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## UPDATE ON PHYTASE UTILIZATION IN SWINE

### MISE À JOUR SUR L'UTILISATION DE LA PHYTASE CHEZ LE PORC

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#### ABSTRACT

The majority of the P in cereal grains and oilseed meals is organically bound as phytic acid or phytate. This form of P is nutritionally unavailable to swine due to the lack of sufficient amounts of phytase in their digestive tract. As a result, swine diets must be supplemented with highly available, inorganic sources of P to meet their P requirements. The poor bioavailability of P in the natural feedstuffs along with high dietary levels of supplemental P result in much higher levels of P in swine manure compared with that of ruminants. Supplementing diets with microbial phytase has been shown to increase the digestibility and bioavailability of P in corn-soybean meal diets, reduce the amount of inorganic P needed to maximize growth and bone mineralization, and markedly reduce P in the manure. Phytase also seems to increase the bioavailability of Ca, Zn, and other divalent cations that otherwise bind to phytate. Some studies suggest that phytase may slightly improve energy and amino acid utilization, but other studies have not shown this response. Commercially available types of phytase (Natuphos<sup>®</sup> G, Ronozyme<sup>®</sup> P, Allzyme Phytase<sup>™</sup>) differ in the type of microorganism used to produce the enzyme, in the method in which they are produced, and in their specific mode of action, optimal pH for maximum activity, and stability during pelleting and storage of feed. Other new types of phytase are being studied experimentally. As low-phytate corn and other grains become commercially available, their use will also provide further avenues for reducing P in swine manure, especially when combined with phytase. Eventual commercialization of low-phytate soybean meal should be an additional bonus. Transgenic crops with cloned phytase genes are on the horizon, and pigs having high levels of phytase in their saliva may someday be practical and accepted. These new technologies offer substantial benefits to swine production by reducing environmental problems associated with excess P excretion.

#### RÉSUMÉ

Le phosphore (P) des grains et des tourteaux d'oléagineuses est en majeure partie fixé par des liens organiques, sous forme d'acide phytique ou de phytate. Cette forme de P n'est pas assimilable par les porcs en raison de la faible quantité de phytase présente dans le tube digestif de ces animaux. Ainsi, pour répondre aux besoins en P des porcs, leurs rations doivent être additionnées de sources de P inorganique et facilement disponible. La faible biodisponibilité du P naturellement présent dans les ingrédients alimentaires jumelée à la forte supplémentation en P des rations se traduit par des concentrations de P beaucoup plus élevées dans le fumier des porcs que dans celui des ruminants. On a démontré que la supplémentation avec de la phytase d'origine microbienne augmente la digestibilité et la biodisponibilité du P des rations à base de maïs et tourteau de soya, réduit la quantité de P inorganique nécessaire pour maximiser la croissance et la minéralisation osseuse et réduit sensiblement le P du fumier. La phytase semble également augmenter la biodisponibilité du Ca, du Zn et d'autres cations bivalents qui, autrement, se fixent au phytate. Selon certaines études, la phytase pourrait légèrement améliorer l'utilisation de l'énergie et des acides aminés; par contre, d'autres études ne sont pas parvenues à ce résultat. Les produits de phytase offerts sur le marché (Natuphos<sup>®</sup> G, Ronozyme<sup>®</sup> P, Allzyme Phytase<sup>MC</sup>) diffèrent par le type de microorganisme utilisé pour produire l'enzyme, par la

méthode avec laquelle ils sont produits, par leur mode d'action spécifique, par le pH auquel ils présentent une activité maximale et par leur stabilité au cours de l'agglomération et de l'entreposage des aliments. De nouveaux types de phytase sont présentement à l'étude. La commercialisation de maïs et d'autres grains pauvres en phytate ouvrira de nouvelles avenues pour la réduction du P dans le fumier de porcs, particulièrement lorsque ces ingrédients seront servis en combinaison avec la phytase. L'arrivée éventuelle sur le marché de tourteau de soya pauvre en phytate devrait être un atout additionnel. Les cultures transgéniques dotées de gènes de phytase clonés ne sont plus loin, et des porcs produisant une salive riche en phytase pourraient un jour être utilisés et acceptés. En permettant une réduction des problèmes environnementaux associés à l'excrétion excessive de P, ces nouvelles technologies comportent des avantages considérables pour la production porcine.

## Introduction

Phosphorus (P) has long been recognized as one of the most important minerals required by livestock and poultry. One of its major roles is that it complexes with calcium (Ca) to give rigidity to bones. In addition, P is an integral part of many organic compounds and it plays important roles in energy and protein metabolism. Almost every series of biochemical reactions that occurs in muscle, blood, and other soft tissues involves this important mineral.

Providing animals with adequate dietary P is not only important for the proper development and maintenance of the skeleton, but it is needed to optimize growth, efficiency of feed utilization, and lean tissue synthesis. The consequences of feeding diets with inadequate P to growing animals can be disastrous. For example, Table 1 shows the results of feeding a P deficient diet to growing-finishing pigs on growth, carcass leanness, and bone mineralization.

**Table 1.** Effects of phosphorus level on pig performance<sup>a</sup>

Item	Adequate diet 0.50% P	Deficient diet <sup>b</sup> 0.32% P
Daily gain, kg	0.77	0.54
Feed:gain	3.08	3.82
Carcass lean cuts, %	58.5	55.9
Bone strength, kg	145	76
Bone ash, %	57.4	52.8

<sup>a</sup>Cromwell et al. (1972). University of Kentucky experiment involving 21 pigs from 17 to 92 kg body weight.

<sup>b</sup>The low P diet consisted of corn and soybean meal without any supplemental inorganic P.

## Phosphorus Requirements

The best source of unbiased information on the P requirements for swine is the National Research Council (NRC, 1998) publication, *Nutrient Requirements of Swine*. The NRC is part of the National Academy of Science, a private organization that was established 140 years ago to advise the Nation on issues of science and technology. For over 70 years, the NRC has established guidelines for the feeding of domestic animals and has published estimates of nutrient requirements for swine since 1944, with the most recent edition (10<sup>th</sup> edition) published in 1998. Table 2 shows the NRC estimates of the P, bioavailable P, and Ca requirements of swine.

For many years, universities and feed companies published recommended nutrient allowances for swine and other livestock and poultry. The general approach has been to use NRC standards as the base, then add extra amounts of most of the nutrients as safety factors. Little attention was paid to "oversupplementing" diets with nutrients as long as it was not overly expensive. Those nutrients that were in excess of the animals' requirements were simply stored in the body tissues or excreted in the manure. However, that situation has changed dramatically during the past several years, especially with respect to

nitrogen (N) and P. Environmental issues relating to water quality have forced livestock and poultry producers to pay much closer attention to their feeding programs so as to limit the amount of N and P in the manure produced by animals.

**Table 2.** Calcium and phosphorus requirements of swine<sup>a</sup>

Stage	Ca (%)	P (%)	Bioavailable P (%)
Growing pigs			
3-5 kg	0.90	0.70	0.55
5-10 kg	0.80	0.65	0.40
10-20 kg	0.70	0.60	0.32
20-50 kg	0.60	0.50	0.23
50-80 kg	0.50	0.45	0.19
80-120 kg	0.45	0.40	0.15
Breeding gilts and sows			
Gestation	0.75	0.60	0.35
Lactation	0.75	0.60	0.35
Boars	0.75	0.60	0.35

<sup>a</sup>NRC (1998).

## Environmental Issues

Environmental challenges in agriculture represent one of the major issues facing the animal industry. Farm animals produce nearly 160 million tonnes of manure annually (Sweeten, 1992; Table 3). Most of the swine manure is produced in confinement units where the nearby land base often is insufficient to accommodate the waste in an environment-friendly manner. Excess N and P in animal manure can contribute to surface and ground water pollution, and N from manure can contribute to aerial ammonia and other gasses, including those with offensive odors.

Pig manure is quite high in both N and P (Table 3). The high N content is attributable to several factors. First, diets are relatively high in crude protein due to the large amounts of amino acids that are needed to support a high rate of lean growth in swine. Second, the dietary protein is not completely digested as the feed passes through the digestive tract which results in approximately 15 to 20% of the dietary N passing out of the pig in the feces. Third, and probably most important, the pattern of the amino acids that are digested from the protein and absorbed into the bloodstream is far from ideal in comparison with the pattern of amino acids that is needed for growth and other functions. Thus, the N from those amino acids that are in excess of the pig's requirements is converted into urea and excreted in the urine.

The major reason for the high concentration of P in swine and poultry manure is that most of the P in cereal grains and oilseed meals is bound in an organic complex called myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate, commonly referred to as phytic acid or phytate. From 55 to 80% of the P in cereal grains and oilseed meals is in this form (Nelson et al., 1968a). In order for nonruminants to utilize the P from phytate, the phosphate groups must first be enzymatically released from the complex, a process that requires the enzyme, phytase. Unfortunately, pigs and poultry do not have sufficient amounts of phytase in their digestive tract to degrade phytate, so most of the P from the grain and oilseed meals is excreted in the feces. In contrast, ruminants utilize phytate quite well because of the abundance of phytase produced by rumen microorganisms.

## Diet Changes to Modify Composition of Manure

A recent publication by the Council for Agricultural Science and Technology reviewed nutritional strategies that can be implemented to reduce the N and P in manure (CAST, 2002). With respect to N, feeding diets that do not have excess protein (amino acids) is one of the most effective means of

reducing N excretion. Reducing dietary protein and adding amino acids is also an effective strategy. Research at our station has shown that feeding pigs a diet with 2% less protein plus 0.15% added lysine is essentially equivalent in nutritional value to a higher protein diet, but it will reduce N excretion by 20 to 30% (Cromwell, 1996). Further reductions in dietary protein are possible if the diet is supplemented with additional lysine along with certain other amino acids such as threonine, tryptophan, and methionine. N excretion can also be reduced by using high quality protein sources with good amino acid profiles, by using feed ingredients that have more highly digestible protein, and by formulating diets on an “ideal protein” basis such that those amino acids that exceed the pig’s requirements are not excessive.

**Table 3.** Quantities of manure, nitrogen, and phosphorus excreted annually by livestock and poultry in the United States (dry matter basis)<sup>a</sup>

Species	Manure (million tonnes)	Mineral Conc. (%)		Excretion (thousand tonnes)	
		N	P	N	P
<b>Ruminants</b>					
Beef cattle	96.6	3.96	1.07	3,828	1,029
Dairy cattle	29.1	3.75	0.79	1,091	230
Sheep	1.8	3.89	0.56	70	10
<b>Nonruminants</b>					
Swine	15.5	4.71	2.97	730	460
Poultry	15.4	5.13	1.62	790	250
<b>Total</b>	<b>158.4</b>			<b>6,509</b>	<b>1,979</b>

<sup>a</sup>Adapted from Sweeten (1992).

Similarly, P excretion can be reduced by feeding diets that do not have excessive levels of supplemental P. For example, feeding a diet with 0.2% more P than needed by growing-finishing pigs (a common practice a few years ago) will result in a 70% greater P excretion compared with feeding P levels that meet NRC (1998) standards. Formulating diets on an “available P” basis rather than a “total P” basis also helps in that it allows one to more precisely meet the P requirements without having overages of nutrients.

Several relatively new technologies now exist that have potential for reducing P excretion by swine. One such technology is the inclusion of microbial phytase in diets. Another is the use of grains and oilseed meals with reduced phytate content. The rest of this paper will address these and other new technologies that have potential for reducing P excretion by swine.

## Phytase

One of the greatest breakthroughs in recent years has been the commercialization of the phytase enzyme for use in swine and poultry feeds and its positive impact on P excretion. Numerous reviews have been written on phytase, some of which include those of Coelho and Kornegay (1996), Kornegay (1999), and Liao et al. (2002).

The technical name for phytase is myo-inositol hexakisphosphate phosphohydrolase. This enzyme acts on the phytate molecule yielding ortho-phosphate and several phosphoric esters ranging from pentaphosphates to monophosphates. Generally the release order is from the 3-position initially, then from the 4, 5, 6, 1, and 2 positions in that order. Phytase is widely distributed in yeasts, fungi, and bacteria. *Aspergillus* (especially *A. ficuum*) microorganisms produce large amounts of this enzyme. In addition, some seeds (rye, wheat, triticale) have relatively high levels of phytase.

The first animal studies with phytase were conducted by Nelson and co-workers 35 years ago. In their initial study, these researchers fed low-P diets containing soybean meal that had been previously treated

with phytase from *Aspergillus ficcum* and reported dramatic responses in growth and bone ash (Nelson et al., 1968b). Subsequently, they added phytase to low-P diets and showed similar responses (Nelson et al., 1971). They pointed out, however, that the enzyme was much too expensive to use in practice.

The discovery of mutant strains of *Aspergillus* that produced high levels of phytase along with the advent of recombinant-derived phytase in the late 1980s and early 1990s stimulated renewed interest in phytase research. Studies by Simons et al. (1990), Jongbloed et al., (1992), Cromwell et al. (1993), Lei et al. (1993ab), and Young et al. (1993) were the first to evaluate these new forms of phytase in pigs. During the past 10 years, more than 80 refereed papers have been published in the *Journal of Animal Science* and *Poultry Science* and over 350 abstracts on phytase have been presented at the annual and sectional meetings of the American Society of Animal Science and Poultry Science Association. Until a few years ago, most of the research has been conducted with Natuphos<sup>®</sup>, a commercial source of phytase produced by *Aspergillus niger* and marketed by BASF (Mount Olive, NJ). This form of recombinant-produced phytase was approved in the USA by the FDA in 1995 for use in animal feeds. Natuphos<sup>®</sup> is also approved for use in Canada.

An overview of the research conducted with phytase conclusively shows that digestibility (i.e., bioavailability) of P in cereal grains and oilseed meals is markedly improved with phytase supplementation. In studies conducted at our university, we found that the bioavailability of P is tripled, from approximately 15% in a corn-soybean meal blend to over 45% when phytase is added to the mix (Cromwell et al., 1995). This means that reduced amounts of supplemental inorganic P are needed in swine diets to maximize growth and bone mineralization. As a result, fecal P is markedly reduced when phytase is included in the diet. These effects are shown in two studies conducted at the University of Kentucky (Tables 4 and 5).

**Table 4.** Effects of phytase in low phosphorus diets on performance and bone strength in finishing pigs<sup>a</sup>

	0.50	0.40	0.40	0.40	0.40
Ca, %: <sup>b</sup>	0.50	0.40	0.40	0.40	0.40
P, %: <sup>b</sup>	0.40	0.30	0.30	0.30	0.30
Phytase, units/kg: <sup>c</sup>	-	-	250	500	750
Daily gain, kg	0.89	0.86	0.85	0.86	0.91
Feed:gain <sup>d</sup>	3.23	3.45	3.33	3.26	3.29
Bone strength, kg <sup>d</sup>	188	163	179	183	190

<sup>a</sup>Cromwell et al. (1997), University of Kentucky. Involved four pens of six pigs per treatment from 57 to 110 kg body weight.

<sup>b</sup>Levels of Ca and P were 0.05% higher during the first 4 weeks of the experiment, from 57 to 80 kg body weight. The levels shown in the table were fed during the final 5 weeks, from 80 to 110 kg. All of the P in the 0.30% P diets was supplied by corn and soybean meal.

<sup>c</sup>Natuphos<sup>®</sup> (BASF, Mt. Olive, NJ).

<sup>d</sup>Treatment effect (P < 0.05).

**Table 5.** Effects of phytase in low phosphorus diets on phosphorus excretion in finishing pigs<sup>a</sup>

	0.50	0.40	0.40	0.40
Ca, %:	0.50	0.40	0.40	0.40
P, %: <sup>b</sup>	0.40	0.30	0.30	0.30
Phytase, units/kg: <sup>c</sup>	-	-	250	500
Dietary P intake, g/day	11.17	8.43	8.53	8.27
Retained P, g/day	3.68	2.03	3.36	3.78
Fecal P excreted, g/day <sup>d</sup>	7.23	6.31	4.86	4.03
Urinary P excreted, g/day	0.26	0.09	0.31	0.46
Total P excreted, g/day <sup>d</sup>	7.49	6.40	5.16	4.49
Reduction in total P excretion, %	-	-	31	40

<sup>a</sup>Pierce et al. (1997), University of Kentucky. The balance experiment involved six pigs (87 kg body weight) per treatment. Feed intake averaged 2.63 kg/day.

<sup>b</sup>All of the P in the low P diets was supplied by corn and soybean meal.

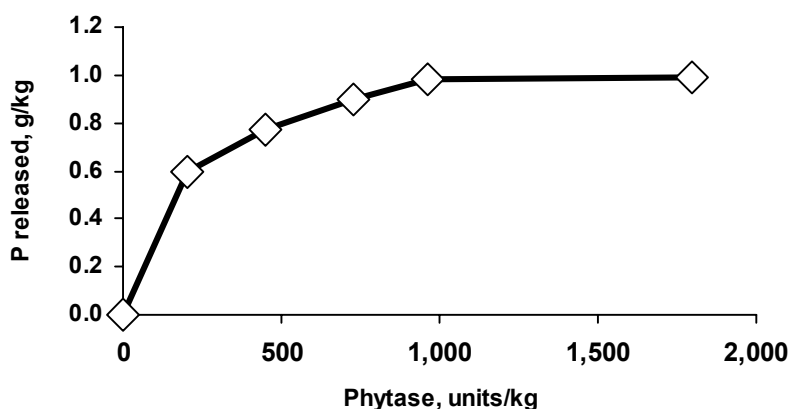
<sup>c</sup>Natuphos<sup>®</sup> (BASF, Mt. Olive, NJ).

<sup>d</sup>Treatment effect (P < 0.01).

The effectiveness of phytase is associated with the amount that is included in the diet. Early studies by Jongbloed et al. (1996) showed that responses in pigs maximized at approximately 1,000 phytase units per kg of diet (Figure 1). More recently, studies have shown that maximum or near-maximum responses can be achieved with lower levels of phytase, especially, when Ca levels are also reduced in the diet. Apparently this is due to the fact that excess Ca ions tend to inhibit the activity of phytase (Lei et al., 1994).

The amount of inorganic P that can be eliminated from the diet when phytase is added can be calculated with regression techniques using bone traits, as illustrated in Figure 2. Early studies indicated that 1,000 units of phytase per kg of diet released approximately 1 g of P per kg of diet (1 g/kg is equivalent to 0.10 of a percentage point). Several of our initial studies supported that relationship. However, more recent tests indicate that less phytase is required to release this amount of P from phytate particularly if dietary Ca is also reduced by 0.05 to 0.10 of a percentage point. The bone strength data of Table 4 in which finishing pigs were fed diets with 0.10% less Ca and P and various levels of Natuphos<sup>®</sup> phytase indicated that 650 units of phytase per kg of diet released 1 g of inorganic P per kg of diet (or 0.10% P). A similar value of 715 phytase units per kg for release of 1 g of P per kg was recently reported by Gainer et al. (2003) for Natuphos<sup>®</sup> phytase. These levels of phytase are more than the 470 to 550 units of phytase per kg proposed by Kornegay (1999), Skaggs and Kornegay (1999), and Robbins et al. (2000), but less than the 930 units of Natuphos<sup>®</sup> phytase per kg recently reported by Augspurger et al. (2003a) to release 1 g of P from phytate per kg of diet.

**Figure 1.** Phosphorus released from phytate with various levels of phytase

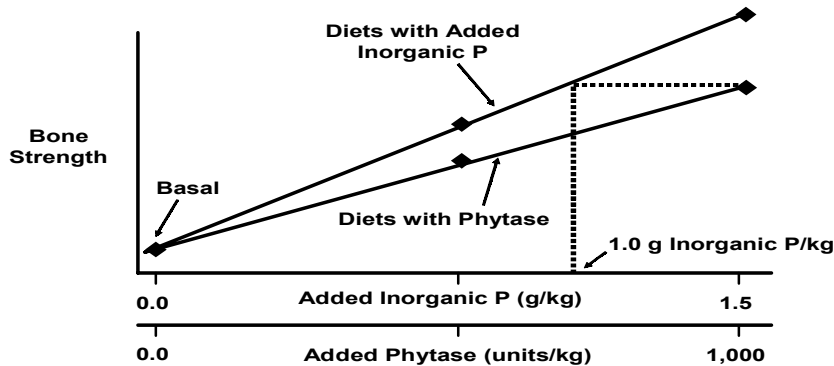


Although less clear, there is evidence that phytase increases the absorption of Ca, Mg, Zn, and other divalent cations due to the disruption of the phytic acid molecule which acts as a chelating agent (Adeola, 1996). Even less clear is the impact of phytase on amino acid availability. Some research suggests that phytase improves the ileal digestibility of amino acids (Mroz, 2002; Liao et al., 2002), but other research has shown no effect on amino acid utilization from phytase additions (Traylor et al., 2001; Adeola, 2002).

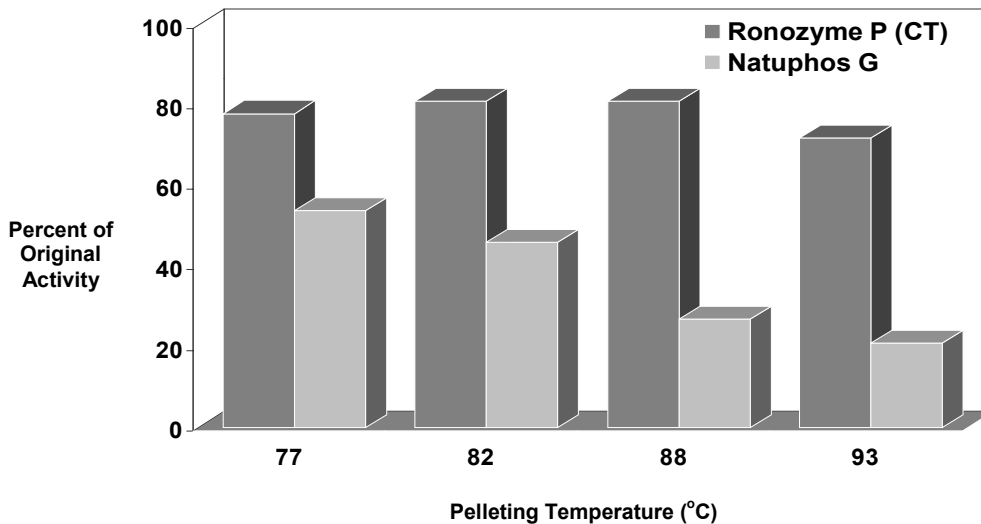
Recently, several new forms of phytase have been commercialized. One of the new phytases released in the USA market approximately two years ago and in Canada several months ago is Ronozyme<sup>®</sup> P (Roche Vitamins, Parsippany, NJ), a phytase produced by *Peniophora lycii*. This particular phytase is a 6-phytase, meaning that it initially removes the P from the 6 position of phytate as opposed to *Aspergillus*-derived phytase (Natuphos<sup>®</sup> G) which initially removes the P from the 3 position. *Peniophora* phytase is most effective at pH 4.5 compared with a slightly higher and broader optimal pH range of 5.5 to 6.5 for *Aspergillus* phytase. A major advantage for Ronozyme<sup>®</sup> P phytase is that it is manufactured using a patented coating process that reduces the loss of activity associated with the high temperatures during the pelleting process as compared with Natuphos<sup>®</sup> phytase (Figure 3). In tests at 63 feed mills, there was an 82% retention of phytase activity following pelleting at an average temperature of 86°C (Ward and

Wilson, 2001). In swine diets, these two forms of phytase were found to be equally effective in several studies (Skaggs and Kornegay, 1999; Robbins et al., 2000; Lynch et al., 2002; Brumm, 2002; Lawrence et al., 2003), but the two sources differed in efficacy in studies by James et al. (2002) and Augspurger et al. (2003a). Various combinations of the two types of phytase to provide a total of 500 phytase units per kg of diet indicated no evidence of synergistic responses as might have been expected, since their mode of action is slightly different (Lawrence et al., 2003).

**Figure 2.** Bioequivalence of phytase and inorganic phosphorus



**Figure 3.** Stability of two sources of phytase to pelleting temperature



Another microbial phytase that is commercially available in the USA and Canada is Allzyme Phytase™ (Alltech, Nicholasville, KY). This phytase, derived from a non-genetically modified strain of *Aspergillus niger* and produced by solid state fermentation, has been shown to be effective (Wu and Ravindran, 2002; Park et al., 2003b). In one study, 500 phytase units per kg released 0.1 percentage unit of P (Park et al., 2003a).

Other new forms of *Escherichia coli* phytase have recently been developed using recombinant and cloning techniques (Rodriguez et al., 1999; Leeson et al., 2000; Augspurger et al., 2003a) and have been shown to be very effective. In one test, an *E. coli* phytase (EcoPhos™, Phytax, Portland, ME) was 1.3 to 2.5 times more effective than *Aspergillus* or *Peniophora* phytases (Augspurger et al., 2003a). In a later study, this same phytase was only slightly more effective than *Aspergillus* phytase (Gainer et al., 2003). Studies with this *E. coli* phytase have shown that 500 to 625 phytase units per kg of diet release 0.10% P (Augspurger et al., 2003b). EcoPhos™ is not presently cleared for use in animal feed.

## Low-Phytate Corn and Soybean Meal

In 1990, Raboy and co-workers identified several mutant genes in corn that suppressed the synthesis of phytic acid in the kernel without affecting the amount of total seed P (Raboy et al., 1990). In 1996, we obtained corn from Pioneer Hi-Bred International (Optimum Quality Grains/Dupont Specialty Grains, Johnston, IA) containing one of these mutant genes (*lpa1*) for animal studies. This corn contained half as much phytate P (0.10 vs 0.20%) and over three times as much inorganic P (0.18 vs 0.05%) as a near-isogenic conventional corn (Table 6). Using slope-ratio procedures, we found that the P in low-phytate corn was about three to four times more bioavailable for pigs than the P in normal corn. Specifically, the low-phytate gene increased the bioavailability of P from approximately 20% in normal corn to over 75% in low-phytate corn (Cromwell et al., 1998). Similar results were also reported by Spencer et al. (2000b) using similar procedures and by Veum et al. (2001) using an *in vitro* procedure.

**Table 6.** Composition of normal and low-phytate corn and normal and low-phytate, low oligosaccharide soybean meal<sup>a</sup>

Item	Normal corn	Low-phytate corn	Normal SBM	Low-phytate SBM
	%	%	%	%
Crude protein	8.50	8.50	53.6	55.3
Lysine	0.23	0.24	3.03	3.18
Methionine	0.15	0.15	0.80	0.83
Calcium	0.01	0.01	0.35	0.36
Total phosphorus	0.25	0.28	0.70	0.77
Phytate phosphorus	0.20	0.10	0.48	0.22
Inorganic phosphorus	0.05	0.18	0.22	0.55
Carbohydrates				
Sucrose			7.22	12.32
Raffinose			0.91	0.55
Stachyose			5.20	0.53

<sup>a</sup>Pioneer Hi-Bred International (Optimum Quality Grains; DuPont Specialty Grains) Johnston, IA. The normal and low-phytate corns were near-isogenic as were the soybeans from which the soybean meals were prepared.

Experiments with both growing and finishing pigs indicated that feeding pigs low-phytate corn-soybean meal diets containing 0.10 to 0.12% less total P than normal results in similar performance and bone mineralization as in pigs fed normal corn-soybean meal diets (Pierce et al., 1998ab). This reduction in total dietary P along with the greater bioavailability of P in low-phytate corn is associated with a 43% reduction in excreted P (Pierce, 1999), as shown in Table 7.

Soybeans that are low in both phytic acid and oligosaccharides have also been produced by the Pioneer Hi-Bred/DuPont group. Both compounds are reduced due to the fact that the synthesis of oligosaccharides (from soluble sugars) and phytic acid use similar metabolic pathways, and the down-regulation of key enzymes in the synthesis pathways of the mutant soybeans affects the amounts of both compounds. In our initial studies with soybean meal produced from low-phytate, low-oligosaccharide soybeans, we found that the mutant soybean meal had approximately half as much phytate P (0.22 vs

0.48%) and more than twice as much inorganic P (0.55 vs 0.22%) as soybean meal from conventional soybeans (Table 6).

Slope-ratio studies conducted at the University of Kentucky have shown that the P in low-phytate soybean meal is approximately 50% bioavailable, compared with 20% in normal soybean meal (Cromwell et al., 2000a). Spencer et al. (2000a) reported similar estimates of P bioavailability in the two soybean meals.

In other studies, pigs fed diets consisting of low-phytate corn and low-phytate soybean meal with no supplemental inorganic P grew as fast and efficiently, had similar bone traits, and excreted 53% less P (Table 8) than pigs fed diets containing conventional corn and soybean meal supplemented with sufficient inorganic P to meet the pig's P requirement (Cromwell et al., 2000b).

**Table 7.** Phosphorus balance of finishing pigs fed corn-soy diets with normal or low-phytate corn<sup>a</sup>

	Normal corn	Low-phytate corn
Total P, % <sup>b</sup>	0.42	0.30
Bioavailable P, % <sup>c</sup>	0.20	0.20
P intake, g/day <sup>d</sup>	11.89	8.66
P retained, g/day	5.31	4.92
P excreted, g/day		
Feces <sup>d</sup>	5.85	3.64
Urine <sup>d</sup>	0.73	0.10
Total <sup>d</sup>	6.58	3.74
Reduction in P excretion, % <sup>e</sup>		43

<sup>a</sup>Pierce (1999), University of Kentucky. A 5-day balance experiment involving five pigs/treatment at approximately 100 kg body weight. Diets contained 0.65% lysine.

<sup>b</sup>Normal corn-soy diet contained 0.12% added P from dicalcium phosphate. Low-phytate corn-soy diet had no additional inorganic P.

<sup>c</sup>Based on the following bioavailabilities of P: normal corn, 20%; low-phytate corn, 75%; soybean meal, 25%; dicalcium phosphate, 100%.

<sup>d</sup>Normal vs low-phytate corn ( $P < 0.01$ ).

<sup>e</sup>Reduction in P excretion compared with pigs fed the normal corn-soy diet.

**Table 8.** Performance of growing pigs fed corn-soy diets with normal or low-phytate corn and normal or low-phytate soybean meal with varying amounts of supplemental inorganic phosphorus<sup>a</sup>

	Normal corn + normal soybean meal			Low-phytate corn + low-phytate soybean meal		
	0.20	0.10	0.00	0.20	0.10	0.00
Supplemental P, %:	0.20	0.10	0.00	0.20	0.10	0.00
Total P, %:	0.56	0.46	0.36	0.59	0.49	0.39
Bioavailable P, % <sup>b</sup>	0.27	0.17	0.07	0.44	0.34	0.24
Daily gain, kg <sup>c</sup>	0.80	0.75	0.63	0.81	0.80	0.79
Feed/gain <sup>c</sup>	2.22	2.30	2.62	2.24	2.16	2.19
Bone traits						
Femur strength, kg <sup>c</sup>	292	219	157	313	305	292
MM strength, kg <sup>c,d</sup>	83	61	42	74	84	80
Relative strength <sup>c,e</sup>	100	74	52	98	102	98
MM ash, % <sup>c</sup>	56.5	54.2	51.7	56.5	56.9	56.2
Fecal P excretion, g/day <sup>f</sup>	7.0	6.2	5.3	5.1	4.0	3.3
Reduction in fecal P excretion, % <sup>g</sup>				27	43	53

<sup>a</sup>Cromwell et al. (2000b), University of Kentucky. The experiment involved eight pens of individually fed pigs from 22 to 49 kg body weight. Dietary lysine was 0.95% and dietary calcium was 0.65%.

<sup>b</sup>Based on the following bioavailabilities of P: normal corn, 20%; low-phytate corn, 75%; normal soybean meal, 20%; low-phytate soybean meal, 50%; dicalcium phosphate, 100%.

<sup>c</sup>Linear effect of added P in normal corn-normal soybean meal diets ( $P < 0.01$ ).

<sup>d</sup>Average of third and fourth metacarpal and metatarsal bones.

<sup>e</sup>Relative to the normal corn diet with the highest level of P. Mean of femur and MM.

<sup>f</sup>Linear effect of added P in both diets ( $P < 0.01$ ).

<sup>g</sup>Reduction in P excretion compared with pigs fed the normal corn-normal soy diet with 0.56% total P.

## **Phytase and Low-Phytate Feedstuffs**

As discussed, the addition of phytase to conventional corn-soybean meal diets and the use of low-phytate corn and low-phytate soybean meal are both very effective means of improving P utilization and reducing P excretion. Research at our station has shown that the combination of phytase and low-phytate feed ingredients is even more effective in improving P utilization and reducing P excretion in pigs. Studies by Pierce and Cromwell (1999ab) with low-phytate corn and normal soybean meal and more recently by Xavier et al. (2003abc) with low-phytate corn and low-phytate soybean meal have clearly shown that phytase is effective when included in diets consisting of low-phytate feedstuffs. For example, P bioavailability was increased from 35% in a 3:1 blend of conventional corn and soybean meal to 64% when phytase was added (Xavier et al., 2003a); whereas, adding phytase to a 3:1 blend of low-phytate corn-soybean meal increased P bioavailability from 79% to 90%. The greater numerical improvement in P bioavailability from phytase addition to the conventional versus the low-phytate corn-soybean meal mix is attributed to the conventional feedstuffs having more substrate (phytate) upon which the enzyme can act. On the other hand, phytase seemed to be equally effective in degrading the phytate P in the two types of corn-soybean meal mixes in that it converted approximately one-half of the unavailable P to an available form.

Tables 9 and 10 show the impact of the combination of phytase addition to diets containing low-phytate corn and low-phytate soybean meal for young, growing pigs. In these studies, feeding pigs low-phytate corn-soy diets with phytase reduced P excretion by 62 to 68% compared with controls fed conventional corn-soy diets without phytase (Xavier et al., 2003bc).

## **Endogenous and Bioengineered Phytase in Plants**

Some crops possess relatively high levels of endogenous phytase in their seeds. This was first shown by McCance and Widdowson (1944) and Mollgaard (1946) who demonstrated that wheat, wheat byproducts (bran, middlings), rye, and to a lesser extent, barley, contain significant amounts of phytase. In studies at the University of Kentucky (Cromwell and Coffey, 1993), we have found a considerably higher bioavailability of P in wheat (50%), wheat middlings (41%), wheat bran (29%), and barley (30%) than that in corn (14%) (Table 11). Wheat bran phytase has also been shown to increase the utilization of P in other feedstuffs in the diet.

Biotechnology has now been used to insert a phytase gene into alfalfa (Ullah et al., 2002) and canola (McHughen, 2000), which greatly increases their phytase content. A recent study by University of Wisconsin researchers showed that alfalfa leaf phytase was effective in increasing P digestibility and reducing P excretion (Saddoris et al., 2003). Commercialization of crops with inserted phytase genes could be important in that it would be an alternative vehicle for supplying phytase to nonruminant diets in order to reduce P excretion.

## **Transgenic Pigs Possessing Salivary Phytase**

Scientists at the University of Guelph (Golovan et al., 2001; Forsberg et al., 2002) have recently produced several lines of transgenic pigs that have high levels of phytase in their saliva. In their studies, the true digestibility of soybean meal P by the transgenic pigs was very high (88 to 99%) and excretion of P was reduced by as much as 75% in weanling pigs (Table 12). They attributed this dramatic response to the much larger amount of enzyme continuously present in the stomach of the transgenic pig due to the copious secretion of saliva when feed is consumed. Consequently, these transgenic pigs may have delivered as much as 200,000 units of phytase to the digestive tract during the consumption of 1 kg of feed. This is considerably more than the normal phytase supplementation of 300 to 1,000 units of phytase per kg of feed. Whether this new finding will become practical remains to be seen, but it certainly opens up a new biological approach for reducing P pollution in animal agriculture.

**Table 9.** Performance of growing pigs fed corn-soy diets with normal or low-phytate corn and normal or low-phytate soybean meal without and with added phytase<sup>a</sup>

	Normal corn + normal soybean meal			Low-phytate corn + low-phytate soybean meal		
Supplemental P, %:	0.20	0.10	0.10	0.10	0.00	0.00
Total P, %:	0.59	0.49	0.49	0.47	0.37	0.37
Phytase, units/kg:	-	-	750	-	-	750
Daily gain, kg <sup>b</sup>	0.75	0.70	0.72	0.79	0.69	0.71
Feed/gain <sup>b</sup>	1.80	1.95	2.01	1.86	1.87	1.88
Bone traits						
Femur strength, kg <sup>b</sup>	285	190	267	278	201	266
MM strength, kg <sup>bc</sup>	71.8	56.2	73.8	78.0	61.7	72.1
Relative strength <sup>bd</sup>	100	73	98	103	78	97
Apparent digestibility of P, % <sup>b</sup>	44	33	49	60	55	70
Fecal P excretion, g/day <sup>b</sup>	4.49	4.45	3.67	2.79	2.11	1.45
Reduction in fecal P excretion, % <sup>e</sup>		-	18	38	53	68

<sup>a</sup>Xavier et al. (2003b), University of Kentucky. The experiment involved six pens of individually fed pigs from 15 to 42 kg body weight. Dietary lysine was 1.05% and dietary calcium was 0.65%.

<sup>b</sup>Treatment effect ( $P < 0.01$ ).

<sup>c</sup>Average of third and fourth metacarpal and metatarsal bones.

<sup>d</sup>Relative to the normal corn diet with the highest level of P. Mean of femur and MM.

<sup>e</sup>Reduction in P excretion compared with pigs fed the normal corn-normal soy diet with 0.59% total P.

**Table 10.** Effects of supplemental phytase in conventional and low-phytate corn-soybean meal diets on phosphorus excretion in growing pigs<sup>a</sup>

	Normal corn + normal soybean meal		Low-phytate corn + low-phytate soybean meal	
Ca, %:	0.55	0.50	0.55	0.50
P, %: <sup>b</sup>	0.48	0.38	0.35	0.35
Phytase, units/kg: <sup>c</sup>	-	750	-	750
Dietary P intake, g/day	9.91	7.72	7.24	7.30
Retained P, g/day	4.71	4.12	4.52	5.32
Fecal P excreted, g/day <sup>d</sup>	4.94	2.97	2.69	1.79
Urinary P excreted, g/day <sup>d</sup>	0.26	0.63	0.04	0.18
Total P excreted, g/day <sup>d</sup>	5.20	3.60	2.73	1.98
Reduction in total P excretion, %		31	48	62

<sup>a</sup>Xavier et al. (2003c), University of Kentucky. The balance experiment involved three pigs (60 kg body weight) per treatment. Feed intake averaged 2.07 kg/day.

<sup>b</sup>All of the P in the low-phytate diets was supplied by corn and soybean meal.

<sup>c</sup>Natuphos® (BASF, Mt. Olive, NJ).

<sup>d</sup>Treatment effect ( $P < 0.01$ ).

## Summary

Several technologies are available to nutritionists to formulate low-P, environmentally friendly diets for swine. Reducing the dietary P level and adding phytase to the diet is presently one of the most effective strategies that can be used to reduce P excretion. As low-phytate corn and other grains become commercially available, their use will also provide further avenues for reducing P in swine and poultry manure. Eventual commercialization of low-phytate soybean meal will be an additional bonus. Transgenic crops with cloned phytase genes are on the horizon, and pigs having high levels of phytase in their saliva may someday be practical and accepted. These and other new technologies provide exciting times for the future of animal agriculture.

**Table 11.** Bioavailability of phosphorus in cereal grains, grain byproducts, high protein meals, and inorganic phosphates for pigs<sup>a</sup>

Feedstuff	Bioavailability of P, % <sup>b</sup>	Feedstuff	Bioavailability of P, % <sup>b</sup>
Cereal grains		High protein meals - plant origin	
Corn	14	Cottonseed meal	1
Grain sorghum	20	Sunflower meal	3
Oats	22	Peanut meal	12
Barley	30	Canola meal	16
Triticale	46	Soybean meal, dehulled	23
Wheat	50	Soybean meal, 44% CP	31
Corn, high moisture	53	Soybean meal, low-phytate	50
Corn, low-phytate	75		
Grain byproducts		High protein meals - animal origin	
Oat groats	14	Meat and bone meal	90
Hominy feed	14	Dried skim milk	91
Corn gluten meal	15	Dried blood meal	92
Rice bran	25	Fish meal	93
Wheat bran	29	Dried whey	96
Brewers dried grains	33	Inorganic phosphates	
Cooked cereal fines	40	Steamed bone meal	82
Wheat middlings	41	Defluorinated phosphate	97
Corn gluten feed	59	Monocalcium phosphate	100
Distillers grains plus solubles	76	Dicalcium phosphate	100
Miscellaneous		Phytase addition	
Soybean hulls	78	Corn-soy diet without phytase	15
Alfalfa meal	100	Corn-soy diet with phytase	45

<sup>a</sup>Based on University of Kentucky research (Cromwell and Coffey, 1993, and other studies).

<sup>b</sup>Relative to the bioavailability of P in monosodium or monocalcium phosphate, which is considered to be 100% bioavailable.

**Table 12.** Ability of transgenic pigs possessing high salivary phytase to utilize phytate phosphorus<sup>a</sup>

Item	Control pigs	Transgenic pigs
Median salivary phytase, units/ml	0	2,420 <sup>b</sup>
True digestibility of soybean meal P, %		
Weanling pigs	49	88
Growing-finishing pigs	52	99
Fecal P, % of dry matter		
Weanling pigs	3.4	0.8
Growing-finishing pigs	3.0	1.3

<sup>a</sup>Adapted from Golovan et al. (2001), University of Guelph.

<sup>b</sup>Saliva from transgenic pigs ranged from 341 to 10,077 phytase units/ml.

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