 mg of Zn can be released per 100 U of phytase for broilers fed corn-soy isolate diets. The P and Zn equivalency values are important for feed industries and animal producers to use

microbial phytase in diet formulation. Academically, the equivalency values made a great contribution to phytase research, because many published reports did not give the values.

Method of Calculating the Equivalency Values

The P and Zn equivalency values of phytase in these studies were calculated by generating the nonlinear or linear equations for the response measurements from graded levels of P or Zn and phytase added. This equation method was more accurate than the way using a single number in the studies of Beer (1992), Simons et al. (1992), and Lei et al. (1993b). In their studies, the response measurements of graded levels of phytase were compared with those from one level of P as a standard. The question is that the response at that P level may be not adequate because of influencing factors such as various animals, diets, and indicators used. On the other hand, using the measurements from one level of phytase to compare those from graded levels of P is not an efficient way, because the efficacy of phytase is nonlinear at most conditions and one value can not be used to predict the responses at other levels of phytase. The generation of the equivalency equation from the functions for P, (or Zn) and for phytase allows for the calculation the P (or Zn) equivalency values of phytase at any points on the line. Further, the use of regression equations allows for the easy incorporation of this information into computer models.

Effects of Phytase on Utilization of Nitrogen, Amino Acids, and Other Minerals

The results from this study indicate that phytase is also effective in improving N and AA digestibility, N retention, and Ca and Zn utilization. This study is the first report about the effects of phytase on AA digestibility in diets for poults. Further research is clearly needed to investigate the effectiveness of microbial phytase on AA, Ca, and trace mineral utilization, especially the mechanism of phytase in pigs and poultry fed diets with various levels of nutrients and protein sources. The data from these experiments provided

important information to establish the model about the comprehensive effects of phytase on P, Ca, trace minerals, and N and AA in the future.

Effects of Phytase on Phosphorus and Nitrogen Excretion

The results clearly demonstrate that phytase significantly decreased P and N excretion from swine and poultry manure. Adding phytase to diets for pigs and poultry provides an important means of environmental protection, because P and N are considered environmental pollutants. Microbial phytase has been used as a feed additive in several countries. It has a great potential to be used in the U.S. It is estimated that in the U.S. swine and poultry annually produce approximately 460, 000 and 250,00 tons of P, and 730,000 and 790,000 tons of N as waste products (Sweeten, 1992). A 30% reduction in P excretion by using microbial phytase would mean about 210,000 tons less P excreted by swine and poultry annually in the U.S. Lenis (1989) reported that lowering the protein level in the diets for growing pigs by 2 percentage units resulted in about a 25% reduction of N excretion. The data from our studies demonstrate that adding phytase to the diets for swine and poultry is also a potential way to reduce N excretion.

The data from these studies provided important information to establish the models of animal production systems and farm nutrient (P and N) management from animal wastes. The current price of microbial phytase (under 500 U/kg diet) is comparable to that of equivalent inorganic phosphate (Yi et al., 1994; Kornegay, 1995). Considering that phytase could decrease the cost of P and N disposal, and the cost of high levels of N, AA, and other minerals, it would be more acceptable to use microbial phytase in the diets for swine and poultry.

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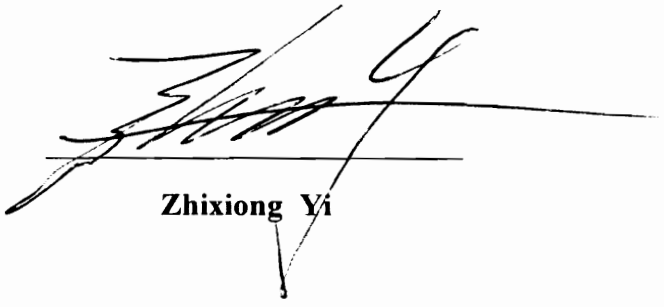

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