Reducing Nutrient Excretion via Improved Nutrient Utilization by Supplementing Pigs and Poultry Diets with Phytase Enzyme

By

Zhibo Zhang

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Approved By

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Dr. Ervin T. Kornegay, Chairman

_____ _____

Dr. D. Michael Denbow Dr. Allen F. Harper

_____ _____

Dr. David R. Notter Dr. Thomas W. Keenan

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Committee Chairman: Dr. E. T. Kornegay

Department of Animal and Poultry Sciences

ABSTRACT

This study was conducted to investigate the efficacy of phytase for improving the nutrient utilization for pigs and poultry. Two experiments, one with broilers and one with pigs, were conducted to compare the efficiency of transgenic microbial (Natuphos®) and plant (Phytaseed®) phytase for enhancing the utilization of phytate P in corn-soybean diets fed to young broilers and pigs, and to evaluate the safety of Phytaseed® phytase. Three levels of the two sources of phytase (250, 500 and 2,500 U/kg of diet) were added to a corn-soybean meal basal diet containing 0.21 and 0.26% nonphytate P, respectively, for broilers and pigs. Forty birds and thirty pigs (8 broilers and 6 pigs, one per cage, from the diet without added phytase and the diets with 500 or 2,500 U/kg phytase from both sources) were randomly selected for gross necropsy and histologic evaluation of liver, kidney and bone tissues. Adding both sources of phytase to the basal diet resulted in similar increases of BW, BW gain, feed intake, gain:feed, apparent digestibilities (retention) of DM, P and Ca, and bone measurements. Phosphorus excretion decreased as phytase addition increased. No significant abnormalities were seen in any of the broilers and pigs necropsied.

In a study with pigs (n=120 and 80, respectively for grower and finisher), the effects of supplemental microbial phytase on crude protein and amino acid utilization of low protein plant-based diets was investigated. During the grower period (32 to 67 kg), diets 1, 2 and 3 contained 14, 13 and 12% crude protein and no added phytase, respectively, and diets 4 and 5 contained 12% crude protein with either 250 or 500 U of phytase/kg of diet, respectively. During the

finisher period (67 to 109 kg), diets 1, 2 and 3 contained 12, 11 and 10% crude protein with no added phytase, respectively, and diets 4 and 5 contained 10% crude protein with either 250 or 500 U of phytase/kg of diet, respectively. At the end of grower phase, two pigs (1 barrow and 1 gilt) were removed from each pen; 12 of the barrows that were removed from diets 1, 3 and 5 were put in metabolism cages for total collection, and the remaining four pigs in each pen continued on test for the finisher phase. At the end of finisher phase, 12 barrows from diet 1, 3, and 5 were put in metabolism cages for total collection. Ileal contents were taken (slaughter technique) from the remaining barrows and the barrows used in metabolism cages. Daily gain increased as protein and phytase levels was added to the lowest protein level. Fecal P and Ca digestibilities improved with added phytase. Phytase addition to basal diet linearly increased ash weight in the grower phase. With the exception of proline and glycine, the digestibilities of the other amino acids were linearly increased with phytase and CP level. Nitrogen excretion was estimated to be reduced by 4.6% when phytase was added to pig diets at a level of 500 U/kg.

In a study with cecectomized roosters, the main effects and interaction of phytase and non-startch polysaccharide enzymes on the nutrient utilization of barley, canola meal, rice bran and soybean meal, and canola-barley (36:64) and soybean meal-barley (27.3:72.7) were evaluated. Phytase supplementation to basal diets increased the utilization of energy, N, total amino acid and most of amino acids in barley, canola meal, and canola-barley and numerically increased energy and N utilization in rice brain, soybean meal and soybean-barley. The magnitudes of improvements in the digestibilities of lysine, arginine, cysteine, serine, and threonine were higher compared with the other amino acids. The true utilization of energy and N, and the digestibilities of total amino acid and of glycine, isoleucine, and histidine in barley quadratically increased with Ronozyme^{TM} B. Phytase addition increased Ca retention in barley, canola and soybean meal, and the soybean meal-barley blend, and increased P retention in barley and soybean meal-barley. Addition of Ronozyme^{TM} B to barley linearly decreased Ca retention and quadratically increased P retention.

In summary, the efficiency of phytase in Phytaseed is equal to that of Natuphos for enhancing the utilization of phytate P in corn-soybean diets, and microbial phytase is effective in improving the utilization of N and amino acid in pigs and N, amino acid, and energy in poultry.

Key words: Pig, Poultry, Phytase, Amino acids, Energy, and Phosphorus

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Chapter 1

Introduction

One of the most striking achievements of the modern poultry and swine industry is their increased productivity using relatively small land areas. This large-scale production has dramatically increased the efficiency of poultry and swine production and provides the consumers with high quality products at low prices. At the same time, however, environmental concerns about animal waste have been raised. Manure disposal is usually accomplished by applying waste water and solids to land as a fertilizer. The use of animal waste as a fertilizer is a classic example of creating both economic and environmental benefits by linking the animal and crop systems. However, excessive application of animal waste to land can cause environmental problems; direct ground and surface water contamination and air pollution. Waste management is becoming the key issue for livestock and poultry industries and will have a potential impact on the structure of these industries such as the farm size and location.

Improvement of nutrient utilization is often a partial solution to the problem of waste management. Improved feed efficiency significantly reduces nutrient levels in the animal manure (Kornegay and Harper, 1997). The efficiency of poultry and swine production is largely dependent on the efficiency of feed utilization, which in turn is determined by the processes of digestion and absorption of nutrients. Nutrient digestion is a hydrolytic process involving mechanical, chemical, and enzymatic actions. Endogenous enzymes in the animal's gastrointestinal tract play a major role. However, swine and poultry do not produce sufficient amounts of some enzymes to hydrolyze all of the components in feed, especially in plant ingredients. This limits the efficiencies of utilization of some nutrients and the productivity of the animals. But the addition of exogenous enzymes to the feed can improve digestion of certain components and thus increase the feed utilization. This can increase the efficiency of production of nonruminants and reduced waste excretion.

Phytate and non-starch polysaccharides (NSP) in plant-based diets are two major antinutrients. Phytate is the chief storage form of phosphorus in cereal grains and legumes. The phytate content of these products varies between 0.50 to 5.20% and accounts from 60 to 90% of the total phosphorus content of the products (Nelson, 1976). The levels of non-starch polysaccharides in cereal grains, such as corn, sorghum, wheat, and barley range from 10 to 30%.

Both phytate and NSP are not only poorly digested by poultry and swine, but they also interfere with the digestion of other nutrients (Oberleas and Moody, 1981; Oberleas, 1973; Henry, 1985; Ravindran and Bryden, 1998). As a result, extra nutrients must be added to the diet to meet the requirements of animals, and excessive phosphorus, calcium, nitrogen and other minerals are excreted. Therefore, environmental concerns are raised because of the potential of pollution.

Various enzyme supplements have been used in an effort to improve the nutritional value of plant-based diets for nonruminants. Many researchers have shown that supplemental microbial phytase improves the bioavailability of phosphorus and other nutrients, and reduces the concentration of these nutrients in animal manure (Coelho and Kornegay, 1996). The use of non-starch polysaccharides (NSP) enzymes as a dietary supplementation has also been shown to have a dramatic effect on the utilization of certain feedstuffs in animal production, particularly in poultry, and especially with those diets that contain cereal grains such as barley, rye, and wheat (Rickes et al.,1962; Hesselmen et al., 1981; Hesselmen and Aman, 1986; Annison, 1992).

Although both phytase and NSP enzymes have established roles in improving the nutritional value of plant ingredients, further research is need in the following areas. First, the use of microbial phytase is not always as economical as the inorganic P that it can replace, and the application of phytase to pelleted feeds requires special equipment that adds cost. Therefore, it is necessary to assess the effectiveness and safety of new sources of phytase, which have the potential to reduce the cost of phytase supplementation. Phytaseed® is a new source of phytase, which was produced in transgenic canola seed expressing the *Aspergillus niger* phytase. Second, phytase has been shown to have positive effect on the digestibility of several critical amino acids, and the equivalency value of phytase for these amino acids must be known for specific recommendations to be made for the practical use of phytase. Third, the addition of phytase or NSP enzymes may improve feed efficiency, but information about the interaction of these two enzymes is limited and not well characterized.

The objectives of studies in this dissertation were 1) to compare the efficiency of phytase in Natuphos® and Phytaseed® for enhancing the utilization of phytate P in low P corn-soybean meal based diets fed to young broilers and young pigs; 2) to determine the safety of Phytaseed® phytase as a dietary supplement for broilers and pigs; 3) to characterize the effect of phytase on protein and amino acids utilization, nitrogen retention, and performance in growing and finishing

pigs, and to determine equivalency values of phytase for protein and amino acids; and 4) to determinate if there are interactive effective of using both phytase and NSP enzyme supplementation of plant-based diets to improve the utilization of key nutrients.

Chapter 2

Literature Review

Phytate

Chemical Structure, Properties, and Metabolisms of Phytate

The chemical name of phytic acids is myoinositol 1, 2, 3, 4, 5, 6-hexaphosphoric acid. It consists of an inositol ring with six phosphate groups and has a chemical formula of $C_6H_{18}O_{24}P_6$. The structure of phytic acid (Figure 2-1) was first proposed by Anderson (Graf, 1986). Phytate is a salt of phytic acid. Phytate exists as different complexes in different seeds. The Ca-Mg-K salt of phytate is the most abundant form and is called phytin (Cosgrove, 1980). Phytate will have accumulated to significant levels by the time a seed has matured (Abernathy et al., 1973; Asada et al., 1969; Makower, 1969). Although phytate is the most abundant inositol phosphate in nature, the exact mechanism of phytate biosynthesis is not fully understood. Evidences favored the proposal for a direct stepwise phosphorylation pathway from myoinositol or myoinositol monophosphate to the myoinositol hexakisphosphate (Mandal and Biswas, 1970). Recent studies indicated that its synthesis from myoinositol is catalyzed by a series of soluble ATP-dependent kinases. The intermediates between myoinositol and phytate are Ins(3)P, Ins(3,6)P2, Ins(3,4,6)P3, Ins(1,3,4,6)P4 and Ins(1,3,4,5,6)P5 (Stephens and Irvine, 1990). Containing high-energy phosphoryl groups, phytate competes for phosphorus and energy with

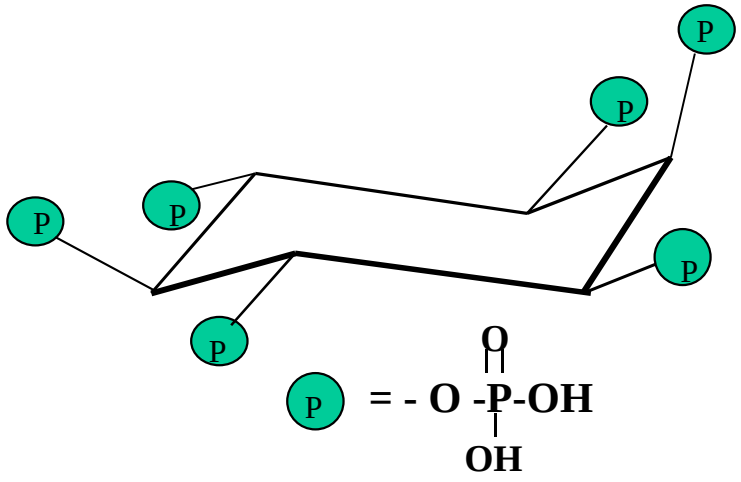

Figure 2-1. The structure of phytic acid in dilute solution (Graf, 1986)

ATP, therefore, the metabolism of the seeds is inhibited and dormancy is induced (Cosgrove, 1980). During germination, phytate in the seeds is degraded by a specific enzyme known as phytase. The phosphorus and other minerals released from phytate meet the biosynthetic requirements in the growing tissues (Laboure et al., 1993; Bartnik and Szafranska, 1986; Reddy et al., 1978).

Phytate Content of Feed Ingredients

Phytate is present in abundance in plants. In general, the levels of phytate vary from 0.50 to 1.89% in cereal grains, from 0.40% to 2.06% in legumes, from 2.00% to 5.20 in oleaginous seeds, and from 0.40 to 7.50% in protein products (Table 2-1). In many seeds, phytate is distributed unevenly and is usually rich in some protein particles (Beal and Mehta, 1985). Chemical analysis of these phytate-rich particles has showed that the levels of K and Mg are much higher than that of Ca (McKenzie-Parnell and Guthrie, 1986). For example, phytin-rich particles from rice grains contain 67.23% phytic acid, 18.89% K, 10.83 Mg and 1.39% Ca on a dry weight basis (Ogawa et al., 1975). Although K and Mg are predominantly high in phytin, a

Table 2-1. Total phosphorus and phytate phosphorus contents in plant ingredients

Ingredients	Total phosphorus, %[a]	Phytate phosphorus, %[b]
Cereals		
Barley	0.34	0.21
Corn	0.26	0.20
Oats	0.34	0.28
Sorghum	0.29	0.22
Triticale	0.33	0.30
Wheat	0.37	0.24
Oilseed meals		
Canola meal	1.01	0.65
Peanut meal	0.62	0.35
Soybean meal	0.67	0.37
Sunflower meal	0.94	0.44

[a]Adapted from Nelson et al. (1968), Reddy et al. (1982), Pointillart et al. 1994, Eeckhout and De Paepe (1994), Ravindran et al., (1995), NRC (Poultry,1994; Swine 1998).

variety of other elements such as Mn, Fe, Zn, and Na are also present. The phytate content and individual mineral level depend upon the species, the tissue regions in seeds, and the soil conditions (Lott, 1985; Miller et al., 1980; Harland and Oberleas, 1987).

Interactions of Minerals, Starch, Protein, and Fat with Phytate

Phytate is an extremely powerful chelating agent. It not only can bind minerals but also organic matter such as protein, starch, and fat. At neutral pH, phytic acid exists as a strong negatively changed molecule that has a potential for binding di- and trivalent cations (Figure 2-2). A variety of minerals including Ca, Mg, Zn, Cu, Fe, Co, Mn and Cr are known to be bound by phytate. Vohra et al (1965) showed that the order of relative stability of phytate-mineral complexes was Cu>Zn>Ni>Co>Mn>Fe>Ca, whereas Maddaiah et al. (1963) found the order of relative stability to be Zn>Cu>Ni>Co>Mn>Fe>Ca. Both results indicated that Cu and Zn have a high affinity for phytate. Later study further demonstrated that the precipitate of the phytate-cation complexes increased as the phytate:cation ratio was elevated (Cheryan , 1980).

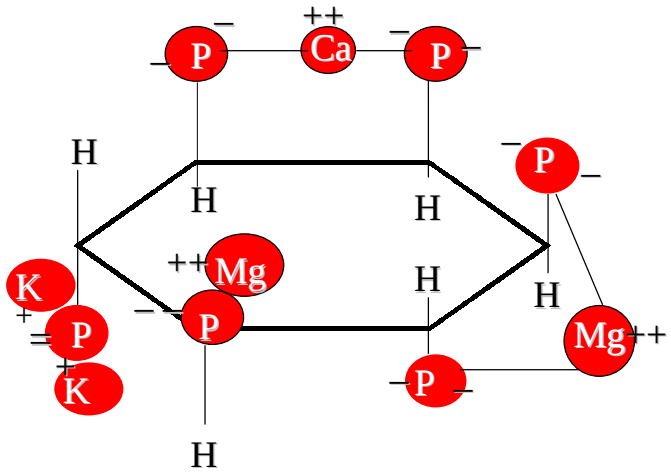

Figure 2-2. Structure of phytic acid chelate at neutral pH

(Ogawa et al., 1975)

Phytate can complex with protein over a wide range of pH values. These complexes can be roughly divided into two categories based on pH values of medium: neutral and acidic (Grynspan and Cheryan, 1983) ((Figure 2-3). Since most plant proteins have isoelectric points near pH 4.0 to 5.0, they are positively charged when the pH is lower than 4.0, and are negatively

charged when the pH is higher than 5 (Champagne and Phillippy, 1989). Under acidic conditions, phytic acid occurs as a negative molecule and forms a complex with protein through the terminal amino group of protein, the ε-amino group of lysine, the imidazole group of histidine, and the guanidyl groups of arginine. At neutral pH, negatively charged phytate molecules form complexes with carboxyl terminal group of protein, and side-chain carboxyl groups of aspartate and glutamate from proteins, via binding the same cations (Champagne, 1988; Champagne et al, 1990).

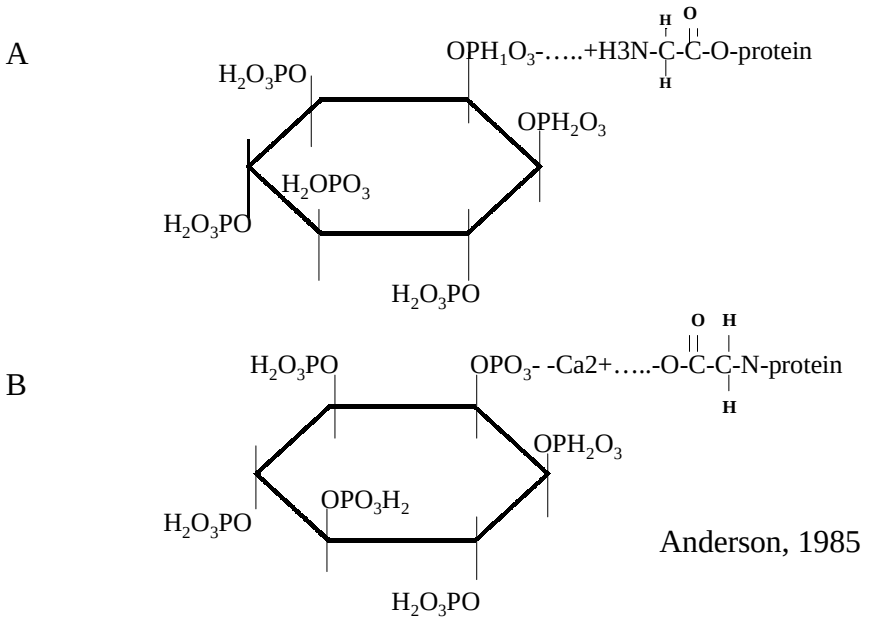

Figure 2-3. Phytic acid-protein complexes at low (A) and neutral pH(B)

Research findings indicated that phytate reduced the solubility of plant proteins (O'Dell and deBoland; 1976; Chen and Morr 1985). For example, removal of phytin from soybean extracts increased the isoelectric point of soy protein by 0.8 units and increased the solubility of this protein. It was also found that interaction with phytate has some effect on protein functionality, especially on some digestive enzymes. This is because most protein functions are dependent on their charge, structure and solubility, and binding with phytate may change these properties (Deshpande and Damodaran, 1989). In addition, phytate was found to interact with starch in the presence of proteins (Maga, 1982), and complex with starch and fat in the presence of calcium (Leeson, 1993; Thompson, 1988) (Figure 2-4).

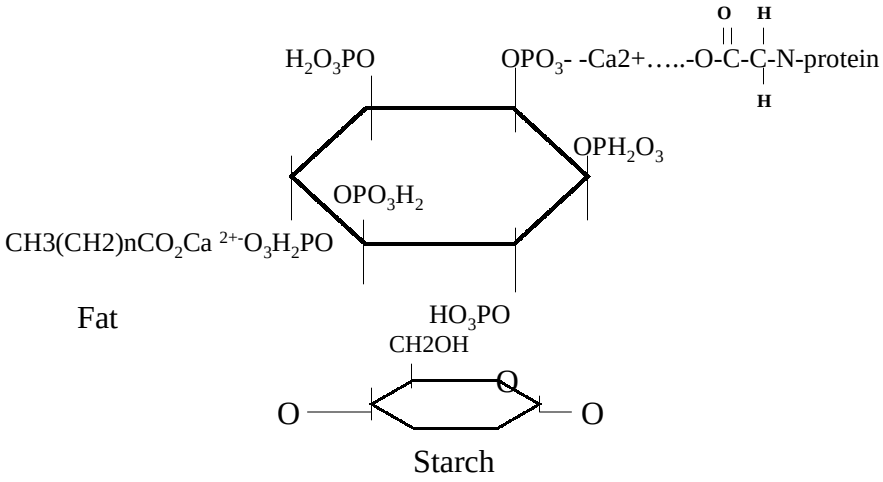

Figure 2-4. Possible interactions with a phytate molecule

(after Thompson, 1986)

Effect of Phytate on the Utilization of Nutrients

Extensive studies have been carried out to evaluate the influence of phytate on the utilization of nutrients (Ravindran et al., 1995a; Pallauf and Rimbach, 1995, 1997; Thompson and Yoon, 1984). Evidence from these studies showed that as an anti-nutrition factor, phytate not only lowers the bioavailability of phosphorus, calcium, and other minerals, but also protein, fat, and starch.

Phosphorus. Phytate is the major storage form of phosphorus in plant, which accounts for 60 to 90% of the total phosphorus of legumes and grains (Maga, 1982). This form of bound phosphate is poorly available to monogastric animals. The availability of total plant phosphorus for swine and poultry is quite variable, ranging from 10 to 60%, with an average value of 33% (Table 2-2). For swine, the phosphorus availability of corn is only 12% while it is 23% for soybean, thus, it is generally accepted that the bioavailability of a corn-soybean diet is 15% (Cromwell, 1989). For poultry, only 30 to 40 % of total plant phosphorus can be used (NRC, 1994, Poultry). The capacity of poultry to digest and absorb phytate P is not well characterized. The value varies from 0 (Nelson, 1967) to 60% of dietary phytate P (Temperton and Cassidy, 1964a,b; Edwards, 1993; Coon and Leske, 1996; Kornegay, 1995).

Several factors are considered to affect the utilization of phosphorus from phytate. For pigs and poultry, the ability to use phytate phosphorus increased with age. In comparison to broilers, adult laying hens have a greater ability to utilize phytate P (Maenz and Classen, 1998). The type of diet also influences the utilization of phytate phosphorus. It was reported that, compared with corn, wheat phosphorus is more available to pig (Pointillart et al., 1984).

Furthermore, the utilization of plant phosphorus is dependent on adequate dietary Ca and P levels, suitable dietary Ca:P ratio, and optimal vitamin D levels (NRC, Swine, 1998; Poultry, 1994).

Table 2-2. Bioavailability of phosphorus and phytase activity in plant ingredients

Ingredients	For swine, %[a]	For poultry, %[b]	Phytase activity, U/kg[c]
Cereals			
Barley	31	36	400
Corn	12	28	15
Oats	23	33	40
Triticale	46	33	1500
Wheat	50	31	700
Oilseed meals			
Canola meal	21	26	15
Peanut meal	12	21	3
Soybean meal, dehulled	25	35	40
Soybean meal, 44% protein	35	40	40
Sunflower meal	3	15	15

[a]Adapted from Cromwell (1992), relative to the availability of P in monosodium phosphate (100%).
[b]Adapted from NRC (Poultry, 1994).

Calcium. Phytic acid also markedly decreases calcium bioavailability (Lonnerdal et al., 1989). Consequently, many studies have shown that phytate binding makes a portion of dietary Ca unavailable to poultry and swine (Soares, 1995). Little is known about the bioavailability of calcium in feedstuffs fed to nonruminants. It is generally assumed that cereal grain-based diets have a very low calcium bioavailability (Cromwell, 1989). Most feedstuffs fed to pigs and poultry are very low in calcium. For example, the calcium level is 0.01 to 0.02% in corn and 0.25 to 0.35% in soybean meal. Thus, the total calcium level of corn and soybean diets for swine and poultry is about 0.05%, which is approximately 5 to 10 % of the total dietary calcium requirement for swine and poultry (0.50 to 0.80 for pig and 0.50 to 1.00 for poultry). Therefore, Ca supplements are required, and because they are relatively cheap, over-supplementation often occurs compared with other nutrients.

Trace minerals. Trace elements are widely distributed in plant ingredients. Legumes and grains are rich sources of manganese and also relatively good sources of copper, zinc, and iron

(Gibson, 1994). It is recognized that absorption, retention and metabolism of most essential minerals such as zinc, iron, and magnesium can be markedly influenced by the presence of phytate (Couzy et al., 1993). It is apparent that the amount of phytic acid is the most important factor that may influence mineral availability, followed by mineral concentration, pH, and the presence of other metallic ions. For example, Nosworthy and Caldwell (1988) and Champagne and Philippy (1989)) reported that the reduced Zn bioavailability was due to the formation of an insoluble complex of phytate-Zn^{2+}-protein (from soy). Gifford and Clydesdale (1990) observed a linear relationship between phytic acid content and the concentration of insoluble complexes of protein and mineral in vitro.

The effect of phytate on the bioavailability of iron has been extensively reviewed. Rossander (1987) showed that phytate appeared to be the major contributor to the reduced availability of iron. These findings agreed with the results reported by Hallberg et al. (1987). They showed that there is a strong semilogarithmic relationship (r=0.99) between the inhibition of iron absorption and the amount of phytate in human diets. It has also been observed that the removal of phytate from soy protein-based diets has a beneficial effect on iron bioavailability (Rodriguez et al., 1985)

The negative effect of phytate on the retention of other minerals was reviewed by Greger (1987), who indicated that decreased Cu and Mg absorption in the intestine appeared to be due to phytate binding.

Organic nutrients. Theoretically, there are three ways that phytate can affect the digestion of organic substances. The effect of phytate could be due to its (1) direct binding with amino acids, fat, and starch (Reddy et al., 1989; Cheryan,1980; Thompson and Serraino, 1986); (2) association with the digestive enzymes which are themselves protein (Yoon et al., 1983; Deshpande and Cheryan, 1984); and 3) chelation of mineral elements required for the activity of digestive enzymes (Caldwell, 1992).

Phytate is known to inhibit α-amylase (Knuckles and Betschart, 1987; Hagenimana et al., 1994), protease (Askar, 1986), and lipases (Knuckles, 1988). By complexing with digestive enzymes, phytate can decrease the activity of trypsin, pepsin, α-amylase, and β-galactosidase. Vaintraub and Bulmaga (1991) reported that phytate inhibited the action of pepsin on protein, but phytate did not inhibited pepsin hydrolysis of a low molecular weight protein. The inhibition

was maximal at pH 2.0 to 3.0 and dropped to zero when the pH was increased to 4.0. Deshpande and Damodaran (1989) found that at pH 3.0, trypsin activity was also strongly inhibited by phytate.

Liener et al. (1994) reported that phytate present in soybeans exerted a negative impact on the nutritional quality of protein by causing pancreatic hypertrophy/ hyperplasia, which ultimately resulted in an inhibition of growth. This happened to humans and piglets with diets containing large quantities of soybean. Knuckles (1988) also observed an inhibitory effect of phytate on lipase activity of pigs.

Two mechanisms have been proposed for the inhibitory effect of phytate on the activity of digestive enzymes. First, since some cations are cofactors for the many digestive enzymes, the interaction with phytate reduces the effective cation concentration, and thereby shifts equilibrium of digestive enzymes toward less active or inactive conformations (Caldwell, 1992). Second, phytate-enzyme interactions reduced the solubility and changed the conformation of the enzymes, thus lowering their apparent activity (Deshpande and Damodaran, 1989).

Similarly, phytate may reduce the digestion and absorption of organic substances by directly binding starches, proteins, and fats, reducing their solubility and the exposure of their active sites for digestion and absorption. Chitra et al. (1995) reported that there was a significant negative correlation between phytic acid concentration and digestibility of protein from soybean, chickpea, and pigeonpea in vitro. These findings were consistent with the results of a similar study by Ene-Obong (1995). In a study with human, Yoon et al. (1983) reported that addition of 2% phytic acid (in the form of sodium phytate) to raw wheat starch reduced the rate of digestion 50% in vitro, and high phytate bread produced a flattened blood glucose response compared with low phytate bread in vivo. It was also noted that removal of phytic acid by fermentation increased the starch digestibility (Sharma and Ketarpaul, 1995; Gupta and Khetarpaul, 1993). Calcium-phytate complexes lowered fat digestibility by forming insoluble soaps with fatty acids in the gastrointestinal tract of poultry and swine (Leeson, 1993; Attech and Leeson, 1984).

Environmental Consequences of Phytate

The reduced bioavailability of phosphorus, calcium, protein, starch, and trace minerals by phytate increases the excretion of these nutrients in manure, which have the potential to cause environmental pollution. Of the nutrients present in animal waste, phosphorus and nitrogen are

of greatest concern (Kornegay, 1995). For example, approximately 30 million tons of manure are excreted annually by poultry and swine in the United States. This includes 6.5 million tons of nitrogen and 2 million tons of phosphorus (Table 2-3). Manure disposal is often accomplished by applying waste water and solids to land as a fertilizer. The use of animal waste as a fertilizer is a classic example of creating both economic and environmental benefits by linking the animal and crop system. However, excessive application of animal waste to land can cause environmental problems.

Table 2-3. Quantities of manure, N and P excreted annually by poultry and swine in the USA[a]

Species	Manure (mil. tons)	Mineral conc.(%) N	Mineral conc.(%) P	Excretion (thous. Tons) N	Excretion (thous. Tons) P
Swine	15.5	4.71	2.97	730	460
Poultry	15.4	5.13	1.62	790	250
Total	30.9	9.84	4.59	1520	710

[a]Adapted from Sweeten (1992)

Nitrogen is mobile in soils, any excess nitrogen will generally leach clear of most soils and cause groundwater pollution. However, since phosphorus tends to build up in soil but when it reaches certain levels on the land, it may move into and contaminate surface water with runoff. Phosphorus and nitrogen are the limiting nutrients for aerobic bacteria. Increased levels of P and N can lead to an increase in alga population, which in turn blocks submerged vegetation from sunlight. This causes death of submerged vegetation and the decomposition of this vegetation depletes the oxygen content in streams, rivers and lakes. As a result, this process adversely affects fish and other aquatic creatures (Adeola and Stutton, 1995; Jongbloed et al., 1997a; Cromwell, 1997).

Strategies to reduce nutrient excretion of poultry and swine have been reviewed by Kornegay and Harper (1996). The ways to reduce the impact of animal production on the environment include genetically improved animals and crops, geographically dispersed production units, nutrient management, and manure management. It was concluded that nutrient management has a greater impact on reduced nutrient excretion. Several strategies are possible for reducing nutrient excretion: 1) avoid excess nutrients in animal rations, the nutrients allowances recommended by feed companies are higher than that suggested by NRC and

universities (Cromwell, 1989); 2) formulate feed on accurate nutrient requirements. For example, formulating diets on available phosphorus and amino acid basis rather than on total phosphorus and protein basis can reduce phosphorus and nitrogen excretion; 3) maintain the correct ratios between nutrients. Improper ratios between nutrients can causes reduced utilization of nutrients and increases waste; 4) use phase feeding and split-sex feeding; and 5) supplement feed with enzymes. Among all the recommended methods, feeding enzymes proved to be the most practical and reasonable approach to environmental issue by animal waste.

Phytase

Definition

Phosphatases, the enzymes that hydrolyze phosphomonoesters, can be divided into alkaline, acid, and protein phosphatases (Vincent et al., 1994). The acid phosphatases can be further subdivided into 1) low molecular weight acid phosphatases (-18,000 Mr) that are found in human and bovine liver, 2) high molecular weight acid phosphatases (-50,000-60,000 Mr) that are found in fungi, and 3) purple acid phosphatases with a binuclear Fe-Fe or Fe-Zn center at the active site that are found in mammals and plants. Phytases, myoinositol hexaphosphate hydrolases, are acid phosphatases capable of hydrolyzing phytic acid to yield inorganic orthophosphate and a series of lower phosphoric esters and finally to free myoinositol (Nayini and Markakis, 1986; Lasztity and Lasztity, 1988; Harland and Morris, 1995). Based on the position in which dephosphorylation of phytate is initiated, phytases can be classed into two main categories, 3-phytase and 6-phytase. The phytases which initiate the dephosphorylation of myoinositol in position 3 are called 3-phytase and predominately are observed in animals and micro-organisms, whereas, 6-phytases, which initiate dephosphorylation at position 6, are mainly present in plants.

Occurrence of Phytase in Plants, Animal and Microorganisms

Phytases are widely distributed in plant and animal tissues, and microorganisms such as fungi, yeast and bacteria. Many species of animals have phytase activity in their small intestinal mucosa and microflora (Davies et al., 1970; Davies and Motzok, 1972; Maenz and Classen, 1998). It is uncertain whether the phytase activity in small intestinal mucosa can be attributed to a specific phytase or nonspecific phosphatases. Evidence favors the proposal that phytase is a different enzyme from phosphatase (Young et al., 1991a,b). Although the levels of phytase

activity in the mucosa vary among species and breeds of chicks and pigs (Su et al., 1984), it is generally accepted that the phytase activity in the mucosa is probably negligible in pigs and poultry. In comparison to the intestinal mucosa phytase, microflora phytase in animal gastrointestinal tract is more capable of hydrolyzing phytate P.

Phytases are known to be present in the seeds of higher plants: cereals, leguminous seeds, and oil meal. Phytase activity of the major feedstuffs used in animal feed is extremely variable: rye, wheat, and triticale have high phytase activity, soybean and corn are low in phytase activity, and oats and sorghum have almost no phytase activity (Barrier-Guillot et al., 1996b; Pointillart et al., 1994; Eeckhout and Paepe, 1994) (Table 2-2). Niziolek (1995) found that phytase was synthesized during maturation and its activity rose remarkably during germination. Published scientific data concerning the effects of germination on chemical composition, anti-nutritional factors, and phytase activity in four legume seeds shows that an increase in phytase activity was accompanied by a concomitant decrease in phytate content. One to two weeks after germination, the phytate content was reduced 64 to 83%, thus increasing the availability of the minerals present in the germinated seeds (Bau et al., 1997; Kyriakidis et al., 1998).

Phytase have been found in fungi, especially Aspergillus (Shich et al., 1969), yeast (Irving and Cosgrove, 1974; Cosgrove et al., 1970), and bacteria (Greaves et al., 1967; Powar and Jagannathan, 1967). Microorganism phytase has been extensively studied. Phytase activity was also detected in rumen microflora (Morse et al., 1992; Yanke et al., 1998). The ability of rumen microflora to hydrolyze phytate is well-documented (Reddy et al., 1989). It is generally considered that 90 % of the phytate P in common feedstuffs can be used by ruminants (Clark et al., 1986).

Commercial Microbial Phytase

Suzuki et al. (1907) was the first to make a preparation of phytase by using liquid extracts from plant tissues. Phytase from the fungus Aspergillus or the bacterium Bacillus subtillis were used to treat digestive problems of animals (Werner, 1982). Although evidence showed that phytase, especially from microbial and plant origin, was very effective in the hydrolysis of phytate, its commercial use was not practical in animal diets because of cost. Commercial phytase has become available due to the development of modern technology and the common awareness of an environmental issue. Microbial phytase for commercial use is made by

overexpressing the phytase gene in a suitable microorganism. For example, commercial microbial phytase includes Natuphos® from BASF, and Novo phytase from Denmark, both of them are produced by overexpressing phytase gene in *A. niger* (Li et al., 1998). Microbial phytases were also produced by tobacco (Verwoerd et al., 1995), and soybean (Li et al., 1997; Denbow et al., 1998). However, most of these are not commercially available.

Molecular Properties of Microbial Phytases

Microbial phytase is a 3-phytase and a high molecular weight acid phosphatase. It consists of 444 amino acids and has a molecular weight of 65,000 Mr (Ullah and Gibson, 1987). Examination of the structure indicated that phytase has a larger α/β-domain and a smaller α-domain. There is a large deep indentation at the surface of the two domains. This deep indentation encompasses the catalytically essential amino acids arginine and histidine, which are believed to be the first two amino acids in the conserved sequence motif RHGXRXP (Kostrewa et al, 1997; Ullah et al., 1988).

As do all other acid phosphatases, microbial phytase hydrolyzes phosphoesters in a two-step mechanism with a phosphorylated active site amino acid as an intermediate (Shute et al., 1988). The basic amino acid region at the active site of phytase creates a favorable electrostatic environment for binding the highly negatively charged substrate phytate. During the hydrolyses,

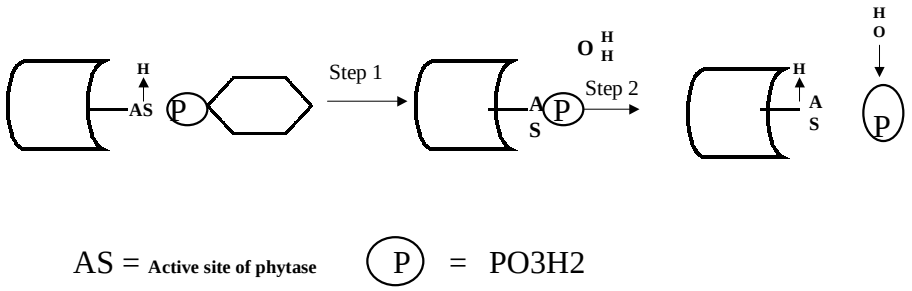

Figure 2-5 The mode of action of a phytase (Shute et al., 1988)

a phosphate group is transferred from phytate to the active site of phytase, and then from the enzyme to water (Figure 2-5). Microbial phytase hydrolyze phytate in step-wise order: 3, 4,5, 6, and 1.

Factors Influencing Phytase Efficacy

Many factors such as temperature, pH, moisture, and some minerals are known to have an effect on the phytase efficiency.

Temperature. Phytase activity linearly increases with increasing temperature and reaches a maximum activity at optimum temperature that varies from 45 ^{0}C to 60 ^{0}C (Kies, 1996).

pH. The optimum pH for phytase activity varies among phytases in the range of 2.2 to 7.8 (Nayini and Markasis, 1986). Most phytases have only one pH-optima while some phytases have more than one pH optima. The microbial phytase as used for Natuphos® has at least two major optima (Irving, 1980), the highest at pH 5.5 and second highest at pH 2.5. Eckhout and de Paepe (1991) reported that phytase was about 60% more active at pH 5.5 than at pH 2.5 and retained most of its activity in the pH range 4.0 to 6.0 and reduced to zero as the pH rose above 7.0 (Figure 2-6).

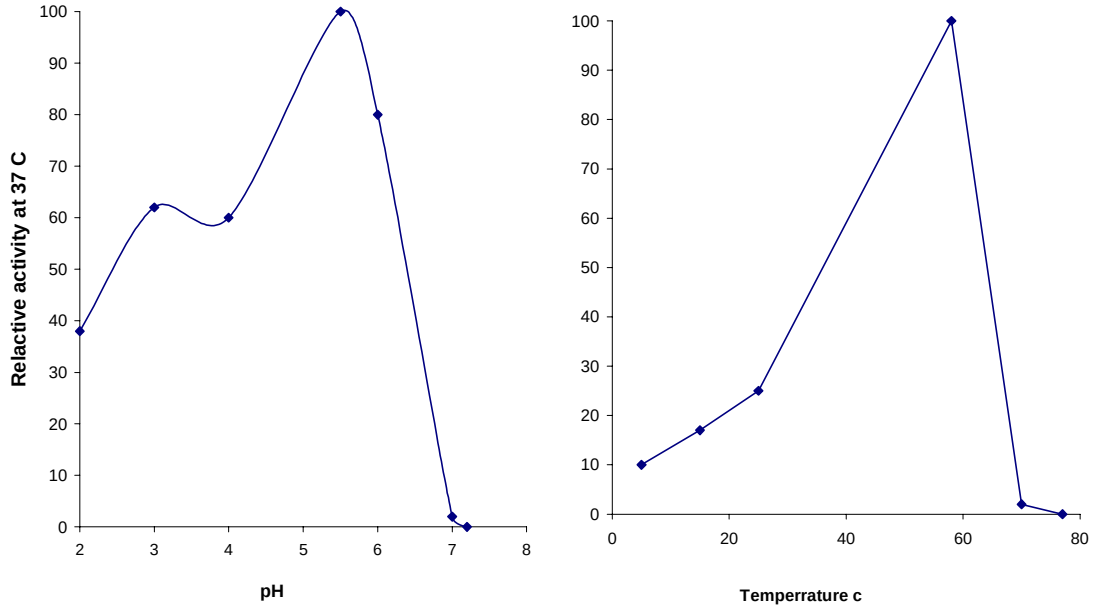

Figure 2-6. pH (A) and Temparature (B) profiles of microbial phytase (after Kies, 1996)

Moisture. Since the process of breaking-down phytate by phytase only works in aqueous solutions, the enzyme starts to work after ingestion by animals due to an increased water level in the digestive tract (Fredlund et al., 1997). It is well established that phytase begin to cleave phytate at about a 25% water content and reach maximum activity when the moisture content is 30%. Study has shown that soaking a diet supplemented 250 U/kg of phytase increased P absorption similar to that obtained in the diet with 500 U/kg of phytase addition when fed dry (Liu et al., 1997).

Minerals. Phytase is noncompetively inhibited by inorganic monophosphate and by Cu^{2+},' Zn^{2+}, Hg^{2+}, Sn^{2+}, Cd^{2+} ions and strongly by F^- ions; it is activated by Ca^{2+}, Mg^{2+} and Mn^{2+} ions (Dvorakova et al., 1997). It is also reported that at high level and in a neutral pH environment, Ca^{2+} interacts with phytate and form insoluble complexes, and these complexes are less subject to attack by phytase (Lantzsch et al., 1992).

Phytase Assay

One phytase unit (FTU) is defined as the amount of enzyme that liberates 1 micromole of inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at 37 ^oC and at pH 5.50. Phytase activity can be assayed as follows. 1) Sample pretreatment: dilute the samples to a phytase activity within the range 0.005 to 0.035 U/ml. 2) Preparation of the standard solutions: prepare a series of solution of standard phytase. 3) Incubation: both sample and standard solution are incubated with sodium phytate and releases inorganic phosphate from its substrate. 4) Termination: after 60-minute incubation, acid molybdate/vanadate is added to stop phytase reaction with phytate and at the same time, form a colored complex with phosphate from phytate. 5) Measurement: the amount of phosphate released by phytase is measure in spectrophotometer and the phytase activity is calculated by the amount of the phosphate.

Criteria for Assessing Phytase Effectiveness in Releasing Phosphorus

Several bioassay methods have been used to evaluate the efficacy of supplemental phytase on bioavailability of phosphorus (Kornegay and Yi, 1996; Yi and Kornegay, 1996). Methods for assessing phytase effectiveness have included performance, bone characteristics, apparent phosphorus digestibility, plasma P level, and alkaline phosphatase activity. For pigs, it was found that bone ash measurements and apparent phosphorus digestibility are most sensitive criteria for assessing phosphorus utilization, performance and shear force and energy of bone

were also sensitive indicators (Yi and Kornegay, 1996), however, alkaline phosphatase activity and serum Ca and P levels are not very responsive to dietary Ca-P changes. For birds, BW gain, toe ash percentage, and apparent retention of P were sensitive criteria for assessing phytase utilization (Kornegay and Yi, 1996).

Use of Phytase in Swine and Poultry Nutrition

The goals of modern animal production should be threefold: environmentally sustainable and sound, nutritional acceptability, and profitability. In studies with poultry and swine, supplemental microbial phytase has been known for its effectiveness in improving the utilization of plant phosphorus, and data from some reports indicated that the availability of Ca, Zn, and amino acids (and N) are also improved (Coehlo and Kornegay, 1996). In addition, it was also concluded that use of phytase is an effective way to keep modern poultry and swine industries environmentally safe as well as economically viable (Kornegay, 1996).

Nutritional Considerations of Using Phytase

Swine

Effects on Bioavailability of Phosphorus

Numerous reports have demonstrated that supplemental phytase was effective in improving performance, the apparent retention of P, plasma alkaline phosphatase activity and bone mineralization by improving hydrolysis of phytate P for pigs (Coelho and Kornegay, 1996). It was shown that addition of phytase to corn-soybean meal diets fed to young pigs improved the phosphorus retention by 10 to 20% (Simons et al, 1990; Jongbloed and Kemme, 1990, Hoppe et al., 1993; Han et al., 1997; Wenk et al., 1993).

Since supplementation of microbial phytase to pig diets results in the release of inorganic phosphate from phytate, the concept of equivalency or replacement values is used to express its activity with reference to the amount of phosphorus from inorganic feed phosphates that can be replaced. The equivalency value of microbial phytase for inorganic P was calculated in several trials with pigs using multiple levels of phytase and phosphorus. Based on the P digestibility, performance, and bone measurements, the relationship between adding phytase and P has been derived. Results showed that young pigs received 1,200 U/kg of phytase utilized dietary P more effectively than pigs fed diet supplemented with 0.21% phosphorus as calcium phosphate, and 1,000 U/kg of phytase activity was equivalent to 0.91 mg of phosphorus from calcium phosphate

(Lei et al., 1993a). Kornegay and Qian (1996) also indicated that addition of 1,400 U/kg phytase to a diet containing 0.16% available phosphorus diet resulted in similar performance to that of pigs fed a diet containing 0.36% available phosphorus.

The equivalency values of phytase for inorganic phosphorus in weaning pig were also evaluated by Kornegay's laboratory (Yi et al., 1996a; Qian et al., 1996a). It was found that nonlinear equations fit relationships between the phytase activity or P level and observed measurements better than with other kinds of equations. The equivalency values of phytase for inorganic phosphorus were calculated by solving these nonlinear response equations. Based on daily gain, phosphorus digestibility, and bone measurements, it was concluded that, addition of 246 to 676 U/kg of phytase to a low-P diet yielded an equivalent of 1g/kg of phosphorus.

The effect of supplemental phytase on the P availability was also studied in growing and finishing pigs. Cromwell et al. (1995a) reported that supplementation of 500 U/kg of phytase to a P-inadequate (0.4/0.3% for growing/finishing, respectively) corn-soybean meal diet improved the rate and efficiency of gain and bone breaking strength equal to that of pigs fed a P-adequate (0.5/0.4% for growing/finishing, respectively) corn-soybean meal diet. The P equivalency values of phytase were also determined by Harper et al. (1997) in growing and finishing pigs. Estimation obtained from their study showed that a P equivalency of 0.64, 1.29, 0.69 and 0.82 g for daily gain, tenth rib shear force, ash weight and ash percentage, with an average 0.86g for 500 U/kg phytase.

Based on a data set from four pig trials, Radcliffe et al. (1998) reported that the equivalency values of 500 U/kg of phytase for inorganic phosphorus was 0.98g for daily gain, 0.99g for bone measurements, and 1.03g for phosphorus digestibility with an average value of 1.0g. Jongbloed et al. (1996) evaluated equivalency values of phytase for inorganic phosphorus by a different approach. Using the dose-response effect of microbial phytase on the apparent total tract digestibility of phosphorus from six pig studies, they concluded that addition of 500 to 2,000 U/kg phytase to corn-soybean diets resulted in 0.8 to 1.0g digestible phosphorus per kg of diet. Using a value of 76% for the digestibility of P in P supplements, the equivalency values of 500 to 2,000 U/kg phytase for inorganic phosphorus is 1.05 to 1.32 g, which is close to the results mentioned above. In summary, it is generally accepted that addition of 500 U/kg phytase to complete feed is equal to a substitution of 0.1 to 0.12% phosphorus in the pig feed.

Since all reports showed that adding phytase linearly increased performance, bone measurements, and P digestibility, several trials were conducted with pigs to determine the optimal levels of supplemental microbial phytase for a low-P, corn-soybean meal basal diet. Conflicting data have been published. For example, Young et al. (1993) indicated that supplemental phytase at 1,000 U/kg of diet did not show further benefit comparing the 500 U/kg phytase of diet, while Cromwell (1995b) reported that supplementation of 1,000 U/kg phytase in corn-soybean diet released enough P for the finishing pigs. Furthermore, Lei et al., (1993b) reported that additions of phytase greater than 1,050 PU/g of basal diet did not improve ADG, ADFI, gain/feed and quadratic relationships between dietary phytase activity and these measures were found and their stationary points were at approximately 1,200 PU/g of basal diet. Later results (Kornegay and Qian, 1996) showed that the maximum responses of these measurements to phytase in pigs were dependent on available phosphorus levels in the diets. Their data demonstrated that maximum responses were observed at a phytase level of 1,050U/kg of diet for 0.07% available phosphorus diets and 700 U for 0.16% available phosphorus diets.

The evaluated equivalency value of phytase for inorganic phosphorus was mainly dependent on total dietary phosphorus and increase in phosphorus digestibility with phytase and this was reviewed by Kornegay (1998). They estimated that at 500 and 1,000 U/kg of phytase was equal to 0.75 and 0.95g digested phosphorus. His conclusion was consistent with the finding of Dungelhoef and Rodehutsscord (1995). They estimated that at 500 and 1,000 U/kg of phytase was equal to 0.71 and 0.91g digested phosphorus.

Effects on the Bioavailability of Calcium

Although most studies were not designed to evaluate the effect of adding phytase on the Ca availability, results of some studies indicated that phytase supplementation of corn-soybean meal diet improved Ca retention. Lei et al. (1993) reported that supplemental phytase to basal diet increased serum Ca concentration of pigs. Mroz et al. (1994) demonstrated improvement in the apparent total tract digestibility of Ca, and total phosphorus by microbial phytase in 45 kg canulated barrows. As a result of supplementation of this enzyme, the retention of Ca and phosphorus of pigs increased 2.2 and 1.9 g/d, respectively. A high positive correlation is generally observed between Ca and P absorption and utilization (Pointillart, 1993; Eeckhout and Paepe, 1991).

The relationship between the addition of phytase and Ca availability was observed in two pig trials reported by Radcliffe and Kornegay (1998) using multiple levels of phytase and Ca. Based on performance, apparent Ca digestibility, and bone measurements, their findings confirmed several previous reports showing that supplemental phytase improved the bioavailability of Ca in corn-soybean diets. Furthermore, adding phytase resulted in linear increase in daily gain, digestible Ca, and tenth rib ash percentage. Using these linear and non-linear equations describing the relationship between phytase and Ca, Ca-equivalency values for 500 U of phytase were estimated to be 1.30, 1.32 and 0.62 Ca, respectively for daily gain, rib ash and digestible Ca with an average of 1.08 g Ca.

Effects on the Bioavailability of Trace Minerals

Apart from the beneficial effects on P and Ca bioavailability, microbial phytase has been shown to improve the bioavailability of trace minerals such as Cu, Zn, and Fe. Based on improved growth rate, increased plasma Zn concentration and alkaline phosphatase activity, addition of phytase to a low P and Zn corn-soybean meal diets enhanced the utilization of Zn by pigs (Lei et al., 1993c; Adeola et al., 1995).

Adeola (1995) indicated that plasma Zn concentration increased when 1,500 U/kg of phytase was added to the diet containing no supplemental Zn. Growth rate, apparent Zn and Cu balance of 9.4 kg pigs were improved with the added phytase. These results indicate that the growth-promoting effect of phytase may be due to an overall increase in the availability of minerals. Similar results was observed by Pallauf et al. (1994) who also reported that the addition of microbial phytase to a diet based on field beans (30%), wheat (28%), peas (25%), and barley (14%) fed to 14 kg piglets enhanced plasma zinc concentration significantly. The concentration of inorganic phosphorus in plasma, zinc digestibility, and magnesium balance tended to be improved.

Murry et al. (1997) indicated that adding microbial phytase to a pearl millet-soybean meal-based diet increased growth rate, apparent digestibility and retention of trace minerals, and bone mineral status of gilts. The addition of microbial phytase (700 or 1,000 units/kg of diet) tended to increase serum Zn concentration.

Effects on the Bioavailability of Organic Nutrients

Numerous experiments have been conducted to investigate the effect of adding phytase on the utilization of organic nutrients such as fat, protein, and carbohydrates. Although the results are variable, it is generally accepted that supplemental microbial phytase has beneficial effect on organic matter availability.

In studies with pigs, Officer and Batterham (1992) and Kahn and Cole (1993) reported total tract digestibility of protein and apparent ileal digestibility were improved 4 to 10 percentage units by phytase addition. These values agreed favorably with findings reported by Mroz et al. (1991), who indicated that the maximum percentage unit improvement in protein digestibility was as high as 13. Biehl and Baker (1997a) showed, based on growth rate and feed efficiency, that the utilization of amino acids was improved 15.5 to 19.5 % when phytase (1,200 U/kg) was added to a low crude protein or high crude protein diet. However, other authors have reported no influence of phytase supplementation on utilization of protein (Nasi, 1990; Pallauf et al., 1994).

Jongbloed et al. (1997b) carried out two trials designed to determine the influence of phytase on the digestibilities of N and amino acids with cannulated pigs. In the first trial, although the apparent ileal digestibilities of amino acids improved when 800 U/kg of microbial phytase was supplemented to the basal diet, but the increase was not significant. However, the apparent total digestibility of N and the retention of N (g/day) were significantly improved by phytase addition. In the second trial, supplemental 900 U/kg of phytase significantly enhanced the apparent ileal digestibilities of most amino acids as well as N. However, the apparent total digestibility of N was not improved by phytase addition. An improvement in DM with phytase was observed in both trials. In conclusion, phytase may have a positive but small effect on digestibility of amino acids and N. The apparent ileal and total tract digestibilities of amino acids may differ in the ability to measure the real value of protein for swine.

Phytase also appeared to improve the retention of energy by increasing digestibility of protein, starch, and fat. In studies with pigs, energy retention was increased 2 percentage units when phytase was supplemented (Ketaren et al., 1993). The inclusion levels and nutrient values for phytase in swine diets were summarized in Table 2-4. Not much information is known about

amino acids equivalency of phytase and phytase effect on fat and carbohydrates. Further research is recommended for specific equivalency values of phytase for these organic nutrients.

Table 2-4. Available nutrients associated with addition of phytase

Nutrients	Per kg feed for swine[a]	Per kg feed for poultry[b]
P, g	1.20	1.10
Ca, g	1.20	1.00
ME, kcal/kg	12.70	15.00
CP, g	20.00	20.00
Lysine, g	0.10	0.20
Methionine, g	0.04	0.04
Cystine	0.03	0.07
Methionine +cystine, g	0.07	0.11
Tryptophan, g	0.02	0.02
Threonine, g	0.04	0.02
Valine, g	0.10	0.02
Isoleucine, g	0.08	0.02
Leucine, g	0.11	0.03
Phenylalanine, g	0.08	0.03
Histidine, g	0.05	0.02
Arginine, g	0.10	0.01

[a]Adapted from Coelho and McKnight (1998) , phytase inclusion rate, 500 U/kg.
[b]Adapted from Dudley-Cash (1998), phytase inclusion rate, 600 U/kg.

Poultry

Effects on the Bioavailability of Phosphorus

Phytase has an established role in releasing a significant portion of the phytate P present in vegetable rations and making it available to broilers (Simons et al., 1990; Huygebaert, 1996; Schoner et al., 1991). Perney et al. (1993) not only demonstrated that phytase added to a low P (0.21%) corn-soybean meal basal diet improved toe and tibia ash and plasma inorganic P, and they also demonstrated that the improvement by phytase addition was equal to supplementation of 0.05 to 0.10% of dietary P. Later, this was observed by Sebastian et al. (1996a,b) and Qian et al. (1996 b,c), who both evaluated the efficacy of microbial phytase on performance and apparent retention of P using three treatments: a normal P level corn-soybean diet, a low-P diet, and a low-P plus phytase diet. Phytase supplementation overcame the depression of feed intake observed for the low P diet, increased the percentage ash of tibia bone, and increased body weight in

chickens. The improvements in low P diet yielded body weights comparable to those obtained on the normal P diet.

Since the addition of microbial phytase to plant-based rations releases inorganic phosphorus from phytate, and improves some parameters related to phosphorus bioavailability, it seems reasonable to express the activity of this enzyme related to the amount of inorganic P that is digested. Linear or nonlinear equations regressed on phytase and P levels can be solved to provide an equivalency or replacement value of phytase for inorganic P. Richter (1993) observed that the growth rate and bone stability expressed as bending and breaking strength of tibia were improved by supplementation of phytase and inorganic P. Based on these observed parameters, 300 U/kg of phytase could replace 0.06 % of inorganic P in the broiler diet.

A review of research devoted to establishing the equivalency value of phytase for inorganic P in broilers fed soybean meal based or corn-soybean meal diets reveals a great deal of variation in results (Kornegay, 1996). Based on P retention and crude ash of broilers fed a corn-soybean meal diet for two weeks, Schoner et al. (1995) indicated that the phytase equivalency value of 1 g P from monocalcium phosphate was 700 and 762 U, respectively. While in another similar trial, Schoner et al. (1993) reported that phytase equivalency of 1g P was 570 U when based on the P retention and 1,050 U when based on BW gain. The average equivalency value was 850 U of phytase for both measurements when broilers were fed the diet for 40 days.

A series of experiments including four broilers (Denbow et al., 1995; Kornegay et al., 1996; Yi et al., 1996b, Qian et al., 1996b) and two turkey (Ravindran et al., 1995b; Qian et al, 1996c) trials designed to evaluated the effectiveness of phytase in replacing inorganic P in soybean based diets and corn-soybean diet were carried out at Virginia Tech. In these trials, graded phytase levels were combined with several levels of dietary P. Defluorinated phosphate was used as the inorganic P source. The responses of multiple levels of phytase and inorganic P on performance, toe ash, tibia ash, apparent retention of P was curvilinear or linear. Nonlinear and linear equations were used to describe these responses. Based on these equations, the equivalency values of phytase for inorganic P were calculated. The results showed that the equivalency value of phytase for 1g of inorganic P ranged from 467 to 922 U.

Further analysis of these equations can give us more information. First, based on the slope values of the curves from these equations, the sensitivity of observed measurements to

phytase level can be evaluated. For example, weight gain, ash percentage and apparent retention of P were sensitive criteria for evaluate P utilization or phytase effectiveness. Second, based on the points of these curves whose slope values are not significantly different from 0, the phytase level that maximized the observed measurements can be calculated.

Effects on the Bioavailability of Calcium

It has been documented that microbial phytase improves the availability of Ca in corn and soybean diet (Schoner et al., 1991, 1993; Kornegay et al., 1996). Sebastian et al. (1996a) reported that phytase supplementation of a low-P diet increased the relative retention of Ca by 12.2% in chickens. This conclusion was confirmed by their later work (Sebastian et al., 1996b) in which microbial phytase supplementation of a low P diet increased growth and relative retention of total P, Ca, and improved bone mineralization in broiler chickens. Microbial phytase increased the plasma P by 15.7% but reduced the plasma Ca by 34.1%.

Based on BW gain and phalanx ash, Schoner et al. (1993) reported that 500 U of microbial phytase were equivalent to 0.035% (0.35g/kg) and 0.056% (0.56g/kg) of Ca, respectively. In a similar study with turkeys, Kornegay and Denbow (1996) reported that body weight, gain:feed ratio, digestible Ca linearly increased as phytase or Ca was added to a basal diet low in Ca (0.6%). Calcium equivalency value for 500 U of microbial phytase were 1.2, 0.7, and 0.7 g Ca for BW gain, gain:feed ratio, and digestible Ca, respectively, with an average value 0.87 g Ca for 500 U of phytase.

Effects on the Bioavailability of Trace Minerals

Results from several studies have shown that additions of microbial phytase to plant-based diets fed to poultry improved the absorption and retention of Zn. Thiel and Weigand (1992) reported that Zn retention was enhanced when 800 U of phytase was added to a low Zn (27 g/kg) diet. In a later study with broilers, Thiel et al. (1993) reported that chicks fed a diet containing 30 g/kg Zn plus 700 U/kg of phytase had the same serum Zn concentration as the chicks fed a diet containing 39 g/kg Zn. Addition of phytase was equal to increasing the dietary Zn level by 30%. These findings were generally in agreement with the results reported by Biehl et al. (1995), who observed that dietary phytase supplementation of a Zn-deficient soy-concentrate diet (13 mg Zn/kg) increased growth rate by 40% and tibia Zn concentration by 160%. Furthermore, based on tibia Zn values obtained from a standard Zn curve, additions of

600 and 1,200 U of phytase per kg diet released 3.8 and 5.5 mg Zn, respectively, of per kg basal diet.

The phytase equivalency value of Zn was evaluated in a trial with young broilers (Yi et al., 1996c) using 3 × 4 factorial arrangement of multiple levels of phytase and Zn supplementation. Adding Zn and phytase to the low Zn basal diet linearly increased BW gain, feed intake, and the amount of Zn retained by broilers. Nonlinear or linear response equations of the effects of Zn and phytase were generated and used to calculate the Zn equivalency values. The average function for estimating Zn equivalency values were developed: $Y = 0.20 + 0.0082X$. Where Y (mg/kg) = amount of Zn released and X (U/kg) = level of microbial phytase. These results indicate that approximately 0.9 mg of Zn was released per 100 U of phytase over the range of 150 to 600 U of phytase.

Effects on the Bioavailability of Organic Nutrients

The influence of supplemental phytase on the utilization of protein or amino acids was evaluated in several poultry studies. Although results were variable between studies, it is generally accepted that adding phytase to poultry diet has positive effect on protein or amino acid utilization. The addition of phytase to diets containing protein and amino acids recommended by NRC (1994) improved the apparent N retention of broilers (Yi et al., 1996d). Biehl and Baker (1997b) reported that adding phytase at 1,200 U/kg to a low protein, amino acids-deficient corn-soybean meal diet improved feed efficiency, but not BW gain. Their later study with adult cecectomized roosters indicated that the average digestibility of amino acids was enhanced two percentage units, but the improvements were not significant. Yi and coworkers (1996d) also reported a positive effect of phytase on feed efficiency of turkeys fed a low protein diet. In addition, they observed that adding phytase to the diets increased the digestibility of N and most of the amino acids. This observation was favorably supported by results of other studies with broilers (Kornegay, et al., 1996; Sebastian et al., 1997). In two recent studies with broilers, Ravindran et al. (1998) reported that phytase supplementation improved apparent metabolizable energy of wheat-sorghum-soybean meal-rice pollard diets by 6% and amino acid digestibility of individual ingredients by 2.2 to 2.5 percentage units. In summary, phytase supplementation increased growth performance, protein, and amino acid digestibilities for both broilers and turkeys, particularly in the low protein, P and Ca diet. The available nutrients associated with the

addition of 600 U/kg phytase is briefly summarized in Table 2-4. Further research should focus on enhancement of organic nutrients in digestibility by phytase addition.

Environmental Considerations of Using Phytase

Phytase supplementation of rations based on primarily plant ingredients improves the utilization of nutrients such as P, Ca, Zn, and amino acids, and reduced the P level in swine and poultry manure. The reduction of nutrient excretion is achieved by improving phytate P utilization which allows for a decrease in total dietary P.

The performances of pigs fed diet containing 0.36% total P plus 1,050 U/kg phytase and pig fed diet containing 0.45% total phosphorus plus 700 U/kg phytase was found to comparable to that of pig fed diet containing 0.61% total P diet (Qian et al, 1996a). This mean that adding 700 and 1,050 U/kg of phytase to 0.36% and 0.45 total P diets can replace 1.6 and 2.6 g inorganic P. Similar magnitudes of reduction in inorganic supplementation were also observed in other pig studies: adding 250 to 500 U of microbial phytase per kg to a typical pig diet can replace 1 to 1.2 g inorganic P (Simons et al., 1990; Beers and Jongbloed, 1991; Pallauf et al., 1992ab; Hoppe et al., 1993). Based on the data from the studies with broilers, the reduction of inorganic supplementation by phytase was as high as 0.18% of the diet (Ravindran et al, 1995b), which means adding 500 to 750 U of phytase can replace 1 to 1.8 g inorganic P. It is generally accepted that addition of 500 to 750 U/kg of phytase to broiler diets and the addition of 250 to 500 U/kg of phytase to pig diets reduced the inorganic P by 0.1 to 0.12%. Using the standard dietary P levels recommended by NRC (Poultry, 1994; Swine, 1998), the dietary P level could be reduce 15 to 30% and 15 to 20%, respectively, for poultry and swine when phytase was added to diet. Based on the feed conversion ratio, feed composition and digestibility, reduction in organic P supplementation by 0.1 % level in diets decreases the P excretion by 36% for pigs and 37% for poultry (Kornegay, 1998).

Supplemental phytase can further decrease the nutrient level of the poultry and pig waste by increasing the utilization of those nutrients in a low P plant-ingredient diet. Supplemental phytase to a low-P pig diet improved the P digestibility by 10 to 20% and the fecal excretion was reduced by 10 to 20% (Jongbloed et al., 1997; Cromwell et al., 1993). Qian et al., (1996bc) and Yi et. al. (1996b) reported similar results in birds, in which the data showed that phytase

enhanced the P digestibility by 10 to 15% and reduced the fecal excretion by 8 to 10%, comparing with a negative diet.

Kornegay (1998) reviewed research addressing the influence of phytase on the P digestion and excretion in pig and bird manure. Thirty two pig experiments and thirteen poultry experiments were used to generate the nonlinear response curve of added phytase on digestibility of P and P level in animal manure. Addition of phytase at 500 U/kg and 1,000 U/kg to low-P diet fed to birds reduced P excretion by 13.3% and 16.7% respectively. For pigs the reduction in P was 15.4% and 18.8%, respectively for 500 and 1,000 U/kg of added phytase.

Using the data from both reductions in P excretion by reducing dietary P level and increasing the bioavailability of P, Kornegay (1998) concluded that addition 500 U/kg of phytase can reduced P by 40 and 50% for poultry and swine. This confirmed several previous studies with pigs (Beers and Jongbloed, 1992) in which the average reduction of P excretion by phytase was 50% and with broilers (Simons et al, 1990; Schoner et al., 1990, 1992) in which the average reduction in P excretion was 40%.

Cost Considerations of Using Phytase

Whether or not using microbial phytase is economical involves several factors: nutrient replacement, freed up space in the ration, and reduced nutrient excretion.

Phytase enzyme added to pig and poultry diets improves the bioavailability of phosphorus as well as other nutrients. Thus, the dietary levels of P, Ca, protein, and energy levels can be lowered when phytase is included in the feeds (Coelho and McKnight, 1998). Using the equivalency value of phytase for these nutrients and the cost of phytase and the nutrients that phytase can replace, the average cost benefit analysis of all nutrients is easily calculated. For example, based on an equivalency 1 g/kg P for 500 to 750U for broiler, 250 to 500 U for pigs and 300 to 500 U for layers, and given a cost of $1.43 per kg of high quality inorganic feed grade P and $1.9 for 500,000 U of phytase, the economic return of per ton feed from using phytase in replacement of inorganic P is -$0.47 to-$1.32 for broilers, $0.48 to -$0.47 for pigs, and $0.13 to -$0.47 for layers. Using phytase is not always as economical as using inorganic P. However, phytase has been found to improve the bioavailability of several critical amino acids and other trace minerals. Although further studies are needed to make specific recommendations, the improvements in amino acid and trace mineral utilization have economic potential. The value of

space freed up in the ration by removing P and Ca varies from species to species and from diets to diets. It is generally accepted that the higher the diet density the more valuable the freed up space. Since high density ingredient are more expensive than the lower energy ingredients, freed up space is probably more important for broiler than for pigs.

Waste disposal costs, although difficult to evaluate, are becoming more and more economically important. Microbial phytase reduced litter P level by 30 to 50% and could reduce waste disposal costs. Based on data from a Virginia turkey farm which produces 65,000 birds annually, Bosch et al., (1998) reported that phytase net return per year was $1435. Coelho and Mcknight (1998) concluded that supplemental phytase reduces feed costs up to $2.50 per ton feed by increasing the availability of P, Ca, protein, amino acids, and energy, and reducing N and P excretion

Dietary Factors that May Influence Phytase Effectiveness

The following factors can affect phytase effectiveness: dietary phytase level, phytate activity, P and Ca concentration, and Ca:P ratio (Kemme et al., 1997; Qian et al., 1996b, 1997). In theory, the relation between the rate (V) of phytase hydrolyzing its substrate, phytate, concentration can be described by the Michealis-Menten equation:

$$V = V_{max} * (\text{phytate})/(K_m + \text{phytate})$$

Since V_{max} and K_m are usually constant, the reaction rate of phytase increases linearly with increase of quantity of enzyme and concentration of phytate. This conclusion is clearly supported by almost all the studies with phytase. The selected measurements such as performance, digestibility, and bone characteristics linearly increased as the dietary phytase level increased (Kornegay and Yi, 1996; and Yi and Kornegay, 1996). In addition, it was also proved that the higher the dietary phytate level, the more efficient the phytase. For example, the equivalency values of phytase for 1g P from defluorinated phosphate was 800 to 939 U when the dietary phytate levels were 0.18 to 0.20% (Denbow et al., 1995; Kornegay et al., 1996), while the equivalency values of phytase for 1g P from defluorinated phosphate was 635 to 760 U when the dietary phytate levels were 0.24 to 0.27% (Yi et al., 1996b; Qian et al., 1996c). This was further confirmed in the trials with turkeys: phytase equivalency value for 1g of P from defluorinated phosphate in 0.22 % phytate P diets was 699 U and was 476 U in 0.27g phytate diets (Ravindran et al., 1995b; Qian et al., 1996b).

It has been established that the nutritional value of phytase is primarily dependent on the release of P from phytate and making it available to monogastric animals. Phosphorus nutrition for poultry and swine rely on dietary P and Ca levels, a suitable Ca:P ratio, and the presence of vitamin D (NRC, Swine , 1998; Poultry, 1994). It is reasonable to conclude that these factors will influence the phytase efficiency. Swine or poultry fed diets with narrow total Ca:total P ratios had better performance, P utilization, and bone mineralization from phytase supplementation. A desirable Ca:P ratio for maximizing the response to phytase is 1.1:1 to 1.4:1 for broiler (Qian et al., 1997) and turkey (Qian et al., 1996d) and 1:1 to 1.1:1 for pigs (Qian et al., 1996a; Lui et al., 1998), which are more narrow than values recommend by NRC (Swine , 1998; Poultry, 1994). This is due to the fact that excess of dietary calcium interferes with phytase and reduces the absorption of P and the phytase efficiency (Kornegay et al., 1998). A dietary P level either too high or too low also reduced the observed responses to phytase (Qian et al., 1996a; Yi et al., 1996a; Harper et al., 1997). The adverse influence of high dietary Ca levels on the utilization of other nutrients was markedly reduced by adding vitamin D_3 to poultry and swine diets (Pallauf and Rimbach, 1995; Biehl and Baker, 1995; Lei et al., 1994).

Practical Use of Microbial Phytase

As an enzyme, phytase loses activity during storage. The loss of phytase activity is 5% per month when stored in the original container at temperature less than 20 ^{o}C. The loss of activity is very fast if the temperature is higher than 40 ^{o}C and relative humidity is above 70%. Mixing phytase with vitamins, minerals, and amino acids can also reduce phytase activity. Prolong mixing time reduces the stability of phytase as much as 5%. Phytase in mash feed loses 10 to 20% of its activity when stored at 20 ^{o}C for 4 to 8 weeks. The activity loss may be as high as 50% at 65 to 75 ^{o}C pelleting temperature. Pelleting temperature above 75 ^{o}C should be avoided since phytase activity may be completely lost.

There are basically two methods that can be used in formulating diets with added phytase. 1). Include phytase as an ingredient with no nutrient value and reduce the P and Ca level according to the amount of Ca and P replaced by phytase. 2). Include phytase as an ingredient

with specific nutrient values. These values are based on the equivalency value of phytase for Ca or P.

Non-starch Polysaccharides

Non-starch Polysaccharides and Their Anti-nutritive Properties

Cereal grains, such as corn, sorghum, wheat, and barley, which make-up 70 to 80 percent of swine and poultry diets, are composed mainly of carbohydrates. The major components of these carbohydrates are starch (70 to 90%), non-starch polysaccharides (10 to 30%), and oligosaccharides (1 to 3%). Sixty to 70 percent of the linkages between glucose in starch are α-(1-4); the other linkages are α-(1-6). These linkages are readily degraded by the endogenous enzymes of monogastric animals (Johnson, 1991). Therefore, the starch in cereal grains can be digested and utilized by poultry and swine. The NSP are poorly digested by poultry and swine because they do not possess the NSP enzymes (Henry, 1985). Although a number of compounds, such as cellulose, hemicellulose, pentosans and glucans, are classified as NSP, the two most important NSP compounds in animal nutrition are water soluble ß-glucans and pentosans (Figure 2-7, 2-8). These polysaccharides are high-molecular weight polymers of monosaccharides linked by bonds that differ from those in starch. For example, the β-(1-3) and β-(1-4) glycosidic bonds

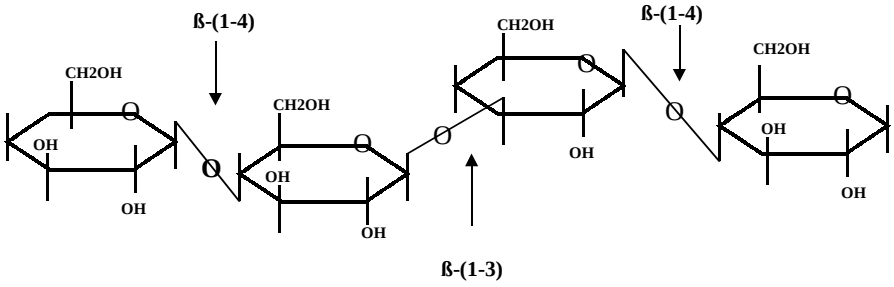

Figure 2-7. ß-(1-3), (1-4)-D-glucans

Annison and Choct, 1992)

in β-glucans can resist the digestion of enzymes which hydrolyze starch (Slominski, 1991). These two dietary carbohydrates often contribute little to meeting the energy requirement of poultry and swine.

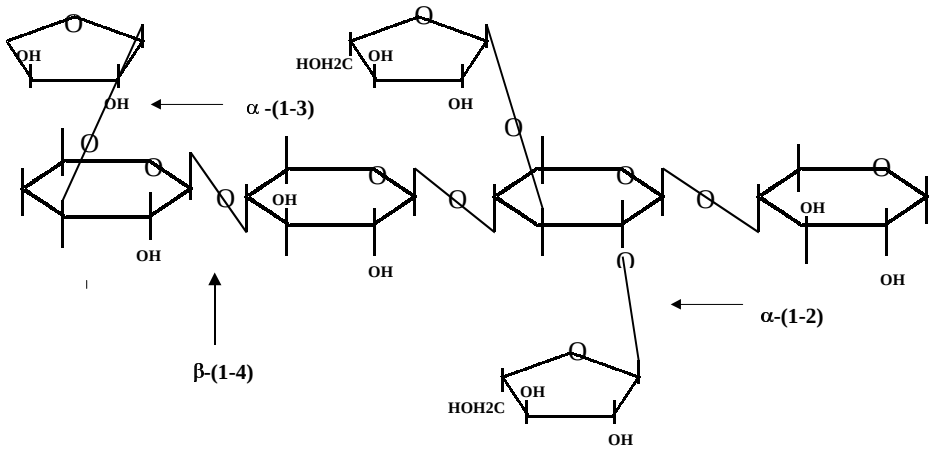

Figure 2-8. α **-(1-2, 3)-L-arabino-**β**-(1-4)- xylans**

(Annison and Choct, 1992)

In addition to their indigestible characteristic, ß-glucans and pentosans have properties which may affect digestion and absorption processes (Coelho, 1997). For example, NSP increase the viscosity of micelles in the G. I. tract and lower the absorptive ability of the small intestine. Also, the negative charges associated with NSP molecules can form a wide variety of insoluble complexes with micelles and protein, thus reducing the absorption of these nutrients (Rickes et al., 1962). Further, NSP have strong water holding capacities which can alter the physical properties of digesta, and reduce the movement of digesta in the intestine (Annison, 1993). Because of these three anti-nutritive properties, ß-glucans and pentosans when present in sufficient concentrations can have adverse effects on the digestive processes of poultry and swine (NRC, Poultry, 1994; NRC, Swine, 1998), and they are considered to be anti-nutrients (Annison,1993; Choct and Annison, 1992a).

Non-starch Polysaccharides Enzymes and Their Modes of Action

The complete breakdown of NSP compounds requires multiple enzymes. Almost all the enzymes used today as feed supplements are produced by fermentation of the selected strains of molds or bacteria (Coelho, 1997). The two most important enzymes are xylanase and β-glucanase; they hydrolyze arabinoxylans and β-glucans, respectively.

The modes of action of enzymes on NSP in broiler diets have been reviewed by Annison (1993). Using a C-14 labeled NSP, Annison and Choct (1993) showed that NSP enzymes could break the main branches of NSP. Because the physical properties of NSP are associated with their long branches, the negative effect of NSP on feed efficiency was removed after the long branches were cleaved. This proposed mode of action was confirmed by results in poultry in which the addition of NSP enzymes increased AME values of the diets and lowered the viscosity of the ileal content (Choct et al., 1994).

Feeding Trial Results

ß-glucanase

Broilers. The addition of ß-glucanase to cereal grain-based diets is very effective in improving feed efficiency and growth rate of broilers. Rickes et al. (1962) demonstrated that addition of ß-glucanase to wheat-based diets improved the performance of broilers. Later studies (Hesselmen et al., 1981; Hesselmen and Aman, 1986) confirmed the findings using barley-based diets. Although research with rye is very limited, studies confirmed that supplementation of ß-glucanase had a similar effect on the performances of the broilers (Peterson and Aman, 1987). Campbell et al. (1989) and Cave et al. (1990) reported that broilers had a better response to ß-glucanase when fed a higher level of ß-glucans in barley- or oats-based diets. Many researchers showed that dietary supplementation of ß-glucanase to wheat-, barley-, or oat- based diets significantly improved the digestibility and absorption of fat, starch, N and amino acids (Wang et al., 1990; Rotter et al., 1989; Edney et al., 1989). Most investigators believe this is due to the reduced viscosity of digesta because ß-glucans are partially hydrolyzed.

Pigs: The response of pigs to ß-glucanase is low and inconsistent. Newman et al. (1993) found that addition of ß-glucanase to a wheat-based diet improved growth rate and feed efficiency of weanling pigs by 11% and 10%, respectively. But, Inborr and Meulen (1993) and

Inborr et al. (1993) showed that although supplementation of ß-glucanase improved the digestibility of NSP, it had no effect on growth rate or feed efficiency.

Xylanase

Broilers. Anti-nutritive activity of arabinoxylans (pentosans) has been reported by Choct and Annison (1992b). When added to the control diet, arabinoxylans lowered apparent metabolizable energy (AME) values; low AME results from the reduced digestibility of starch, protein, and fat (Rogel et al., 1987). The anti-nutritional activity of arabinoxylans will be reduced if arabinoxylans are hydrolyzed before they are added to the diets. The addition of xylanase to wheat-based diets improved growth rate, feed conversion of the birds, and AME values of the feed. Similar results were reported for barley and oats (Annison, 1992). The improved performances resulted from higher retention of macronutrients, such as starch, protein, and lipids, and also of minor nutrients, such as calcium, phosphorus, and zinc (Annison, 1992). Further investigation of the relationship between xylanase dose and broiler performance revealed that the performance of broilers would reach a peak at 0.4 to 0.6 percent xylanase inclusion in the diet. (Morgan et al., 1993). At enzyme inclusion levels exceeding the optimum, there are negative effects on feed efficiency and weight gain (Schutte, 1990).

Pigs. Research in pigs is very limited. Bedford et al. (1992) added high level of xylanase to a rye diets and found no effect in pigs. These results were confirmed by Thacker et al. (1992).

Interaction between Phytase and Other Additives on Utilization of Nutrients

Phytase Plus Organic Acids

Fumaric and citric acid are the most common acidifiers that have been reported to improve gain and feed efficiency in weanling pigs. The mode of acidifier action is not fully understood. However, scientists believed that it is due to the reduction in stomach pH by acidification. Low pH in gastrointestinal tract may increase the activity of digestive enzymes, reduce coliforms and pathogens, and decrease the rate of stomach emptying, thus increasing digestion time. In theory, acidification of a swine diet may lower dietary pH and in turn the pH of the stomach digesta to provided a optimal pH for phytase. This could potentially increase the effectiveness of microbial phytase, which has its highest optimal pH at 5.0 to 5.5. However, the results are inconsistent. For example, although it was shown that the addition citric acid linearly reduced the stomach pH of pigs, the interaction of phytase and acidifier was not significant

(Radcliffe, et al., 1998). On the other hand, other studies with pigs indicated supplemental formic acid and phytase increased daily gain by 10 and 12%, respectively, and using the additives together improved gains by 26% (Best and Gill, 1998). The interaction between the phytase and another organic acid, lactic acid was also observed by Jongbloed et al., (1997), who indicated supplementation of this acid increased that apparent P digestibility by 14% compared with that of the control diet with only added phytase.

Combination of NSP Enzymes and Phytase

Like other enzymes, the efficacy of the phytase and NSP enzymes is influenced by several dietary factors. One major factor is the time that the enzymes act on their substrate. The efficacy of enzymes will be reduced if they have less time in contact with their substrate. Phytate and NSP are large molecules and they may reduce the time that other enzymes act on their substrate in the G. I. tract. Phytase and NSP enzymes can break down the molecules of non-polysaccharides and phytate and can also increase the efficiency of other enzymes. Only a few studies have been conducted to evaluate the interaction of phytase and other enzymes. Wenk (1993) reported that there may be a negative interaction between carbohydrase and phytase. It is important to investigate this further.

Summary

Addition of phytase and non-starch polysaccharide enzymes to certain cereal-based diets can 1) remove anti-nutritive factors and 2) improve the utilization of nutrients by broilers and pigs. Although the responses of pigs and poultry to these enzyme preparation are positive, further research on cheaper source phytase, amino acids, energy equivalency of enzymes, and using both enzymes together in broiler and pig diets needs to be conducted.

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Chapter III

Comparison of Genetically Engineered Microbial and Plant Phytase for Young Broilers

ABSTRACT This study was conducted to compare the efficiency of genetically engineered microbial (Natuphos®) and plant (Phytaseed®) phytase for enhancing the utilization of phytate P in corn-soybean diets fed to young broilers, and to evaluate the safety of Phytaseed® phytase. Three levels of the two sources of phytase (250, 500 and 2,500 U/kg of diet) were added to a corn-soybean meal basal diet containing 0.46% total P, 0.21% nonphytate P, and 0.92% Ca. There were eight cages per treatment (8 birds per cage for wk 2-3, and 7 birds for wk 4-5), except the diet without added phytase that had 16 cages. Body weight and feed consumption were recorded weekly on a cage basis. During wk 5, cage fecal samples were collected for determination of the apparent digestibilities of DM, Ca and P. At the end of wk 5, all birds were killed, and the left and right toes were removed for determination of toe ash weight and percentage. Forty birds (8 broilers, one per cage, from the diet without added phytase and the diets with 500 or 2,500 U/kg phytase from both sources) were randomly selected for gross necropsy and histologic evaluation of liver, kidney and bone tissues. Adding both sources of phytase resulted in similar increases ($P < 0.05$) of BW, BW gain, feed intake, gain:feed, apparent digestibilities (retention) of DM, P and Ca, and toe measurements. Phosphorus excretion decreased as phytase addition increased. No significant abnormalities were seen in any of the 40 broilers necropsied. Further, the fit of a nonlinear function revealed that most measurements reached a plateau at 2,500 U/kg. Based on performance, bone characteristics and digestibilities of P, Ca, and DM of young broilers, the efficiency of Phytaseed® phytase was similar to that of Natuphos® phytase for enhancing the utilization of phytate P in corn-soybean. General necropsy and histologic examination of liver, kidney and tibial tissues revealed no adverse effects of phytase source and level.

Key Words: Phytase, Broiler, and Phosphorus.

INTRODUCTION

Most of the recent studies with phytase have used Natuphos®, a microbial phytase produced by overexpressing the *Aspergillus ficuum* phytase gene in *Aspergillus niger*. Many researchers have shown that supplemental microbial phytase improves the bioavailability of P

and other nutrients, and reduces the concentration of these nutrients in poultry manure (Coelho and Konergay, 1996). The poultry industry is slowly adopting the use of supplemental phytase, but microbial phytase is not always as economical as the inorganic P that it can replace, and the application of phytase to pelleted feeds requires special equipment. Compared with microbial phytase, a genetically modified phytase in plant has some advantages: 1) foreign genes can be easily transferred and expressed in plants such as canola and tobacco, 2) plants use solar energy and have large biomass accumulation, and 3) the phytase in the plant has no contamination with animal pathogens (Glick and Pasternak, 1994). Furthermore, with increasing environmental concern on manure management, the use of phytase in animal and poultry diets to improve the utilization of P, Ca, and even amino acids and starch will rise. Therefore, it is necessary to assess the effectiveness and safety of new sources of phytase. Phytaseed® is a new source of phytase, which was produced in canola seed by expressing the same phytase gene that was expressed in *Aspergillus niger* for the production of Natuphos®. The objectives of this study were two-fold: 1) to compare the efficiency of two phytase sources, Natuphos® and Phytaseed®, for enhancing the utilization of phytate P in low P corn-soybean meal based diets fed to young broilers, and 2) to determine the safety of Phytaseed® phytase as a dietary supplement for broilers.

MATERIALS AND METHODS

Birds and Feeding Management. Following a 7-d adjustment, commercial male broilers (n = 576) were used in a 4-wk test to investigate the effect of adding Natuphos®[1] or Phytaseed®[2] to a soybean-meal based diet for young broilers. The care and treatment of the birds followed published guidelines (Consortium, 1988).

On the day of hatching, chicks were wing-banded and randomly assigned to batteries (nine birds/cage) with raised wire bottom floors. The batteries were in an environmentally controlled room. At the end of the 7-d adjustment, birds were equalized to eight per cage, weighed, and put on test for 4 wk. There were eight cages per treatment, except the diet without added phytase which had 16 cages. Dietary treatments were randomly assigned to cages within each replicate (block). At the end of wk 2 on test, birds were moved from starter batteries to

[1] Microbial phytase: Natuphos 6000™ produced by *Aspergillus niger* genetically modified with Aspergillus ficuum phytase gene. BASF Corp. Mt. Olive, NJ, USA.
[2] Phytase produced from canola seed genetically modified with *Aspergillus ficuum*,phytase gene. BASF Aktiengesellschaft, Offenbach, Germany.

grower batteries for the remaining 2 wk. The number of birds per cage was reduced to seven per cage at this time. Birds had ad libitum access to feed and water at all times, and were routinely checked twice daily with attention given to feeders, waterers, temperature and condition of birds.

Dietary Treatments. Two sources of phytase (Natuphos® and Phytaseed®) were fed at three levels of phytase activity (250, 500 and 2,500 U/kg of diet). A low P corn-soybean meal diet containing 2.5% (25 g/kg) milled canola, either as Phytaseed canola or Westar canola, was used for all treatments (Table 3-1). A basal diet (less milled canola) was formulated and mixed common basal diet ensured that all dietary treatments contained the same proportion and quality of ingredients except for the Phytaseed canola or Westar canola. Westar canola was used as a carrier for making a Natuphos phytase premix containing 103 U/g of phytase. Diet 1 served as the negative control, containing no added phytase from Natuphos or Phytaseed. Diets 2, 3, and 4 contained 250, 500, and 2,500 U/kg of Natuphos® phytase, respectively. Diets 5, 6, and 7 contained 250, 500, and 2,500 U/kg of Phytaseed® phytase, respectively. Based on an initial assay of the phytase activity of Phytaseed (103 U/g), Phytaseed canola replaced milled Westar canola on an equal weight basis to obtain the desired levels of phytase. A later phytase assay of Phytaseed conducted when all diets were assayed for phytase showed that Phytaseed contained 78 U/g. The assayed phytase activity of the diets is shown in Table 3-2. The original calculated levels of phytase were used in data analyses because we believe, based on assayed phytase activities of the diets, that the original assay of phytase activities of Phytaseed more nearly represents the true activities. All diets were fed as a mash.

Sampling and Analysis. Body weight and feed consumption were recorded weekly on a cage basis, and mortality was recorded daily. During the fourth week on test (fifth week of age), a 3-d Ca and P balance study was carried out. Excreta from each cage was stored in plastic bags at -20° C for subsequent drying in a forced-air oven at 65° C. Dried excreta and 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 by the vanadomolybdate procedure, and Ca was determined with an atomic absorption spectrophotometer (AOAC, 1990). Apparent retention (percentage of intake) of P and Ca was calculated using the direct method. At the end of the test, all surviving chicks were killed

TABLE 3-1. Composition of the basal diet

Ingredients	g/kg
Corn (8.5% CP)	484.1
Soybean meal (44% CP)	415.0
Milled canola seed (25% CP)[a]	25.0
Stabilized fat	40.0
Limestone[b]	17.9
Defluorinated phosphate[c]	3.0
Vitamin premix[d]	2.0
Trace mineral premix[e]	2.0
Salt	4.0
DL-Methionine	2.0
Corn starch-dextrose	5.0
Total	100.0
Calculated to contain	
Crude protein	230.0
Lysine	13.6
Methionine + cystine	9.4
Calcium	9.2
Total phosphorus	4.6
Nonphytate P	2.1
Ca:tP ratio	2.0
Ca:nP ratio	4.4

[a]Milled Phytaseed canola, milled Westar canola, and a milled Westar-Natuphos premix were used in the appropriate amounts to supply the desired phytase activity. See Table 3-2 for dietary treatments.

[b]Ground limestone (Luttrel Co., Luttrel, TN, 38% Ca).

[c]Coronet industry, Inc., Plant City, FL, 30% Ca, 18% P.

[d]Supplied (per kg diet): retinyl acetate 908 µg, cholecalciferol 66 µg, DL-α-topheryl acetate 26 mg, menadione sodium bisulphite complex 0.75 mg, riboflavin 7.5 mg, D-calcium pantothenate 9.7 mg, niacin 26.4 mg, cyanocobalamin 0.011 mg, choline chloride 1012 mg, D-biotin 0.31 mg, pteroylmonoglutamic acid 3.1 mg, thiamin-HCl 8 mg, pyridoxine-HCl 3.1 mg, ethoxyquin 50 mg, and virginiamycin 2.9 mg.

[e]Supplied per kilogram of diet: Mn 88 mg, Zn 95 mg, Fe 100 mg, Cu 12.5 mg, I 4 mg, and Se 0.6 mg.

The basal diet was then used to make individual treatments as shown in Table 3-2. The use of a

TABLE 3-2. Dietary treatments and assayed phytase levels

	Dietary Treatments						
		Natuphos (U/kg)			Phytaseed (U/kg)		
	Basal	250	500	2500	250	500	2500
Ingredients Diets	1	2	3	4	5	6	7
Westar canola (%)	2.50	2.26	2.02	0.07	2.26	2.02	0.07
Phytaseed canola (%)[a]	0	0	0	0	0.24	0.48	2.43
Phytase/Westar premix[b]	0	0.24	0.48	2.43	0	0	0
Phytase activity (U/kg)							
Calculated	0	250	500	2500	250[c]	500[c]	2500[c]
Assayed	<70	300	530	2580	240	540	1970

[a]The phytase level of Phytaseed canola was initially assayed to be 103 U/g. This activity was used for diet formulation. A later assay of Phytaseed showed 78 U/g.

[b]Due to the small amount of phytase required in the diets, a Natuphos/Westar premix was prepared as follows: 200 g phytase (Natuphos 6000™, -assayed 6240 U/g) was premixed with 11,916 g of Westar canola to provide 103 U of phytase per g of Natuphos/Westar canola).

[c]These expected values would be less if the lower phytase activity of Phytaseed was used.

for collection of toe samples that were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left and right toes were pooled separately within pens, but were averaged for statistical analysis. The pooled samples were dried to a constant weight at 100° C and then ashed in a muffle furnace at 600° C for 4 h.

Eight birds, one per cage from diets 1, 3, 4, 6, and 7, were randomly selected at the termination of the test for gross necropsy by Dr. Calvin Larsen. This included histological examination of any suspicious tissue, as well as histological examination of the tibia. The histologic examination was performed by Dr. Hugo P. Veit. Dietary treatments were unknown to both Dr. Larsen and Dr. Veit during gross and histologic evaluations and scoring.

Statistical Analysis. Data for all measurements and calculations were analyzed by the GLM procedures of SAS (1990). Cage was the experimental unit, except for general necropsy and histologic examination of tissues where the individual bird was the experimental unit. The main effects included in the model were treatment and replicate. Contrasts were used to test differences between the two sources of phytase, between the basal and the other diets, and between the 250 and 500 U/kg diets versus the 2,500 U/kg diets (across phytase sources). Contrasts were also used to test the interaction between the two sources of phytase and the linear and quadratic effects (we acknowledge that phytase levels were unequally spaced). Because both sources of phytase were found to elicit similar responses for the various measurements, the data were output by phytase levels (across phytase sources), and nonlinear and linear equations were derived for the phytase response for the various measurements taken. The model for non-linear equations was as follows: $Y = a(1\text{-}be^{-kx})$. The sensitivities of the response measurements (Y) to phytase supplementation (X) were defined as the magnitude of change in measurement value against phytase level and calculated as follows: $Ys = (1\text{-}be^{-kx})/(1\text{-}b)$ ($Y_0 = a(1\text{-}b)$, when X=0 and $Yx = a(1\text{-}be^{-kx})$, when X=x). Based on the response values of these equations (Ys), the sensitivities of the response measurements (Y) to phytase supplementation (X) were evaluated. The stationary points of $Y = a(1\text{-}be^{-kX})$, estimates of dietary phytase activity at which maximum responses of observed measures are expected to occur, were calculated from the first derivative of $1\text{-}be^{-kX}$, Based on X= (ln(0.00001/bk)/-k when Y' = 0.00001.

RESULTS

Comparison of Sources. During wk 2-3, wk 4-5, and overall, the BW gain, feed intake, and gain:feed ratios were not significantly different for the diets with Phytaseed phytase compared with the diets with the same level of Natuphos phytase (Table 3-3). Furthermore, there were no main effect differences between broilers fed the two phytase sources for apparent retention (%) of P and Ca, digestibility (%) of DM, and toe measurements (Tables 4). The probability values were always greater than 0.20, and with an average 0.43.

Phytase Levels. Across both phytase sources, average BW gain and gain:feed ratio increased ($P < 0.01$) as the dietary phytase level increased (Table 3-5). Feed intake was not significantly increased across phytase levels because of an unexplained decrease at 500 U/kg of added phytase level, although feed intake of all phytase diets was larger than that of the basal

diet. The fit of the nonlinear equation was very good for BW gain and gain:feed ratios (Table 3-6). These findings indicate that the magnitude of the response per unit of phytase decreased as the level of phytase increased.

The apparent retention (%) of P and Ca, digested P and Ca, and toe ash weight and toe ash as a percentage of dried toes increased ($P < 0.05$ for all the measurements) as the level of phytase increased (Table 3-5). Nonlinear equations were the best fit for these measurements (Table 3-6), indicating that the magnitude of the response to additional phytase decreased as the level of phytase increased. However, dry matter digestibility was similar among phytase levels, although the numerical phytase response was largest at 2,500 U/kg compared with 250 and 500 U/kg (Table 3-4). Excreted P and Ca decreased ($P < 0.01$) with phytase supplementation.

Gross Pathologic and Histopathologic Examination. Gross findings for 40 birds (diets 1, 3, 4, 6, 7) necropsied at the end of the test were incidental to euthanasia. A few histologic liver and kidney changes were noted, but appeared unrelated to treatments (Table 3-7). Specifically, there were variable granulomas and/or lymphoid nodule size or numbers within the liver and kidney tissues. This could represent variable exposures to unidentified antigens. The slightly higher lipid vacuole scores in hepatocytes for broiler fed Natuphos® at 500 U/kg and Phytaseed® at 2,500 U/kg was probably indicative of their high performance. Overall, no significant disease symptoms were seen in any of the 40 birds necropsied, and there were no detectable differences in the various tibial measurements.

DISCUSSION

Comparison of Sources. Based on the performance, P and Ca retention and excretion, digestibility of DM, and bone characteristics, the efficiency of phytase from the two sources, Natuphos® phytase and Phytaseed® phytase, were equally effective (Table 3-3 and 3-4). Similar findings were also observed in a pig study using the same two phytase products (Zhang et al., 1998). Phytase occurs widely in microorganisms, plants, and certain animal tissues. Depending on their own phytase, some yeasts grow well on media in which sodium phytate is the sole source of inorganic phosphate (Lambrechts et al., 1992), some cereal by-products improve the availability of phytate phosphorus (Pointillart, 1991), and animals have a variable ability to use

TABLE 3-3. Body weight, BW gain, feed intake, and gain per feed ratios of young broilers in response to Natuphos or Phytaseed supplementation

	Dietary Treatments[a]							
		Natuphos (U/kg)			Phytaseed (U/kg)			
	Basal	250	500	2500	250	500	2500	
Items Diets	1	2	3	4	5	6	7	MSE[b]
Body weight (g)								
Initial	129	129	127	130	130	126	129	4.8
Final	1371	1437	1454	1606	1449	1488	1564	32.0
BW gain (g)								
Week 2-3[c,d]	502	506	518	560	512	528	572	31.0
Week 4-5[d,e]	740	802	809	916	807	834	863	39.2
Week 2-5[d,e]	1242	1308	1327	1476	1319	1362	1435	70.6
Feed intake (g)								
Week 2-3[c,d]	726	741	749	780	733	758	803	36.0
Week 4-5[e]	1763	1873	1734	1898	1823	1796	1776	78.4
Week 2-5[f,g]	2489	2614	2483	2678	2556	2554	2579	120.8
Gain:feed ratio (g/kg)								
Week 2-3[d]	692	682	692	718	699	696	713	21.2
Week 4-5[d]	420	428	467	483	443	464	486	27.2
Week 2-5[d,e]	500	501	534	552	516	533	557	25.4

[a]Each treatment mean represents eight cages containing eight birds per cage except Diet 1 which represents 16 cages containing eight birds per cage.

[b]MSE = the root mean square error. SEM = MSE/$\sqrt{n}$, where n = 16 (Diet 1) or n = 8 (all other diets) pens. All comparisons between Natuphos® and Phytaseed® had P values greater than 0.30.

[c]Basal diet less than all others ($P < 0.01$).

[d]The 2500 U/kg diets were larger than 250 and 500 U/kg diets ($P < 0.001$).

[e]Basal diet less than all others ($P < 0.001$).

[f]Basal diet less than all others ($P < 0.05$).

[g]The 2500 U/kg diets were larger than 250 and 500 U/kg diets ($P < 0.05$).

TABLE 3-4. DM digestibility, retention of Ca and P, digested and excreted Ca and P, and toe measurements of young broilers in response to Natuphos or Phytaseed supplementation

	Dietary Treatments[a]							
		Natuphos (U/kg)			Phytaseed (U/kg)			
	Basal	250	500	2500	250	500	2500	
Items Diets	1	2	3	4	5	6	7	MSE[b]
Digestibility and retention coefficients								
DM (%)	69.6	69.9	69.7	70.8	69.5	70.0	70.2	1.60
P (%)[c,d]	48.8	52.3	54.1	58.3	51.8	53.8	56.0	3.02
Ca (%)[e]	34.5	34.1	37.2	39.2	34.9	34.6	37.8	5.51
Digested P and Ca[f]								
P (%)[c,d]	0.22	0.24	0.25	0.27	0.24	0.25	0.26	0.01
Ca (%)[e]	0.32	0.32	0.35	0.37	0.33	0.32	0.35	0.05
Excreted P and Ca per bird								
P (g/d)[g,h]	0.208	0.206	0.187	0.183	0.202	0.193	0.186	0.01
Ca (g/d)	0.543	0.575	0.520	0.542	0.554	0.558	0.534	0.05
Toe measurements								
Ash (g)[c]	0.428	0.503	0.506	0.525	0.479	0.528	0.566	0.08
Ash (%)[c,d]	11.1	11.2	11.6	12.4	11.4	11.6	12.7	0.27

[a]Each treatment mean represents eight cages containing eight birds per cage except Diet 1 which represents 16 cages containing eight birds per cage.

[b]MSE = the root mean square error. SEM = MSE/ $\sqrt{n}$, where n = 16 (Diet 1) or n = 8 (all other diets) pens.

[c]Basal diet less than all others ($P < 0.01$).

[d]The 2500 U/kg diets were larger than 250 and 500 U/kg diets ($P < 0.01$).

[e]The 2500 U/kg diets were larger than 250 and 500 U/kg diets ($P < 0.05$).

[f]Digested Ca and P (digestibility time dietary P or Ca level) as a percentage of the diet.

[g]Basal diet more than all others ($P < 0.01$).

[h]The 2500 U/kg diets were smaller than 250 and 500 U/kg diets ($P < 0.01$).

TABLE 3-5. Performance, digestibility and retention coefficients, digested and excreted nutrients, and toe measurements of broilers in response to phytase supplementation

		Phytase (U/kg) [a]			
Items	Basal	250	500	2500	SEM
Performance (wk 2-5)					
BW gain (g)[b]	1243	1314	1344	1456	16.7
Feed intake (g)	2489	2585	2518	2629	30.0
Gain:feed ratio (g/kg)[b]	500	508	534	554	6.4
Digestibility and retention coefficients					
DM (%)[c]	69.6	69.7	70.3	70.5	0.4
P (%)[b]	48.8	52.0	54.0	57.2	0.76
Ca (%)[d]	34.5	34.5	35.9	38.5	1.38
Digested P and Ca[e]					
P (%)[b],	0.220	0.240	0.250	0.265	0.003
Ca (%)[d]	0.320	0.320	0.335	0.360	0.01
Excreted P and Ca[f]					
P (%)[b]	0.240	0.220	0.210	0.195	0.003
Ca (%)	0.600	0.600	0.585	0.560	0.01
Toe measurements					
Ash (g)[d]	0.428	0.491	0.517	0.546	0.02
Ash (%)[d]	11.12	11.31	11.62	12.55	0.07

[a]Each treatment mean represents 16 cages containing seven to eight birds per cage. Phytase means represents eight cages of birds fed Natuphos® and eight cages of birds fed Phytaseed®.

[b]Phytase effect ($P < 0.01$).

[c]Phytase effect ($P < 0.07$).

[d]Phytase effect ($P < 0.05$).

[e]Digested Ca and P as a percentage of the diet.

[f]Excreted Ca and P as a percentage of the diet.

TABLE 3-6. Nonlinear equations for the effect of phytase (across phytase source) on various measurements[a]

Performance (wk 2-5)

BW gain (g) = $1463.44(1 - 0.1493e^{-0.00130X})$, r^2 =0.99

Feed intake (g) = poor fit of both nonlinear and linear models.

Gain:feed (g/kg) = $556.51(1 - 0.1056e^{-0.00146X})$, r^2 = 0.95

Digestion coefficients

DM (%) = poor fit of both nonlinear and linear model.

P (%) = $57.226(1 - 0.1464e^{-0.00190X})$, r^2 = 0.99

Ca (%) = $40.557(1 - 0.1537e^{-0.000452X})$, r^2 = 0.97

Digested P and Ca[b]

P (%) = $0.263(1 - 0.1464e^{-0.00190X})$, r^2 = 0.99

Ca (%) = $0.379(1 - 0.1537e^{-0.000452X})$, r^2 = 0.97

Excreted P and Ca per bird[c]

P (%) = $0.197(1 + 0.1954e^{-0.00190X})$, r^2 = 0.99

Ca (%) = $541(1 + 0.1077e^{-0.000452X})$, r^2 = 0.97

Toe measurements

Ash (g) = $0.5454(1 - 0.2147e^{-0.00295X})$, r^2 = 0.99

Ash (%) = $12.958(1 - 0.1434e^{-0.00060X})$, r^2 = 0.99

[a]Where X = phytase level (U/kg).

[b]Digested Ca and P as a percentage of the diet.

[c]Excreted Ca and P as a percentage of the diet.

TABLE 3-7. Liver, kidney and tibial tissue evaluation of young broilers in response to Natuphos or Phytaseed supplementation.

	Dietary Treatments[a]				
		Natuphos (U/kg)		Phytaseed (U/kg)	
	Basal	500	2500	500	2500
Items Diets	1	3	4	6	7	SEM[b]
Liver[b]						
Granulomars	0.3	0	0	0	0	0.11
Lymphoid nodules	0.3	0.1	0.1	0.4	0.5	0.20
Hepatacellular lipid vacuoles[c]	0.3	0.8	0.3	0	1.1	0.23
Kidney[b]						
Lymphoid nodules	0.3	0.1	0.1	0.4	0.5	0.20
Tubular hyperplasia	0.4	0.3	0.4	0	0.3	0.20
Bone parameters						
Cortical bone density[d]	2.1	2.1	2.1	2.1	2.2	0.15
Trabecular bone density[d]	2.1	1.7	1.8	2.1	2.1	0.15
Cartilage thickness[e]	2.4	2.1	1.9	2.0	2.8	0.30
Cartilage development						
Proliferative zone[f]	1.6	2.0	1.8	1.6	1.9	0.23
Hypertrophy zone[g]	1.6	1.5	1.4	1.8	1.4	0.18
% of cartilage in trabecular bone[h]	1.4	1.4	1.1	1.1	1.3	0.16

[a]Eight birds per dietary treatment.

[b]The following scores were used for kidney and liver tissues: 0 = Not remarkable or within normal limits, 1 = Slight change(s), 2 = Moderate change(s), and 3 = Severe change(s).

[c]Treatment effect ($P < 0.05$).

[d]1 = slight, 1.5 = sl/moderate, 2.0 = moderate, 2.5 = mod/severe, 3.0 = severe.

[e]1 = below 1 high power field, 2 = equal to 1 high power field, 3 = greater than on 1 high power field.

[f]1 = narrow proliferative zone (PZ), 2 = normal PZ, 3 = wide PZ.

[g]1 = random hypertrophy zone, 2 = partially oriented hypertrophy zone.

[h]1 = 20% cartilage or more, 2 = less than 20% cartilage.

phosphorus from phytate (Maenz and Classen, 1998). However, the activities of these phytases are relatively low.

For more than 50 years it has been known that plant phytase had the ability to hydrolyze phytate (McCance and Widdowson, 1944; Hill and Tyler, 1954) and its effectiveness for improving phytate P digestibility in pigs and poultry has been clearly shown (Nelson, 1967; Newton et al., 1983; Bagheri and Gueguen, 1985). Nelson et al. (1971) was one of the first to report that addition of a crude phytase prepared from *Aspergillus niger* improved the P availability of a corn-soybean diet. A recent study with Finase®[3] (500 U/kg), Lei et al (1993) showed that phytase supplementation improves P bioavialbility of basal diets by 50%. Cromwell et al. (1995) demonstrated that adding 500 U/kg of Allzyme phytase®[4] (50 U/kg) to a low P (0.30%) diet improved performance and bone breaking strength of pigs to levels that approached those of pigs fed an adequate P (0.50%) diet. But these phytases cannot be widely used in animal industry due to high cost.

More active and cost-effective sources of phytase were developed by cloning the phytase gene into plants and microbes. Using Natuphos® phytase, Simons et al. (1990) showed that the availability of P in low P diet for broilers increased to over 60%. Pen et al. (1993) reported that transgenic tobacco seeds improved phosphorus utilization by broilers. Denbow et al. (1998) also showed that the either Natuphos® or transformed soybean seeds[5] improved growth performance of broilers fed low P diets.

Use of phytase instead of adding inorganic P to animal diets is not always economical (Kornegay, 1998a). The availability of a cheaper or more effective phytase source would enhance a more rapid application of phytase in pig and poultry industries. Since the efficiencies of Natuphos® phytase and Phytaseed® phytase are the same, and if plant phytase is cheaper than microbial phytase, then the use of phytase in swine and poultry diets may be increased.

Phytase Levels. Based on the data averaged across sources, addition of 250, 500, 2,500 U/kg of phytase to the basal diet resulted in increases of BW, BW gain, gain:feed ratio, P and Ca retention and excretion, and toe measurements. The non-linear response equations of graded

[3] Microbial phytase produced by Aspergillus niger, Alko Ltd. Biotechnology, Rajamaki, Finland.
[4]Microbial phytase produced by Aspergillus niger, Alltech, Nicholasville, KY.
[5]Soybeans transformed with *Aspergillus niger* gene. Agracetus, Middletown, WI 53502

levels of phytase were developed for these measurements. The r^2 values for the non-linear response equations on BW gain gain:feed, Ca and P digestibility, and ash weight and percentage were greater than 0.94, which means that all were good indicators of phytase effectiveness (Table 3-6).

Over the total 28-day trial, compared with the basal diet (0.19% nP and 0.46% tP), supplemental phytase of 250, 500, or 2,500 U/kg, averaged across sources, increased BW gain 5.7, 8.1, or 17.1%, gain:feed 3.9, 6.8, or 11.8%, and feed intake 1.2 3.9, to 5.6%, respectively. Increased BW gain was a result of both an increase in feed intake and an improvement in gain:feed ratio. Results also showed that the response of birds to phytase appeared after two weeks on test and lasted until the end of the test (Table 3-3). Simons et al. (1990), Saylor et al. (1991), and Kornegay et al. (1997) have reported improvements in feed efficiency when phytase was supplemented to low P broiler diets. In contrast, other researchers reported that gain:feed ratios of broilers were unaffected by phytase supplementation (Denbow et al., 1995; Schoner et al., 1991).

Phosphorus may be the most critical component in poultry waste that could leave the site of application and cause environmental pollution (Kornegay, 1995). Feeding phytase appears to be a practical and reasonable approach to address this issue. In this study, addition of phytase to lower P basal diet increased the P retention 6.6 to 17.2%, improved P digestion 9.1 to 20.5%, and lowered P excretion 8.3 to 18.8%. (Table 3-4). These findings generally confirmed the earlier work by Yi et al. (1996), which showed that dietary supplementation of 250, 500, and 1,000 U/kg phytase improved the P retention by 8.3 to 22.0% when compared with the negative control. Much larger reductions in P excretion were observed when phytase supplemented groups are compared with positive control diets. Based on the results of several studies, Kornegay (1998b) suggested that when an appropriate level (500 to 750 U/kg) of phytase was included in the diet and the dietary P level needed was reduced 0.1 percentage unit below recommended levels (NRC, 1994), P excretion was reduced 31.8 to 35.7% compared with recommended levels.

Based on the response curve for P retention in this study, the maximum value occurred at 1,500 U /kg phytase. This result agrees with the findings from the review of Kornegay (1998b), although the calculated digested P value from his curve is much higher. For example, improvements in digested P were 0.37 g at 500 U/kg and 0.55 g at 1,000 U/kg phytase levels in

his review compared with 0.26 g at 500 U/kg and 0.37 g at 1,000 U/kg phytase levels in this study (Figure 3-1). Further analysis of digested P curves showed that the magnitude of increase in digested P with phytase addition was almost the same (Figure 3-2). Both curves indicated that the magnitude of improvements in digested P per unit of phytase decreased as phytase levels

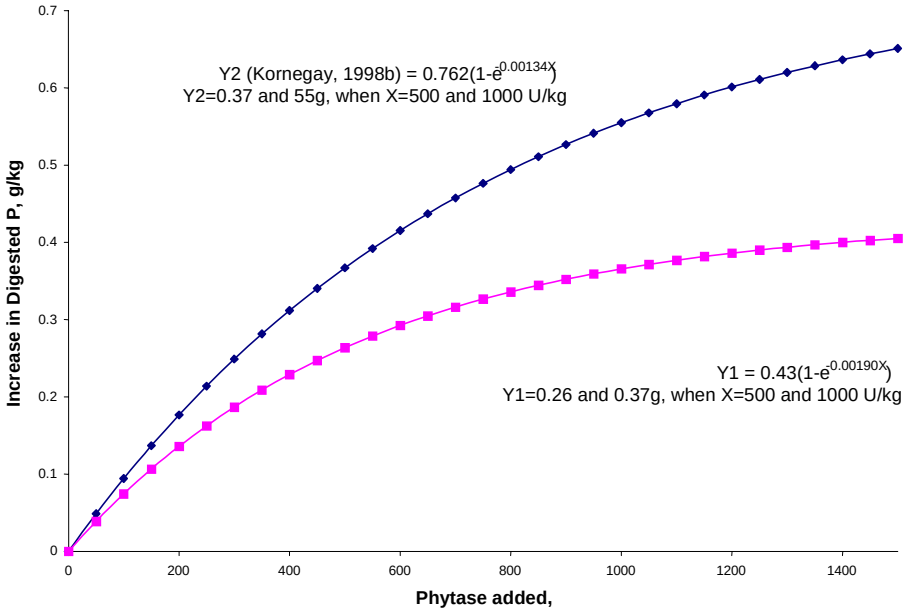

Figure3-1. Increase in Digested P (g/kg)

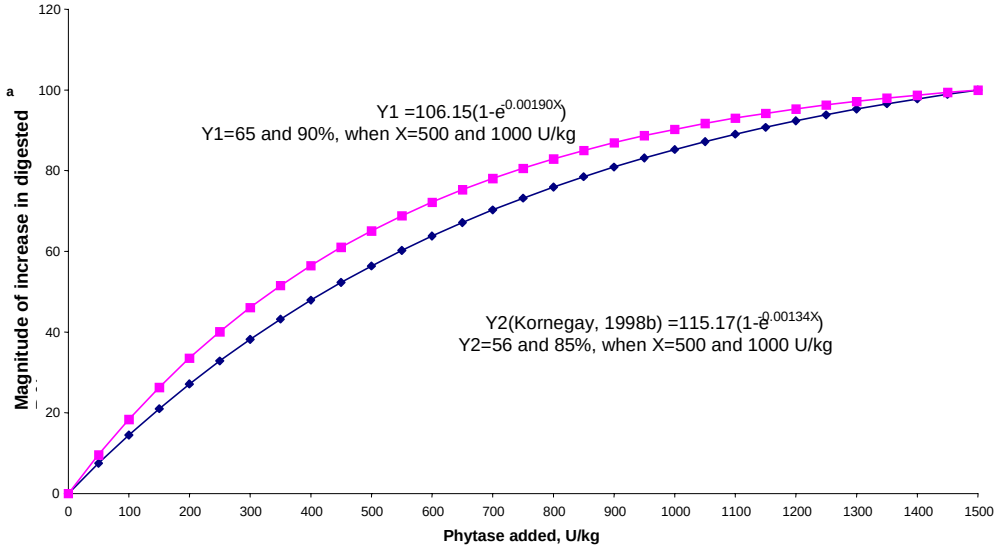

Figure3-2 Magnitude of increase in digested P by phytase

[a]base on assumption that the amount of digested P by 1500 U/kg of phytase(0.65g for his curve and 0.41g for this curve) is 100% in both set.

increased. For example, addition of 500 and 1,000 U/kg of phytase liberated 65 and 90% of P released by 1,500 U/kg of phytase in this study, and 56 and 85% from Kornegay's result.

Addition of phytase to the basal diet resulted in a decrease in P excretion in this study. This result agrees with the findings by Kornegay (1998b). Based on the exponential equations derived from both data sets (Figure 3-3), the reduced P excretion with phytase in this study was smaller than the calculated value from the response curve regressed on phytase level reported by Kornegay (1998b). His data indicated that adding 500 and 1,000 U/kg phytase to low P diets decreased P excretion by 0.34 and 0.52 g per kg of diet compared with 0.23 and 0.33g in this study.

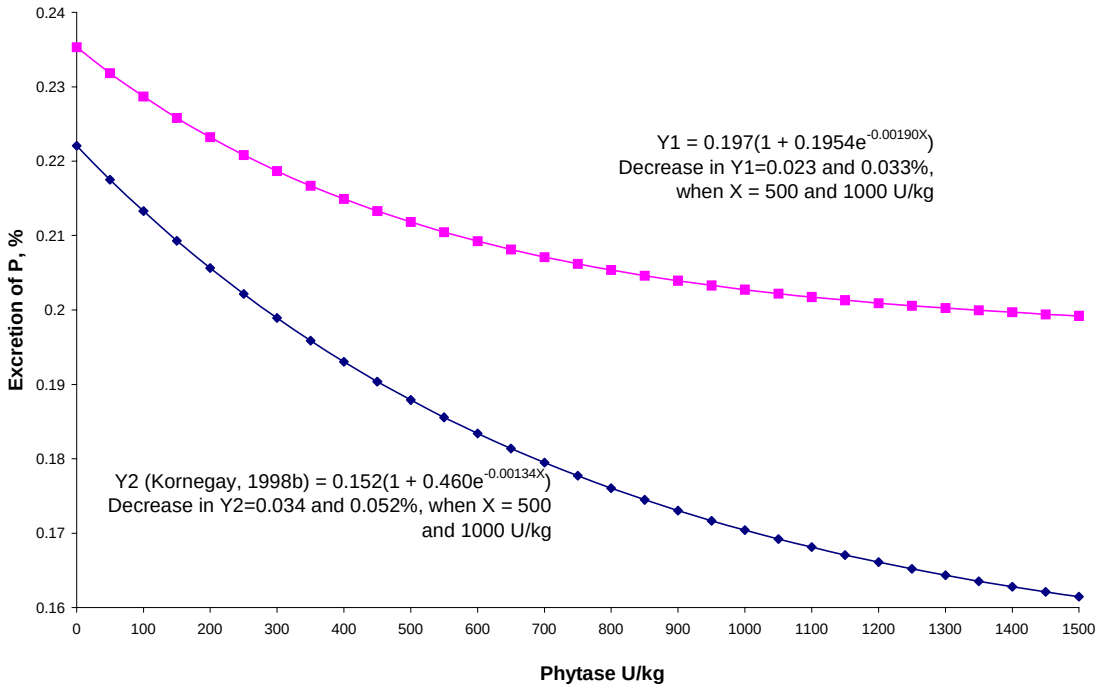

Figure3-3. Decrease P excretion as a percentage of the diet

Although our study was not designed to test the effect of phytase on DM digestibilities, there was a trend for an improvement in DM digestibility as the phytase level increased; the difference between the basal diet and other treatments was numerically higher (P=0.07). Supplemental phytase can have positive effects on DM digestibility by releasing bound organic nutrients such as protein and starch (Dudley Cach, 1998). However, the magnitude of response is small compared with that of P and the phytase level for maximum DM digestibility is not known. For example, Ravindran and Bryden (1997) indicated that there was no further improvement in

DM digestibility when phytase levels greater than 400 U/kg of diet were fed. Yi et al. (1996) observed that DM digestibility increased as phytase level increased to 1,050 U/kg of diet. The results of this study showed that a higher level phytase may be needed in order to detect a difference in the digestibility of DM between treatments.

Adding phytase to the basal diet improved the retention of Ca in all diets except diet 2. It is now well established that Ca utilization is enhanced when phytase is added to the diet. The results of a turkey study reported by Kornegay et al. (1996) showed that based on BW gain, gain:feed ratio, and digested Ca, the addition of 500 U/kg of microbial phytase to corn-soybean meal diet was equal to adding 1.2, 0.70, and 0.70 g Ca/kg to the diets, respectively, with an average Ca equivalent value of 0.87 g Ca for 500 U of phytase. In a study with broilers, Schoner et al. (1994) also demonstrated that 500 U of microbial phytase was equivalent to 0.35g/kg Ca as measured by BW gain and 0.56g/kg as measured by phalanx ash.

The response of toe measurements to added phytase supports the results of added phytase on performance and P retention. Birds fed the basal diet without phytase had low ash weight and ash percentage of toe. Adding 250 to 2,500 U/kg of phytase increased ash weight from 14.7 to 27.6% and percentage of toe ash from 1.7% to 12.9%, respectively (Table 3-5). The magnitude of the increase in bone measurements from added phytase is comparable to results obtained from other studies using broilers (Yi et al., 1996; Denbow et al., 1998; Perney, 1993) and turkeys (Kornegay and Skaggs, 1998).

Since bone acts as the reservoir of minerals, primarily P and Ca, measurements such as toe ash weight and toe ash percentage are often used as criteria to evaluate the P bioavailability of feedstuffs and the effectiveness of phytase for digested P. Although these two measurements, as well as performance and P digestibility, give a good fit when regressed on phytase level, the nonlinear equations in this study showed that the sensitivity of these measurements to phytase were different (Table 3-6). Based on the response values of observed measurements derived from these equations for evaluating sensitivity, toe ash weight and P retention coefficient were the most sensitive criteria for assessing the efficacy of phytase. Overall BW gain and gain:feed ratio were moderately sensitive indicators, and better than Ca retention coefficients and toe ash percentage. This finding differs from that of previous studies from our laboratory which showed

that toe ash percentage was equally sensitive to ash weight for assessing P bioavailability or effectiveness of microbial phytase (Kornegay and Yi, 1996).

Gross Pathologic and Histopathologic Examination. Our data suggest there are no safety concern when genetically engineered phytase from *A. niger* or canola seed is supplemented at levels 3 to 5 times the recommended dosage. This finding is based on the lack of any abnormal and detrimental gross pathologic and histopathologic lesions, and the fact that performance, P retention and bone mineralization of broilers continued to increase to the highest level of 2,500 U/kg. This finding was consistent with the results reported by Kornegay and Skaggs (1998). They observed no negative effects when turkeys were fed 10,000 U/kg of supplemental phytase for 4 weeks. The number and size of granulomas and lymphoid nodules in animals usually increase when exposed to antigens. In our study, various granulomas and (or) lymphoid nodules were observed within the liver and kidney tissues. But these abnormalities appeared unrelated to treatments. The number and size of lipid vacuoles in liver may increase due to the high performance of animals and this theory probably explains the higher lipid vacuole scores in hepatocytes for broilers fed Natuphos at 500 U/kg and Phytaseed at 2,500 U/kg. Previous reports (Schoknecht and Pond, 1993; Anugwa and Pond, 1989) also showed that long term feeding of a high protein diet was associated with structure changes of liver, and kidney of growing swine. All levels of phytase increased performances of the birds, and did not produce diseases. Since the activity of phytase in the gastrointestinal tract is minimal after the stomach (Liebert et al., 1993), it is unlikely that phytase will cause damage to the liver and kidney.

IMPLICATIONS

Based on performance, toe measurements, retention and excretion of P and Ca, and digestibility of DM of broilers, the efficiency of phytase in Phytaseed® was equal to that of Natuphos® for enhancing the utilization of phytate P in corn-soybean diets. The fact that the response to 2,500 U/kg of phytase was greater than the response to 250 and 500 U/kg, and that general necropsy and histologic examination of liver, kidney and tibial tissues revealed no adverse effects of phytase source and level suggest no concern about the safety of phytase as a feed additive.

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Chapter IV

Comparison of Genetically Engineered Microbial and Plant Phytase for Young Pigs

ABSTRACT Ninety-six crossbred pigs with an average weight of 9.0 kg were used in a 5-wk trial to compare the efficiency of genetically engineered microbial (Natuphos®) and plant (Phytaseed®) phytase for enhancing the utilization of phytate P in corn-soybean diets fed to young pigs, and to evaluate the safety of Phytaseed® phytase. Three levels of the two sources of phytase (250, 500 and 2,500 U/kg of diet) were added to a corn-soybean meal basal diet containing 0.35% total P, 0.09% available P, and 0.50% Ca. There were six pens per treatment (one barrow and one gilt/pen), except the diet without added phytase that had 12 pens. Pen feed consumption and BW were recorded weekly. During wk 5, pen fecal samples were collected for determination of apparent digestibilities of DM, Ca and P. At the end of wk 5, all barrows were killed, and the 10th rib on both sides was removed for determination of shear force and energy. Thirty pigs (six from the diet without added phytase and the diet with 500, and 2,500 U/kg phytase from both sources) were randomly selected for gross necropsy and histologic evaluation of liver, kidney, and bone tissues. Adding both sources of phytase increased ($P < .05$) daily gain, gain:feed, apparent digestibilities of DM, P and Ca, and 10th rib measurements. Phosphorus excretion reduced with phytase addition. Feed intake was only influenced by phytase levels during wk 4-5. No significant abnormalities were seen in any of the 30 pigs necropsied. The fit of a nonlinear function revealed that most measurements were reaching a plateau at 2,500 U/kg. In summary, based on performance, bone measurements, and digestibilities of P, Ca, and DM of young pigs, the efficiency of Phytaseed® was similar to that of Natuphos® for enhancing the utilization of phytate P in corn-soybean. General necropsy and histologic examination of tissues indicated no toxic effect of phytase.

Key Words: Phytase, Pigs, and Phosphorus.

INTRODUCTION

The efficiency of a microbial phytase from a genetically engineered *Aspergillus niger* has been extensively studied for practical use since it became available (Coelho and Kornegay, 1996). Studies have demonstrated that dietary supplementation of microbial phytase enhances the utilization of minerals, such as P, Ca, and Zn (Kornegay and Qian, 1996; Yi et al., 1996; Harper et al., 1997), and, at high dietary levels, may also enhance the utilization of organic

nutrients such as amino acids (Mroz et al., 1994; Kemme et al., 1995). However, microbial phytase is not always as economical as the inorganic P that it can replace (Kornegay, 1995). It is necessary to develop cheaper sources of phytase and assess the effectiveness and the safety of new sources of phytase. Phytaseed® is a new source of phytase, which was produce in canola seed by expressing the same phytase gene that was expressed in *Aspergillus niger* for the production of Natuphos®. Compared with microbial phytase, a genetically modified phytase in plants has some advantages, which may lower the cost of phytase: 1) the foreign genes can be easily transferred and expressed in plants such as canola and tobacco, 2) plants use solar energy and have large biomass accumulation, and 3) the phytase in the plant has no contamination with animal pathogens (Glick and Pasternak, 1994). The objectives of this study were two-fold: 1) to compare the efficiency of phytase in Natuphos® and Phytaseed® for enhancing the utilization of phytate P in low-P corn-soybean diets fed to young pigs, and 2) to determine the safety of Phytaseed phytase as a dietary supplement for young pigs.

MATERIALS AND METHODS

Animals, Housing and Management. After a week of adjustment following weaning, 96 crossbred pigs with an average weight of 9.0 kg were randomly assigned from outcome groups based on gender and body weight to the seven dietary treatments shown in Table 4-1. There were 12 replicate pens of one castrated male and one female for the basal diet, and six replicate pens for the other diets. Twice as many pens of pigs were used for the basal diet to provide a better baseline for evaluating the efficiency of phytase in Natuphos® and Phytaseed®. Pigs had ad libitum access to feed and water (automatic waterers) at all times and were routinely checked twice daily with attention given to feeders, waterers, environmental temperature, and condition of pigs. The care and treatment of the pigs followed published guidelines (Consortium, 1988).

Dietary Treatments. The two sources of phytase (Natuphos®[6] and Phytaseed®[7]) were fed at three dietary levels of phytase activity (250, 500 and 2,500 U/kg of diet). The active phytase gene, which originated from *Aspergillus ficuum*, was the same in both Natuphos, produced from *Aspergillus niger*, and Phytaseed, produced by genetically engineering canola seed (rapeseed).

[6] Microbial phytase: Natuphos 6000™, produced by Aspergillus niger genetically modified with the Aspergillus Ficuum phytase gene. BASF Corp. Mt. Olive, NJ, USA.
[7] Phytase produced from canola seed genetically modified with *Aspergillus Ficuum* phytase gene. BASF, Aktiengesellschaft, Offenbach, Germany.

A corn-soybean meal diet (less milled canola) was formulated to contain a deficient level of P, a low level of Ca, and an adequate level of other nutrients based on NRC (1998) recommendations (Table 4-2). The basal diet was mixed to contain all ingredients common to all treatments. This basal mix was used with Phytaseed canola or Westar canola, or a Natuphos/Westar premix to make the seven dietary treatments (Table 4-1). Thus, all dietary treatments contained 2.5% (25 g/kg) milled canola from either Phytaseed canola or Westar canola. This ensured that all diets contained the same proportion and quality of all ingredients

TABLE 4-1. Dietary treatments and assayed phytase levels

	Dietary Treatments						
		Natuphos (U/kg)			Phytaseed (U/kg)		
	Basal	250	500	2500	250	500	2500
Ingredients Diets	1	2	3	4	5	6	7
Westar canola (%)	2.50	2.26	2.02	0.07	2.26	2.02	0.07
Phytaseed canola (%)[a]	0	0	0	0	0.24	0.48	2.43
Phytase/Westar premix[b]	0	0.24	0.48	2.43	0	0	0
Phytase activity (U/kg)							
Calculated	0	250	500	2500	250[c]	500[c]	2500[c]
Assayed	<70	270	530	2560	210	460	2000

[a]The phytase level of phytaseed canola was initially assayed to be 103 U/g. This activity was used for diet formulation. A later assay of Phytaseed showed 78 U/g.

[b]Due to the small amount of phytase required in the diets, a Natuphos/Westar premix was prepared as follows: 200 g phytase (Natuphos 6000™) was premixed with 11,916 g of Westar canola to provide 103 U of phytase per g of Natuphos/Westar canola).

[c]These expected values would be less if the lower phytase activity of Phytaseed was used.

TABLE 4-2. Composition of the basal diet

Ingredients	%
Corn (8.5% CP)	62.70
Soybean meal (44% CP)	32.20
Milled canola seed (25% CP)[a]	2.50
Limestone[b]	1.20
Salt	0.30
Selenium premix[c]	0.05
Trace mineral premix[d]	0.10
Vitamin premix[e]	0.25
L-lysine HCl (78%)	0.10
Corn starch-dextrose	0.40
Chromic oxide - starch mixture[f]	0.20
Total	100.00
Calculated to contain	
Crude protein	19.50
Lysine	1.10
Calcium	0.50
Phosphorus (total)	0.35

[a]Milled Phytaseed canola, milled Westar canola or a milled Westar/Natuphos premix was used in the appropriate amount to supply the desired phytase activity. See Table 4-1 for Individual treatments.

[b]Ground limestone (Tenn. Luttrel Co., Limestone Div., Luttrel, TN, 38% Ca).

[c]Supplied 0.3 mg Se per kilogram of diet.

[d]Supplied per kilogram of diet: Mn, 60mg; Zn, 150 mg; Fe, 175 mg; Cu, 17.5 mg; I, 2mg.

[e]Supplied (per kg of diet): retiny acetate, 1514 μg; cholecalciferol, 110 μg; dl-α-topherol acetate, 22 mg; riboflavin, 4.4 mg; niacin, 22 mg, choline chloride, 440 mg; d-pantothenic acid, 22 mg; d-biotin, 0.44 mg; cyanocobalamin, 22 μg; menadione dimethylprimidinol bisulfite, 2.2 mg.

[f]The ratio of chromic oxide to dextrose was 1 to 3 (0.05% chromic oxide in the diet).

except for ingredients added to make individual diets. Based on an initial assay of phytase activity (103 U/g) by BASF, Phytaseed replaced milled Westar canola on an equal weight basis to obtain the desired levels of phytase. A later phytase assay of Phytaseed conducted when all diets were assayed for phytase showed that Phytaseed contained 78 U of phytase activity per gram. The assayed phytase activities of the diets are shown in Table 4-1. The source of microbial phytase was Natuphos 6000^{TM}. Due to the small amount required in the diets, Natuphos was premixed with Westar canola just prior to diet preparation (Table 4-1). Commercial vitamin and trace mineral premixes supplied supplemental vitamins and trace minerals.

Sampling and Analysis. Pen body weight and feed consumption were recorded weekly for calculation of average body weight gain, feed intake and gain:feed ratios. All diets contained 0.2% of a chromic oxide - starch premix (25% chromic oxide) providing 0.05% chromic oxide in all diets. Pen fecal samples were collected during wk 5 of the 5-wk test. Approximately equal amounts of feces were collected from each pen twice daily (morning and evening) on three alternate days. Collections were pooled by pen and frozen at -20°C in airtight plastic bags for subsequent analyses. After thawing, fecal samples were dried in an oven at 65°C. The dried fecal samples, along with representative samples of diets were ground to pass through a 1-mm sieve and analyzed for DM according to AOAC procedures (1990). Fecal and feed samples were wet-digested using nitric acid and perchloric acid, and total P concentrations were assayed colorimetrically using the vanadomolybdate procedure. Calcium and Cr concentrations of digested fecal and feed samples were determined with a Perkin-Elmer atomic absorption spectrophotometer (Model 5100 PC, Perkin-Elmer, Norwalk, CT). The apparent digestibilities of DM, Ca and P were calculated using the indicator method (Wenderley et al., 1985).

At the end of wk 5 on test, all barrows (six per treatment) were killed, and the 10th rib on both sides was removed and frozen. The soft tissues of the rib samples were removed and the bones were then refrozen and used later for shear force determination as described by Combs et . (1991). The shear force and energy of the 10th rib were determined using an Instron Universal Testing Machine (Model 1123, Instron Corp., Canton, MA). Bones were thawed immediately before testing to prevent desiccation. 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 12 h.

Thirty pigs from diets 1, 3, 4, 6 and 7 (six/diet) were randomly selected at the termination of the trial for gross necropsy. Dr. Hugo P. Veit performed the swine necropsies and evaluated the liver, kidney and bone samples from all animals. Tissues grossly examined included skin, hooves, tongue, esophagus, trachea, heart, lungs, stomach, intestines, colon, rectum, spleen, liver, pancreas, kidneys, ribs, and either the left or right tibia which was sectioned at the proximal end for histologic evaluation. Liver, kidney and tibial sections were sectioned and fixed in 10-fold volumes of 10% phosphate buffered formalin. Tibial tissues were decalcified, and all tissues stained with hematoxylin and eosin. Dietary treatments were unknown to Dr. Veit during all gross and nueroscopic evaluation and scoring procedures. The following scores were used for kidney and liver tissues: 0 = Not remarkable or within normal limits, 1 = Slight change(s), 2 = Moderate change(s), and 3 = Severe change(s).

Bone parameters were evaluated using the following scoring system: cortical bone, 1 = thin, 2 = normal, 3 = thickened; trabecular bone, 1 = thin, 2 = normal, 3 = thickened; physis: 1 = thin, 1.5 = thin to normal, 2.0 = normal, 2.5 = normal to thick, 3.0 = thick, and physis, 0 = well organized, 0.5 = well organized to slightly disorganized, 1.0 = slightly disorganized, 1.5 = slight to moderately disorganized, 2.0 = moderately disorganized.

Statistical Analysis. Data for all measurements and calculations were analyzed by GLM procedures of SAS (1990) using pen as experimental unit, except for scores assigned during histologic examination, where individual pig tissue scores were used. The effects included in the model were treatment and replicate. Contrasts were used to test differences between the two sources of phytase, between the basal and the other diets, and between the 250 and 500 U/kg diets versus the 2,500 U/kg diets (across phytase sources). Contrasts were also used to test the interaction between the two sources of phytase and the linear and quadratic effects (We acknowledge that phytase levels were unequally spaced). Because both sources of phytase were found to elicit similar responses for the various measurements, the data was output by phytase levels (across phytase sources), and nonlinear equations were derived for the response of phytase for the various measurements.

The models for non-linear equations was as follows: $Y = c + bX + aX^2$ and $Y = a(1\text{-}be^{-kx})$. For $Y = c + bX + aX^2$, the slope of the response curve regressed on phytase levels was obtained from the first derivatives of these quadratic equations ($Y' = b + 2aX$). The stationary points,

estimates of dietary phytase activity at which maximum responses of observed measures were expected to occur were calculated from X = - b/2a, when Y' = 0. The maxima of $Y = a(1-be^{-kx})$ were calculated from the first derivatives of $1-be^{-kx}$ (coefficient was not included in order to eliminate the effect of different measurements) as X = (ln(0.00001/bk))/(-k) when Y' =0.00001. The sensitivities of the response measurements (Y) to phytase supplementation (X) were defined as the magnitude of change in measurement value against phytase level and calculated as follows: $Ys = (1-be^{-kx})/(1-b)$ ($Y_0 = a(1-b)$ when X=0 and $Yx = a(1-be^{-kx})$ when X=x). Based on the response values of these equations (Ys), the sensitivities of the response measurements (Y) to phytase supplementation (X) were evaluated.

RESULTS

Comparison of Sources. Daily gain, daily feed intake and gain:feed ratios were similar for pigs fed the two phytase sources (Table 4-3). Also, apparent digestibilities (%) of P, Ca and DM, 10th rib measurements, and P and Ca excretion were not different between the two-phytase sources (Table 4-4). The probability values were greater than $P < 0.25$ and averaged $P < 0.72$. There were no interaction(s) between the sources of phytase for any of the performance, digestibility, excretion, or 10th rib measurements.

Phytase Levels. Daily gain were increased ($P < 0.05$) as the level of the phytase increased across the phytase sources during weeks 4-5, and overall (Table 4-5). Feed intake was only significantly ($P < 0.01$) influenced by phytase levels during wk 4 and 5. Gain:feed ratio was increased as the phytase levels increased during wk 1 to 3 ($P < 0.05$), wk 4 and 5 ($P < 0.01$) and overall ($P < 0.05$). Nonlinear equations fit the daily gain, daily feed intake and gain:feed ratio data well during all periods except wk 1 to 3 for daily feed intake (Table 4-6).

Apparent digestibilities of DM, Ca, and P were increased ($P < 0.01$) as the level of phytase increased (Table 4-5). All 10th rib measurements also increased ($P < 0.05$ to 0.001) as the phytase level increased. P and Ca excretion decreased with phytase supplementation (Table 4-5). The nonlinear equation was the best fit for P digestion, rib ash weight, ash percent, force and energy and the quadratic equation was the best fit for daily P and Ca excretion (Table 4-6). Both nonlinear and linear equations fit the data for Ca digestion. The goodness of fit of the nonlinear equation suggests that the magnitude of the response to phytase supplementation was greater per unit of phytase at the lower levels. For daily gain during wk 4

TABLE 4-3. Body weight, daily gain, daily feed intake and gain:feed ratio of young pigs in response to Natuphos or Phytaseed supplementation

	Dietary Treatments[a]							
		Natuphos (U/kg)			Phytaseed (U/kg)			
	Basal	250	500	2500	250	500	2500	
Items Diets	1	2	3	4	5	6	7	MSE[b]
Body weight (g)								
Initial	8,929	8,978	9,042	8,963	8,951	9,001	8,985	110
Final[c,d]	22754	24763	25282	25833	24806	24891	26590	1330
Daily gain (g)								
Week 1-3	364	385	391	401	388	375	433	54
Week 4-5[c,d]	445	557	581	612	558	583	617	53
Week 1-5[c,d]	395	451	464	482	453	454	503	38
Daily feed intake (g)								
Week 1-3	667	687	682	671	674	689	681	83
Week 4-5[c]	911	998	1,004	1,079	1,050	1,064	1,085	106
Week 1-5[c]	760	806	805	827	818	832	836	68
Gain:feed ratio (g/kg)								
Week 1-3[d]	550	560	581	596	577	545	637	62
Week 4-5[c]	497	559	579	567	534	547	568	49
Week 1-5[d,e]	525	558	576	582	555	546	603	42

[a]Each treatment mean represents six pens containing two pigs each (1 barrow and 1 gilt) except the Diet 1 which represents 12 pens with two pigs (1 barrow and 1 gilt) per pen.

[b]MSE = the root mean square error. SEM = MSE/$\sqrt{n}$, where n = 12 (Diet 1) or n = 6 (all other diets) pens. All comparisons between Natuphos® and Phytaseed® had P values greater than 0.27.

[c]Basal diet less than all others ($P < 0.001$)

[d]The 2500 U/kg diets were larger than 250 and 500 U/kg diets ($P < 0.05$).

[e]Basal diet less than all others ($P < 0.01$)

TABLE 4-4. DM, Ca, and P digestibility, digested Ca and P, Ca and P excretion, and rib characteristics of young pigs in response to Natuphos or Phytaseed supplementation

	Dietary Treatments[a]							
		Natuphos (U/kg)			Phytaseed (U/kg)			
	Basal	250	500	2500	250	500	2500	
Items Diets	1	2	3	4	5	6	7	MSE[b]
Digestibility coefficients								
DM (%)[c]	86.0	86.1	87.5	87.2	86.4	87.9	86.4	1.0
P (%)[d,e]	23.1	37.1	46.2	57.7	31.8	50.3	60.8	5.6
Ca (%)[d,e]	59.0	61.6	64.8	74.2	61.3	62.5	76.9	6.1
Digested P and Ca[f]								
P (%)[d,e]	0.081	0.130	0.162	0.203	0.112	0.177	0.213	0.02
Ca (%)[d,e]	0.297	0.310	0.326	0.373	0.308	0.314	0.387	0.03
Daily P and Ca excretion								
P (g)[d,e]	2.05	1.77	1.53	1.23	1.97	1.46	1.15	0.23
Ca (g)[d,e]	1.58	1.57	1.44	1.02	1.60	1.57	0.97	0.31
Tenth rib measurements								
Ash wt (g)[d,e]	0.809	0.959	0.987	1.561	0.973	1.000	1.457	0.178
Ash (%)[d,e]	38.4	41.1	43.1	48.5	40.5	42.6	48.3	2.26
Force (N)[d,e]	454	541	572	754	520	545	799	91
Energy (N-mm)[d,e]	567	701	764	1190	698	728	1101	156

[a]Each treatment mean represents six pens containing two pigs each (1 barrow and 1 gilt) except the Diet 1 which represents 12 pens with two pigs (1 barrow and 1 gilt) per pen.

[b]MSE = the root mean square error. SEM = MSE/$\sqrt{n}$, where n = 12 (Diet 1) or n = 6 (all other diets) pens. All comparisons between Natuphos® and Phytaseed® had P values greater than 0.40.

[c]Basal diet vs all others ($P < 0.01$)

[d]Basal diet vs all others ($P < 0.001$)

[e]The 2500 U/kg diets vs 250 and 500 U/kg diets ($P < 0.01$).

[f]Digested Ca and P as a percentage of the diet.

TABLE 4-5. DM, Ca, P digestibility coefficients, rib characteristics, performance of young pigs in response to phytase supplementation

		Phytase (U/kg)[a]			
Items	Basal	250	500	2500	SEM
Performance (wk1-5)					
Daily gain (g)[b]	395	452	459	492	11
Daily Feed intake (g)	760	812	819	831	20
Gain:feed ratio (g/kg)[c]	525	557	563	592	12
Digestibility coefficients					
DM (%)[b]	86.0	86.3	87.7	86.8	0.29
P (%)[d]	23.1	34.4	48.2	59.3	1.60
Ca (%)[b]	59.0	61.5	63.7	75.6	1.80
Digested P and Ca[e]					
P (%)[d]	0.081	0.121	0.169	0.208	0.010
Ca (%)[b]	0.297	0.309	0.320	0.380	0.010
Daily P and Ca excretion					
P (g)[d]	2.05	1.88	1.49	1.19	0.020
Ca (g)[d]	1.58	1.58	1.50	1.02	0.086
Bone measurements					
Ash wt. (g)[c]	.809	.966	.994	1.517	0.514
Ash (%)[b]	38.4	40.8	42.8	48.4	0.65
Force (N)[c]	454	530	558	776	27
Energy (N-mm)[c]	567	700	746	1145	45

[a]Each treatment mean represents 12 pens containing two pigs per pen. Phytase means represent six pens of pigs fed Natuphos® and six pens of pigs fed Phytaseed®.

[c]Phytase effect ($P < 0.05$).

[b]Phytase effect ($P < 0.01$).

[d]Phytase effect ($P < 0.001$).

[e]Digested Ca and P as a percentage of the diet.

TABLE 4-6. Nonlinear equations for the effect of phytase across phytase source) on various measurements[a]

Performance (wk1-5)

Daily gain (g) = $490.53(1 - 0.1905e^{-0.00279X})$, $r^2 = 0.97$

Daily feed (g) = $829.05(1 - 0.0824e^{-0.00498X})$, $r^2 = 0.99$

Gain:feed (g/kg) = $592.39(1 - 0.110e^{-0.00181X})$, $r^2 = 0.97$

Digestion coefficients

DM (%)= poor fit of both nonlinear and linear model.

P (%) = $59.887(1 - 0.627e^{-0.00199X})$, $r^2 = 0.98$

Ca (%) = $86.172(1 - 0.315e^{-0.00038X})$, $r^2 = 0.99$

Digested P and Ca[b]

P (%) = $0.210(1 - 0.627e^{-0.00199X})$, $r^2 = 0.98$

Ca (%) = $0.433(1 - 0.315e^{-0.00038X})$, $r^2 = 0.99$

Daily P and Ca excretion

P (g) = $0.140(1 + 0.9405e^{-0.00199X})$, $r^2 = 0.98$

Ca (g) = $0.067(1 + 6.43e^{-0.00038X})$, $r^2 = 0.99$

Rib measurements

Ash wt, (%) = $1.979(1 - 0.583e^{-0.00037X})$, $r^2 = 0.99$

Ash (%) = $49.20(1 - 0.2200e^{-0.00105X})$, $r^2 = 0.99$

Force (N) = $861.309(1 - 0.467e^{-0.00062X})$, $r^2 = 0.99$

Energy (N-mm) = $1331.73(1 - 0.567e^{-0.00056X})$, $r^2 = 0.99$

[a]Where X = phytase level (U/kg)

[b]Digested Ca and P as a percentage of the diet.

TABLE 4-7. Liver, kidney and tibial tissue evaluation of young pigs in response to Natuphos or Phytaseed supplementation[a]

		Dietary Treatments				
		Natuphos (U/kg)		Phytaseed (U/kg)		
	Basal	500	2500	500	2500	
Items Diets	1	3	4	6	7	SEM[b]
Liver scores[b]	0	.7	0	.2	.3	.15
Kidney scores[b]	0	0	0	0	0	---
Bone parameters						
Cortical bone density[c]	1.7	2.1	1.9	2.0	2.1	0.20
Trabecular bone density[d]	1.5	1.9	1.9	1.5	2.0	0.20
Physeal thickness[e]	2.3[g]	1.3	1.3	1.3	1.3	0.20
Physeal development[f]	1.0[g]	0.2	0.3	0.3	0.4	0.23

[a]Six pigs per treatment mean.

[b]Liver and kidney scoring system: 0 = not remarkable or within normal limits;; 1 = slight change(s); 2 = moderate change(s); 3 = severe change(s).

[c]1 = thin cortical bone, 2 = normal cortical bone, 3 = thickened cortical bone

[d]1 = thin trabecular bone, 2 = normal trabecular bone, 3 = thickened trabecular bone

[e]1 = thin, 1.5 = thin to normal, 2.0 = normal, 2.5 = normal to thick, 3.0 = thick physis

[f]0 = well organized, 0.5 = well organized to slightly disorganized physis, 1.0 = slightly discorganized physis, 1.5 = sl. To moderate disorganized physis, 2.0 = moderately disorganized physis

[g]Basal diet greater than others ($P < 0.01$).

and 5, gain:feed ratios during wk 1 to 3 and overall, P and Ca digestion, and all tenth rib measurements, the phytase response was greater at 2,500 U/kg compared with 250 and 500 U/kg (Tables 3 and 4). Neither nonlinear nor linear equations fit the data for DM digestion.

Gross Pathologic and Histopathologic Examination. Gross findings for the 30 pigs necropsied at the end of the test were incidental to euthanasia (random, slight pulmonary petechial or ecchymotic hemorrhage and/or congestion of the lungs and/or spleen). A few random lung lesions were seen in two pigs (unrelated treatments - basal and 2,500 U/kg of phytase from Phytaseed®) amounting to very minimal resolved chronic pneumonia (bronchial). Three pigs had small, congenital kidney cysts (basal, 2,500 U/kg of phytase from Natuphos®, and 500 U/kg of phytase from Phytaseed®). Such cysts are commonly seen in about 10% of the pig population, hence, no treatment effect was suspected. Overall, no significant disease symptoms were seen grossly or microscopically in any of the 30 pigs necropsied. This included bone morphology from microscopic examination (Table 4-7). Phytase supplementation of basal diets, regardless of source or concentration caused slightly (nonsignificant) thicker cortical tibial bone, wider trabecular bone (500 U/kg Phytaseed treatment excepted), and a significantly thinner and more well organized physis. All of these effects are compatible with a more robust (normal) bone development, compared with pigs fed the basal diet.

DISCUSSION

Comparison of the sources. Based on the performance, measurements of apparent digestibility of P and Ca and dry matter, and bone characteristics, the efficiency of Natuphos® phytase and Phytaseed® phytase in improving the utilization of nutrients in the diets was not different (Table 4-3 and 4-4). These findings are in agreement with that of a broiler study using the same two phytase products (Zhang et al., 1998). Phytase has a wide distribution in microorganism, plants, and certain animal tissues. Depending on their own phytase, some yeasts grow well on media in which sodium phytate is the sole source of inorganic phosphate (Lambrechts et al., 1992), some cereal by-products improve the availability of phytate P (Pointillart, 1991), and animals have a variable ability to use phytate P (Maenz and Classen, 1998). However, the activities of these phytases are relatively low.

For more than 50 years it has been known that plant phytase had the ability to hydrolyze phytate (McCance and Widdowson, 1944; Hill and Tyler, 1954) and its effectiveness for improving phytate P digestibility in pigs and poultry has been clearly shown (Nelson, 1967; Newton et al., 1983; Bagheri and Gueguen, 1985). Nelson et al. (1971) was one of the first to reported that addition of a crude phytase prepared from *Aspergillus niger* improved the P

availability of a corn-soybean diet. In a recent study with Finase®[8] (500 U/kg), Lei et al (1993) showed that phytase supplementation improve the P bioavialbility of basal diet by 50%. Cromwell et al. (1995) demonstrated that adding 500 U/kg of Allzyme phytase®[9] (50 U/kg) to a low P (0.30%) diet improved performance and bone breaking strength of pigs to levels that approached those of pigs fed an adequate P (0.50%) diet. But these phytases cannot be widely used in the animal industry due to high cost.

More active and cost-effective sources of phytase were developed by cloning the phytase gene into plants and microbes. Using Natuphos® phytase, Simons et al. (1990) showed that the availability of P in low-P diet for broilers increased to over 60%. Pen et al. (1993) reported that transgenic tobacco seeds improved phosphorus utilization by broilers. Denbow et al. (1998) also showed that the either Natuphos® or transformed soybean seeds[10] improved growth performance of broiler fed low-P diets.

Using phytase instead of inorganic phosphorous is not always economically favorable (Kornegay, 1998a). The availability of a cheaper, more effective phytase source would enhance a more rapid application of phytase in pig and poultry nutrition. Since the efficiencies of Natuphos® phytase and Phytaseed® phytase are the same and plant phytase should be cheaper than microbial phytase, the obstacle of using phytase in feed industry may become smaller.

Phytase Levels. Across sources of phytase, supplementing the low-P basal diet with 250, 500, or 2500 U/kg of phytase improved performance, increased digestibilities of DM, P, and Ca and rib measurements, and reduced P excretion (Table 4-6). All of the observed measurements showed good fit when regressed on phytase level. However, further analysis of these curves indicated that the sensitivities of these measurements to phytase supplementation differed. Based on the response values of these equations for evaluating the sensitivity of observed measurements to phytase, P digestibility and daily gain were found to be the best indicator. Rib measurements including bone shear force and energy, and ash weight and percentage were also sensitive criteria. Gain:feed ratio, Ca digestibility, and daily feed intake appeared insensitive to phytase supplementation. These findings are consistent with other studies in pigs (Dellaert et al., 1991; Cromwell et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996).

[8] Microbial phytase produced by Aspergillus niger, Alko Ltd. Biotechnology, Rajamaki, Finland.
[9]Microbial phytase produced by Aspergillus niger, Alltech, Nicholasville, KY.
[10]Soybeans transformed with *Aspergillus niger* gene. Agracetus, Middletown, WI 53502

Over the total 28-day trial, across phytase sources, compared with the basal diet that had the same level of total P (0.35%), supplemental phytase levels of 250, 500, or 2500 U/kg increased body weight 8.9 to 15.2%, BW gain 14.4 to 24.6%, feed intake 6.8% to 9.3%, and gain:feed ratio 6.1 to 12.8%. These responses to phytase were more evident after two weeks on test and lasted until the end of the test (Table 4-3). Increased BW gain was due to an increase in feed intake and an improvement in the gain:feed ratio. As a powerful chelating agent, phytate may lower the availability of essential minerals and may also reduced the utilization of organic nutrients (Reddy et al., 1989; Couzy et al., 1993; Thompson and Yoon, 1984). The improvement in feed efficiency with phytase supports this hypothesis.

Since waste P can leave the site of application and cause contamination of water supplies, P in the animal manure is a major environmental concern (Sharpley et al., 1994). Phytase appears to increase the utilization of P and allows reduction of the amount of inorganic P supplementation in feed, with a resulting significant decrease in P excretion. In this study, addition of phytase to the low P basal diet lowered the dietary total phosphorus from 0.60% (P level recommended by NRC, 1998) to 0.35% and reduced the total dietary P level by 41.7%. Supplemental phytase levels of 250, 500, or 2500 U/kg to the basal diet increased the P retention 48.9 to 156.7 % compared with the basal diet without phytase, and reduced P excretion by 8.3% to 42.0% (Table 4-4). If it is assumed that P retention of pigs fed an adequate P feed (0.60%) is 45%, addition of these three levels of phytase to the basal P diet in this study reduced P excretion

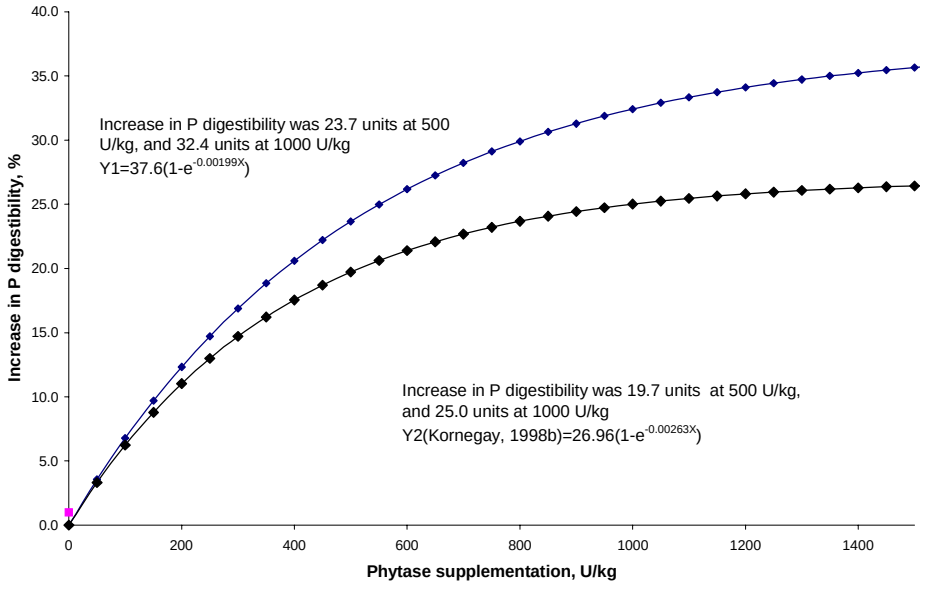

Figure 4-1 Increase in P digestibility by phytase supplementation

by 24.5 to 53.1% compared with an adequate P diet.

The improvement in P digestibility with phytase was higher in the basal diet without inorganic P addition than that suggested by Kornegay (1998b) (Figure 4-1). For example, the enhancement of P digestibility by phytase was 23.7 percentage units at 500U/kg and 32.4 percentage units at 1,000 U/kg of phytase addition in our study compared with 19.6 percentage units at 500 U/kg and 25.6 percentage units at 1,000 U/kg of phytase level in the Kornegay' (1998b) review, in which most data were based on low P diets. The average dietary P level was 0.38% compared with 0.35% in this study. The response curve of increase in digested P in both data sets showed the same trends as that of increase in P digestibility (Figure 4-2). For example, the increase in digested P was 0.83g at 500 U/kg and 1.13g at 1,000 U/kg of phytase level in this study, and 0.75g at 500 U/kg and 0.95g at 1,000 U/kg of phytase level in his curve. The increase in digested P is equal to dietary P multiplying the increase in P digestibility with phytase. Since the total dietary P of both data sets were almost same, the different in digested P values of these two data sets was mainly due to that in the P digestibility.

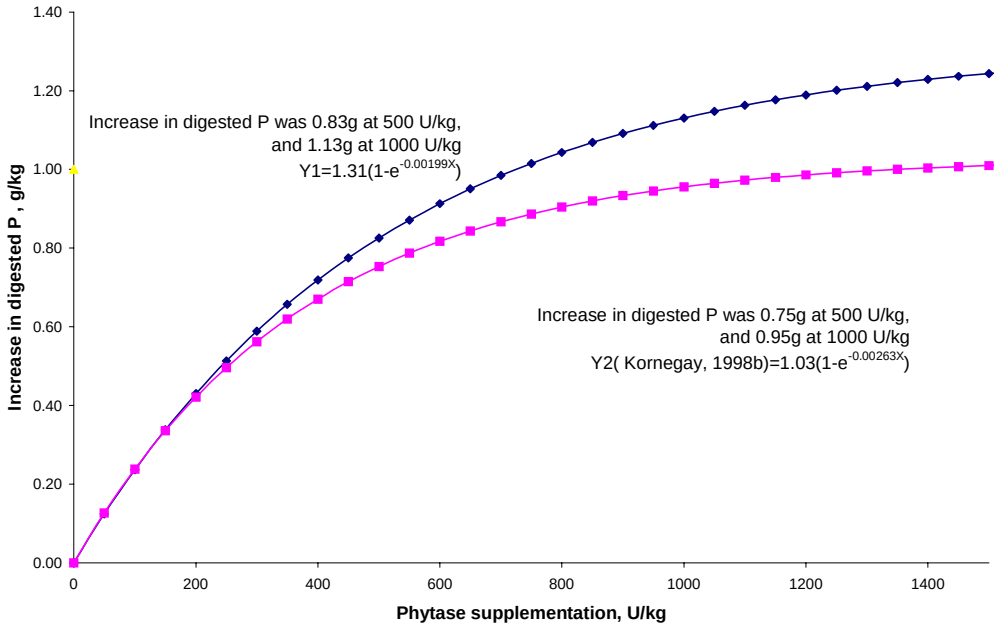

Figure 4-2 Increase in digested P by phytase supplementation

Addition of phytase resulted in a reduction of P excretion in this study, which agrees with the findings by Kornegay (1998b) (Figure 4-3). Based on the exponential equations derived from both data sets (Figure 4-3), the reduced P excretion with phytase in this study was larger than the

calculated value from the response curve regressed on phytase level reported by Kornegay

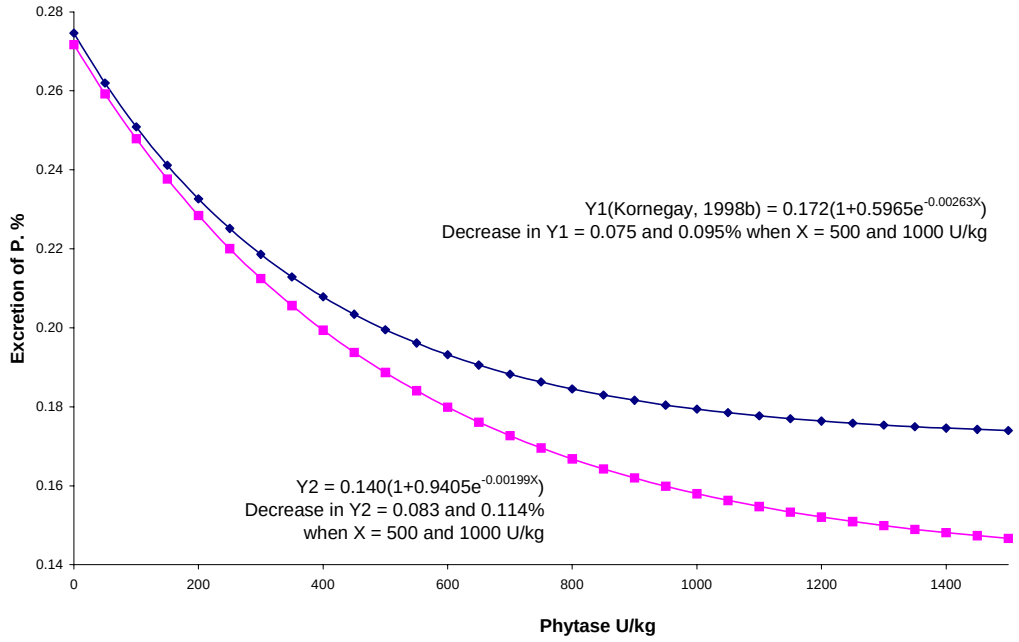

Figure 4-3. Decrease P excretion as a percentege of diet

(1998b). His data indicated that adding 500 and 1,000 U/kg phytase to low P diets decreased P excretion by 0.75 and 0.95 g per kg of diet compared with 0.83 and 1.14g in this study.

Supplemental phytase increased apparent total tract digestibility of Ca (Table 4-4). Digested Ca increased from 0.295g/kg to 0.378g/kg and Ca excretion of pigs was reduced by 6.3% to 35.4%. It is now well established that Ca utilization is enhanced when phytase is added to the diet (Kornegay et al., 1996). Furthermore, Radcliffe and Kornegay (1998) reported that based on performance, bone characteristic and Ca retention of pigs, the addition of 500 U/kg of microbial phytase to corn-soybean meal was equal to adding 0.78 to 1.08 g of Ca. In a study with broilers, Schoner et al., (1994) demonstrated that 500 U of microbial phytase was equivalent to 0.35g of Ca as measured by BW gain and .56g as measured by phalanx ash.

Theoretically, phytase may increase the digestibility of DM by releasing organic nutrients such as protein and starch bound by phytate (Yoon et al., 1983; Thompson and Serraino, 1986), however, results of practical feeding studies are inconclusive. For example, most investigators found supplemental phytase had no or little effect on total tract digestibility of DM (Simons et al., 1990; Jongbloed et al., 1992; Yi et al., 1996; O'Quinn et al., 1997; Ketaren et al., 1993). In contrast, Mroz et al. (1994) reported a significant increase in apparent digestibility of DM with phytase addition. The results of our study supported the finding of Mroz et al (1994). The

similarity of both trials was that the basal diet contained no added inorganic P. In conclusion, P levels in the basal diet may be a factor that influences whether or not the improvements in DM digestibility are significant. When dietary P in the basal diet (control) is low, the chance to detect the enhancement in DM digestibility due to phytase (treatment) becomes greater.

It is generally accepted that ash measurements are more responsive to dietary P than performance criteria, and breaking force and energy of ribs are even better indicator of P level than ash weight and percentage (Peo, 1995). The rib measurements of this study showed that breaking force and energy of ribs were more sensitive to phytase effectiveness than rib ash weight and percentage. However, ADG was found to be a better indicator of the effectiveness of phytase addition than all rib measurements. These findings were consistent with other studies with pig (Kornegay and Qian, 1996, Cromwell et al., 1993; and Lei et al., 1993).

Since the addition of phytase resulted in increases in the performances, digestibilities of DM, P, and Ca, and rib measurements of pigs, the phytase levels for maximizing these responses can be estimated from curves of these non-linear functions. Daily feed intake approached the maximum at a phytase level of 746 U/kg, while daily gain and feed efficiency reached a plateau at 1,424 and 1,653 U/kg of phytase levels, respectively. Thus, when the level of phytase was smaller than about 746 U/kg, the improved BW gain was due to the increase in feed intake as well as the improvement in gain:feed ratio gain. When the level of phytase was greater than 746 U/kg, the improved BW gain was due to the increased feed efficiency only. These results strongly suggested that feed efficiency was improved with phytase in two stages. First, phytase improved P availability and thus increased the utilization of other nutrients, and consequently feed efficacy was improved. It was reported that P is involved in the control of appetite and feed intake (Underwood, 1981). During this stage, as the level of phytase increased, more P was released from phytate and was absorbed by pigs, and the appetite of pigs was increased and food intake improved. Second, as the phytase continued to increase, feed intake reached a plateau, and the improved daily gain was purely due to the increased availability of nutrients. The following evidences further supported this hypothesis. The maximum apparent P digestibility seems to occur at phytase levels 2,425 U/kg of diet. In addition, daily gain reached its stationary points at phytase level greater than 2,500U/kg during the week 1 to 3 while at about 1,424 U/kg

during the week 4 to 5. This was because the younger pig requires a higher level of P in the diet (NRC, Swine, 1998).

The level of P and Ca for maximal bone-ash content and bone strength is about 0.1 percentage unit higher than that for maximal performance (NRC, 1998, Swine). The data from ash percentage of rib and bone shear force and energy supported this conclusion. Our data showed that phytase level for maximizing bone mineralization was greater than 2,500 U/kg (8,301, 2,990, 5,429, and 6,175 U/kg of phytase respectively for ash weight, ash percentage, and shear force and energy, based on the assumption that these equation are still valid when phytase levels are greater than 2,500 U/kg), which is much higher than that phytase level for maximal performance. The digestibility of Ca also reached its plateau at a phytase level greater than 2,500 U/kg (6,533 U/kg), which showed further evidence that bone mineralization depends on both adequate P and Ca supply.

Gross Pathologic and Histopathologic Examination. The supplementation of genetically engineered phytase generally improves the utilization of phytate P in the feed of plant origin, and our data suggest that there are no safety concerns when this product is used at levels 3 to 5 times the recommended dosage. Our findings are based on the lack of any abnormal and detrimental gross pathologic and histopathologic lesions associated with treatments and the fact that performance continues to increase to the highest level of 2,500 U/kg. Since phytase would be degraded in the intestinal tract of pigs, and its activity in the lower small intestine is negligible (Kornegay and Yi, 1996), it is not likely that phytase could cause damage to the liver and kidney.

Our results showed that adding phytase did not increase trabecular bone density, it did improve the bone structure. The improvements on bone development by adding phytase were also observed in broilers and turkeys (Qian et al., 1996a,b).

IMPLICATIONS

Based on performance, bone measurements, retention of P and Ca, and digestibility of DM of pigs, the efficiency of phytase in Phytaseed was equal to that of Natuphos for enhancing the utilization of phytate P in corn-soybean meal diets. Nonlinear equations usually fit the data best. General necropsy and histologic examination of liver, kidney and bone tissues revealed no adverse effects of phytase source and level and the response to 2,500 U/kg of phytase was greater than the response to 250 and 500 U/kg which further suggests no toxic effect of phytase.

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Chapter V

Influence of Phytase on Ileal Amino Acid and Protein Digestibilities, and Total Tract Nitrogen, Phosphorus and Calcium Nutrient Excretion in Growing and Finishing Pig

ABSTRACT One hundred and twenty crossbred pigs (equal barrows and gilts in 20 pens) were used to investigate the effects of supplemental microbial phytase on crude protein and amino acid utilization of low-protein plant-based diets. During the grower period (32 to 67 kg), diets 1, 2 and 3 contained 14, 13 and 12% crude protein and no added phytase, respectively, and diets 4 and 5 contained 12% crude protein with either 250 or 500 U of phytase/kg of diet, respectively. During the finisher period (67 to 109 kg), diets 1, 2 and 3 contained 12, 11 and 10% crude protein with no added phytase, respectively, and diets 4 and 5 contained 10% crude protein with either 250 or 500 U of phytase/kg of diet, respectively. The Ca and P level was .56 and .50 and .47 and .40%, respectively for the grower and finisher periods. At the end of grower phase, two pigs (1 barrow and 1 gilt) were removed from each pen; the12 barrows that were removed from diets 1, 3 and 5 were put in metabolism cages for total collection, and the remaining four pigs in each pen continued on test for the finisher phase. At the end of finisher phase, 12 barrows from diet 1, 3, and 5 were put in metabolism cages for total collection. Ileal contents were taken (slaughter technique) from the remaining barrows and barrows used in metabolism cages. Individual pig body weights and pen feed consumption were measured weekly. Fecal collections were taken during the last 10 d of the grower and finisher phases. Daily gain linearly increased as protein or phytase was added to the lowest protein level ($P < 0.05$). Fecal P and Ca digestibilities improved with added phytase ($P < 0.05$). Phytase addition to the basal diet linearly increased metacarpal ash weight in grower phase. With the exception of proline and glycine, the digestibilities of the other amino acids were linearly increased ($P < 0.05$) with added phytase. The digestibility of all amino acids, except glycine, were linearly increased ($P < 0.10$ to 0.01) as the dietary CP level was increased. Nitrogen excretion was estimated to be reduced 6.1% when phytase was added to pig diets at a level of 500 U/kg.

Key words: pig, amino acids, nitrogen, and crude protein.

INTRODUCTION

Theoretically, phytate may have deleterious effects on the utilization of protein and (or) individual amino acids by monogastrics in the following ways: (1) electrostatic linkages with amino acids and complexing with peptide and protein which consist of amino acids (Yoon et al., 1983; Reddy et al., 1989; Cheryan, 1980; Thompson and Serraino, 1987), (2) inhibition of proteolytic enzymes which are themselves protein (Yoon et al., 1983; Sharma et al., 1987; Deshpande and Cheryan, 1984), and 3) chelation of mineral elements required for the activity of digestive enzymes (Caldwell, 1992). However, published results from practical studies are limited and lack consistency. For example, Mroz et al. (1994) and Kemme (1998) reported that adding phytase improved the apparent digestibility of protein in pigs. In contrast, Ketaren et al. (1993) found that supplementation of phytase to the diet of young pigs did not affect the CP digestibility, although it did increase protein deposition and N retention. Other studies also showed phytase addition increased the apparent N absorption, and even weight gain and feed efficiency of pigs (Yi, et al, 1996 a, b). Therefore, further studies are needed. The objectives of this study were to evaluate the effect of phytase on protein and amino acids utilization, nitrogen retention, and performance in growing and finishing pigs, and to determine equivalency values between phytase and protein.

MATERIALS AND METHODS

Experimental Procedures

Growing-Finishing Study.

One hundred twenty crossbred pigs averaging 32 kg BW were assigned to five treatments described in Table 5-1 (four replicate pens of three barrows and three gilts per treatment) to investigate the effects of supplemental microbial phytase on crude protein and amino acid utilization of low-protein plant-based diets. Pigs were randomly assigned to treatments from groups based on gender and body weight. Littermates were balanced across treatments. At the end of the growing phase, two pigs (one barrow and one gilt) were removed from each pen to provide an optimum stocking density during the finishing phase. Twelve of the barrows (four/treatment) removed from diets 1, 3, and 5, were used in a N balance trial. The remaining four pigs (two barrows and two gilts) in each pen continued on test during the finisher phase.

During the grower period (32 to 67 kg), Diets 1, 2 and 3 contained 14, 13 and 12% crude protein and no added phytase, respectively, and Diets 4 and 5 contained 12% crude protein with 250 and 500 U of added Natuphos®phytase[11]/kg of diet, respectively. During the finisher period (67 to 109 kg), Diets 1, 2 and 3 contained 12, 11 and 10% crude protein with no added phytase, respectively, and Diets 4 and 5 contained 10% crude protein with 250 and 500 U of added phytase/kg of diet, respectively. The major ingredients were corn and soybean meal. A mixture of cornstarch and dextrose was used to reduce the crude protein levels of Diets 2 through 5 so that amino acid ratios were kept constant over all crude protein levels. Composition and nutrient analysis of Diets 1, 2, and 3 are shown in Table 5-1, and analyzed composition of amino acid and protein (N X 6.25) is shown in Table 5-2.

Individual pig body weights and pen feed consumption were measured weekly. Growth performance criteria included ADG, ADFI and feed:gain ratio.

Digestibility determination. Fecal collections were taken during the last 10 d of the grower and finisher phases. At least 6 d before and throughout each collection period, 0.05% chromic oxide was added to all diets as an indicator. During each collection period, fresh fecal grab samples were taken from pigs within each pen. Each pen was sampled six times with alternate morning and afternoon sampling times over a 6 d period. The fecal samples from each collection period were pooled, sealed in plastic bags and stored at -20°C until they were dried in a forced air oven at 65°C.

Ileal digesta. Ileal contents were collected by the slaughter technique from all barrows at the end of finishing period; immediately for those not used in the metabolism trials, and at the end of the metabolism trials for the remainder. The last 50 cm of the small intestine was removed and carefully cleaned with water. The contents were gently squeezed out and immediately frozen at -80°C until they were freeze dried and ground for subsequent analysis. Five-gram samples of digesta and diets were sent to the University of Missouri[12] for amino acid (less tryptophan), N, and dry matter determination. Chromium, Ca, and P were determined in our laboratory as subsequently described.

[11] Microbial phytase: 600 U/g, BASF Corp. Mt. Olive, NJ, USA.
[12]Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO 65211.

Table 5-1. Composition of the diets used in the phytase/amino acid grower-finisher and metabolism studies

	Dietary treatments[a]					
	Grower phase[b]			Finisher phase[b]		
Ingredients, %	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Ground corn	81.38	75.57	69.83	87.54	80.25	72.95
Soybean meal (44% CP)	16.10	14.95	13.80	10.40	9.53	8.67
Limestone	0.93	0.90	0.87	0.82	0.79	0.75
Biophos[c]	0.79	0.90	1.00	0.44	0.55	0.67
Dextrose	0	3.44	6.85	0	4.04	8.08
Starch	0	3.44	6.85	0	4.04	8.08
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Cr_2O_3-dextrose mixture[d]	0.20	0.20	0.20	0.20	0.20	0.20
Selenium premix[e]	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix[f]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix[g]	0.20	0.20	0.20	0.20	0.20	0.20
Calculated analysis[h]						
Calcium, %	0.55	0.55	0.55	0.44	0.44	0.44
Total phosphorus, %	0.50	0.50	0.50	0.40	0.40	0.40
Available phosphorus, %	0.23	0.25	0.27	0.15	0.17	0.19
Crude protein, %	14.00	13.00	12.00	12.00	11.00	10.00
Total lysine, %	0.67	0.62	0.57	0.52	0.47	0.43
ME, kcal/kg	3435.00	3457.00	3480.00	3453.00	3483.00	3506.00
Assayed						
Calcium %	0.57	0.55	0.56	0.47	0.47	0.47
Phosphorus, %	0.50	0.50	0.50	0.40	0.40	0.40

[a]Phytase containing diets 4 and 5 (not shown) were prepared from diet 3 except that 0.042% and 0.084% Natuphos® phytase premix (600 U of phytase/g, BASF Corp., Mt. Olive, NJ) replaced dextrose for diets 4 and 5, respectively. Intended phytase levels for diets 4 and 5, were 250 and 500 U/kg, respectively.

[b]Grower diets were fed from a BW mean of 32 to 67 kg. Finisher diets were fed from a BW mean of 67 to 101 kg.

[c]Biophos: a chemical mixture of monocalcium and dicalcium phosphates for poultry and livestock feed (Mallinckrodt Veterinary, Inc. Mundelein Illinois).

[d]a mixture of chromic oxide and dextrose with a ratio1:4.

[e]Supplied 0.3 mg Se per kilogram of diet.

[f]Supplied per kilogram of diet: Zn, 75 mg; Fe, 88 mg; Mn, 30 mg; Cu, 8 mg; I, 2 mg.

[g]Supplied per kilogram of diet: retiny acetate, 1211 μg; cholecalciferol, 88 μg; dl-α-topherol acetate, 18 mg; riboflavin, 3.5 mg; niacin, 18 mg, choline chloride, 352 mg; d-pantothenic acid, 18 mg; d-biotin, 0.35 mg; cyanocobalamin, 18 μg; menadione dimethylprimidinol bisulfite, 1.8 mg.

[h]Calculated values are based on NRC (1998) nutrient levels in corn, soybean meal and dextrose and guaranteed levels in Biophos (21% P and 15% Ca) and ground limestone (Tenn Luttrel Co., Limestone Div., Luttrell, TN, 38% Ca). Available P values are based on NRC (1998) data and assume P bioavailabilities of 15% in corn, 38% in soybean meal and 100% in Biophos.

Table 5-2. Analyzed amino acid and crude protein composition of basal diets used in the finisher phase of the grower-finisher, metabolism studies

Item	Finisher diets [a]		
	Diet 1	Diet 2	Diet 3[b]
	---------- % ----------		
Amino acid			
Aspartic acid	1.14	1.08	1.04
Threonine	.46	.43	.41
Serine	.53	.51	.49
Glutamic Acid	2.19	2.08	1.99
Proline	.84	.79	.75
Glycine	.51	.48	.45
Alanine	.72	.68	.63
Cystine	.26	.24	.22
Valine	.61	.56	.51
Methionine	.22	.20	.19
Isoleucine	.50	.47	.43
Leucine	1.21	1.15	1.07
Tyrosine	.38	.36	.35
Phenylalanine	.61	.58	.55
Histidine	.34	.32	.31
Lysine	.60	.57	.54
Arginine	.77	.71	.68
Cystine + Methionine	.48	.44	.41
Crude protein	11.80	11.00	10.10
AA protein	12.21	11.54	11.10
Dry Matter	86.33	86.56	86.66

[a]The analyzed Ca and P content was 0.47 and 0.40%, respectively for all three diets.

[b]For Diets 4 and 5, phytase was added at levels of 250 and 500 U/kg, respectively.

Metabolism Trials.

Period 1: At the end of the grower phase of the grower-finisher study, a balance trial was conducted using four barrows from each of diets 1, 3, and 5 fed the finisher diets. Pigs were housed in stainless steel metabolism crates and given a 15-d adjustment to the crates followed by a 5 or 6-d collection period. While in the metabolism crates, pigs in Trial 1 were fed the finisher diets at 8% of their metabolic BW ($BW^{.75}$), and pigs in Trial 2 were fed the finisher diets at 7.5 % of their metabolic BW. Feces were collected once each day and frozen at -20°C. The total weight of the urine was measured, and 10% of the urine was collected and kept acidic (pH <4.0) by adding 25% HCl.

Periods 2 and 3: At the end of the finisher phase, a balance trial with two collection periods was conducted using four barrows from each of diets 1, 3, and 5. The first 6-d total collection was immediately followed by a second 6-d total collection. Barrows continued on the same diet. Collection of feces and urine followed procedure described in period 1.

Bone Collection.

The third metacarpal bones in the left front foot from all the slaughtered barrows were removed and frozen. The soft tissues of the bone samples were removed and the bones were then refrozen and used later for shear force determination as described by Combs et al. (1991). The shear force and energy of the metacarpal bones were determined using an Instron Universal Testing Machine (Model 1123, Instron Corp., Canton, MA). Bones were thawed immediately before testing to prevent desiccation. 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 12 h.

Laboratory Processing and Analysis

Fecal samples were dried (60°C) in the forced-air oven and ileal samples were freeze dried. The dried fecal samples from the grower and finisher phase, and metabolism trials, along with representative samples of diets were ground to pass through a 1-mm sieve and analyzed for DM according to AOAC procedures (1990). Fecal and feed samples were wet-digested with nitric and perchloric acid, and the total P concentrations were assayed colorimetrically using the vanadomolybdate procedure (AOAC 1990). Calcium and Cr concentrations were determined with a Perkin-Elmer atomic absorption spectrophotometer (Model 5100 PC, Perkin-Elmer, Norwalk, CT). Fecal, urine, and feed samples were digested with sulfuric acid, and the total CP

concentrations were assayed using the Kjeldahl procedure (AOAC 1990). The apparent digestibilities of Ca, N, and P were calculated using both the direct and the indirect methods in the metabolism trials. Samples were analyzed for N content using standard methods (AOAC, 1990). Diets and ileal digesta were hydrolyzed in 6 N HCl and analyzed for amino acid content using HPLC.

Statistical Analysis

The performance and fecal data of the grower, finisher, and combined phases were analyzed using the GLM procedure of SAS (1990) with pen means serving as the experimental unit. The two phases were analyzed separately and then combined. The model included replicate and treatment. The ileal digesta data were analyzed using the GLM procedure of SAS (1990) with pig serving as the experimental unit. The model included replicate and treatment. Linear contrasts were used to test the effect of increasing phytase levels or decreasing protein levels and to test the differences between treatment means.

The data from the metabolism trials were analyzed using the GLM procedure of SAS (1990) with pig serving as the experimental unit. The model included period and treatment. Nonorthogonal contrasts were used to test significant differences between the three treatment means.

In the growing-finishing study, a linear function was derived for protein levels (diets 1 through 3) and for phytase levels (diets 3 through 5) with the model: $Y = a + bX$; where Y = the response measurements; X = protein (0, 1, and 2 percentage units) or phytase addition (0, 250 or 500 U/kg of phytase) to the 10% CP basal diet (diet 3). The linear equation for phytase was solved for 500 U/kg of phytase, and the product was set equal to the protein equation and solved. The product was protein equivalent value of 500 U/kg phytase. In digestibility study (CP, N, and amino acids), the linear function was derived for protein levels (diets 1 through 3) and for phytase levels (diets 3 through 5) as described above. The equivalency value of phytase for individual nutrients was based on the digested nutrient (the digestibility time by the dietary nutrient level). The absolute value of the slope (b) from each linear equation represents the responsive magnitude of amino acid digestibility to protein or phytase supplementation.

RESULTS

Growing and Finishing Study

Performance. During the grower phase, daily gain, daily feed intake and feed to gain ratios were not different (P <0.05) among protein and phytase treatments (Table 5-3). However, in the finisher phase ($P < 0.01$) and overall ($P < 0.001$), daily gain linearly increased as phytase or protein was added to the lowest protein level. Some numerical differences were observed in daily feed intake and feed:gain ratios, but differences were not significant ($P > 0.05$). For cumulative daily gain, the addition of 500 U/kg of phytase compensated for a 0.85 percentage unit in CP level (diet 1) which would be near NRC (1998) recommended amounts (Table 5-7).

Total tract digestibilities. During both the grower and finisher phases and for the combined phases, P digestibility increased ($P < 0.05$) as phytase level increased (Table 5-4). The digestibility of Ca tended to be increased ($P = 0.10$) in the grower phase, and was increased ($P < 0.05$) in the finisher phase and combined phases with phytase supplementation.

Dry matter digestibility was increased ($P < 0.05$) as phytase was added to the low protein diet in the grower, finisher and combined phases. Crude protein (N x 6.25) digestibility was only numerically increased in the grower phase, but was significantly increased in the finisher phase, and for the combined phases.

The digestibility of P and Ca were only numerically lower for the lowest level of protein compared with the highest level during the grower phase, but were linearly decreased ($P < 0.01$ to 0.10) as the protein level decreased in the finisher phase and for the combined phases. Dry matter digestibility was similar among protein levels in all phases. Crude protein digestibility increased ($P < 0.10$ to 0.01) as the crude protein level increased in all phases.

Digestibility of CP increased 4.9 percentage units as the CP level increased from 10 to 12% and increased 5.1 percentage units when 500 U/kg of phytase was added to the 10% CP diet. Using crude protein digestibility, the equivalency of 500 U/kg of phytase for reducing CP was 0.67 percentage units for finisher phase (Table 5-7).

Metabolism Trials

Apparent digestibility of P, Ca, DM, and N were directly determined (Table 5-5) and were estimated indirectly using chromic oxide as an indirect marker (Table 5-5). Only diets 1, 3,

and 5 (Table 5-1) from the finisher phase of the grower-finisher study were used in the metabolism trials.

Table 5-3. Daily gain, daily feed intake and feed:gain ratio of growing-finishing pigs fed different protein and phytase levels

	Dietary treatments[a, b]					
Diets	1	2	3	4	5	
CP, %	14/12[c]	13/11	12/10	12/10	12/10	
Phytase, U/kg	0	0	0	250	500	SEM
Initial weight (kg)	32.9	33.0	33.1	33.1	33.0	0.07
Grower end wt (kg)	67.2	68.6	67.1	65.9	66.7	0.59
Finisher end wt (kg)	104.0	102.4	99.9	99.9	102.9	1.51
Daily gain (kg)						
Grower phase	0.83	0.82	0.82	0.79	0.81	0.01
Finisher phase[d, e]	1.06	0.97	0.94	0.99	1.04	0.02
Overall[f, g, h]	0.91	0.88	0.86	0.87	0.89	0.01
Daily feed intake (kg)						
Grower phase	2.45	2.42	2.39	2.28	2.36	0.06
Finisher phase	3.73	3.35	3.42	3.37	3.41	0.13
Overall	2.90	2.76	2.76	2.68	2.73	0.06
Feed:gain ratio						
Grower phase	2.99	2.95	2.93	2.89	2.91	0.094
Finisher phase	3.51	3.46	3.64	3.43	3.28	0.095
Overall	3.19	3.16	3.20	3.11	3.06	0.067

[a]Each treatment mean in the grower phase (41.5 d average) represents four pens containing 6 pigs each (3 barrows and 3 gilts), and each treatment mean in the finisher phase (34.8 d average) represents four pens containing 4 pigs each (2 barrows and 2 gilts).
[b]The Ca and P levels were 0.56/0.40% for the grower period and 0.44/0.40% for the finisher period, respectively.
[c]Growing and finishing phase, respectively.
[d]Linear protein effect (diets 1, 2 and 3, $P < 0.01$).
[e]Linear phytase effect (diets 3, 4 and 5, $P < 0.01$).
[f]Linear protein effect ($P < 0.001$).
[g]Linear phytase effect ($P < 0.001$).
[h]Linear protein vs linear phytase effect ($P < 0.05$).

Table 5-4. Influence of phytase and protein on digestibility of phosphorus, calcium, dry matter and protein of growing and finishing pigs

	Dietary treatments[a, b]					
Diets	1	2	3	4	5	
CP, %	14/12[c]	13/11	12/10	12/10	12/10	
Phytase, U/kg	0	0	0	250	500	SEM
Grower phase						
P, %[d]	62.7	62.5	61.5	66.7	68.3	1.22
Ca, %[e, f]	67.2	60.8	63.1	64.8	66.5	1.40
DM, %[g]	87.3	87.8	87.9	88.1	89.0	0.33
CP, %[h]	80.0	79.5	77.9	78.7	79.7	0.66
Finisher phase						
P, %[d, f]	45.1	41.2	40.1	45.0	47.4	1.74
Ca, %[f, g, h]	58.7	53.5	53.3	56.3	60.3	1.75
DM, %[g]	86.4	86.6	86.6	86.9	87.9	0.34
CP, %[d, f, l]	78.1	76.4	73.2	74.7	78.3	0.65
Phases combined						
P, %[d, h]	53.9	51.9	50.8	55.9	57.8	1.07
Ca, %[d, f, g]	63.0	57.2	58.2	60.6	63.4	1.07
DM, %[d]	86.9	87.2	87.2	87.5	88.5	0.24
CP, %[d, e, f]	79.1	77.9	75.6	76.7	79.0	0.49

[a]Each treatment mean in the grower phase represents four pens containing 6 pigs each (3 barrows and 3 gilts), and each treatment mean in the finisher phase represents four pens containing 4 pigs each (2 barrows and 2 gilts).
[b]The Ca and P levels were 0.56/0.40% for the grower period and 0.44/0.40% for the finisher period, respectively.
[c]Growing and finishing phase, respectively.
[d]Linear phytase effect (diets 3, 4 and 5, $P < 0.01$).
[e]Linear protein trend (diets 1, 2 and 3, $P < 0.10$).
[f]Linear protein vs linear phytase effect ($P < 0.05$).
[g]Linear phytase effect ($P < 0.05$).
[h]Linear protein effect ($P < 0.05$).
[l]Linear protein effect ($P < 0.01$).

Table 5-5. Influence of phytase and protein level on the digestibility of phosphorus, calcium, dry matter, and N and N utilization of finishing pigs during the metabolism phase of the grower-finisher study

	Dietary treatments[a,b]			
Diets	1	3	5	
CP, %	12.0	10	10	
Phytase, U/kg	0	0	500	SEM
Direct				
P, %	46.6[x]	38.7[y]	48.0[x]	1.93
Ca, %	63.0[x]	53.3[y]	63.3[x]	1.95
DM, %	90.3	90.3	90.5	0.28
N, %	86.8[x]	84.7[y]	86.0[x]	0.58
N_u, %	51.6[xy]	46.6[y]	55.6[x]	2.27
Indirect				
P, %	46.2[x]	42.1[y]	47.2[x]	1.58
Ca, %	62.9[x]	55.9[y]	63.2[x]	1.40
DM, %	90.2	90.8	90.4	0.22
N, %	86.8[x]	85.4[y]	85.9 [xy]	0.39

[a]Each treatment mean represents 12 pens.

[b]The Ca and P levels were 0.56/0.40% for the grower period and 0.44/0.40% for the finisher period, respectively.

[xy]Means in the same row with different superscripts differ ($P < 0.05$).

Direct method. During the combined phases, the digestibilities of P, Ca and N were increased ($P < 0.05$) when the CP level was increased (diet 1 vs 3) and increased ($P < 0.05$) when phytase was added to the low protein diet (diet 3 vs 5). Nitrogen utilization (N_u) was also increased ($P < 0.05$) when phytase was added to the low protein diet and was numerically higher for the adequate protein diet. Nitrogen digestibility increased 2.1 percentage units as the CP level increased from 10 to 12% and increased 1.3 percentage units as 500 U/kg of phytase was added to the 10% CP diet. Nitrogen retention as a percentage of intake increased 5.0 percentage units as CP increased from 10 to 12% and increased 9.0 percentage units when 500 U/kg of phytase was added to the 10% CP diet. Dry matter digestibility was not different during any of the periods and the combined periods for protein or phytase treatments.

Indirect method. Calcium and P digestibilities generally increased as dietary protein increased and were increased when phytase was added to the low protein diet. There appeared to be less of an effect of phytase on N digestibility when the indirect method was used (Table 5-5). As observed with the direct method, DM digestibility was similar for all diets.

When measured by the direct method, digestibilities of P increased 9.3 percentage units and Ca increased 10.0 percentage units as 500 U/kg of phytase was added to the low CP diet. Using the indirect method, P digestibility increased 5.1 percentage units and Ca increased 7.3 percentage units as phytase was added.

Ileal Digestibilities of Finishing Pigs

Minerals, DM and N digestibilities. The digestibility of P was increased 9.5 percentage units ($P < 0.05$) when 500 U/kg of phytase was added to the low protein diet, but increasing the dietary CP level did not affect P digestibility (Table 5-6). Calcium and DM digestibilities were not influenced by protein or phytase additions.

Nitrogen and total amino acid (sum of all amino acids) digestibilities were increased ($P < 0.01$) by increasing the dietary CP level and by adding phytase to the low protein diet (Table 5-6). Ileal digestibility of N increased 10.5 percentage units as the CP level increased from 10 to 12% and increased 9.4 percentage units when 500 U/kg of phytase was added to the 10% CP diet. Total amino acid digestibility increased 7.5 percentage units as the CP level increased from 10 to 12% and increased 6.8 percentage units when 500 U/kg of phytase was added to the 10% CP diet. The equivalency of 500 U/kg of phytase was 0.29 percentage units and 0.84 percentage units CP, respectively, for N and total amino acid digestibilities (Table 5-7).

Amino acid digestibilities. With the exception of proline and glycine, the digestibilities of the other amino acids were linearly increased ($P < 0.05$) when phytase was added to the low protein diet (Table 5-6). All amino acids, except glycine, were linearly increased ($P < 0.05$) as the dietary CP level was increased. When the equivalency of added phytase for reduced CP was calculated for each of the amino acids and averaged (weighted equally), 500 U/kg of phytase were equivalent to 0.80 percentage units of CP compared to the NRC CP level (diet 1). Based on the magnitude of improvements in amino acid digestibility (slope value of linear equation), there was little difference between essential and nonessential amino acids (Table 5-7).

Bone Measurements

During the grower phase, metacarpal weight, ash percentage, and shear force and energy were not different (P >0.05) among treatments. During the finisher phase, addition of phytase or

Table 5-6. Influence of phytase and protein on ileal digestibility of nutrients of finishing pigs

Diets[a,b]	1	2	3	4	5	
CP, %	12	11	10	10	10	
Phytase, U/kg	0	0	0	250	500	MSE[c]
DM, %	78.5	79.1	77.7	79.2	77.7	2.1
TotalAA, %[d, e]	83.1	81.2	75.6	77.8	82.4	3.4
P, %[d]	34.8	30.2	30.1	36.5	39.6	3.7
Ca, %	55.5	46.0	57.0	52.7	64.4	4.2
N, %[d, e]	78.0	75.9	67.5	70.5	76.8	4.2
Threonine, %[f, g]	75.0	72.2	64.8	68.0	75.3	4.7
Valine, %[d, g]	82.0	78.4	71.5	73.1	80.3	4.0
Isoleucine %[f, g]	83.3	80.3	72.4	74.2	81.9	4.5
Leucine, %[f,g]	86.2	82.4	78.7	80.1	82.8	3.5
Phenylalanine, %[d, g]	85.3	82.6	77.0	79.6	83.7	3.6
Histidine, %[d, g]	85.5	83.5	79.0	80.8	85.0	3.1
Lysine, %[f, g]	83.5	79.4	72.0	74.4	84.0	5.0
Arginine, %[f, g]	88.1	86.5	81.6	83.2	87.9	2.9
Meth + Cystine, %[f, h]	82.1	76.1	72.2	74.4	80.0	4.8
Aspartic acid, %[f, g]	82.1	80.9	74.5	76.8	83.3	3.7
Serine, %[d,f]	80.6	79.6	75.0	77.6	81.9	3.7
Glutamic acid, %[f, h]	87.4	84.8	80.6	83.0	84.7	3.6
Proline, %[h]	80.3	77.8	77.0	78.4	79.8	5.2
Glycine, %	64.7	70.7	60.3	60.7	66.0	6.6
Alanine, %[e, g]	82.7	77.8	72.9	74.3	79.5	4.3
Cystine, %[e, g]	78.1	71.7	68.8	71.2	77.2	5.5
Methionine, %[f,l]	86.7	81.4	76.1	78.2	83.2	5.5
Tyrosine, %[d,g]	81.3	78.3	71.9	74.6	79.7	4.2

[a]Treatment mean represents six barrows in Diets 1, 4, five barrows in Diets 2 and 5, and eight barrows in Diet 3.
[b]The Ca and P levels were 0.56/0.40% for the grower period and 0.44/0.40% for the finisher period, respectively.
[c]SEM = the root mean square error (MSE)/$\sqrt{n}$, where n = 6 (Diets 1, 4), n = 5 (Diets 2 and 5) or n = 8 (Diet 3).
[d]Linear phytase effect (diets 3, 4 and 5, P < 0.01).
[e]Linear protein effect (diets 1, 2 and 3, P < 0.01).
[f]Linear phytase effect (P < 0.001).
[g]Linear protein effect (P < 0.001).
[h]Linear phytase trend (P < 0.10).
[l]Linear phytase effect (P < 0.05).

Table 5-7. Daily gain, fecal CP digestibility and ileal digestibility equations and protein equivalency value developed for the effect of protein and phytase

	Protein (0, 1, 2 percentage unit increase)	Phytase (0, 250, 500 U/kg)	Equivalency[a]
Daily gain, lb/d			
Finisher phase	0.93 + 0.060X, r^2 = 0.92	2.12 + 0.0003X, r^2= 0.87	1.68
Overall	0.86 + 0.025X, r^2= 0.97	1.89 + 0.0001X, r^2= 0.87	0.85
Fecal CP digestibility, %			
Finisher phase	75.8 + 1.75X, r^2 = 0.97	72.9 + 0.0102X, r^2= 0.97	0.67
Overall	73.5 + 2.45X, r^2= 0.97	75.4 + 0.0068X, r^2= 0.97	0.26
Ileal digestibility, %			
N	68.6 + 5.25X, r^2 = 0.96	67.0 + 0.0183X, r^2= 0.95	0.29 (0.042)
Total AA	76.2 + 3.75X, r^2= 0.94	75.2 + 0.0136X, r^2= 0.92	0.84 (0.84)
Amino acid digestibilities, %			
Aspartic acid	75.4 + 3.8X, r^2 = 0.87	73.8 + 0.018X, r^2= 0.93	1.08 (0.043)
Threonine	65.6 + 5.1X, r^2= 0.94	64.1 + 0.021X, r^2= 0.95	1.05 (0.021)
Serine	75.6 + 2.8X, r^2= 0.88	74.7 + 0.014X, r^2= 0.98	0.97 (0.019)
Glutamic acid	80.9 + 3.4X, r^2= 0.98	80.7 + 0.008X, r^2= 0.99	0.53 (0.048)
Alanine	72.9 + 4.9X, r^2= 0.99	72.7 + 0.013X, r^2= 0.99	0.58 (0.029)
Cystine	68.2 + 4.7X, r^2= 0.95	68.2 + 0.017X, r^2= 0.94	0.76 (0.015)
Valine	72.1 + 5.3X, r^2= 0.97	70.6 + 0.018X, r^2= 0.88	0.59 (0.030)
Methionine	76.1 + 5.3X, r^2= 0.99	75.6 + 0.014X, r^2= 0.95	0.68 (0.007)
Isoleucine	73.2 + 5.5X, r^2= 0.94	71.4 + 0.019X, r^2= 0.89	0.61 (0.024)
Leucine	78.7 + 3.8X, r^2= 0.99	78.5 + 0.008X, r^2 = 0.99	0.37 (0.031)
Tyrosine	72.5 + 4.7X, r^2= 0.95	78.5 + 0.008X, r^2 = 0.97	1.44 (0.014)
Phenylalanine	77.5 + 4.2X, r^2= 0.96	76.8 + 0.013X, r^2 = 0.98	0.68 (0.020)
Histidine	79.4 + 3.3X, r^2= 0.95	78.6 + 0.012X, r^2= 0.95	0.87 (0.009)
Lysine	72.6 + 5.8X, r^2= 0.97	70.8 + 0.024X, r^2= 0.89	1.00 (0.030)
Arginine	82.2 + 3.3X, r^2= 0.92	81.1 + 0.013X, r^2 = 0.93	0.77 (0.023)
Average (each amino acid weighed equally)			0.80

[a]For daily gain, the linear equation for phytase was solved for 500 U/kg of phytase, and the result was set equal to the protein equation and solved. The result was the protein equivalent value of 500 U/kg of phytase. For the digestibility of CP, the equations were based on the digested nutrients (digestibility time by dietary level of CP). The value in the parentheses is the percentage units of this nutrient that can be replaced by 500 U/kg of phytase.

Table 5-8. Influence of phytase and protein on bone measurements of growing and finishing pigs

	Dietary treatments					
Diets	1	2	3	4	5	
CP, %	14/12	13/11	12/10	12/10	12/10	
Phytase, U/kg	0	0	0	250	500	MSE[a]
Grower phase[b]						
Bone weight, g	8.66	7.83	8.00	6.85	8.71	1.32
Ash weight, g	5.41	5.09	5.06	4.79	5.38	0.67
Ash, %	38.5	39.4	38.8	41.3	38.2	0.29
Force, N	3817	3171	3675	3546	3571	609
Energy, N-mm	11504	9510	12288	8667	9949	3414
Finisher phase[c]						
Bone weight	11.20	10.18	11.36	9.74	10.36	1.06
Ash weight, g	7.30	7.35	7.65	7.08	7.32	0.74
Ash, %[d]	39.4	41.9	40.3	42.0	41.3	2.35
Force, N[d]	4026	4717	4811	3952	4362	579
Energy, N-mm	14001	16115	17989	11569	15222	5604

[a]MSE = the root mean square error. SEM = MSE/$\sqrt{n}$.
[b]Mean in the grower phase represents 8 pigs for treatment 1, and 5 and 4 pigs for treatment 2 and 4, and 9 pigs for treatment 3.
[c]Mean in the finisher phase represents 8 pigs except that treatment 2 that represents 7.
[d]Linear phytase effect ($P < 0.05$).

protein to the basal diet resulted in decreases ($P < 0.05$) in metacarpal weight and shear force (Table 5-8).

DISCUSSION

Results of this study indicate that daily gain of pigs in the finisher phase and the combined grower-finisher phase linearly increased as phytase or protein was added to lowest protein level. Addition of 250 to 500 U/kg of phytase to basal diet improved the daily gain by 4.3 to 10% in the finisher phase and 0.5 to 3.7% in the overall phase. The improvement in daily gain of pigs with 1 and 2 percentage units protein addition to 10% CP basal diet was 2.8 to 12% in the finisher phase and 1.5 to 5.6% in the overall phase. Using the linear equations derived from the daily gain by phytase level and daily gain by protein level of diet, addition of 500 U/kg

of phytase to the basal diet (10% CP) was equivalent to a 11.68% CP; 1.68 percentage units increase in CP compared with the 10% CP level.

The improved gain, feed intake, and the gain:feed ratio resulting from phytase supplementation have been reported in many studies (Cromwell et al., 1993; Lei et al., 1995; Harper et al., 1997). However, most of the restoration of performance in pigs due to phytase reported by these authors is based on the comparison with the low-P basal diets. These data indicated that the improvement of animal performance by phytase couldn't be explained only by the improved digestion of P and Ca; utilization of organic nutrients may also be involved, although the improvement attributed to organic nutrient enhancement may be small. In a recent study with broilers, Biehl and Baker (1997) reported that supplementing 1200 U/kg of phytase to an amino acid deficient corn-soy diet improved feed efficiency and daily gain. Jongbloed et al. (1996) reported that supplementation of 800 U/kg phytase to an adequate P diet improved feed efficiency of pigs by 6.0%. As a powerful chelating agent, phytate may lower the availability of essential minerals and may also reduce the utilization of organic nutrients (Reddy et al., 1989; Couzy et al., 1993; Thompson and Yoon, 1984). The improvement in performance with phytase in these studies supports this hypothesis.

Potassium, Mg and Ca are predominantly high in phytin-rich particles from feed ingredients (Ogawa et al., 1975). With phytase addition to plant-origin diets, Ca as well as P is released. A positive correlation exists between the digestibility of these two macrominerals (Poinitillart, 1993; Eckhout and de Paepe, 1996; Kornegay et al., 1998). Findings in this study confirmed this conclusion. The enhancement of apparent P digestibility was 11.1% in the grower phase, 18.2% in the finisher phase, and 13.8% overall with 500 U/kg of phytase addition to basal diet. The improvement in P digestibility during finisher phase for pigs fed the basal diet with added phytase was close to that observed in the metabolism trials, which was 24.0% by direct method and 12.1% by indirect method, with an average of 18.1%. The improvement of apparent Ca digestibility was 18.1% in the grower phase, 5.3% in the finisher and 13.1% overall with 500 U/kg of phytase addition. The increase in Ca digestibility of finisher basal diet with phytase was close to that of metabolism trails, which was 18.8% in direct method and 13.1% in indirect method, with an average of 16.1%. The results from the metabolism trials showed that addition

of 2 percentage units protein to basal diet (10% CP) improved the Ca and P digestibilities by an amount compatible to supplemental 500 U/kg of phytase.

Bone measurements such as toe ash weight and percentage and shear force and energy are often used as criteria to evaluate the P bioavailability of feedstuffs and the effectiveness of phytase for digested P, although the sensitivity of these measurements are different (Zhang et al., 1998ab). In the grower phase of our study, the ash measurement tended to improve with protein and phytase addition, however the shear force and energy showed the opposite trend. Bone, in addition to minerals, also contains a number of different organic components, which have important roles in its supporting function. The shear force and energy of bone depend on not only minerals but also organic composition (Wasserman et al., 1993). Overmineralization of bones may make them becomes brittle, and this may be the case in this study. Kornegay and Thomas (1981) reported that high dietary P and Ca do not necessarily improve structural soundness of pigs. In the finisher phase, the bone measurements tended to decrease with protein and phytase addition. Compared with other treatments, pigs fed the basal diet had a low daily gain and less P may have been deposited in the muscle and more P may have accumulated in bone.

As an anti-nutritional factor, phytate not only lowers the bioavailability of P, Ca, and trace minerals, but also protein, fat, and starch. Phytate is known to inhibit α-amylase (Knuckles and Betschart, 1987; Hagenimana et al., 1994), protease (Askar, 1986), and lipase (Knuckles, 1988). Similarly, phytate may reduce the digestion and absorption of these organic nutrients by directly binding starch, protein, and fat. Chitra et al. (1995) indicated that there may be a negative correlation between phytic acid concentration and protein digestibility of protein ingredients. It may be speculated that phytase will elicit a positive effect on the utilization of organic nutrients by cleaving the bond of phytate. However, the results of practical feeding studies were inconclusive. For example, some investigators found supplemental phytase had no or little effect on total tract digestibility of DM (Simons et al., 1990; Jongbloed et al., 1992; Yi et al., 1996; O'Quinn et al., 1997; Ketaren et al., 1993). In contrast, Mroz et al. (1994) reported a significant increase in apparent digestibility of DM with phytase addition. His findings were confirmed by the result of a later study (Fandrejewski et al., 1997). In our study, the total tract DM digestibility linearly increased with phytase supplementation to basal diet. However, the

results of metabolism trials and the ileal digestibility did not indicate the same trend. The findings of this study generally supported the speculation that phytase may improve the digestibility of DM, but the magnitude of improvement is small, inconsistent and hard to detect (Ravindran et al., 1997). The result from this study also indicated that dietary CP level had an effect on the digestibility of DM.

The apparent fecal CP digestibility in the present study linearly responded to phytase as well as protein supplementation in the finisher phase and overall. The data from the direct method in metabolism trial further confirmed this finding. Based on these observations, the improvement in CP digestibility of basal diet with 500 U/kg of phytase is close to that of a two percentage units increase in protein level. Since the hindgut microflora have effects on amino acid excretion, it is generally accepted that ileal protein digestibility is more indicative of relative nutritional value of protein than fecal protein digestibility. However, the results of these two measurements from our study agreed with each other.

Except for glycine and proline, supplementation of both phytase and protein to basal diet resulted in linear improvement of other amino acid digestibilities. The improvements in amino acid digestibility ranged 0.4 to 12 percentage units, depending on the amino acid, dietary protein level, and phytase supplementation level. Based on the average values of improvements in amino acid digestibilities, supplemental 1 to 2 percentage units protein to basal diet improved amino acid digestibilities by 5.5 to 8.6 percentage units, and addition of 250 U/kg and 500 U/kg of phytase to basal diet improved amino acid digestibilities by 2.2 to 7.6 percentage units.

The process of protein digestion can be divided into two stages: pepsin cleaves protein into oligopeptides in the stomach with pH at 2 to 4 and pancreatic enzymes further hydrolyze peptides in the duodenum with pH at 6 to 7 (Reeds and Beckett, 1996). It has been found that phytate may negatively affect protein utilization as follows: at low pH (3.5 to 5.0), phytate may bind with α-NH_2 terminal groups, ε- NH_2 groups of lysine, guanidyl groups of arginine, and histidine residues in protein (Barre and van Houot, 1965); at neutral pH (6.0 to 7.0), phytase and protein may form complexes with the carboxyl terminal groups and with the side-chain carboxyl groups of aspartate and glutamate. Based on these observations, the magnitude of improvements in amino acid digestibilities of charged side chains with phytase are higher compared with that of non-charged side chains, and polar, uncharged side chains groups are higher than that of

nonpolar, aliphatic groups and aromatic groups. However, the results in this study did not indicate this trend.

Since linear equations fit data well when amino acid digestibility was regressed on phytase or protein levels (r^2 >0.88, Table 5-7), the magnitudes in improvements of amino acid digestibilities can be evaluated by the slope of the linear equations. Based on the slope values of these linear equations, the improvements in digestibilities with phytase were highest for lysine followed by threonine, isoleucine, aspartic acid, and valine. The improvements in amino acid digestibilities with protein were highest for lysine followed by isoleucine, valine, methionine and threonine. Interestingly, some similarity of enhancement in amino acid digestibilities with phytase was observed in a broiler study by Ravindran et al. (1997), who reported that the order of the improvement of amino acid digestibility from high to low is threonine, phenylalanine, leucine, isoleucine, and valine.

The comparison between the amino acid composition of the basal diet, highest protein level diet, and ideal protein showed that the first six limiting amino acid for the basal diet and the highest protein level diet was lysine, threonine, isoleucine, methionine, histidine, and valine. This is very close to the first five amino acid with highest improvement in digestibilities with protein or phytase addition. This evidence suggested that the magnitude of improvements in amino acid digestibility with protein or phytase strongly depend on whether this amino acid is limiting in the diet.

The reduced bioavailability of phosphorus, calcium, protein, starch, and trace minerals by phytate increases the excreted level of these nutrients in manure, which has the potential to cause environmental pollution. Of the nutrients present in animal waste, N is of greatest concern (Kornegay, 1995). Adding phytase could reduce N excretion by pigs and broilers (Cromwell and Coffey, 1991; Yi et al., 1996). In this study, phytase addition to the lowest CP diet improved the digestibility coefficients by 3.4, 9.2, and 6.8 percentage units, respectively for fecal CP digestibility of overall phase and ileal digestibility of N and total amino acid. Using the average improvements in digestibility, addition of 500 U/kg of phytase reduced N excretion by 6.1%. Since supplementation of microbial phytase to pig diets results in the improved protein utilization, the concept of equivalency is used to express its activity with reference to the amount of protein that can be replaced. The equivalency value of 500 U/kg phytase for protein is 0.85,

0.84, and 0.80 percentage units, respectively for overall daily, and total and average amino acid digestibility. Using an equivalency value, addition of 500 U/kg phytase to basal diet (10% CP) can allow increase of dietary CP by 0.83 percentage units; from 10% to 10.83%.

IMPLICATIONS

Phytase addition to a CP deficient corn-soybean meal diet improved the utilization of protein, nitrogen, and increased the digestibility of most amino acids by pigs. Based on the result, the addition of 500 U/kg of phytase to the basal diet was equal to increasing the dietary CP level by 0.83 percentage units, and the N excretion was reduced by 5.1%.

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Chapter VI

Evaluation of Phytase and Non-starch Polysaccharide Enzymes added alone and in Combination on Nutrient Utilization of Individual and Mixtures of Feedstuffes with Adult Cecectomized Roosters

ABSTRACT The effects phytase and NSP enzymes added alone or in combination on the nutrient utilization of barley, canola meal, rice bran, soybean meal, a canola meal-barley blend and a soybean meal-barley blend were investigated in six experiments with 72 cecectomized roosters. Canola meal-barley and soybean meal meal-barley blends were formulated based on a 20 % protein level with ratios of 36:64 and 27.3:72.7, respectively. Barley and rice bran were used in Trials 1 and 2, respectively, with added microbial phytase (0 and 900 U/kg) and Ronozyme^{TM} B (0, 220, 440 g/kg) in a 2×3 factorial arrangement. Soybean meal and canola meal were used in Trials 3 and 4, respectively, with added phytase (0 and 900 U/kg) and Ronozyme^{TM} VP (0, 350, 700 g/kg) in a 2×3 factorial arrangement. Soybean meal meal-barley and canola meal-barley blends were used in Trials 5 and 6, respectively, with added phytase (0 and 900 U/kg) and NSP enzymes (0, 440 g/kg Ronozyme^{TM} B, 700 g/kg Ronozyme^{TM} VP, and a combination of 440 g/kg Ronozyme^{TM} B and 700 g/kg VP) in a 2×4 factorial arrangement. Phytase supplementation increased the utilization of energy, N, total amino acid and most of the amino acids in barley, canola meal, and canola meal-barley ($P < 0.05$) and numerically increased energy and N utilization in rice brain, soybean meal and soybean meal meal-barley. The side chains in amino acids played an important role in improvements in the digestibilities of amino acids with phytase. Compared with the other amino acids, lysine, arginine, cysteine, serine, and threonine had a larger magnitude of improvements in digestibilities with phytase. The true utilization of energy and N, and digestibilities of total amino acid and glycine, isoleucine, and histidine in barley quadratically increased ($P < 0.05$) with Ronozyme^{TM} B. Phytase addition increased Ca retention in barley, canola meal, soybean meal, and the canola meal-barley and soybean meal meal-barley blends, and increased P retention in barley and the soybean meal-barley blend. Addition of Ronozyme^{TM} B to barley decreased ($P < 0.05$) Ca retention and quadratically increased P retention. In summary, phytase and suitable NSP enzyme supplementation increased AME, TME, N retention, and amino acids digestibility in barley, canola meal, rice bran, soybean meal meal-barley and canola meal-barley blends.

Key words: ingredients, amino digestibility, energy, and roosters.

INTRODUCTION

Phytate and non-starch polysaccharides (NSP) in plant ingredients are known to have a negative effect on the utilization of nutrients. They are not only indigestible, but also interfere with the digestion and absorption of other nutrients, thus reducing the feed efficiency of plant ingredients used for non-ruminants (Annison, 1993; Ravindran et al., 1995; Pallauf and Rimbach, 1997). Enzyme supplemented in certain cereal-based blends have been proven to be effective in improving the utilization of nutrients by degrading antinutritional factors. For example, addition of NSP enzymes to wheat-based diets significantly improved the digestibility of nutrients and the performance of broilers (Annison, 1991; Choct et al., 1996; Fuente et al., 1998). The beneficial effects of phytase supplementation to plant ingredient diets also have been illustrated (Denbow et al., 1995; Ravindran et al., 1995; Kornegay et al., 1996). However, information about the combination of these two enzymes on nutrient utilization is very limited. In pigs, Wenk et al., (1993) reported that there was possibly a negative interaction between carbohydrase and phytase. The objectives of this study were to investigate the main effects of phytase, and NSP enzymes, and any interactions between phytase and NSP enzymes on nutrients, and especially organic nutrients, utilization of cereal grains, protein supplements, and blends of cereal grains and protein supplements using adult cecectomized roosters suggested by Sibbald (1986).

MATERIALS AND METHODS

Dietary treatments. Barley, canola meal, rice bran and soybean meal were obtained from various companies in the United States. Two blends, canola meal-barley and soybean meal-barley were formulated based on a 20 % protein level. The ratios for canola meal-barley and soybean meal meal-barley were 36.0:64.0 and 27.3:72.7, respectively. Composition of individual ingredients and blends are showed in Table 6-1.

Barley and rice bran were used in Trials 1 and 2, respectively, with two levels of microbial phytase[13] (0 and 900 U/kg) and three levels of Ronozyme™ B[14] (0, 220, 440 g/kg) in a

[13]Phytase: Natuphos® (analyzed activity was 482U/g) was supplied by BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234.
[14]Ronozyme™ B (estimated activities of Fungal β-glucanase, Pectinase, and Hemi-cellulase were 50 FBG/g, 5,000 PSU/g, and 125 kVHCU/g, respectively) was supplied by Roche Vitamins & Fine Chemicals.

2×3 factorial arrangement of treatments. Soybean meal and canola meal were used in Trials 3 and 4, respectively, with two levels of phytase (0 and 900 U/kg) and three levels of Ronozyme™ VP[15] (0, 350, 700 g/kg) in a 2×3 factorial arrangement of treatments. A blend of soybean meal

Table 6-1. Composition of ingredients and blends for cecectomized rooster study

	Ingredients and blends					
Item	Barley	Canola meal	Rice bran	Soybean meal	Canola meal barley	Soybean meal barley
DM, %	88.9	90.0	90.4	89.6	89.1	89.5
GE, kcal/kg	3887	4277	3765	4149	3917	3963
CP, %	8.8	34.5	16.8	41.6	19.0	18.4
Ca, %	0.76	0.65	3.86	0.33	0.45	0.26
P, %	0.35	1.11	2.21	0.57	0.66	0.42

and barley was used in Trial 5, and a blend of canola meal and barley was used in Trial 6. Two levels of phytase (0 and 900 U/kg) and four levels of NSP enzymes (0 or 440 g/kg Ronozyme™ B; 700 g/kg Ronozyme™ VP; and a combination of 440 g/kg Ronozyme™ B and 700 g/kg VP) were used with both blends in a 2×4 factorial arrangement of treatments.

Birds. Seventy-two cecectomized roosters were used in six Precision-Feed Cecectomized Rooster Assays (Sibbald, 1976). The adult Single Comb White Leghorn cockerels were cecectomized at approximately 25 wk of age; the ceca, believed to be main sites of intestinal microflora, were removed according to the procedure of Parsons (1985). After 24 h of fasting, the birds were anesthetized by injection of sodium pentobarbital[16] into the wing vein at the level of 0.4 ml per kilogram live weight. Half of the dose was given rapidly and then the remainder of the dose was administered carefully according the responses of birds. Feathers were removed from the abdomen and the area was disinfected with iodine. A 2.5-cm transverse incision was

[15]Ronozyme™ VP (estimated activities of Fungal xylanase, α-amylase, and Endo-β- glucannase were 600 FXU/g, 50 KNU/g and 90 EGU/g, receptively) was supplied by Roche Vitamins & Fine Chemicals.
[16]Ten grams of sodium pentobarbital, 20 ml ETOH (70%), 40 ml propylene glycol, and 140 ml water.

made posterior to the last rib on the left side. The twin ceca were carefully pulled out through the opening. Then the ceca were tied-off near the ileal-cecal junction using absorbable surgical thread[17] and the distal ends of ceca were teased free from the intestine. The intestine and the ceca stubs were returned to the body cavity. The muscle and the skin layer were closed with surgical thread. After the surgery, an antibiotic solution, Baytril®[18] was injected intramuscularly to birds twice a day at the level of 0.6 ml per kilogram live weight for three days.

Birds were given a 10-wk recovery after the surgery before being used. They were housed individually in cages with raised wire floors and were kept in an environmentally controlled room and subjected to a 16:8 h light:dark period. Before the start of the experiment, feed and water were supplied ad libitum. Surgical and animal care procedures followed published guidelines (Consortium, 1998)

The 72 birds were divided into two sets of 36 each. The first set was used in Trial 1. Thirty cecectomized roosters (five/diet) were selected for precision feedings. After a 24 hr fast, all the birds received a 30-g crop intubation of the ground feedstuffs fortified with the respective exogenous enzymes. The birds were confined to individual collection cages and excreta were collected in plastic trays (45.4 by 35.2 cm) at approximately 24 hr, and again at exactly at 48 hours after feeding. Two birds were used as feed-deprived controls to estimate the endogenous excretion. The other roosters of Set 1 were used as replacements if precision feeding of some birds was not successful. The second set of birds was used in Trial 2 in the same way as the first set of birds. Trials 3, 4, 5, and 6 were conducted by using these two set birds alternatively. The birds were give 10 days rest after each collection, except that 6 birds had only a 3-d rest in Trial 6. Excreta from each cockerel was freeze dried, weighed, ground through a 60-mesh screen and then frozen for later determination of P, Ca, DM, N and GE. All the ingredients and blends were also analyzed for DM, GE, CP (N×6.25), P and Ca (AOAC, 1984). Five-gram excreta and diets samples from barley, canola meal, and canola meal-barley were sent to the University of Missouri[19] for amino acid (less tryptophan), N, and dry matter determination. The composition of the diets assayed is showed in Table 6-2.

[17] Surgical thread: 3-O PDS (polydioxanone suture) was produced by Ethicon.
[18]Baytril®(each ml contains enrofoxacin 22.7 mg, n-butyl alcohol 30 mg, potassium hydroxide for pH adjustment and water for injection) was supplied by Bayer Corp., Shawnee Mission, Kansas 66201.
[19]Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO 65211.

Table 6-2. Analyzed amino acid and crude protein composition of basal diets

Item	Barley	Canola meal	Canola meal barley
	---------- % ----------		
Amino acid			
Aspartic acid	0.58	2.60	1.38
Threonine	0.32	1.53	0.75
Serine	0.36	1.32	0.80
Glutamic Acid	1.87	6.18	3.63
Proline	0.84	2.20	1.37
Glycine	0.39	1.81	0.91
Alanine	0.41	1.62	0.86
Cystine	0.22	0.97	0.51
Valine	0.46	1.94	0.95
Methionine	0.15	0.76	0.37
Isoleucine	0.29	1.45	0.71
Leucine	0.62	2.66	1.41
Tyrosine	0.25	1.03	0.51
Phenylalanine	0.43	1.54	0.83
Histidine	0.21	1.04	0.51
Lysine	0.32	2.13	0.93
Arginine	0.45	2.32	1.10
N	1.48	6.01	3.17
Total AA	8.38	33.53	17.97
Dry Matter	88.77	91.76	91.73

Calculations and Statistical Analysis. The apparent and true availability of protein and amino acids, minerals (Ca and P), and energy of these feedstuffs were calculated by the method of Sibbald (1986).

$AX = IX – (FX + UX)$

$TX = IX – (FX + UX) + (F_mX + U_mX + U_eX)$

Where: AX is the apparent available nutrient X;

TX is the true available nutrient X;

IX is the amount of X placed in the fed-birds;

(FX + UX) is the amount of X excreted by the fed-birds; and

($F_mX + U_mX + U_eX$) is the amount of X excreted by the fasted bird.

All data were analyzed using the GLM procedure of SAS (1990). Data from the six trials were analyzed by two-way ANOVA with a model that included dietary phytase level, dietary NSP enzyme concentration, and their interaction. Bird was the experimental unit. Orthogonal contrasts were used to separate the differences between the main effects of NSP ($P < 0.05$).

RESULTS

Phytase effect. Phytase supplementation to the basal diet increased ($P < 0.05$) all the observed measurements in barley, and all the observed measurements except Ca and P retention in canola meal, and all the observed measurements except P retention in canola meal-barley blend. The true retention of Ca in soybean meal (Table 6-4) and P in soybean meal meal-barley blend (Table 6-5) were improved with phytase ($P < 0.05$). The improvements in N retention and TME were 8.0 and 29 kcal/kg, 7.2 and 58 kcal/kg, and 7.9% and 34 kcal/kg, respectively for rice bran, soybean meal and the soybean meal-barley blend ($P > 0.05$).

With the exception of aspartic acid, glycine, methionine and isoleucine, supplementation of phytase to basal barley and canola meal resulted in improvements ($P < 0.05$) in the true digestibilities of other amino acids and total amino acid (Table 6-6 to 6-7). Except for aspartic acid, glycine, alanine, methionine, isoleucine, leucine, tyrosine, and phenylalanine, phytase addition to canola meal-barley improved other amino acids digestibilities (Table 6-8). The improvements in the true digestibilities of amino acids ranged from 1.4 to 23.3 percentage units, and the enhancement in total amino acid was 3.4, 6.3, and 7.2 percentage units, respectively for canola meal, canola meal-barley, and barley. The magnitudes of improvements in amino acid digestibility did not show any different between the essential or non-essential amino acids.

Non-starch polysaccharide enzyme effect. Addition of Ronozyme B to barley quadratically ($P < 0.05$) increased digestibility of DM and N, and AME and TME of barley diet (Table 6-3). Non-starch polysaccharide enzyme supplementation, either Ronozyme B (0.70g/kg) or Ronozyme VP (0.44g/kg) alone, to canola meal-barley basal blend deceased ($P < 0.05$) the digestibility of DM and energy utilization. However, addition of both Ronozyme VP and B to canola meal-barley basal blend resulted in no difference ($P > 0.05$) in N and energy utilization (Table 6-5). Non-starch polysaccharide enzyme supplementation to rice bran, canola meal, soybean meal, soybean meal-barley resulted in numerically increased digestibilities of DM and N, and AME and TME. Non-starch polysaccharide enzyme supplementation decreased Ca

retention in barley, and numerically decreased Ca retention in rice bran, canola meal, and canola meal-barley, and numerically increased Ca retention in soybean meal and soybean meal-barley. Addition of NSP enzymes quadratically increased the P retention in barley (Table 6-3, 6-4, 6-5).

The digestibilities of total amino acid, histidine, isoleucine, glycine, and cystine in barley quadratically improved with Ronozyme B addition ($P < 0.05$). Addition of Ronozyme B to canola meal-barley had a better effect on lysine digestibility than that of Ronozyme VP ($P<0.05$)

The interaction between the phytase and NSP enzymes. Without phytase, Ronozyme B quadratically ($P < 0.05$) increased the digestibilities of alanine, valine, cysteine, and total amino acid in barley, but did not show this trend with phytase. Without phytase, Ronozyme VP quadratically decreased ($P < 0.05$) the digestibilities of threonine, proline, valine and isoleucine in canola meal, but did not show this trend with phytase (Table 6-6 and 6-7).

Table 6-3. The main effect of phytase and Ronozyme B on true retention of phosphorus, calcium, N and energy, digestibilities of DM, and AME and TME of barley and rice bran[a]

Item	Phytase, U/kg[b]			Ronozyme B, g/kg[c]			
	0	900	SE	0	0.35	0.70	SE
Barley							
DM, %	63.8	76.2	2.2*	66.2	74.8	69.1	2.6[q]
N, %	28.0	105	18.3*	53.3	102	44.5	19.2[q]
Energy, %	67.1	75.9	1.59*	69.0	74.3	71.1	1.9[q]
AMEn[d], kcal/kg	1968	2310	61.8*	2042	2248	2126	75.7[q]
TMEn[e], kcal/kg	2607	2948	61.8*	2681	2887	2765	75.7[q]
Ca, %	-324	-144	36.8*	-187	-190	-325	45.0[l]
P, %	12.4	48.6	10.5*	16.8	50.7	18.9	12.9[q]
Rice bran							
DM, %	44.3	47.4	2.5	44.1	50.8	42.6	3.0
N, %	41.0	49.0	12.4	46.6	63.0	25.1	15.2
Energy, %	54.5	58.0	1.6	56.0	58.8	53.9	1.9
AMEn[d], kcal/kg	1414	1543	58.9	1471	1575	1389	72.0
TMEn[e], kcal/kg	2052	2182	58.9	2110	2214	2027	72.0
Ca, %	-11.6	-4.4	3.8	-6.1	-5.5	-12.5	4.7
P, %	48.9	53.4	2.2	51.1	50.9	51.4	2.7

[a]True retention = $(IX - FX + FX_f)/IX$, where IX = the amount of intake, FX = amount excreted by the fed bird, respectively.
FX_f = amount excreted by the fasted bird.
[b]Each treatment mean represents fifteen birds.
[c]Each treatment mean represents ten birds.
[d]AMEn = $IX - (FX + 8.22*N_f)$, where N_f = total N excreted by the fed birds.
[e]TME = $IX - (FX + FX_f + 8.22*N_f)$, where N_f = total N excreted by the fed birds.
*Phytase or Ronozyme effect ($P < 0.05$).
[q]Quadratic Ronozyme B effect ($P < 0.05$).
[l]Linear Ronozyme B effect ($P < 0.05$).

Table 6-4. The main effect of phytase and Ronozyme VP on true retention of phosphorus, calcium, N and energy, and digestibilities of DM, and AME and TME of canola meal and soybean meal[a]

Item	Phytase, U/kg[b]			Ronozyme VP, g/kg[c]			
	0	900	SE	0	0.22	0.44	SE
Canola meal							
DM, %	27.9	36.7	2.8*	31.3	30.1	35.4	3.5
N, %	0.01	25.9	8.4*	9.0	12.0	17.9	10.3
Energy, %	43.6	49.5	1.9*	44.7	44.8	48.4	2.3
AMEn[d], kcal/kg	1230	1477	81.0*	1357	1275	1429	99.3
TMEn[e], kcal/kg	1868	2116	81.0*	1995	1913	2068	99.3
Ca, %	-30.7	-12.1	8.7	-20.6	-35.2	-8.4	10.6
P, %	-13.1	0.3	4.8	-7.0	-8.2	-4.0	5.8
Soybean meal							
DM, %	37.0	39.2	2.3	38.5	37.1	38.7	2.8
N, %	16.6	23.8	5.5	21.1	21.1	18.4	6.7
Energy, %	51.8	53.2	1.5	52.2	50.7	54.5	1.9
AMEn[d], kcal/kg	1510	1568	62.7	1528	1463	1626	76.9
TMEn[e], kcal/kg	2149	2207	62.8	2167	2102	2265	76.9
Ca, %	-195	-136	17.2*	-169	-194	-132	20.9
P, %	2.9	9.9	6.5	4.6	9.7	4.9	8.0

[a]True retention = $(IX - FX + FX_f)/IX$, where IX = the amount of intake, FX = amount excreted by the fed bird , respectively.
FX_f = amount excreted by the fasted bird.
[b]Each treatment mean represents fifteen birds.
[c]Each treatment mean represents ten birds.
[d]AMEn = $IX - (FX + 8.22*N_f)$, N_f = total N excreted by the fed birds.
[e]TME = $IX - (FX + FX_f + 8.22*N_f)$, N_f = total N excreted by the fed birds.
*Phytase or Ronozyme effect ($P < 0.05$).

Table 6-5. The main effect of phytase and NSP enzymes on true retention of phosphorus, calcium, N and energy, digestibilities of DM, and TME of canola meal-barley and soybean meal meal-barley[a]

Item	Phytase, U/kg[b]			Ronozyme (VP/B), g/kg[c]				
	0	900	SE	0/0	0.44/0	0/0.70	0.44/0.70	SE
Canola meal-barley								
DM, %	58.6	63.5	1.5*	64.4	58.6	56.6	64.6	2.1*[q]
N, %	75.4	79.8	0.9*	78.7	76.7	76.1	79.0	1.2
Energy, %	62.5	66.3	1.1*	66.2	62.6	62.0	66.8	1.5*[q]
TMEn[d], kcal/kg	2448	2597	41.5*	2593	2452	2430	2616	58.7*[q]
AMEn[e], kcal/kg	1810	1959	41.5*	1954	1814	1791	1978	58.7*[q]
Ca, %	-67.1	-37.4	7.7*	-37.8	-86.6	-80.5	-4.1	10.9*[q]
P, %	21.6	29.2	3.6	31.2	19.4	17.6	33.3	5.1
Soybean meal meal-barley								
DM, %	69.3	70.8	1.7	69.1	68.4	71.3	71.5	2.5
N, %	84.2	92.1	9.0	80.4	71.8	94.7	106	12.7
Energy, %	72.0	72.8	1.3	71.6	71.6	72.4	74.0	1.8
TMEn[d], kcal/kg	2852	2886	50.9	2836	2838	2868	2935	71.9
AMEn[e], kcal/kg	2214	2248	50.9	2198	2199	2230	2296	71.9
Ca, %	11.3	37.4	9.2*	8.4	24.5	27.6	36.8	13.1
P, %	13.1	38.5	8.2*	15.6	20.1	31.9	45.6	11.5

[a]True retention = $(IX - FX + FX_f)/IX$, where IX = the amount of intake, FX = amount excreted by the fed bird , respectively.
FX_f = amount excreted by the fasted bird.
[b]Each treatment mean represents twenty birds.
[c]Each treatment mean represents ten birds.
[d]AMEn = $IX - (FX + 8.22*N_f)$, N_f = total N excreted by the fed birds.
[e]TME = $IX - (FX + FX_f + 8.22*N_f)$, N_f = total N excreted by the fed birds.
*Phytase or Ronozyme effect ($P < 0.05$).
[q]Main effect of Ronozyme VP plus B is greater than that of either VP or B alone ($P < 0.05$).

Table 6-6. The main effect of phytase and Ronozyme B on the true digestibilities of total AA and amino acids of barley[a]

Item	Phytase, U/kg[b]			Ronozyme B , g/kg[c]			
	0	900	SE	0	0.35	0.70	SE
	……..%……						
Aspartic acid	72.0	76.5	1.9	72.6	77.4	72.7	2.4
Threonine	63.3	70.5	2.2*	65.5	69.6	65.7	2.7
Serine	76.4	83.7	1.8*	79.1	81.4	79.7	2.2
Glutamic acid	85.2	88.4	0.9*	85.9	88.0	86.5	1.1
Proline	82.9	86.8	1.1*	83.9	85.8	85.0	1.3
Glycine	-39.9	-38.5	18.0	-76.8	-1.7	-39.0	22[q]
Alanine[i]	64.3	74.4	2.0*	67.3	70.6	70.2	2.5
Cysteine[i]	74.1	82.8	1.8*	76.7	84.0	74.7	2.2
Valine[i]	76.5	82.5	1.4*	77.6	81.5	79.4	1.8
Methionine	81.8	84.4	1.9	83.2	83.6	82.6	2.3
Isoleucine	84.8	87.1	1.5	84.8	89.2	84.0	1.8[q]
Leucine	83.4	88.1	1.3*	84.8	87.1	85.5	1.5
Tyrosine	82.9	88.0	1.3*	84.5	87.6	84.3	1.6
Phenylalanine	84.0	88.3	1.2*	85.1	87.5	85.9	1.4
Histidine	74.4	81.2	1.7*	76.8	82.3	74.4	2.0[q]
Lysine	48.1	71.3	7.8*	67.1	67.3	44.7	9.6
Arginine	67.2	84.3	6.6*	78.1	82.9	66.3	8.1
Total AA[i]	69.2	76.4	1.8*	70.3	77.8	70.4	2.2[q]

[abc]See Table 6-3.
*Phytase effect ($P < 0.05$).
[q]Quadratic Ronozyme B effect ($P < 0.05$).
[i]Interactive effect of phytase and NSP enzyme ($P < 0.05$).

Table 6-7. The main effect of phytase and Ronozyme VP on the true digestibilities of total AA and amino acids canola meal[a]

Item	Phytase, U/kg[b]			Ronozyme VP, g/kg[c]			
	0	900	SE	0	0.22	0.44	SE
	…….%						
Aspartic acid	84.1	86.5	0.9	86.3	84.4	85.2	1.0
Threonine[i]	77.7	82.3	1.2*	81.2	78.4	80.2	1.5
Serine	79.0	83.0	1.2*	82.1	79.7	81.3	1.5
Glutamic acid	90.2	92.2	0.5*	91.6	90.7	91.3	0.7
Proline[i]	80.5	84.5	1.1*	83.8	80.9	82.6	1.3
Glycine	21.9	17.2	5.7	21.5	18.8	18.4	0.7
Alanine	85.8	88.7	0.8*	88.0	86.3	87.4	1.0
Cysteine	78.1	81.4	1.3*	81.1	77.6	80.7	1.6
Valine[i]	84.7	88.2	0.8*	87.4	85.5	86.4	1.0
Methionine	91.5	92.6	0.7	92.2	92.6	91.3	0.9
Isoleucine	86.6	88.1	0.7	88.5	86.6	86.9	0.9
Leucine[i]	88.8	90.8	0.6*	90.7	89.0	89.7	0.9
Tyrosine	86.5	88.5	0.8*	88.0	86.7	87.8	0.9
phenylalanine	88.8	90.9	0.6*	90.5	89.2	90.0	0.7
Histidine	84.0	86.9	0.9*	85.6	85.1	85.6	1.1
Lysine	79.4	85.7	1.9*	83.4	82.6	81.6	2.3
Arginine	81.2	87.4	1.9*	82.2	86.8	83.9	2.4
Total AA	78.5	81.9	0.9*	81.0	79.8	79.8	1.1

[abc]See Table 6-4.
*Phytase effect ($P < 0.05$).
[i]Interactive effect of phytase and NSP enzyme ($P < 0.05$).

Table 6-8. The main effect of phytase and NSP enzymes on the true digestibilities of total AA and amino acids of canola meal-barley[a]

Item	Phytase, U/kg[b]			Ronozyme (VP/B), g/kg[c]				
	0	900	SE	0/0	0.44/0	0/0.70	0.44/0.70	SE
Aspartic acid	80.9	82.6	0.9	81.2	81.9	81.6	82.5	1.2
Threonine	75.2	79.2	1.0*	77.2	76.5	77.9	77.4	1.4
Serine	77.6	81.2	1.1*	78.4	80.4	79.3	79.4	1.5
Glutamic acid	89.0	90.4	0.5*	89.3	89.9	89.4	90.3	0.6
Proline	81.0	83.7	0.8*	82.1	82.5	81.7	83.2	1.1
Glycine	-20.9	6.5	7.5	12.0	-2.1	-36.7	-2.0	10.6
Alanine	83.5	85.0	0.9	82.5	85.2	84.1	85.3	1.3
Cysteine	72.4	77.6	1.2*	74.3	75.9	74.4	75.3	1.7
Valine	80.3	83.2	0.9*	82.3	79.2	81.4	83.9	1.2
Methionine	90.1	89.2	0.5	89.6	90.3	90.6	89.0	0.9
Isoleucine	81.3	83.4	0.9	82.2	79.5	82.7	85.1	1.3
Leucine	86.1	87.7	0.8	85.7	87.6	86.5	87.8	1.0
Tyrosine	84.1	85.5	0.9	83.1	85.7	83.7	86.8	1.3
Phenylalanine	86.4	87.9	0.7	86.0	87.9	86.4	88.3	1.0
Histidine	81.4	84.9	0.7*	84.5	81.4	82.5	84.0	1.0
Lysine	82.7	87.1	0.7*	86.6	79.5	86.7	86.8	2.5[q]
Arginine	85.3	87.9	0.9*	86.2	85.0	87.3	87.8	1.3
Total AA	58.1	63.4	1.5*	63.7	58.1	56.9	64.2	2.1

[abc]See Table 6-5.
*$P < 0.05$.
[q]Ronozyme B has a better effect than VP ($P < 0.05$).

DISCUSSION

The primary aim of this experiment was to examine the influence of phytase and NSP enzymes on nitrogen, amino acids and energy utilization of barley, canola meal, rice bran, soybean meal, canola meal-barley and soybean meal diets.

Phytase effect Our data indicate that the addition of 900 U/kg of phytase to cereal grains, protein supplements, and blends of cereal grains and protein supplements has a positive effect on the digestibilities of dry matter and utilization of energy, although the magnitude of improvements varied not only between the ingredients but also within the individual ingredient. For example, phytase supplementation improved the digestibility of DM, retention of energy, and AME and TME in barley, canola meal, and canola meal-barley blend. The enhancement was 4.9 to 12.4 percentage units, 3.8 to 8.7 percentage units, 149 to 320 kcal/kg, and 149 to 324 kcal/kg, respectively for digestibility of DM, retention of energy, AME and TME. Addition of phytase to rice bran, soybean meal, and soybean meal-barley blend improved DM digestibility 1.5 to 3.1 percentage units, energy retention 0.8 to 3.5 percentage units, AME 34 to 129 kcal/kg, and TME 34 to 130 kcal/kg, however, the increases were not significant.

As a powerful chelating agent, phytate may reduce the digestion and absorption of organic substances by directly binding starch, protein, and fat, reducing their solubility and the exposure of their active sites for digestion and absorption. For example, Yoon et al. (1983) reported that addition of 2% phytic acid (in the form of sodium phytate) to raw wheat starch reduced the rate of digestion 50% in vitro, and high phytate bread produced a flattened blood glucose response compared with low phytate bread in vivo. Calcium-phytate complexes lowered fat digestibility by forming insoluble soaps with fatty acids in gastrointestinal tract of poultry and swine (Attech and Leeson, 1984; Leeson, 1993). It was also noted that removal of phytic acid by fermentation increased the starch digestibility (Sharma and Ketarpaul, 1997; Gupta and Khetarpaul, 1993). Base on these observations, it was concluded that phytate may have a negative effect on the energy utilization of diets. Thus, phytase inclusion can increase the energy utilization of plant-based diets that contain phytate.

Rojas and Scott (1969) were the first to report the beneficial effect of phytase on the AME values of cottonseed and soybean meals for chicks. In a later study, Miles and Nelson (1974) also reported that a crude phytase product from *Aspergillus ficuum* improved the AME

values of cottonseed meal and wheat bran by 340 and 500 kcal/kg, respectively. However, the improvement in AME with this phytase product was not observed in soybean meal. Recently, investigators confirmed the beneficial effect of phytase on the utilization of energy, although the improvements might be small. It was reported that phytase addition to cereal-based diets improved the AME by 110 to 210 kcal/kg (Farrell et al., 1993; 1994; Ravindran et al., 1998). The results of our study generally agree with some of the observation described above. For example, in our study, addition of 900 U/kg of phytase to soybean meal did not improve the energy utilization by roosters, and the improvement in AME value with phytase was 149 to 320 kcal/kg for other ingredents or blend.

The results of this experiment indicated that the addition of 900 U/kg of phytase to individual ingredients or blends of ingredients had a positive effect on the retention of N and digestibilities of amino acids and total amino acid, although the improvements were variable between the ingredients and within individual ingredients. For example, phytase supplementation significantly improved the utilization of N, digestibilities of amino acids and total amino acid for barley, canola meal, and canola meal-barley blend. The improvements in N were also observed in soybean meal, rice bran, and soybean meal meal-barley blend, however the enhancement was not significant.

The process of protein digestion can be divided into two stages: pepsin cleaves protein into oligopeptides in the stomach with pH at 2 to 4 and pancreatic enzymes further hydrolyze peptides in duodenum with pH at 6 to 7 (Reeds and Beckett, 1996). It is found that phytate may negatively affect protein utilization as follows: at low pH (3.5 to 5.0), phytate may bind the basic groups such as α-NH_2 terminal group, ε- NH_2 of lysine, histidine, and guanidyl groups of arginine in protein (Barre and van Houot, 1965); at neutral pH (6.0 to 7.0), phytase and protein may form complex with the carboxyl terminal groups and with second carboxyl groups of aspartate and glutamate. Based on these observations, the magnitude of improvements in amino acid digestibilities of basic and acidic amino acids should be higher compared with other amino acids. However, the results in this study did not indicate this trend.

Of the seventeen observed amino acids, the rank of the magnitude of improvement in digestibilities by phytase for the first eight amino acid was lysine, arginine, alanine, threonine, cysteine, serine and valine in barley; lysine, arginine, alanine, threonine, serine, proline, cysteine,

valine, and histidine in canola meal; and cysteine, lysine, threonine, serine, histidine, valine, proline, and arginine in canola meal-barley. In contrast to the theory stated above, aspartic acid and glutamic acid were ninth and fourteenth in barley, tenth and fourteenth in canola meal, and tenth and fifteenth in canola meal-barley. In addition, the magnitude of improvement in lysine and arginine were higher than that of histidine. The results of this study indicated that the side chains of the amino acids might play an important role in forming complexes with phytate, and thus the utilization of these amino acids may be reduced.

In the stomach, with a pH at about 3.5, since the pK_R value of arginine, lysine and histidine are 12, 10, and 6, respectively, most portions of these two amino acids were positively charged. On the other hand, the pK_R value of aspartic acid and glutamic acid are 3.65 and 4.25, respectively, about 85% glutamic acids are uncharged, and 50% aspartic acids are negatively charged. In the small intestine, with a pH at 6 to7, most lysine and arginine are still positively charged, most histidine is unchanged, and most aspartic and glutamic acids are negatively charged. Based on the situation described above, lysine and arginine can be easily bound by phytate in both stomach and small intestine while histidine only in the stomach, and aspartic acid can be bound by phytate via cations in both stomach and small intestine while glutamic acid only in small intestine. This may explain why the rank of the magnitude of improvement in amino acid digestibilities by phytase supplementation was lysine and arginine, histidine, and aspartic and glutamic acids. This strongly suggests that the effect of phytate on amino acid digestion plays a more important role in the stomach rather than in the small intestine, and a more important role for basic amino acids than for acidic amino acids. The reasons may be as the follows: compared with positively charged amino acids, negatively charged amino acids can not bind phytase directly, and they need a cation through which they can bind phytate. In addition, phytase activity in the small intestine decreases greatly due to the high pH value and hydrolysis of the digestive enzymes compared with that in the stomach.

The magnitudes of improvement in serine, cysteine, and threonine were next to that of lysine and arginine, the sulfhydryl group of cysteine and the hydroxyl groups of serine and threonine are relatively active, and may bind phytate via cations.

Non-starch polysaccharide effect Cereal grains, such as sorghum, wheat, and barley contain 10 to 30% non-starch polysaccharides (NSP). These dietary carbohydrates often

contribute little to meeting the energy requirement of poultry. Besides, they may affect digestion and absorption of other nutrients. These deleterious effects can be reduced or completely reversed with suitable enzyme supplementation (Rogel et al., 1987; Annison 1992). Many researchers have shown that dietary supplementation of NSP enzymes to wheat-, barley-, or oat-based diets improved performance, AME and digestibility of N and amino acids (Rotter et al., 1989; Edney et al., 1989; Almirall et al., 1995; Bedford et al., 1998). However, this beneficial effect was only observed when Ronozyme B was added to barley in our study.

The effects of NSP enzyme on AME and TME as well as N retention or amino acid digestibilities depend on the enzyme, enzyme level, and ingredients or blends. The results from this study suggested that addition of Ronozyme B to barley diet resulted in a quadratic increase in AME and TME. This was also observed in the canola meal-barley blend which showed that addition of 0.70g/kg VB or 0.44 g/kg VP reduced the AME and TME. Schutte (1990) reported that there are negative effects on feed efficiency and weight gain of broilers when enzyme inclusion levels exceeded the optimum. The antinutritional properties of NSP are associated with their long branches, and their negative effects on the digestion and absorption were reduced after the long branches were cleaved. However, an overdose of NSP enzymes hydrolyzed NSP to monosaccharides, which even have a greater ability to hold water than NSP, thus causing more serious problems to digestion and absorption of broilers.

Interaction between Phytase and NSP Enzymes The information about interaction of phytase and NSP enzymes is very limited. Wenk et al. (1993) reported that there is possibly a negative interaction between carbohydrase and phytase on nutrient utilization. In this study, however, positive interactions between phytase and NSP enzymes in N, total amino acid, and some amino acids were only observed in barley, and canola meal-barley blend. Further studies are needed for clarifying the interaction of phytase and NAP enzymes on nutrient utilization.

True metabolizable energy system is a simple and rapid assay for measuring available energy in feedstuffs, but it may not be very good method to evaluate mineral utilization because excess minerals are excreted with feces whereas excess energy is deposited as fat (Sibbald, 1982). Based on this principle, the larger the mineral requirement of the cecectomized birds or the lower the mineral levels in the feedstuffs, the better the chance of obtaining accurate estimates of mineral bioavailability. The results from this study were in agreement with the

conclusion by Sibbald. Addition of 900 U/kg of phytase improved Ca retention in barley, soybean meal-barley, and soybean meal diets which contained 0.17, 0.26, and 0.33% Ca, respectively. In contrast, the improvements in Ca retention were not significant for canola meal-barley, canola meal, and rice bran which contained 0.45, 0.65, and 3.86% Ca, respectively. For the P digestibility, the enhancements with phytase were observed in the barley and soybean meal -barley which have 0.35 and 0.42% dietary P level, and not in the other ingredients or blends which have 0.57 to 2.2% dietary P level.

Non-starch polysaccharides have the ability to bind polyvalent mineral ions and may have a negative effect on the bioavailability of some minerals. For example, corn bran could bind sufficient Ca in upper gastrointestinal tract to impact Ca absorption to a small extent under certain dietary regimes (Laszlo, 1991). Spiller et al. (1986) also showed that high dietary fiber levels reduced the transit time of digesta through the gastrointestinal tract and increased Ca excretion in human. However, the effect of NSP enzymes and interactive effect of phytase and NSP enzymes on the utilization of minerals did not show as clear a pattern as that of phytase. Based on these observations, it may be concluded that the TME system may not be a good method to evaluate mineral utilization.

IMPLICATIONS

Phytase and NSP enzyme supplementation increased AME, TME, N retention, and amino acids digestibility in barley, canola meal, and canola meal-barley, and energy and N utilization of rice bran, soybean meal-barley and blends. However, their effects on the utilization of minerals did not show as clear a pattern as that on organic nutrients. Positive interactions between phytase and NSP enzymes in N, total amino acid, and some amino acid digestibility were only observed in barley, and canola meal-barley blend. True metabolizable energy system is a simple and rapid assay for measuring available energy and amino acid digestibility in feedstuffs, but may be not a very good method to evaluate the mineral utilization.

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Chapter VII

General Discussion and Implications

Sources of Phytase

Different genetically modified phytases have been produced since recombinant DNA technology became available. This provides abundant enzyme for the feed industry. However, using phytase instead of inorganic phosphorous is not always economically favorable. Since the results from this study showed that the efficiencies of Natuphose® phytase and Phytaseed® phytase are the same, and phytase from transgenic plant should be cheaper than microbial phytase, so this would foster a more rapid application of phytase in pig and poultry nutrition.

Safety of High Dose of Phytase

Genetic modification of organisms (GMOs) by recombinant DNA technology (transgenesis) entails the introduction of a cloned gene into the genome of a cell. It will be present in the germ lines of the organism and it may be possible to establish true strains of microbes and plants. Overexpression of the gene of interest in a suitable microorganism or plant provides abundant supply of cost-effective enzyme for the feed industry, but also cause an impact on evaluating the safety of GMOs. For phytase, the dietary exposure of broilers and pigs to phytase will rise, and it is necessary to assess whether phytase would be harmful for the animals. Our data suggest there are no safety concerns when genetically engineered phytase from *Aspergillus niger* or canola seed is supplemented at levels 3 to 5 times the recommended dosage. This finding is based on the lack of any abnormal and detrimental gross pathologic and histopathologic lesions, and the fact that performance, P retention and bone mineralization of broilers continued to increase to the highest level of 2,500 U/kg.

Sensitivity Indicators for Evaluating Phytase Efficiency

Our data show that the correlation (R^2) between the measurements such as digestibilities of Ca and P, performance of poultry and swine, and bone characteristics and phytase level are greater than 0.85, which means that all of the observed measurements have good fit when regressed on phytase level. The sensitivities of the response measurements (Y) to phytase supplementation (X) should be defined as the magnitude of change in measurement value against phytase level. For the linear equation $Y = a + bX$, quadratic equation $Y = c + bX + aX^2$, and Mitcherlich equation $Y = a(1-be^{-kx})$, the sensitivity of the measurement is the first derivatives of

these equations, b and (2aX + b), respectively, for the linear and quadratic equations, and Ys = $(1\text{-}be^{-kx})/(1\text{-}b)$ for the non-linear equation. Based on the response values of these equations for evaluating the sensitivity of observed measurements to phytase in our study, P digestibility and daily gain are found to be the best indicator. Bone measurements including bone break energy and force, ash weight, and ash percentage are also sensitive criteria. Gain:feed ratio, Ca digestibility, and daily feed intake appear insensitive to phytase supplementation.

Methods for evaluating the maximum effective dose

The maximum effective dose is defined for phytase as the dose above which no improvement in efficacy is obtained. The maximum effective dose for the quadratic equation is the stationary point. The stationary points of $Y = c + bX + aX^2$ is calculated from the first derivatives ($(Y' = b + 2aX)$ of this equation: $X = - b/2a$, when $Y' = 0$. Based on the results of this study, the methods to estimate the maximum effective dose for equation $Y = a(1\text{-}be^{-kx})$ are calculated from these first derivatives of $1\text{-}be^{-kx}$ (coefficient a was not included in order to eliminate the effect of different measurements) based $X = (\ln(0.00001/bk))/(-k)$ when $Y' = 0.00001$. This is based on the principle that when the slope of the curve at a certain point is small enough, the dose of phytase above this point will produce a minimal improvement in the measurements.

Effects of Phytase on Utilization of Nitrogen, Amino Acids and energy

The results from this study clearly show that supplemental microbial phytase has a positive effect on N retention, amino acid digestibilities, and energy utilization, although these improvements depend on the species, type of diet, and phytase level. Data from these experiments indicate that side chains of the amino acids are a major factor that may affect the improvements in digestibilities of amino acids.

Effects of NSP enzyme on Utilization of Nitrogen, Amino Acids and energy

The effects of NSP enzyme on energy utilization, N retention or amino acid digestibilities depend on the type of enzyme, enzyme level, and ingredients. The results from this study show that Ronozyme B improves the nutrient utilization of barley. However, an overdose of Ronozyme B has a negative effect on the utilization of nutrients. Addition of Ronozyme VP to protein ingredients does not show any beneficial effects on the nutrient utilization.

VITA

Zhibo Zhang, son of Jie Zhang and Wenhua Gao, was born on January 19, 1965 in Chifeng (Red Mountains), Inner Mongolia, China. In 1985, He received his BS in Animal Science at the China Agriculture University, China. Then, he was a faculty member of Inner Mongolia Agriculture College, where he taught Animal Nutrition in Department of Animal Science and Biostatistics in the Veterinary School. In 1987, he returned to the China Agriculture University to continue his education where he got his Master's Degree in Animal Nutrition and Feed Sciences in 1990. As a research scientist and a manager of customer service, he worked in Beijing Feed Stuff Institution (subsidiary of Beijing Feed Co.) from 1990 to 1995 for development of new products, feed quality control, feed formulation, and customer services. He was awarded a Pratt fellowship to study for a Ph.D. in Animal Nutrition at the Virginia Polytechnic Institute and State University in the fall of 1995.

The author is a member of the American Society of Animal Science and Poultry Science Association. He has received a number of academic awards, including a *Scientific Advancement Award* (top priority) from the Chinese Ministry of Commerce, recognizing his contribution to the development and application of animal nutrition in China.

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Zhibo Zhang