EFFECTS OF A SOLID-STATE FERMENTED PHYTASE ON PHOSPHORUS UTILIZATION AND NUTRIENT DIGESTIBILITY IN GROWING PIGS

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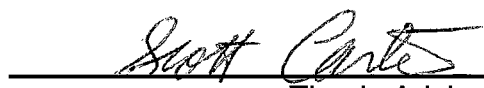

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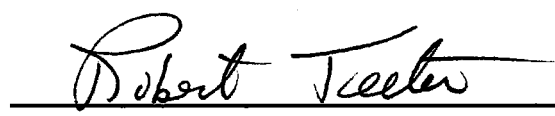

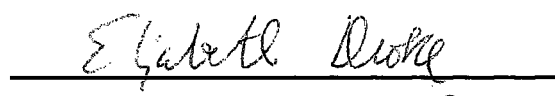

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Introduction

Phytate (myo-inositol 1,2,3,4,5,6 hexa, dihydrogen phosphate or IP6) is the major form of phosphorus in cereal grains and oilseed meals (Reddy et al., 1982). Approximately 60 to 70% of the phosphorus in corn and soybean meal is in the form of phytate (Nelson et al., 1968; NRC 1998). In ruminant animals, phytic P in feed is utilized by microorganisms in the rumen (Reid et al., 1947). However, monogastric animals such as pigs and chicks can not utilize the phytate form of P efficiently due to the lack of endogenous phytase that hydrolyzes phytic P (Taylor, 1965; Peeler 1972). Therefore, inorganic sources of phosphorus have been routinely added to diets for non-ruminant animals to supply sufficient levels of dietary phosphorus, which can lead to significant amounts of phosphorus excreted to the environment.

During the past decade, dietary phytase, myo-inositol hexaphosphate phosphohydrolases (EC 3.1.3.8 and EC 3.1.3.24), has been added to swine diets to improve phosphorus availability, and ultimately, to decrease the amount of phosphorus excreted to the environment (Lei et al., 1993; Cromwell et al., 1995; O'Quinn et al., 1997).

Recently, environmental problems associated with phosphorus accumulation in soils have become a large issue confronting swine producers. Because of the high nutrient content of manure and its fertilizer value, land application has been the major means of handling manure. However, the overall

quality of water can be negatively affected by land application of excess nitrogen and phosphorus (Correll, 1999). Excess phosphorus applications result in excess buildup of phosphorus in soil and in surface runoff water into streams, lakes, and rivers. Phosphorus is the most limiting nutrient for aquatic plant growth, so as the level of phosphorus in these bodies of water increases, so does the growth of algae and other aquatic vegetation (Pierzynski et al., 1994; Sharpley et al., 1994). Decomposition of such vegetation can lead to general deterioration of water quality, a process called "eutrophication" (Crenshaw and Johnson, 1995). For these reasons, the addition of dietary phytase with decreasing additions of inorganic phosphorus is beneficial. There are several types of phytase already available in the market, and new phytase sources are currently being developed. Most of the phytase in the market is produced by submerged microbial fermentation (SmF). Recently, solid-state fermentation (SSF) technology has been utilized as an alternative to produce microbial phytase. Therefore, the purpose of these experiments was to determine the effects of the addition of a solid-state fermented phytase complex to low available P, corn- and barley-soybean meal diets on growth performance, excretion and digestibility of nutrients (dry matter, P, N, and gross energy), bone traits, and tissue accretion rates in growing pigs.

CHAPTER I

Literature Review

General Review on Phosphorus

Phosphorus is the most abundant mineral element in the animal body along with calcium. Phosphorus is an essential mineral for the animal (NRC, 1998). A major role of phosphorus involves mineralization of bone. Also, phosphorus is located in every cell in the body with important functions such as structural components of phospholipids in membranes, energy storage in form of phosphate diester bonds, osmotic balance, buffering, and activating enzymes via phosphorylation. Importantly, the formation or cleavage of phosphorus bonds is essential for energy transfer reaction within cells.

Chemical properties. Phosphorus is the 15^{th} element and has a molecular weight of 30.97. The primary source of P is from rock phosphate [$(Ca_5(PO_4)_3F$ or $CaF_2 3Ca_3(PO_4)_2$].

All living matter contains P, but it exists as phosphate (PO_4). Pure phosphorus is too reactive to be found free in nature and ignites spontaneously when exposed to air. Thus, ortho-phosphates (PO_4) are the forms that are the base unit for metabolism. In biological systems, phosphate is maintained in a ratio of dibasic (HPO_4^{-2}, mw = 95.97) or monobasic ($H_2PO_4^{-1}$, mw = 95.97) ion

complexes depending on pH (pH =pKa + log (HPO_4^{-2} / $H_2PO_4^{-1}$)). At pH 7.4, a pKa value for phosphate in physiological solution is 6.8 (Groff et al., 1995). Therefore, the concentration of HPO_4^{-2} is four times greater than $H_2PO_4^{-1}$ in physiological solution. Concentrations of P in intracellular and extracellular fluid are less tightly regulated than Ca concentrations. Serum P concentration varies throughout the day and is influenced by age, sex, diet, pH, and hormones (Broadus, 1999). About 90% of serum P is in an ionic form and only 10% is associated with proteins.

Digestion, Absorption, and Transport. Regardless of its dietary form, most phosphorus is absorbed in its inorganic form. Organically bound phosphorus is hydrolyzed enzymatically in the lumen of the small intestine and released as inorganic phosphate. Alkaline phosphatase functions at the brush border of the enterocyte to free phosphorus from its bound form. Although alkaline phosphatase can release the bound phosphorus, phosphorus associated with phytic acid is not bioavailable (Groff et al., 1995).

The major sites of P absorption are distal segments of the small intestine. Net secretion of P was observed in the large intestine (Partridge, 1978). Phosphate is absorbed as inorganic P from both dietary inorganic sources and from organic sources after hydrolysis by phophatases in the enterocytes (Jongbloed, 1987), and as a structural part of organic compounds such as phospholipids. In terms of absorption mechanism, active, saturable and passive, nonsaturable transport systems are utilized for phosphate absorption in the small

intestine (Breves and Schroder, 1991). Vitamin D as calcitrol stimulates phosphate absorption with independent effects of vitamin D on Ca absorption (Crenshaw, 2001). Also, sodium is required for vitamin D-meditated P absorption (Crenshaw, 2001). A vitamin D-responsive, Na-P cotransport system has been identified in the small intestine of the rat and rabbit (Crenshaw, 2001). In addition, a Na-independent diffusion mechanism has been described for cellular P uptake (Crenshaw, 2001). Higher rates of active P absorption are found in the jejunum (160 nmol/cm^2/h) than in the duodenum (40 nmol/cm^2/h). There is very little absorption of P in the ileum (Crenshaw, 2001).

Magnesium, aluminum, and calcium intake impair phosphorus absorption (Groff et al., 1995; Allen and Wood, 1994). Phosphorus absorption may be reduced by dietary magnesium and, conversely, a deficiency of luminal magnesium enhances the absorption of phosphate. Aluminum and calcium are thought to form a complex within the gastrointestinal tract to render each other unavailable for absorption (Allen and Wood, 1994). A wide calcium to phosphorus ratio lowers phosphorus absorption, resulting in reduced growth performance and bone calcification, especially when pigs are fed marginal levels of phosphorus (NRC, 1998). On the other hand, the ratio is less critical if the diet contains excess phosphorus. A suggested ratio of total calcium-to-total phosphorus ratio is between 1:1 to 1.25:1 or 2:1 to 3:1 based on available phosphorus (Jongboed, 1987; Ketaren et al., 1989; Qian et al., 1996).

Homeostasis of phosphorus. Regulation of P homeostasis is dependent on mobilization of bone reserves and the regulation of renal excretion and intestinal absorption. When animals are fed marginal levels of P, the proportion of dietary P absorbed increases in the intestine, and renal P reabsorption is unregulated to minimize urinary loss of P (Groff et al., 1995). Parathyroid hormone (PTH), calcitonin (CT), and 1,25-$(OH)_2D_3$ are also involved in P homeostasis. Hyperphosphatemia is associated with decreased circulating levels of 1,25-$(OH)_2D_3$. Increases in circulating levels of 1,25-$(OH)_2D_3$ can lead to hypophosphatemia (Holick, 1999).

Availability. Most inorganic forms of P are highly available. The P in rock phosphate has the same availability as that of dicalcium phosphate, but rock phosphate contains high levels of fluorine (Kragstrup et al., 1989). Orthophosphoric acid (H_3PO_4) must contain no more than 100 ppm fluorine, because fluorine is toxic to animals (Kragstrup et al., 1989). Animals fed raw rock phosphate may lead to fluorine toxicity (Kragstrup et al., 1989). Natural ingredients used in animal diets provide sufficient quantities of fluorine to meet minimum requirements (Nelson, 1983)

The availability of plant sources of P is low due to phytate P in plant cells. Monogastric animals do not synthesize phytase at large quantities to break down phytate. Bioavailability of phytic P from plant sources varies widely from 10 to 60% (Crenshaw, 2001). These variations may be partially explained by intrinsic phytase activity in some feed ingredients. Therefore, the addition of exogenous

phytase to feeds may be beneficial for increasing bioavailability of P in plant sources.

Functions. Phosphate is of prime importance in the development of skeletal tissue, which in itself accounts for 85% of the total phosphorus store. In bone, phosphorus is part of calcium phosphate ($Ca_3[PO_4]_2$) and the crystal, hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$), which is laid down on collagen in the ossification process of bone formation (Groff et al., 1995). Bone from mature animals consist of water (45%), ash (25%), protein (20%), and fat (10%) (Lian et al., 1999). Phosphorus accumulation in bone as crystals of hydroxyapatite ($Ca_{10}(PO_4)_6$) consists of Ca and P in a nearly constant ratio of 2.2:1 (Lian et al., 1999). One element will not be deposited or reabsorbed without the other. Bone ash consists of 36 to 39% Ca and 17 to 19% P (Hayes, 1976). The concentration of Ca and P in bone ash does not change in response to extreme shifts in nutrient intake, but the total amount of ash accumulation varies with nutrient status (Hayes, 1976). Certain forms of P play important roles in the inhibition of mineralization, especially, in soft tissue. Rusell and Rogers (1999) reported that pyrophosphate inhibited crystallization of Ca salts and formation of new hydroxyapatite crystals.

Phosphorus that is not part of bone is found in either extracellular fluids or intracellularly. Within cells, phosphorus is involved in a host of processes. Phosphorus is of vital importance in intermediary metabolism of nutrients, contributing to the metabolic potential in the form of high-energy phosphate

bonds, such as ATP, and through the phosphorylation of substrates. Phosphorus is also an important component of the nucleic acids (DNA and RNA). Phosphorus alternates with pentose sugars to form the linear backbone of those macromolecules (Groff et al., 1995).

Phosphate also functions in acid-base balance. Filtered phosphate reacts with secreted hydrogen ions, releasing sodium ions in the process: [Na_2HPO_4 + $H^+ \rightarrow NaH_2PO_4 + Na^+$](Groff et al., 1995). This action increases pH. The sodium ion may be reabsorbed through the kidney tubule under the influence of aldosterone (Groff et al., 1995). Other functions of P include structural components of phospholipids and proteins, reactive ligand in active sites of enzymes and transport proteins, and osmotic balance. Also, phosphate is involved in both aerobic and anaerobic energy metabolism. Phosphate in bone serves as a reservoir to buffer changes in plasma and intracellular P. Recently, Kegley et al. (2001) reported that dietary P affects immune function.

Requirements. The estimations of dietary requirements for maximum growth rate and feed efficiency of pigs from 3 to 120 kg have been published (NRC, 1998). These requirement estimations were determined by maximum growth rate and feed efficiency. In NRC (1998), traditional modeling procedures were not used to estimate the requirements for minerals and vitamins. Instead, estimates were made from empirical experiments. Exponential equations were then used to fit the midpoint of these weight groups by the equation:

Requirement = $e^{a + b(\ln BW) + c(\ln BW)2}$

The individual coefficients (Table 1.1) for the prediction equation can be found in NRC (1998). The levels of Ca and P that result in maximum growth rate are not necessarily adequate for maximum bone mineralization.

Table 1.1. Coefficients used in the growth model to predict Ca and P requirements for pigs of various body weights[a].

	Coefficients			
	a	b	c	R^2
Ca (%)	0.0658	-0.1023	-0.0185	.99
P, total (%)	-0.2735	-0.0262	-0.0244	.99
P, available (%)	-0.0557	-0.4160	0.0050	.99

[a] NRC, 1998

Estimates were made on a dietary concentration basis for six weight ranges of pigs (Table 1.2) and for gestating and lactating sows. Previous studies (Mahan et al., 1980, Crenshaw et al., 1981) indicate that the requirements for maximum bone strength and bone ash content are at least 0.1 percentage unit higher than that for maximum rate and efficiency of gain.

Table 1. 2. Dietary P requirements of growing pigs allowed a*d libitum* access to feed[a]

	Body weight (kg)					
	3-5	5-10	10-20	20-50	50-80	80-120
Ca, %	0.90	0.80	0.70	0.60	0.50	0.45
Total P, %	0.70	0.65	0.60	0.50	0.45	0.40
Avail P, %	0.55	0.40	0.32	0.23	0.19	0.15
Ca:total P	1.28:1	1.23:1	1.16:1	1.20:0	1.11:1	1.16:5
Ca:avail. P	1.63:1	2.00:1	2.18:1	2.60:1	2.63:1	3.00:1

[a] NRC, 1998

Based on total ingredient costs, P is the most expensive mineral added to swine diets. Therefore, growth performance and feed efficiency are the best response criteria to determine the P requirement, which helps to formulate diets on a least cost basis. However, because of the increased pressure to reduce the amount of P in excreta, the amount of total dietary P has to be reduced and diets should be formulated based on an available P basis rather than total P.

Deficiency Symptoms. Signs of phosphorus deficiency are similar to those of Ca or vitamin D deficiency, which include depressed growth and poor bone mineralization resulting in rickets in young pigs and osteomalacia in older pigs. A marginal deficiency of P affects growth and protein deposition (Vipperman et al., 1974). Conditions involving sever diarrhea, malabsorption, or a decrease in renal P reabsorption may decrease serum, but not intracellular P levels (Crenshaw, 2001). Sows in Ca or P deficiency status exhibit a paralysis of hind legs (posterior paralysis). This problem occurs most frequently in sows producing high levels of milk toward the end or just after the termination of lactation (NRC, 1998). Also, P deficiency causes disturbances in oxygen dissociation from hemoglobin due to a decrease in the formation of 2,3-diphosphoglycerate, which regulates the release of oxygen from hemoglobin (Allen and Wood, 1994). Phosphorus deficiency has also been associated with myopathy, weakness, cardiomyopathy, and neurologic problems (Allen and Wood, 1994).

Toxicity Symptoms. Signs of P toxicity include an increased incidence of urinary calculi, osteodystrophia fibrosa, and metastatic calcification in soft tissue (NRC, 1998). Excess levels of Ca and P may reduce performance of pigs (Hall et al., 1991), and the effect is greater when the Ca:P ratio is increased. However, pigs can tolerate fairly high dietary levels of phosphorus if the Ca:P ratio is narrow. Excess P can be harmful if dietary Ca is marginal.

Bioavailability of Phosphorus. In human studies, the true absorption efficiency of P from a mixed diet has been estimated to range from 70 to 90% (Groff et al., 1995). In swine, generally the purchased feed-grade phosphate source is considered to be 100% available regardless of whether it is dicalcium or mono-dicalcium phosphate (Baker, 2001). The estimated phosphorus bioavailability in commercial defluorinated phosphate was 85% (Coffey et al., 1994) to 90% (Cromwell, 1992).

Phosphorus in plant sources is relatively unavailable (Table 1.3) but some feed ingredients such as wheat and barley contain intrinsic phytase activity which may increase P bioavailability (Nelson, 1967). However, corn-soybean meal based diets are the most common in swine industry. Phosphorus bioavailability in a corn-soybean diet is considered to be about 25% due to phytate-P (Erdman, 1979; Cromwell, 1992). Estimated P bioavailability in corn and soybean meal was 14% and 23 to 31%, respectively, relative to mono-dicalcium phosphate (Cromwell, 1992). The P in high-moisture corn or grain sorghum is considerably more available than that in the dry grain (Ross et al., 1983).

Table 1.3. Phosphorus bioavailability in feedstuffs[a]

Feedstuffs	Bioavailability (relative to mono-dicalcium phosphate)
Corn	14%
Low-phyate corn	77%
Soybean meal	23-31%
Wheat and wheat by-product	29- 49%
Rice bran	25%
Cottonseed meal	1%
Peanut meal	12%
Dried whey, blood meal, fish meal	100%
Meat and bone meal	67, 90%

[a] Adapted from Cromwell (1992, 1998) and Traylor and Cromwell (1998).

CHAPTER II

General Review on Phytate

Phytic acid (Phytate, myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the major storage form of phosphate and inositol in almost all seeds (Reddy et al., 1982). Most of the phosphorus in cereal gains and oilseed meals exist as phytate-P (Table 2.1), which is not available to swine due to lack of endogenous enzyme activity. Phytate is a naturally-occurring compound. It complexes with protein as well as mono- and divalent cations.

Table 2. 1 The concentration of phytate in feedstuffs[a] .

Feedstuff	Phytate, g/kg
Corn	7.44
Sorghum	7.44
Wheat	5.67
Soybean meal	16.67
Canola meal	26.24
Sunflower meal	27.30
Cottonseed meal	32.98
Wheat middlings	27.30
Rice polishings	27.66

[a]Adapted from Ravindran et al. (1999)

Phytate exist as a complex salt termed phytin in plant tissues (Cosgrove, 1980; Reddy et al., 1982). The physiological roles for phytate include its role as a mineral storage compound, an energy source, an initiator of dormancy, and as a mineral storage site (Raboy, 1990). Phytin is widely distributed in plant seeds

and grains, as well as in other organs such as roots and tubers and vegetative tissue (Roberts and Loewus, 1968), pollen (Jackson et al., 1982), and reproductive structures (Helsper et al., 1984). Phytin content in the seed ranges from 0.5 to 1.89% in cereals and from 0.4 to 5.2% in legumes and oilseeds (Reddy et al., 1982). This concentration of phytate-P may account for up to 88% of the total phosphorus in the seed.

Phytate rapidly accumulates during the ripening period of seeds and cereals. It usually is localized to the aleurone particles in grains, to the aleurone layer in cereals, and to protein bodies in the endosperm and cotyledons of legumes and oilseeds (Pernollet, 1978). Also, the embryo, aleurone layer, endosperm, cotyledons, or scutellum have been identified as sites of phytin biosynthesis and localization (Pernollet, 1978). However, corn is an exception to the typical localization pattern seen in monocots. About 88% of phytic acid in corn is found in the germ rather than the aleurone layer. (Scott and Loewus, 1986).

Storage sites and form of phytate. Phytate exists as different complexes in different seeds. In corn, phytate is located primarily in the germ in a water soluble form. Complexation with proteins which were either water soluble or whose isoelectric point were above or below the pH of water would render solubility (Ravindran, 1996). Otherwise, in legumes, phytate has been shown to be associated with protein. Soybeans have no specific site of localization of the phytate molecule (deBoland et al., 1975). Phytate in peanuts is concentrated in

substructures within the protein body membrane (Saio et al., 1977). Phytate in cereal grains is less well defined, but it is contained in significant concentrations both in the bran and germ, and thought to be in a Ca-Mg complex (Pomeranz, 1973). Phytate in sesame seeds appears to be the most unique and least soluble of all seeds (Oberleas, 1983). In barley, phytate is found in the form of a Ca-Mg salt (Pomeranz, 1973).

Effects of phytate on animal nutrition

Phytase activity has been found in the intestine of a variety of animals: broiler and laying hens (Maenz and Classen, 1998), rats, rabbits, guinea pigs, hamsters (Cooper and Gowing, 1983), calves (Bitar and Reinhold, 1972), and pigs (Moser et al., 1982; Pointillart et al., 1984). In addition, Hu et al. (1996) reported that there was hydrolytic enzyme activity towards IP3, less for IP4, and least for IP5 and IP6 in the pig intestinal mucosa. Also, these authors found that the jejunum had the highest activity and the duodenal activity was higher than the ileal. Ketaren et al. (1993) suggested the introduction of microbial phytase to pig feeds is likely to increase the concentrations of IP3-IP5 in the small intestine relative to IP6. Recently, transgenic pigs have been shown to have significant levels of salivary phytase activity, and the transgenic pigs can utilize phytic acid in soybean meal (Golovan et al., 2001)

Even though there is evidence of existence of intestinal phytase, monogastric animals (except the transgenic pigs) do not express sufficient endogenous phytase activity to breakdown the phytate molecule. So, the

phosphorus associated with the phytate molecule is not available for pigs. Therefore, inorganic sources of phosphorus are routinely added to meet the requirement of pigs for optimal growth. As a result, the unavailable form of phosphorus is excreted, which potentially can lead to environmental pollution problems.

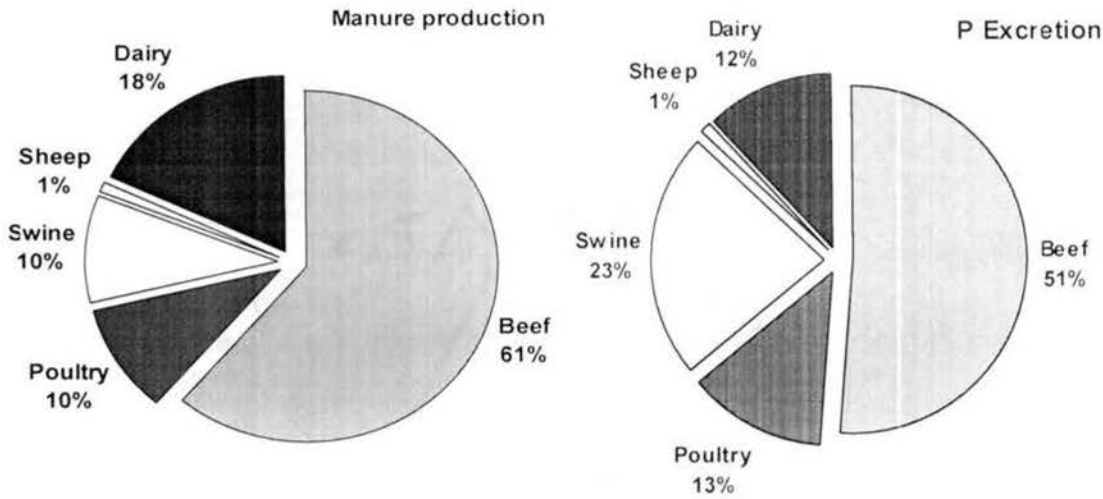
Pig manure contains large amounts of phosphorus (Table 2.2 and Figure 2.1). One of the major reasons for this is that biologically unavailable phytate-P in feedstuffs is not digested and absorbed.

Table 2.2. Average nitrogen and phosphorus contents of manure samples collected by Arkansas producers (Daniel et al., 1998)

	Nitrogen	P_2O_5	Phosphorus	N/P_2O_5
Broiler litter, lb/ton	56	54	23.6	1.04
Dairy manure, lb/1,000gal	6	4	1.75	1.50
Swine manure, lb/1,000gal	14	13	5.68	1.08

In the case this excreted phosphorus in the manure is not properly managed, it may be released to waterways via leaching through the soil or erosion, which may lead to eutrophication of waterways (Crenshaw and Johnson, 1995).

Figure 2.1. Animal livestock manure production and phosphorus excretion in United States (adapted from Crenshaw and Johnson, 1995)



Effects of phytate on nutrient utilization

Phytic acid is the acid form of the anion, phytate. Phytate is crystalline and white, and it turns pink when irradiated (Harland and Oberleas, 1996). Phytate is present in all plants and behaves as a chelating agent. The phytate molecule was designed to claw (chelate) and hold minerals to be released as the growing plant matured. Ca, Co, Cu, Fe, Mg, Mn, Ni, Se, and Zn are reported to be chelated by phytic acid (Harland and Oberleas, 1996). This chelating agent (phytate) has received much attention because of its ability to bind minerals and amino acids from other feed ingredients and from biological fluids in the animal, which negatively affects the bioavailability of nutrients for animals.

Phytate:Protein Interactions. Phytate in plant cells complexes with protein as well as mono- and divalent cations (Ravindran, 1996). Because of its anionic

properties, phytic acids can bind with amino acids and proteins at a pH that is below their isoelectric point. As a result, phytate forms strong complexes with some proteins which prevent proteolysis. Carmovale et al. (1988) reported that in vitro digestibility of protein was decreased by the addition of phytic acid to several protein sources (peas, whole flour, protein concentrate, protein isolate, lactoalbumin, casein, serum albumin and zein) after 1 hr at room temperature. This study indicates that phytic acid negatively affects protein digestibility in vitro. Mroz and Jongbloed (1998) proposed that the presence of phytate-rich diets interferes with optimal amino acid utilization from intact proteins by formation of indigestible protein-phytate complexes, by inhibition of digestible enzymes, and by depressed absorption of nutrients from the small intestine. In vitro studies have shown that phytate inhibits many proteolytic enzymes because of the formation of protein-phytate complexes (Caldwell, 1992).

Phytate:Calcium Interactions. In the presence of phytate and added calcium, interference with mineral absorption occurred as a result of the formation of insoluble complexes (Sandberg et al., 1993). Dietary supplementation of calcium in rapeseed diets decreased phytate hydrolysis in the colon of pigs, but not in the stomach or small intestine (Sandberg et al., 1993).

Phytate:Carbohydrate-Glycemic Index Interactions. A negative relation between phytate intake and glycemic index (blood glucose response) of cereal and legume foods consumed by healthy humans has been reported by Yoon et

al. (1983). In another study in humans, removal of phytate from navy bean flour increased glycemic index compared to that of whole bean flour (Thompson et al., 1987). In vitro, when wheat or bean starch was incubated with Na phytate, hydrolysis of starch was retarded, but digestion was restored when Ca was added with the Na phytate (Yoon et al., 1983; Thompson et al., 1984). Phytate has been shown to inhibit α-amylase activity (Desphande and Cheryan, 1984) and to form Ca-phosphate-phytate complexes with carbohydrates (Thompson and Yoon, 1984).

Phytate:Mineral Interactions. Phytate is a polyanionic species that under appropriate conditions may complex with a variety of cationic minerals: Ca, Zn, Cu, Mn, Mg, Co, and Fe (Oberleas and Harland, 1996), including several nutritionally important elements. Phytic acid binds approximately two thirds of intrinsic phosphorus in plant feedstuffs (Cosgrove, 1980), and forms insoluble complexes with dietary di- and trivalent cations (Erdman, 1979; Maga, 1982). Phytate has been reported to suppress the availability of some cations such as calcium, iron, and zinc (Lonneral et al., 1989; Ali and Harland, 1991; Reddy et al., 1997). Also, other studies have shown that phytate in the feedstuff negatively affected the utilization of phosphorus and calcium, and the addition of exogenous phytase diminished the adverse effect of phytate (Qian et al., 1996; Liu et al., 1998, 2000; Cromwell et al., 1993).

Methods to Reduce/Destroy Phytate Molecules in Feedstuffs

It is difficult to remove phytate without changing the structure of the protein, the nutrient content, the organoleptic, and solubility characteristics. Grinding and milling processes can break down the phytate structure (Larsen, 1993). Also, the pelleting process can damage the phytate structure in the feedstuffs (Larsen, 1993). Early studies showed that wheat stored at high moisture content and temperature increased inorganic phosphate and decreased phytate (Glass and Geddes, 1946).

Thermo-process. The heating (dry or moist heat, baking, simmering, boiling, autoclaving) is effective to some degree. Through thermoprocessing, the phytate molecule can be hydrolyzed, thus rendering it less effective as a chelating agent with other nutrients such as minerals and amino acids (Bayley and Thomson, 1969; Bayley et al., 1975).

Autoclaving and Roasting. This process can reduce phytate content in legumes dramatically with an increase in total phosphorus. The heat processing causes hydrolysis or decomposition of phytic acid. Consequently, the phosphate group is released from phytate (Hernadez-Unzon and Ortega-Delgado, 1989).

Fermentation. Sour-dough leavening involves the use of microorganisms to improve the flavor, texture, aroma, and digestibility of foods. The production of

organic acids during the process lowers the pH of the seed and activates the phytase component (Harland and Oberleas, 1996)

Malting Process. This process involves a series of steps like steeping, soaking, germination, and kilning that converts the hard insoluble grain of the cereal into soft, sweet kernels. Steeping allows the seed to absorb moisture and also activates the intrinsic phytase enzyme (Harland and Oberleas, 1996). Adequate moisture retention in the seed allows it to germinate and as a result hydrolytic enzymes are synthesized. Activation of phytase from the inactive form initiates phytate hydrolysis. However, germinated seeds not only contain phytase but also the enzymes necessary for synthesis of phytate and therefore some phytate is also synthesized during the process of germination. The most significant increase in protein content was observed for 120 hours in tap water, and the most appreciable decrease in phytate content occurred in seeds treated with 0.20 kGy dose (irradiation) and germinated for 120 hours. Kilning involves the drying and roasting/curing to bring out the color and flavor of the individual feeds. Irradiation and germination collectively as well as independently had a negative effect on the total phytate content of soybeans (Harland and Oberleas, 1996)

These methods are used for utilizing the naturally-occurring phytase in the feeds to hydrolyze the phytate. Most cereal grains (wheat, corn, barley, oats and rice) contain intrinsic phytase. All of these processes are time, temperature, and pH-dependent.

Chemical process. There was a significant reduction of phytate in soybean and cottonseed meals through the use of heat, enzyme, acid extraction, precipitation by divalent cations, and subsequent washing with a 1N HCl solution (Han, 1988). These improvements are not of sufficient magnitude to be economically justified in most current feeding programs in the United States (Crenshaw, 2001)

Enzymatical Process (Phytases). Microbial phytases, phytase-rich bacteria, and yeast are added to feeds to hydrolyze the phytate and minimize mineral binding. Different grains have different levels of intrinsic phytase activity with the highest levels in rye, followed by wheat, barley, and oats (Peers, 1953). Previous studies showed addition of microbial phytase improved bioavailability of phosphorus of pigs fed corn or sorghum-based diets (Cromwell et al., 1993; Jongbloed et al., 1992; O'Quinn et al.,1997; Kemme et al.,1999; Nasi et al., 1999; Sand et al., 2001; Augspurger et al., 2003).

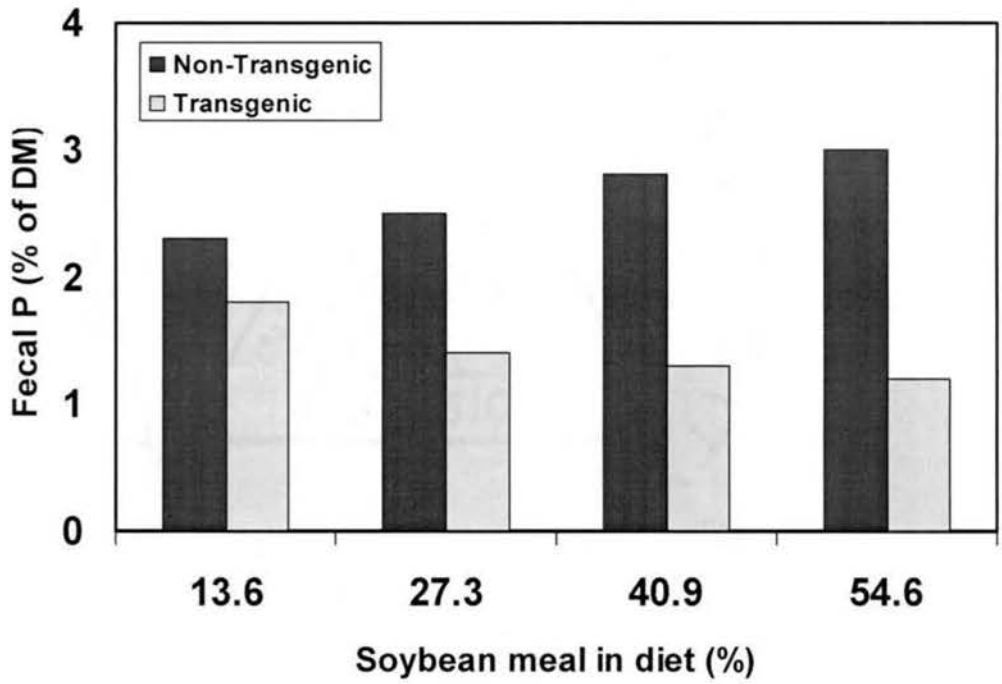
Bioengineering in Plants. Some crops possess relatively high levels of endogenous phytase in their seeds. This was first shown by McCance and Widdowson (1944) and Mollgaard (1946) who demonstrated that wheat, wheat byproducts (bran, middlings), rye, and to a lesser extent, barley, contain significant amounts of phytase. Studies (Cromwell and Coffey, 1993) show a considerably higher bioavailability of P in wheat (50%), wheat middlings (41%),

wheat bran (29%), and barley (30%) than that in corn (14%). Wheat bran phytase has also been shown to increase the utilization of P in other feedstuffs in the diet (Cromwell and Coffey, 1993). 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.

Bioengineering in Pigs. University of Guelph researchers (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 these 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 (Figure 2.2). 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 phytase unit 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.

Figure 2.2. Total fecal phosphorus content from non-transgenic pigs and transgenic pigs fed different levels of soybean meal as the sole source of dietary phosphorus. (adapted from Golovan et al., 2001)



CHAPTER III

General Review on Phytase

Justification for Phytase Use

Both surface and ground water can be negatively affected by applying excess nutrients, especially phosphorus, to the soil. Phosphorus can be adsorbed onto soil particles and leach into ground water. Also, it can erode into stream, lakes, and rivers. Phosphorus is the most limiting nutrient that regulates aquatic plant growth (Sharpley et al., 1994); therefore, phosphorus in water stimulates the growth of algae and other aquatic vegetation. Decomposition of such vegetation can lead to a general deterioration of water quality, a process called eutrophication (Crenshaw and Johanson, 1995). Therefore, the addition of exogenous phytase can be beneficial. The addition of phytase to low P diets can reduce total dietary levels of P by improving the bioavailability of P, resulting in decreased P excretion.

Definition of phytase

Phytases (myo-inositol hexaphosphate phosphohydrolases) comprise a family of enzymes that catalyze the stepwide removal of inorganic orthophosphate from phytic acid as well as from a variety of natural and synthetic phosphorylated substrates accepted by nonspecific acid phosphatase (Ginson

and Ullah, 1990). Phytase activity from rice bran was one of the first enzymes exhibiting phosphomonoesterase activity to be characterized (Suzuki et al., 1907) and several comprehensive reviews on phytase have been reported (Cosgrove, 1980; Graf, 1986).

Two classes of phytases (3-phytase and 6-phytase) are recognized by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB). 3-Phytase (EC 3.1.3.8) initially removes orthophosphate from the 3-positon of phytic acid. 6-Phytase (EC 3.1.8.24) catalyzes the removal of orthophosphate from the 6-position of phytic acid. Successive dephosphorylations result in intermediates from inositol mono- to tetra-phosphate and free myo-inositol. The 3-phytases are characteristic of the phytase found in microorganism and the filamentous fungi (Nys et al., 1996; Pandey et al., 2001). The 6-phytases are found in plants such as wheat (Irving, 1980).

Phytases (6-phytase) in Plants

Phytase activity has been found in variety of plants and feedstuffs (Table 3.1). Plant phytases have a specific pH for optimum enzyme activity. Most of the plant phytases (6-Phytase) have maximum enzyme activity at approximately pH 5.0 with values reported for wheat, wheat bran, barley, corn, and bean of pH 5.2, 5.0, 5.2, 5.6 and 5.3, respectively (Radcliffe, 2000). The optimum pH for soybean phytase occurs between pH 4.5 and 4.8 (Nayini and Markakis, 1986).

The maximum activity of phytase activity has been observed at 50° C, with a range of 45 to 57 °C (Irving, 1980).

Table 3.1. Intrinsic phytase activity in feedstuffs (Adapted from Radcliffe, 2000).

Feedstuff	Phytase activity, U/kg
Rye	4,900[1], 4,132-6,127[2]
Triticale	1,500[1], 1,475-2,039[2]
Wheat	700[1], 915-1,581[2]
Barley	400[1], 408-822[2]
Wheat Bran	1,200[1], 1,180-5,208[2]
Corn	0-46[2]
Soybeans (heated)	0-188[2]
Soybean meal, 44%	0-120[2]
Soybean meal, 48%	0-20[2]
Canola meal	16[1], 100[2]
Sorghum	24[2]
Wheat middling	1,900[1], 4,381[2]

[1]Pointillart (1994).
[2]Eeckhout and De Paepe (1994).

Microbial Phytases (3-phytase)

Microbial phytase has been reported to have two peaks of activity at pH 2.5 and pH 5.0 to 5.5 (Shieh et al. 1969; Irving and Cosgrove, 1974; Siomons et al., 1990). Aspergillus phytase has 50% more activity at pH 2.5 compared to pH 4.5 (Simons et al., 1990). Natuphos® (BASF) phytase has been reported to have more enzyme activity at pH 5.5 compared to pH 2.5 (Beudeker, 1990). There was no phytase activity at pH 7 or higher.

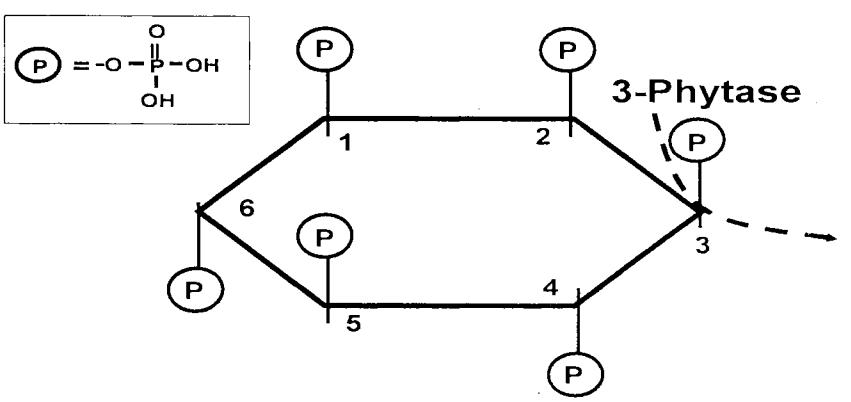
Currently, three microbial phytases are available on the market. Natuphos is phytase produced by Gist-Brocades (Dflft, The Netherland) and marketed by BASF (Mount Olive, NJ). Novo Nordisk (Bagsvaerd, Denmark) produces a genetically-modified phytase in Europe, which is marketed by Roche (Basel,

Switzerland) in the U.S. Also, Alltech, Inc (Nicholasville, KY) produces and markets a solid state fermented phytase (SSF Phytase) known as Allzyme®.

Mode of Action

Phytase is a phosphatase which is able to catalyze the hydrolysis of a phosphate-ester. Phytase can cleave off a phosphate group from the phytate molecule. There are many phosphatases. Some of them play an important role in the metabolism of plants and animals, such as ATP-ase. The phosphatases can be classified as alkaline or acid depending on its optimal pH value. There are two main types of phytase known, as 3- and 6-phytase, which have different modes of action (Kies, 1996). For example, 3-phytase initiates the dephosphorylation of phytate at the 3-position (Figure 3.1), whereas 6-phytase start the dephosphorylation at the 6-position (Kies, 1996).

Figure 3.1. Chemical structure of phytate and mode of action of microbial phytase (Adapted from Nys et al., 1996).



Myo-inositol hexakis dihydrogen phosphate

The mechanism of removal phosphate from phytate is a ping-pong mechanism (Shute et al., 1988). This means a phosphate group is transferred from the substrate to the enzyme, and then from the enzyme to water. Phytases hydrolyze phosphate groups from phytate in a very specific way. Venekamp et al. (1995) using NMR-techniques found that microbial phytase broke down phytate in a certain order. Aspergillus phytase starts to hydrolyze the phosphate group at the 3 position. After cleaving off the phosphate group from the 3 position, phytase then catalyzes the stepwise hydrolysis of phosphate groups from the 4, 5, 6 and 1 position, producing inositol phosphate-5 (IP5), IP4, IP3, IP2, and IP1 (Venekamp et al., 1995). *Aspergillus* phytase cleaves off the last phosphate (position 2) group from IP1 at a very slow rate (Kies, 1996). However, some acid phosphatases are known to hydrolyze the phosphate group from IP1. Therefore, the addition of acid phosphatases may be beneficial. However, Nasi et al. (1995) reported a lack of synergistic effect of the addition of acid phosphatase and microbial phytase on phosphorus digestibility.

Site of phytase activity

The optimal pH of phytase from *Aspergillus ficuum* has two response peaks at pH 5.0 to 5.5 and pH 2.5 (Shiel et al. 1969; Irving and Cosgrove, 1974; Simons et al., 1990). The variation of pH in the gastrointestinal tract of animals may affect the activity of phytase in the diets. The acidity of the stomach lumen ranges from pH 1.0 to 4.5 (Chessen, 1987), and the luminal pH of the gastrointestinal track increases from the duodenum to the terminal ileum. The

pH of the stomach digesta of pigs is 3.4 to 4.8, which is much lower than that in the small intestine (pH 6.4 to 7.2; Eidelsburger et al., 1992; Risley et al., 1992). The duodenal pH immediately following a meal was 5.7, after which it gradually decreased to pH 3.3 (Eidelsburger et al., 1992; Risley et al., 1992). It is generally accepted that the duodenal pH is approximately 4.8 (Eidelsburger et al., 1992; Risley et al., 1992). The jejunum, which represents approximately 90% of the total length of the small intestine, has a mean pH of 5.5 to 6.9, while the ileum has a mean pH of 7.0 to 7.4 (Eidelsburger et al., 1992; Risley et al., 1992). Therefore, the stomach of pigs is the site of greatest phytase activity. An early study by Gueguen et al. (1968) reported that plant phytase from activated wheat bran hydrolyzed phytate-P mainly in the stomach of pigs. Lantzsch et al. (1992) reported that phytate-P from corn was mainly degraded in the stomach and upper small intestine. Other studies have shown that approximately 50% of the phytate-P from a corn-soybean meal diet was degraded in the stomach of pigs and an additional 9% in the duodenum and jejunum (Jongbloed et al., 1992). However, no phytase activity was detected in the ileum (Jongbloed et al., 1992). Also, Jongbloed et al. (1992) reported that 85% of added phytase activity (1,565 U/kg) was detected in the duodenal digesta of growing and finishing pigs fed a corn-soybean meal diet. No phytase activity was observed in the ileal digesta. Yi and Kornegay (1996) reported that phytase activity in the digesta decreased from the stomach to the upper small intestine to the lower small intestine when phytase activity was measured 3 h after ingestion of feed. Phytase activity, as a percentage of the total dietary phytase activity, was found to be 51% in the

stomach, 31% in the upper small intestine, and 5% in the lower small intestine (Yi and Kornegay, 1996). Therefore, the pH of stomach digesta is favorable for optimum phytase activity; however, the pH in the lower small intestine is not favorable for phytase activity.

In chickens, Liebert et al. (1993) reported that 25 to 50% of added phytase activity was detected in the contents of the crop and that 10 to 25% of added phytase activity was detected in the proventriculus when 500 or 1,000 PU/kg was added to corn-soybean meal diets. No phytase activity was detected in the small intestine of the chicken. This study indicates the main sites of phytase are the crop and the proventriculus.

Sources of Phytase

Dietary phytase can be produced from several sources of plant, animals, and microorganisms. Microbial phytase is the major source of exogenous phytase in commercial production and commonly used in feed industry. The source and fermentation methods are listed in Table 3.2.

Bacterial phytase. Several bacterial strains (wild and genetically modified) such as *Lactobacillus amylovorus, E. coli, B. subtilis, B. amyloliquefaciens, Klebsiella sp.*, etc., have been utilized for phytase synthesis. Sreeramulu et al. (1996) evaluated 19 strains of lactic acid-producing bacteria of the genera *Lactobacillus* and *Streptococcus* for the production of extra-cellular phytase. Also, phytase was produced from E.coli.. Sunitha et al. (1999) optimized the

medium for recombinant phytase production by E. *coli* BL21 using response surface methodology. A genetically modified *B. subtilis* produced extra-cellular phytase and the yield was 100-fold higher than the wild type *B. amyloliquefaciens* DS11 (Kim et al., 1999).

Yeast phytase. Phytase production using yeast cultures has generally been carried out in submerged fermentation systems. The strains used include *Schwanniomyces castelli, S. occdentalis, Hansenula polymorph, Arxula adeninivorans, Rhodotorula gracilis*, etc. Mayer et al. (1999) developed an efficient process for low-cost production of phytase using *Hanenula polymorpha*. Glucose or glucose syrups were used as the main carbon sources during fermentation. Compared with the process using glycerol the use of glucose led to a reduction of more than 80% in raw materials cost. In addition, exceptionally high concentrations of active enzyme were obtained in the medium, with phytase representing over 97% of the total accumulated protein.

Fungal phytase. Several types of fungal cultures are employed for the production of phytase in submerged fermentation (SmF) or solid-state fermentation (SSF) systems. Ahmad et al. (2000) used a maize starch-based medium for the production of phytase in SmF using *Aspergillus* niger. Activity of the enzyme was found to be 0.075 phytase units per min per ml of the crude culture filtrate at pH 5.5 and 40 °C. Extra-cellular phytase produced by *Aspergillus sp.* showed a five-fold higher activity in liquid culture when compared

with cultures of *A. ficcum* NRRL3135. Solid state fermentation also has been used for phytase production using strains of *Aspergillus sp.* Ebune et al. (1995a) used canola meal for phytase production by *A. ficcum*. Optimum substrate moisture was 64%. Age of the inoculum has a profound effect on enzyme synthesis by the culture. Alasheh and Duvnjak (1995) found 53% to 60% moisture as the optimum when a strain of *A. carbonarius* was used on canola meal.

Table 3.2. Source and production methods of microbial phytase[a]

Micro-organism	Method	Substrate
Bateria		
Bacillussp.	SmF[b]	Maltose
B. amyloliquefaciens	SmF	Complex medium
B. amyloliquefaciens		
B. subtilis		
Enterobacter sp.	SmF	Complex medium
E.coli	SmF	Complex medium
Lactobacillus amylovorus	SmF	Glucose
Yeasts		
Arxula adeninivorans	SmF	Complex medium
Hansenula polymorpha	SmF	Glucose
Schwanniomyces castellii	SmF	Wheat bran, cotton flour
Fungi		
Aspergillussp.	SmF	Complex medium
A. carbonarius	SSF[c]	Canola meal
A. ficuum	SSF	Canola meal
A. ficuum	SmF/SSF	Glucose, canola meal
A. niger	SmF	Maize starch
A. niger	SmF/SSF	Complex/wheat bran

[a] Adopted from Pandey et al. (2001)
[b] Submerged fermentation method
[c] Solid State fermentation method

Production techniques for microbial phytase

Phytase can be produced from a host of microorganisms including bacteria, yeast, and fungi. During the past several decades, the use of filamentous fungi for the production of commercial enzymes has dramatically increased, and phytase is no exception. Submerged fermentation (SmF) has largely been employed as the production technology. Recently, solid state fermentation (SSF) has been used for microbial phytase and has gained interest for the production of primary and secondary metabolites (Pandey, 1991, 1992, 1994).

Techniques of SmF as well as SSF have been used for the production of phytases. Type of strain, culture conditions, nature of the substrate, and availability of the nutrients are critical factors affecting the yield and should be taken into consideration for selecting a particular production technique. For example, a filamentous fungus in SmF is exposed to hydrodynamic forces, but in SSF, the surface of the solid particles acts as the matrix for culture (Pandey, 1994). Papagianni et al. (1999) investigated qualitative relationships between medium composition, *Aspergillus niger* morphology, and phytase production in SmF and SSF. These authors found that media composition and fungal morphology greatly affected phytase production in submerged fermentation and the addition of wheat bran and a slow releasing organic phosphate source enhanced *Aspergillus niger* growth and phytase production.

Submerged fermentation method (SmF)

Approximately 90% of all industrial enzymes are produced by a submerged fermentation process. In this process, the microbes (fungi or bacteria) are submerged in nutrient-rich liquid media contained in a batch fermenter. The conditions inside the fermenter (pH, temperature, and oxygen) are tightly controlled to maximize microbial growth. When the fermentation process is terminated, enzyme-rich liquid media is dried, processed, and standardized. The attractive feature of this process is high production rate when genetically-modified organisms are used (Filer, 2001).

Solid State fermentation method (SSF)

Background. The use of filamentous fungi for production of commercial products has increased over the decades and the production of enzymes by submerged fermentation has been established. Recently, solid state fermentation (SSF) methods have generated more attention because they offer several economical and practical advantages which include higher product concentration, improved product recovery, very simple cultivation, decreased water output, lower capital investment, and lower plant operation costs (Becerra and Gonzales, 1996). In the SSF system, microbes ferment a solid substrate. Solid state fermentation is not a new concept, and its history can be traced back to bread making in ancient Egypt (Filer, 2001). Also, the Japanese used a SSF method make dietary protein from waste material (Filer, 2001). Composting, silage production, cheese ripening, and mushroom cultivation are modern

examples of SSF process. Solid state fermentation has been used widely in Asia in the production of certain foods and beverages, such as sake and soy sauce (Filer, 2001). However, the SSF process has not been considered as a method for the production of enzymes.

In a review, Pandey et al. (2000) discussed the potential of SSF for the development of bioprocesses such as bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, biotransformation of crops and crop-residues for nutritional enrichment, biopulping, and production of value added products, such as biologically active secondary metabolites, including antibiotics, alkaloids, plant growth factors, enzymes, organic acids, biopesticides, and aromatic compounds. The SSF system, which during the past two decades was termed as 'low-technology' systems appears to be promising for the production of value-added 'low volume-high cost' products such as biopharmaceuticals (Pandey et al., 2000). Use of agro-industrial residues offers potential advantages of SSF system.

Procedure of SSF system. Establishing a SSF system begins with the screening, selection, isolation, and propagation of a microbial culture that produces large amounts of the desired enzyme. In some cases, it produces more than 400 times the normal amount (Filer, 2001). The selected cultures are used to inoculate the SSF system. Production of the microbes used to inoculate the system is done on a small scale.

Bacteria, yeast, and fungi that can grow on solid substrates are viable candidates. However, filamentous fungi, such as *Aspergillus niger*, have the greatest advantages in SSF systems that produce enzymes for feed applications. The next step is the preparation of the substrate. The solid state fermentation process can utilize minimally processed by-products such as wheat or rice bran, and other inexpensive, readily available materials such as soyflour as a substrate. Often the ratio of protein:carbohydrate source is the determining factor in enzyme yield (Filer, 2001). The substrate must be heat and pressure sterilized to destroy any harmful microbes especially yeast and their spores. The moisture content of the substrate is adjusted to 45 to 50% (Filer, 2001). One of the important features of SSF is the low free moisture content of the substrate. Most of the water is bound to the substrate which maximizes the exposure of the microbes to air. By this process, microbial activity is stimulated. Consequently, the production rate of an enzyme is high. In addition, the low moisture reduces drying time and energy costs during downstream processing (Filer, 2001).

The selected microbial culture is seeded into the sterilized substrate and mixed to create a material called "koji", a Japanese term. Usually, 500 mL of starter culture can inoculate 1 ton of substrate. The koji is loaded into a reactor and allowed to ferment. There are three basic types of reactors: tray, packed-bed, and agitated (Filer, 2001).

The tray reactor is the most widely used. It consists of a series of 35 x 20 cm sterilized stainless steel trays. Thin layers of culture are covered, placed on a stainless steel rack, and housed in a controlled environment (90% humidity). To

produce feed-grade phytase, the fermentation process usually takes 5 to 7 days. After the fermentation is complete, the material is dried, ground, standardized, and packaged. Alternatively, the fermented material can be extracted to produce a liquid enzyme product. A facility with 10,000 trays can make a sufficient quantity of enzyme in one year to supplement 6 to 8 million tons of feed (Filer, 2001).

Packed-bed and agitated reactors are being developed to increase enzyme production capacity. However, over-heating, aeration, and condensation inside the vessel are challenges. Also, in deep-bed systems, certain layers could become deficient in oxygen (Filer, 2001).

Advantages of using SSF system. There is evidence that enzymes produced through solid-state fermentation are qualitatively different from those produced by submerged fermentation. Solid state fermentation systems that use non-genetically modified microbes often produce high activities of the desired enzymes as well as lower but substantial activities of other enzymes, known as "side activities" (Filer, 2001). For example, an SSF-phytase product had measurable protease and beta-glucanase activity (Filer, 2001) (Table 3.3).

Microbial feed-grade SSF phytase has greater stability during storage than SmF phytase. A glucosidase produced by SSF was found to be more heat-tolerant than a traditional phytase (SmF phytase). Also, SSF protease had a significantly greater protein digestion capacity than one produced by submerged fermentation process (Filer, 2001).

Table 3.3. Enzyme activities of phytase produced by SSF and SmF[a]

Enzyme	Submerged fermentation	Solid-state fermentation
Phytase (PU/g)	1,900	1,900
α-amylase (FAU/g)	-	300
β-glucanse (BGU/g)	-	2,700
Protease (HUT/g)	-	9,300
Xylanase (XU/g)	-	500
Cellulase (CMCU/g)	-	390

[a]Adapted from Filer (2001).

Effects of Exogenous Phytase on Swine

In corn and soybean meal diets, about two-thirds of the phosphorus is bound as phytic acid and is poorly available to pigs because pigs can not utilize phytate-P in feedstuffs (NRC 1998). Thus, inorganic phosphorus such as dicalcium phosphate is routinely added to rations to supply sufficient phosphorus for proper growth. Adding inorganic sources of phosphorus can satisfy the animal's requirement, but indigestible phytate-P is still excreted to the environment. However, the amount of excreted P can be significantly decreased by inclusion of microbial phytase in the diets, which releases P from phytate (Table 3.4).

Table 3.4. Potential reduction in excretion of nitrogen and phosphorus by various nutritional strategies in swine.

Strategy	Reduction in nutrient excretion
Formulation closer to requirements	10 – 15% for N and P
Reducing feed spillage/waste	1.5% for all nutrients for every 1% reduction
Pelleting	5% for N, P, Zn, Cu
Fine particle size (700-1,000um)	5% for N, P, Zn, Cu
Use of highly digestible feed ingredients	5% for N and P
Reduced variability by quality control	10-20% for P; 20-40% for N
Reduced protein/amino acid supplementation	9% for every reduction in dietary CP
Low-phytate corn	25-50% for P
Phytase/low P	2-5% for N, Zn; 20 - 30% for P
Phytase/low phytate corn	2-5% for N, Zn; 20 - 40% for P
Phytase/enzyme cocktail	2-5% for N, Zn; 20 - 40% for P
Phytase/1,25$(OH)_2D_3$	2-5% for N, Zn; 20 - 60% for P
Phytase/probiotics	2-5% for N, Zn; 20 - 40% for P
Cellulase, Xylanases, Pentosanase, β-glucanse	5% for N, P
Phase feeding	5-10% for N, P
Split-sex feeding	5-8% for N
Reducing micromineral/organic minerals	Up to 50% for Zn, Cu, Mn

Adapted from Ferket et al. (2002).

The magnitude of the response to microbial phytase has been shown to be influenced by the source of phosphorus, dietary level of available phosphorus, the amount of phytase added, and the ratio of Ca to P (Lei et al, 1994; Liu et al., 1998;Qian et al., 1996; and Kornegay, 1996). Also, previous studies indicate microbial phytase releases calcium (Mroz et al., 1993; Radcliffe et al., 1995), zinc (Lei et al., 1993a; Pallauf et al., 1994), and some amino acids (Kemme et al., 1995) that may be bound to phytic acid.

Effects of Microbial Phytase on P Utilization by Swine. Approximately 60 to 70% of the P in plant sources that are commonly used in pig diets is associated with phytate. Phytate-P is not available for absorption (Cromwell, 1992; Ravindran et al., 1994, 1995). An early study by Nelson et al. (1968) demonstrated that the addition of phytase can release P from phytate. During the past decades, dietary phytase has been extensively studied. Addition of microbial phytase has been shown to catalyze the hydrolysis of the phytate molecule, releasing P from phytate (Jongbloed et al., 1992, 1996; Cromwell et al., 1993b; Kornegay 1995).

Dietary microbial phytase has been added to a variety of feedstuffs and has been shown to improve bioavailability of P in different types of diets. Supplemental microbial phytase improves the bioavailability of P in corn- (Cromwell et al., 1995), oat- (Bruce and Sundstol, 1995), wheat-, triticale (Dungelhoef et al., 1994), and barley-based (Campbell and Bedford, 1992; Valaja et al., 1998; Nasi et al., 1999) diets for swine. Microbial phytase also has been

shown to affect growth performance of pigs fed low P diets by increasing average daily gains, primarily due to an increased feed intake (Simons et al., 1990; Beer and Jongbloed, 1992; Kornegay and Qian, 1996; Yi et al., 1996). In other studies, the addition of microbial phytase decreased P excretion by 25 to 50% by increasing P digestibility or retention and by decreasing the total level of P in the diets (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993b; Kornegay and Qian, 1996; Yi et al., 1996). Also, increases in bone breaking strength or shear force have been observed in several studies with pigs when microbial phytase was added to low P diets (Cromwell et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996; Radcliff and Kornegay, 1998; Park et al. , 2003abc). These results indicate dietary microbial phytase improves P utilization in the diet. The recent phytase studies were summarized in Table 3.5.

Phytase and Low-Phytate Feedstuffs. The addition of dietary 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 (Table 3.4). Previous studies have 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. In these studies, feeding pigs low-phytate corn-soybean meal diets with phytase reduced P excretion by 62 to 68% compared with controls fed conventional corn-soy diets without phytase (Xavier et al., 2003bc).

Calcium Effects on Phytase. The greater the calcium content of the diet the poorer the efficiency of phytase activity (Fisher, 1992; Lei et al., 1994; Sebastian et al., 1996). Calcium is thought not only to precipitate phytate but also to interact with soluble substrate, as a result reducing the susceptibility of the subtrate to enzymatic attack (Sebastian et al., 1996). Use of chelators, such as citrate, which removes the calcium from soluble phytate complexs, are possible methods to increase the apparent activity of phytase (Zyla et al., 1996; Boling et al., 1998; Maenz et al., 1999). Not only the absolute calcium content but its ratio to phosphorus content in the diet affect phosphorus absorption (Liu et al., 1998,2000; Qian et al., 1996). In addition, vitamin D has been shown to

affect phytate utilization. Increasing vitamin D and decreasing calcium improved phytate utilization even in the absence of phytase addition (Edwards, 1992 and Fisher, 1992).

Phytase Effects on Weanling Pigs. The effectiveness of Aspergillus niger phytase in improving the utilization of phytate-P in corn-soybean meal diets (Lei et al., 1993; Leunissen and Young, 1992; Adeloa et al., 1995; Radcilffe et al., 1998; Roberson, 1999; Zhang et al, 2000), semipurified diets (Kornegay et al., 1996), and pearl millet soybean meal diets (Murry et al., 1997) for weanling pigs has been reported. The effect was linear over a range of dietary phytase activity from 0 to 750 PU/g of corn-soybean meal diets (Lei et al., 1993a). Another study by Lei et al. (1993b) indicated that supplements of 1,200 PU/g maximized utilization of phytate-P and possibly removed the need for inorganic P supplementation. A recent study showed that supplementing phytase (1,000PU/kg) to the low P diets, significantly improved average daily gain. A significant additional effect was observed when acetic acid was added to the diet (Valencia et al., 2002). Also, these authors reported that the apparent digestibility of P and Ca were increased by phytase or acetic acid, and by phytase and acetate supplementation, but average daily feed intake was not different among treatments. This study showed that an additional effect of acetic acid combined with phytase in promoting better mineral digestibility. The improved performance observed in this study seems to support the hypothesis that young weanling pig may be unable to adequately digest certain nutrients.

Due to the lack of adequate levels of hydrochloric acid in the stomach (Jongbloed et al., 1992), addition of citric acid can improve both protein digestion and enhance phytase activity by lowering gastric pH. In this study, phosphorus excretion decreased by 44% with the addition of phytase (up to 1,000 PU/kg) and it was further reduced to 51% when acetic acid (1%) was also added with phytase. However, Radcliffe et al. (1998) reported the additions of citric acid and phytase to weanling pigs were each beneficial, but no synergistic effect was found. These authors also observed that phytase addition (up to 750 PU/kg) did not affect performance, but linearly increased rib shear force, shear energy, dry bone weight, ash weight, ash percentage, and Ca and P digestibility. The addition of citric acid reduced dietary pH and stomach pH, and improved ADG, feed efficiency, and Ca digestibility.

In low phytate corn, Sand et al. (2001) reported that the percentage of P digested and retained was improved and fecal P excretion lowered by feeding low phytate corn. In this study, the addition of 600 phytase units per kg to high available P corn-based diets further improved P digestibility and reduced P excretion in pigs. This study indicated that formulation of diets with low phytate corn with dietary phytase can maximize P utilization and minimize P excretion.

Effects of Phytase on Growing- Finishing pigs. During the past decade, numerous experiments have been conducted which demonstrated that dietary microbial phytase can partly render phytate-P available to growing-finishing pigs (Jougbloed et al, 1992, 2000; Cromwell et al., 1993b,1995; Harper et al., 1997;

Kemme et al., 1997, 2000) . A number of studies clearly show a consistent positive effect of microbial phytase on P and Ca bioavailability. The addition of microbial phytase to corn-soybean meal diets for growing-finishing pigs has been shown to increase the availability of P and Ca (Jougbloed et al, 1992; Cromwell et al., 1993b,1995; Harper et al., 1997; Kemme et al., 1997; Jongbloed et al., 2000). The addition of microbial phytase to feed usually results in the enhancement of the digestibility of phytate-P up to 40% (Jongbloed et al., 1996) indicating 60% of the phytate-P is still unavailable to pigs. Jongbloed (1987) reported that lowered intestinal pH increases the solubility of P and phytate and improves P absorption in the small intestine. In addition to their effect on intestinal pH, supplementary organic acids can also bind various cations along the intestine and may act as chelating agents (Radvindran and Kornegay, 1993).

The efficacy of microbial phytase is shown to be pH-dependent (Simons et al., 1990) and the highest activity was observed at two pH optima (5.0 to 5.5 and 2.5). A large portion of digesta leaves the pig stomach shortly after feeding and has a pH that is too high for optimal microbial phytase (Jongbloed et al., 1992). Therefore, feed acidification may reduce the rate of gastric emptying (Mayer, 1994). Kemme et al. (1999) reported that supplementation of a grower-finisher diet with 3% lactic acid not only had a positive effect on the digestibility of total P, but also a synergistic interactive effect with phytase. Similar effects were reported by Jongbloed et al. (2000). However, Radcliff et al. (1998) reported that addition of citric acid (1.5 or 3.0%) improved P digestibility, but there was no significant interaction between microbial phytase and citric acid.

Effects of Phytase on Amino Acids. Mroz and Jongbloed (1998) proposed that the presence of phytate-rich diets interferes with optimal amino acid utilization from intact protein by formation of indigestible protein-phytate complexes, by inhibition of digestive enzymes, and by depressed absorption of nutrients from the small intestine. The effect of dietary phytase on crude protein or amino acid utilization by pigs seems to be inconsistent. Radcilffe et al. (1999) reported that the addition of microbial phytase has been shown to increase availability of amino acids. However, Traylor et al. (2001) reported that addition of phytase up to 1,500 PU/kg did not improve ileal digestibility of amino acids provided by soybean meal, but improved Ca and P utilization by growing pigs fed soybean meal based diets. Also, Nasi et al. (1995) reported that addition of 1,000 PU/kg did not improve apparent total tract digestibility of crude protein when pigs were fed a barley-rapeseed meal-based diet. Other studies (Ketaren et al., 1993; Mroz et al., 1994) have shown that protein deposition and (or) protein retention were increased with microbial phytase.

In terms of energy utilization, Johnston (2000) reported that the addition of microbial phytase increased GE digestibility in pigs fed a corn-soybean meal diet. Also, Williams (2001) reported that phytase increased starch digestibility in pigs fed corn-soybean meal diets. However, a recent study by Shelton et al. (2003) showed that the addition of 500 PU/kg did not have an effect on average daily gain, daily feed intake, gain:feed, longgissimus muscle area, 10th-rib fat depth, or energy availability.

Table 3.5. Summary of recent phytase experiments.

Reference	Initial BW	Diets	Phytase	Activity	ADG	ADFI	G/F	Digestibility	Bone	Others
Augspurger et al., 2003	8.4 kg	Corn-SBM	*E. coli* Phytase	400	↑	NA	↑	NA	↑ Fibula Ash	.108 %P was released by 400 PU
Traylor et al, 2001	25 kg (canulation)	Semi-purified	Natuphos (*Aspergillus niger*)	500-1500	NA	NA	NA	No effect on App & ileal AA dig. ↑ Ca, P	NA	
Sands et al., 2001	9.25 kg 14 kg	Corn-SBM w/high aP	Natuphos (*Aspergillus niger*)	600	↑	NS	NS	↑ Ca retention, P digestibility	NA	↑ Plasma P
Stahal et al., 2000	11.0 kg	Corn-SBM	Yeast	300 – 1,200	↑ (P=.15)	NS	NS	NA	NA	↑ Plasma P
Matsui et al., 2000	10.5 kg	Corn-SBM	Yeast or *Aspergillus niger*	1,000- 4,000 (yeast) 1000 (Asp.)	↑ (Yeast< Aspergillus) 4,000 (Y) = 1,000 (A)	NS	NS	NA	↑ tibial density, tibial P	↑ serum P, (yeast had less stability)
Zhang et al., 2000	9.0 kg	Corn-SBM	*Aspergillus niger*	250, 500, 2,500	↑	NS	↑	↑ DM, P, Ca	↑ 10th rib	No toxic at 2,500 PU
Kemme et al., 1999	37kg (Cannulated pigs)	Maize-SBM	*Aspergillus niger* +Lactic acid	900 + 30g/kg (lactic acid)	NA	NA	NA	↑ DM, P, Ca, Mg, N, amino acid (no interaction)	NA	
Nasi et al., 1999	36 kg	Maize-, barley-SBM	*Trichoderma reesei* + (acid phosphase)	1100 + (500, 4800, 7900, 19300)	NA	NS	NS	↑ Ash, P	NA	↑ P retention, ↑ Ca retention (Maize-SBM)
Han et al., 1998	9.9kg 12.3 kg 10.7kg	Corn-SBM-WM	Natuphos Cereal (WM)	1,200 300+WM+citric acid 1.5%	↑	↑	↑	NA	↑ MT strength	↑ Plasma P

Table 3.5 (Continued)

Reference	Initial BW	Diets	Phytase	Activity	ADG	ADFI	G/F	Digestibility	Bone	Others
Liu et al., 1998	23 kg	Corn-SBM	Natuphos (*Aspergillus niger*)	500 w/Ca:tP	↑	NS	↑	↑ P, Ca	↑ MC strength, ash	
Radcliffe et al., 1998	7.4 kg n=96 9.6 kg n=96	Corn-SBM	Natuphos (*Aspergillus niger*)	250,500,750 + citric acid	NS	NS	NS	↑ DM, P, Ca	↑ 10thrib, ash	
O'Quinn et al., 1997	50 kg 80 kg	Sorghum-SBM	Natuphos	300, 500	↑	↑	NS	↑ ileal total P, Ca No effects on DM, GE, N dig.		NS carcass CP, fat water (300 was optimum)
Liu, et al., 1997	18.7 kg	Corn-SBM	Natuphos	250, 500	↑	↑	↑	↓ fecal P (40%), 49 % w/soaking	NA	
Harper et al., 1997	18.6 kg 29.5 kg	Corn-SBM	Natuphos	250, 500	↑	↑	↑ growing phase, overall	↑ P No effects Ca,DM	↑ 10th rib	No effects on carcass
Kemme et al., 1997	10 kg 30 kg Sows	Corn,WM, SBM, Tapioca meal	*Aspergillus niger*	500	↑ growing - finishing	↑	↑ growing-finishing	↑ P, ↑ Ca except sow	NA	
Murry et al., 1997	10.9 kg	Pearl Millet-SBM	microbial	700, 1,000	↑	NS	↑	↓ fecal P trend N, Ca Zn	↑ bone mineral density	↑ serum P trend Zn

Table 3. 5. (Continued)

Reference	Initial BW	Diets	Phytase	Activity	ADG	ADFI	G/F	Digestibility	Bone	Others
Han et al., 1997	10 kg 51 kg	Corn-SBM	*Aspergillus ficuum* Cereal (WM)	1,000	↑	NS	NS	↑ retained P, N, Ca	↑ MC-MT strength	↑ serum P
Yi et al., 1996	7.5 kg n=96	semipurified	Natuphos (*Aspergillus niger*)	350,700,1050,1400	↑	↑ or NS	↑	↑ P	↑ 10^{th}rib, MC	
Cromwell et al., 1995	38.3 kg 13.0 kg n = 115	Corn-SBM	Allzyme(*Aspergillus niger*)	500 250,500, 1,000, 2,000	NS ↑	NS ↑	↑ ↑	NA ↓ P excretion	↑ MT-MC, femur	↑ aP (23%) made aP
Cromwell et al., 1995	24.2 kg 21.6 kg 15.3 kg n = 162	Corn-SBM	Natuphos (*Aspergillus niger*)	250, 500, 1,000	↑ ↑ ↑	↑ ↑ ↑	NS NS ↑	NA ↓ P excretion	↑ MT-MC, femur ash	↑ aP (29%) made aP
Adeola et al., 1995	9.4 kg n = 48	Corn-SBM (.63% tP)	Natuphos (*Aspergillus niger*)	1,500 + Zn 100ppm	↑	NS	↑	↑ Ca, P, Cu, Zn (w/Zn + phytase)	NA	↑ Plasma P, Mg, Zn(w/o Zn)
Lei, et al., 1993	7.61 kg 6.39 kg n = 62	Corn-SBM	*Aspergillus niger*	750, 1,050 1,250 1,350	NS	NS	NS	↓ fecal P (55%)	NA	
Cromwell et al., 1993	26.4 kg 33.6 kg 18.6 kg 20.7 kg n=225	Corn-SBM	FINASE(*Aspergillus niger*)	250, 500, 1000	↑ ↑ ↑	↑ NS ↑	↑ NS NS	NA	↑ MT, MC, Femur	↑ aP (43%) made aP

Phosphorus Equivalency Value of Microbial Phytase

In order for swine producers to efficiently use microbial phytase as a supplement, accurate equivalency values of phytase for P is important. Ideally, in studies designed to develop equivalency values, multiple levels of P should be fed without added phytase and multiple levels of phytase should be fed at a low level of dietary P in order to develop response equations for both P and phytase. These response equations can be set equal to one another and solved to determine the P equivalency of phytase. In general, linear response ($Y = a + bX$) or an asymptotic curve ($Y = a(1\text{-}be^{-kX})$), where Y = response and X = the level of P or phyase, have provided the best fits for phytase and P responses in corn-soybean meal based diets (Kornegay et al., 1998). However, Jongbloed et al. (1996) reported that a logistic curve provided a better fit to the response of P and phytase in a Dutch practical diet. The P equivalency value for pigs fed 500 PU/kg reported in literature range from 0.06 to 0.25% available P (Table 3.6).

Factors which may influence these estimates include the basal level of P, the response criteria used, and most importantly the ratio of Ca: total P. Phosphorus absorption has been shown to be impaired if the Ca to P ratio is too wide (NRC, 1998). Qian et al. (1996) reported that there was a negative effect of widening Ca to P ratio in excess of 1.2:1 on microbial phytase efficacy in pigs, which may be the result of poorer P absorption due to a wide Ca to P ratio rather than a decrease in phytase effectiveness. Excess Ca may also bind to the phytate molecule, making it insoluble and therefore unavailable for exposure to phytase in the gastrointestinal tract.

Table 3.6. Phosphorus equivalency of phytase in Corn-Soybean meal diets

Response Criteria	Equation	Equivalency [a] (%)
ADG	$Y= 4.062-3.865e^{-0.00095X}$	.17 [b]
	$Y = 3.362 - 3.380e^{-0.002666X}$	.25 [b]
	$Y = 0.0654 - 0.0741\ e^{-0.00839X}$	.06 [c]
	$Y = 0.084 + 0.002X$	.10 [d]
	$Y = 1.19 - 1.25\ e^{-0.0050X}$	.11 [d]
	$Y = 0.277 - 0.274\ e^{-0.000797X}$	.09 [e]
	$Y = 0.0977- 0.0988\ e^{-0.0035X}$	.08 [e]
	$Y = 0.176 + 0.00213X$	.12 [f]
	$Y = 0.033 + 0.0032X$	.16 [f]
10^{th} rib ash, %	$Y = 1.848 - 1.926\ e^{-0.0045X}$	.17 [b]
	$Y = 1.629 - 1.806\ e^{-0.0036X}$	.13 [b]
10^{th} rib strength	$Y = 0.348 - 0.357\ e^{-0.00082X}$	.11 [c]
	$Y = -0.0243 + 0.0014X$	.07 [d]
Metacarpal ash, %	$Y = 1.112- 0.1118\ e^{-0.0029X}$	.09 [e]
	$Y = 0.1127 - 0.1184\ e^{-0.00095X}$	.04 [e]
Metacarpal strength	$Y = 0.0057 + 0.000005X$	.03 [e]
	$Y = 0.0862 - 0.0891\ e^{-0.0014X}$	.04 [e]
	$Y = 0.00097 + 0.00149X$	.08 [f]
	$Y = 0.0788 + 0.002X$	.09 [f]
P Digestibility	$Y = 2.631 - 2.965\ e^{-0.00108X}$	.12 [b]
	$Y = 1.564- 1.735\ e^{-0.00284X}$	.11 [b]
	$Y = -0.087Ln(-6.718 + 7.713\ e^{-0.000199X})$	.12 [c]
	$Y = -0.464Ln(0.888 - 0.0014\ X)$	.08 [d]
	$Y = 0.1552 - 0.1489\ e^{-0.00198X}$	.10 [e]
	$Y = -0.112 + 0.0037X$	.18 [f]
	$Y = -0.22 + 0.0038X$	.17 [f]

[a] The P equivalency value for pigs for 500 PU/kg
[b] Kornegay and Qian, 1996
[c] Harper et al., 1997
[d] Radcliffe and Korenegay, 1998
[e] Skaggs, 1999
[f] Rice et al., 1999

In studies by Kornegay and Qian (1996), Jongbloed et al. (1996), and Yi et al. (1996b) only two levels of P were fed, and the response of various criteria to P was assumed to be linear. The Ca:P ratio was 2:1 in studies by Kornegay and Qian (1996) and Yi et al. (1996b). However, in a study by Jongbloed et al.

(1996) the Ca:P ratio ranged from 1.94 to 2.5:1. Harper et al. (1997) used three levels of P and maintained a Ca:P ratio at 1.2:1 to 1.4:1 in all growing-finishing diets. These authors found that 500 PU of microbial phytase released 0.96 g of P/kg of diet. For weaning pigs, Radcliffe and Kornegay (1998) determined the P equivalency value of microbial phytase. The addition of microbial phytase to a low P diet improved ADG, rib shear force, ash, and digestibility of P. In this study 500 PU/kg of diet was equivalent to 0.84 g of P/kg of diet (.084% P).

Data from 52 experimental have been used to estimate the P equivalency value of dietary microbial phytase (Kornegay et al., 1998). These authors used data from these experiments to generate a response curve for P digestibility, digested P (g/kg), and P excretion as influenced by phytase and P. The addition of dietary phytase increased P digestibility, but the magnitude of this response is dependent on diet type, total P content of the diet, phytate P content of the diet, Ca:P ratio, and age and physiological status of the pig. The actual data was plotted to generate the curve. For basal diets, with no added phytase, P digestibility ranged from 8.4 to 63%. This wide range is due to the inclusion of plant feedstuffs with intrinsic phytase activity in some studies, differences in the Ca:P ratio, differences in the inclusion level of inorganic P, and differences in the phytate P level of the diet. This variation continues as phytase is added to the diets and causes the relatively poor fit (r^2=.47) of the response curve calculated in this review. However, if the equation and observed value are adjusted by calculating the percentage unit improvement in P digestibility as phytase is added to the diet, then the variation is decreased. Based on the review by Kornegay et

al. (1998), 500 PU of phytase/kg of diet will release 0.75 g of P. If this number is divided by the estimated bioavailability of inorganic P (76.7%) then 500 PU/kg can replace 0.98 g of P from the inorganic P source, which is in agreement with the value from Harper et al (1997) and Radcliffe and Kornegay (1998).

In summary, phytate content in feed ingredients varies. The availability of phytate to exogenous microbial phytase hydrolysis varies from ingredient to ingredient (Ravindran et al., 1999). For practical purposes, a significant safety margin needs to be employed in estimation of the phosphorus contribution as a result of phytate hydrolysis induced by microbial phytase. The consequence of overestimation for the benefit of the phytase are dramatic. van Tuijl (1998) reported the estimates of P release as a result of the addition of phytase seems to be over-optimistic when translated into a commercial production system. Nevertheless, the benefit in reducing pollution is clear.

CHAPTER IV

Experiment 1

Effects of a Solid-State Fermented Phytase on Growth Performance, Phosphorus Excretion, Bone traits and Tissue Accretion Rates of Growing Pigs fed Low P, Corn-Soybean Meal Based Diets

ABSTRACT: Forty-two barrows (avg BW = 19.9 kg) were used in a 33-d study to determine the effects of the addition of a solid-state fermented phytase complex (Allzyme SSF; Alltech, Inc) to low P, corn-soybean meal diets on growth performance, P excretion, bone traits, and tissue accretion rates. Pigs were blocked by weight and ancestry, and randomly allotted to one of seven dietary treatments (6 pigs/trt). A basal diet consisted of corn and soybean meal and was adequate in all nutrients, except Ca and P. This diet contained 0.34% total P (0.07% available P), all of which was provided by corn and soybean meal. Treatments were the basal, the basal plus monosodium phosphate (MSP) to provide 0.05, 0.10, and 0.15% added available P, and the basal plus enzyme to provide 250, 500, and 1,000 PU/kg. All diets were formulated to 0.95% total lysine and a Ca:total P ratio of 1.2:1. Pigs were housed individually in metabolic chambers with ad libitum access to feed and water. There were two 5-d total collection periods (d 10-15 and d 25-30) during the 33-d study. At the end of the 33-d study, all pigs were killed and the femurs and 3rd/4th metacarpals and metatarsals (MM) were extracted. The remainder of the carcass was ground for

ash and P analysis. Average daily gain and G:F increased (linear, $P < 0.05$) with addition of MSP or SSF. However, ADFI was not affected ($P > 0.29$) by either addition of MSP or SSF. The addition of 500 or 1,000 PU/kg to the low P, corn-soybean meal diet increased ADG and G:F similar to that for the highest level of MSP. Dry matter, N, and energy digestibility were not different ($P > 0.10$) among treatments, but digestibility of P increased (linear, $P < 0.01$) with addition of MSP or SSF. Compared to the basal diet, additions of SSF decreased P excretion (3.06 vs 2.48, 2.36, 1.68 g/d) by 19.3, 23.3, and 45.4%, respectively. Bone breaking strength (BS) of MM and femurs and ash (%) increased (linear, $P < 0.01$) with increasing MSP or SSF. Based on average BS and ash, addition of 250, 500, or 1,000 PU/kg was equivalent to 0.066, 0.120, and 0.140% available P, respectively. For the carcass, the contents (%) and accretion rates of water, protein, and fat were not affected ($P > 0.10$) by either MSP or SSF. The content (%) and accretion of P and ash increased (linear, $P < 0.01$) with addition of MSP and SSF. The increase in bone strength and carcass P associated with increasing SSF was similar to that for MSP addition. These data indicate that the addition of a solid-state fermented phytase improves growth performance and P utilization, and markedly reduces P excretion of pigs fed low P, corn-soybean meal diets.

Key Words: Pigs, Phytase, Bone strength, Phosphorus excretion

Introduction

Phytate (myoinositol 1,2,3,4,5,6 hexa, dihydrogen phosphate) is the major form of P in cereal grains and oilseed meals (Reddy et al., 1982). Approximately 60 to 70% of the P in corn and soybean meal is in the form of phytate (Nelson et al., 1968; NRC 1998). Monogastric animals such as pigs and chicks can not utilize phytate P efficiently due to the lack of endogenous phytase that hydrolyzes phytic P (Taylor, 1965; Peeler 1972). Therefore, inorganic sources of P have been routinely added to diets for non-ruminant animals to supply sufficient levels of P which can lead to significant amounts of P excreted to the environment. Recently, environmental concerns related to excess P excretion have become a major issue confronting swine industry. Due to these concerns, decreasing addition of inorganic P and the addition of dietary phytase may necessary to reduce concerns related to P excretion. Many efforts have been made to decrease P excretion by improving P bioavailability in the feedstuff. Dietary phytase has been added to swine diets to improve P utilization and decrease the amount of P excretion into the environment (Jongbloed 1987; Lei et al., 1993; Cromwell et al., 1995; O'Quinn et al., 1997).

There are several types of phytase already available in the market and new phytase sources are being developed. Most of the phytase in the market is produced by submerged microbial fermentation (SmF). Recently, solid-state fermentation (SSF) technology has been used as an alternative to produce phytase. Therefore, the purpose of this study was to determine the effects of the addition of a solid-state fermented phytase complex (Allzyme SSF; Alltech, Inc)

to low P, corn-soybean meal diets on growth performance, P excretion, bone traits, and tissue accretion rates in growing pigs.

Materials and Methods

Animals, Diets, and Treatments. A total of 42 barrows (Crossbred, Yorkshire, Hampshire) with an average BW of 19.9 kg were used in a 33-d study to investigate the effects of phytase addition on growth performance and bone strength of pigs fed corn-soybean meal-based diets. Pigs were blocked by body weight and allotted randomly to one of seven dietary treatments in a randomized complete block design.

All diets were corn-soybean meal based (Table 4.1). Diet 1 served as the basal diet and was composed of corn and soybean meal. Corn and soybean provided 0.34% total P and 0.07% available P in the basal diet. Monosodium phosphate (MSP) was added to the basal diet in increasing amounts to provide 0.05, 0.10, and 0.15% available P (Diet 2 to 4). Diets 5 to7 were as Diet 1 with additions of 250, 500, or 1,000 phytase units (PU)/kg of diet. All experimental diets were formulated to contain 0.95% lysine. All nutrients met or exceeded NRC (1998) standards except Ca and P. The Ca: total P ratio in all diets was fixed at 1.2:1.

In this experiment, pigs were individually housed in metabolic chambers in an environmentally-controlled room. The chambers were specially designed for the total, but separate collection of feces, urine, and wasted feed. Each chamber

had a galvanized steel mesh floor and one stainless steel self-feeder and one nipple waterer. Beneath the floor, a five-quart plastic container was used to collect urine. All pigs were allowed ad libitum access to feed and water. All diets were fed in meal form.

Collection and Analyses. There were two 5-d collection periods (d 10 to 15 and d 25 to 30) during the 33-d study. Feces were collected every morning during the collection periods from the 1-mm screen under the chamber. The collected feces were immediately weighed and placed in a plastic bag, and frozen (-20 °C). At the same time, refused feed was collected and weighed.

Initially, frozen fecal samples were dried in a forced-air oven for 4 d at 55°C before grinding. Partially dried feces and diets were ground through a 1-mm screen using a Wiley Mill (Standard Model No.3; Arthur H. Thomas Co., Philadelphia, PA). Dry matter content of diets and feces were determined by drying at 100°C for 24 h (AOAC, 1998). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1998) using an automated analyzer (FOSS Tecator, 2020 Digestor, 2400 Kjeltec Analyzer; Hoganas, Sweden). Total phosphorus content in feces and diets was determined by a gravimetric quinolinium molybdophosphate method (AOAC, 1998).

At the end of the experiment, all pigs were slaughtered. Following scalding, scraping, and evisceration, the hot carcass was weighed. The front and rear feet were removed and frozen (-20 °C). Also, the femurs from each pig were excised and placed in a plastic bag and frozen (-20 °C). Later, the feet

were allowed to thaw and autoclaved at 120 °C and 15 psi for approximately 7 min. Following autoclaving, the MC and MT were extracted and cleaned of extraneous tissue, and frozen. Later, the MC and MT were allowed to thaw overnight before breaking strength analyses. Breaking strength of the MC and MT were determined using an Instron Universal Testing Machine (Model 4502, Instron, Canton, MA) by procedures of Cromwell et al. (1972). Bones were placed on supports 3.8 cm apart for the MC and MT, and 8.1 cm apart for femurs. Breaking strength was defined as the amount of force (kg) required to break the bone when placed in a horizontal position. The breaking strength of MC and MT were averaged. The marrow from the fourth MC of the right foot of each pig was removed and the bones were soaked in petroleum ether for 24 h to remove fat. After fat extraction, the bones were dried overnight at 100 °C, weighed, and ashed for 48 h at 600 °C. Both femurs from each pig were cleaned of all extraneous tissues and weighed. The outside diameter of the femurs was measured at the midshaft by averaging two measurements taken 90° to each other, using a caliper. Breaking strength of the femurs were performed as previously described for the MC and MT.

At the start of the experiment, 6 pigs were initially slaughtered for the determination of initial body composition. At the end of the experiment, all pigs were weighed and slaughtered at the Oklahoma State University meat lab. The carcasses were weighed and placed in box and stored at –20 °C for grinding and lab analyses. The carcasses were cut into small pieces with a band saw and ground three times through a 64-mm screen using a commercial meat grinder

(Auto Grinder, Model 801GHP, Astria, OR). During the grinding process, dry ice was added to prevent water loss. After grinding, approximately 500 g of well-mixed sample was collected in a plastic container and stored in a -20 °C freezer. Carcass sub-samples were freeze-dried (Virtis Freezemobile 12SL;Gardiner, NY). The frozen carcass sub-samples were ground using the same procedure for feed and feces. Nitrogen and phosphorus content in the carcass was determined by the same procedures described in feed and feces analysis.

Accretion Rate. Accretion rates of the chemical components (water, protein, lipid, phosphorus and ash) of the carcass were determined by a "comparative slaughter" procedure as described by Carter and Cromwell (1998). Six pigs from the same contemporary group as the experimental pigs were harvested at the start of experiment for the determination of initial body composition. The weight of the pigs used to estimate initial carcass composition ranged from 14.5 to 25.5 kg BW. The carcasses from the initial pigs were processed the same as that described for the experimental pigs. Carcass composition was regressed on BW for each of the initial pigs to obtain a prediction equation to estimate the initial carcass composition for each of the experimental pigs based on its initial BW. Accretion rates of the chemical components (water, protein, lipid, phosphorus and ash) were determined by subtracting the estimated initial composition from the final composition and dividing by the number of days on test for each pig.

Statistical Analyses. Data were analyzed as a randomized complete block design using procedures described by Steel et al. (1997) with initial BW as the blocking criterion. The model included the effects of block (rep), treatment, and block × treatment (error). The model for digestibility included the effects of block (rep), treatment, period and treatment × period. The effects of MSP and phytase supplementation were tested for linearity and curvilinearity using orthogonal polynomial contrasts. For the four levels of phytase, polynomial coefficients for unequally spaced treatments were generated using the ORPAL matrix function of the IML procedure of SAS. In addition, a nonorthogonal contrast was used for testing treatments between SSF phytase and control diet. In all cases, pig served as the experimental unit.

Results

Analyzed total P contents in the diets were 0.37, 0.43, 0.48, 0.52, 0.37, 0.37, and 0.37% for the 7 dietary treatments, respectively. These were somewhat higher than the calculated value; however, the incremental increase in P was similar to calculated values. All diets without SSF phytase had similar levels of total P content.

Growth Performance. Average daily gain was increased (linear, $P < 0.03$) by the addition of monosodium phosphate (Table 4.2). Among the pigs fed diets with SSF phytase, ADG was linearly increased ($P < 0.05$) as SSF phytase increased from 0 to 1,000 units/kg. Adding 500 or 1,000 PU/kg of SSF phytase to the basal diets increased ADG and gain:feed by 17% and 9%, respectively,

compared with basal diet. However, with increasing phytase level from 500 to 1,000 PU/kg, there was no further improvement in ADG. Average daily feed intake for 33 d was not affected ($P > 0.29$) by addition of monosodium phosphate or SSF phytase. Compared with pigs fed the basal diet, ADG ($P < 0.03$) and gain:feed ($P < 0.08$) for pigs fed SSF phytase supplemented diets was greater.

Excretion and Digestibility. There was no interaction ($P > 0.5$) between treatment and period; therefore, the data were pooled. Excretion and absorption of dry matter, energy, and nitrogen were not affected ($P > 0.89$) by either the addition of monosodium phophate or SSF phytase (Table 4.3). Among the pigs fed monosodium phosphate, the amount of P excretion was similar, but percent P excretion relative to P intake was decreased by addition of monosodium phosphate. For the pigs fed SSF phytase, the amount of P excretion via feces decreased (linear, $P < 0.01$) as SSF phytase increased. The level of 1,000 PU/kg improved P digestibility by 26% units compared to the basal diet. Also, supplementation of SSF phytase linearly decreased ($P < 0.01$) P excretion. Compared with the basal diet, P excretion via the feces was dramatically reduced by 22.8% and 45.9% with addition of 500 and 1,000 PU/kg, respectively. Also, digestibility of P was improved linearly ($P < 0.01$) from 44% to 70% when SSF phytase was added up to 1,000 unit/kg of diet.

Bone characteristics. For bone breaking strength, the addition of monosodium phosphate to the basal diet increased ($P < 0.01$) breaking strength

of the metacarpals, metatarsals, and femurs (Table 4.4). When SSF phytase was added, bone (metacarpal, metatarsal and femurs) breaking strength also increased (linear, $P < 0.01$). Pigs fed diet supplemented with 1,000 PU/kg had the maximum bone breaking strength. However, adding SSF phytase from 500 to 1,000 PU/kg did not increase bone breaking strength as much as from 0 to 500 PU/kg. Metacarpal-metatarsal and femur breaking strengths of pigs fed the basal diet with monosodium phosphate and SSF phytase were regressed based on total P and available P intake. The bone breaking strength fit very well ($R^2 = 0.99$) when linearly regressed based on total P (Figure 4.1). Compared with the basal diet, pigs fed diets containing SSF phytase had greater ($P < 0.01$) bone breaking strength and ash.

For physical characteristics of the bone (Table 4.4), the weight of metacarpals ($P < 0.06$), metatarsals ($P < 0.01$), and femurs ($P < 0.01$) were increased by addition of monosodium phosphate. Also, the addition of SSF phytase increased (linear, $P < 0.01$) bone weight (metacarpal, metatarsal, and femurs). The diameter of the femurs was increased by the addition of MSP ($P < 0.02$) and dietary SSF phytase supplementation ($P < 0.04$). Ash content in the metacarpals increased (linear, $P < 0.01$) with increased level of monosodium phosphate and SSF phytase.

Carcass composition and accretion. For carcass composition (Table 4.5), the percentages of water, protein (N × 6.25) and fat were not affected ($P > .66$) by addition of MSP or SSF phytase. However, the percentages of ash and P

were increased (linear, $P < 0.01$) by addition of MSP or SSF phytase. Accretion rates (g/d) of water, protein, and fat were similar ($P > 0.10$) among the dietary treatments. However, the accretion rates of P and ash increased (linear, $P < 0.01$) as MSP or SSF phytase increased. The accretion rates of P and ash for pigs fed diets with SSF phytase were greater ($P < 0.01$) than that for pigs fed the basal diet.

Discussion

The natural storage form of P in almost all plants exists as phytate (Cosgrove, 1980). About 60 to 70% of the P is organically bound in the form of phytate in cereal grains, grain by-products, and oilseed meals (Lelson et al., 1968; Lolas et al., 1976), which is poorly available to the pig (Taylor, 1965; Peeler, 1972; Cromwell, 1979) due to lack of phytase activity. Therefore, inorganic P is routinely added to feeds to supply sufficient P for growth, which cause excretion of unavailable P to the environment. During the past decade, microbial dietary phytase has been developed and utilized in the feed industry. Yet, nutritionists are looking for cheaper and better sources of phytase.

In terms of production method, most of dietary microbial phytases in the market are produced by the submerged fermentation method. Also, genetically modified microbes are commonly used to produce large volumes of commercial dietary phytase. An early study by Han et al. (1987) demonstrated that cultivation of microbes on a solid substrate was an economical method for phytase production. Recently, increased interest has been given to SSF

methods and this method has been applied to produce commercial scales of microbial phytase because of their biological, practical and economical advantages, which includes higher product concentration, improved product recovery, simplicity of cultivation equipment, decreased wastewater output, lower capital investment, and lower plant operation cost (Becerra and Gonzalez, 1996). Another advantage is that the phytase complex from SSF has a significant amount of side enzyme activity such as β-glucanase, protease, xylanase, cellulase (Filer, 2001), which is an attractive advantage from a nutritional standpoint.

As we expected, P digestibility was dramatically improved by addition of SSF phytase. Among the pigs fed MSP, the amount of daily fecal P excretion was similar, but P excretion based on % P intake was decreased as MSP increased. Also, supplementation of SSF phytase decreased daily fecal P excretion. Addition of 500 and 1,000 PU/kg reduced fecal P excretion by 22.8% and 45.9%, respectively, compared with the basal diet. Note that pigs fed the highest levels of SSF phytase (1,000 PU/kg) had similar ADG and gain:feed with those fed highest level of added available P from MSP. Also, digestibility of P was improved linearly from 44% to 70% as SSF phytase increased up to 1,000 PU/kg of diet. These results are similar with previous studies with SmF phytase (Cromwell, 1995, 1996).

Addition of SSF phytase up to 1,000 PU/kg improved bone breaking strength. Similar results consistently have been found in previous research (Han et al.,1997, 1998; Liu et al., 1998; Cromwell et al., 1993, 1995; O'Quinn et al.,

1997, Zhang et al, 2000). Even though there was a linear increase in P digestibility, a quadratic improvement in bone breaking strength by SSF phytase was observed which is somewhat different than other studies. Ca:total P was fixed at 1.2:1 in this study. Phytase was added to the basal diet from 250 to 1,000 PU/kg with fixed Ca (0.41%) levels. It seems Ca might have been limited for bone mineralization in this experiment. A previous study showed that excessively wide Ca:total P ratio decreased P absorption, and growth performance of weanling (Qian et al., 1996) and growing-finishing pigs (Liu et al., 1994, 1998). Also, previous studies indicated that Ca:total P ratio had positive effects on phytase. Liu et al. (2000) reported that lowering Ca:total P ratio in diets containing phytase from 1.5:1 to 1.0:1 increased the apparent absorption (% and g/d) of P in the small intestine, but Ca absorption was not affected. Therefore, when formulating diets with dietary phytase, lowering Ca:total P ratio is critical for maximizing P absorption and effects of phytase. However, in other studies, Ca levels were fixed at the NRC standard with decreased total P levels (Cromwell et al., 1993,1995) resulting in wide Ca:total P ratios (1.66:1 to 1.88:1). Even though there were positive phytase effects on P absorption, the intensity of phytase might be diminished by relatively high Ca content in diets.

Because SSF phytase contains significant side enzyme activity (Filer, 2001) the addition of SSF phytase complex has the potential to improve digestibility of other nutrients besides P. Phytic acid is known to inhibit enzymes such as α-amylase, trypsin, tyrosinase, and pepsin (Erdman and Poneros, 1989; Dvorakova, 1998; and Ebune et al., 1995). Thus, addition of phytase can

improve digestibility of nutrients resulting in greater growth (Liu et al., 1998). In our experiment, ADG and gain:feed were increased when SSF phytase (up to 1,000 units/kg) was added to low P, corn-soybean meal diets. Adding 500 units/kg phytase to the basal diet dramatically increased ADG, and from 500 to 1,000 PU/kg there was no further improvement. Average daily feed intake was not affected by addition of MSP or SSF phytase, which is similar to previous studies with phytase from SmF (Cromwell, 1995ab, 1998). However, other studies have not shown improvements in gain:feed (Lei et al., 1993; Han et al. 1997; Radcliffe et al. 1998). The increased level from 0 to 500 PU/kg dramatically increased gain:feed ratio, but there was little further improvement from 500 PU/kg to 1,000PU/kg, indicating 500 PU/kg is the optimum level for growth performance. Similar responses have been reported in previous studies (Cromwell et al, 1995ab; O'Qunn, 1997; and Harper et al., 1997).

Also, we did not find any improvement in digestibility of dry matter, protein, and energy. In this study, we fed a corn-soybean meal diet which was relatively low in fiber content, and pigs can readily utilize the nutrients from this type of diet. In agreement, a recent study by Traylor et al. (2001) reported that the addition of phytase did not improve the utilization of amino acids provided by soybean meal.

These data indicate the addition of SSF phytase to low P, corn-SBM based diets improves the utilization of phytate P in corn and soybean meal. Based on our results, it seems that 500 PU/kg is the optimum level when pigs are fed corn-SBM based diet without inorganic P addition. Addition of SSF phytase up to 1,000 PU/kg improved P bioavailability resulting in improved growth, bone

strength, and decreased P excretion. Similar results have been found in previous research with SmF phytase from mutant and recombinant-derived phytase (Joongbleod et al.,1992; Lei et al., 1993; Cromwell et al., 1993, 1994; O'Quinn et al., 1997, Zhang et al, 2000). Addition of microbial phytase, mostly from submerged fermentation, improved the bioavailability of P in corn-soybean meal diets (Zhang et al., 2000; Han et al., 1997; Liu et al., 1998; Cromwell et al., 1993, 1995a, b) and P equivalency value of phytase (500 PU/kg) based on bone breaking strength ranged from 0.27 to 0.93 g/kg (Skaggs, 1999; Rice et al., 1999). In our study, 1.74 g/d of P was liberated from corn and soybean meal by the addition of SSF phytase (500 PU/kg), which was equivalent to 0.11% available P in the diet (Table 4.6) based on average bone strength and ash content. These data indicate solid state fermented phytase is similar or better than that from submerged fermentation. Based on equivalency values of 250, 500, and 1,000 PU/kg were used to generate prediction equation of SSF phytase (Figure 4.2). The prediction equation of P equivalence value of SSF phytase was $Y= -2E-07x^2 + 0.0003X$ (Y=available P, X= phytase activity, PU/kg). By using this equation, the P equivalency value can be predicted from 0 to 1,000 PU/kg, which can be used in formulating diets with SSF phytase.

This study shows the addition of SSF phytase to low P, corn-soybean diets have similar effects that have been found in previous studies with SmF phytase, which indicate that the solid-state fermentation method is an alternative way to produce a quality microbial phytase.

Implications

In the present study, the addition of 1,000 PU/kg to low P, corn-soybean meal diets maximized growth performance, phosphorus utilization, and bone traits. Yet, 500 PU/kg was equivalent to 0.11% P and appear to be the optimum level for growing pigs fed low P, corn soybean meal diets. This study indicate that the solid state fermented phytase method can be used as an alternative way to produce microbial dietary phytase.

Table 4.1. Composition of experimental diets, as-fed basis (Exp.1)

	Diet						
Total P, %	0.34	0.39	0.44	0.49	0.34	0.34	0.34
Available P, %	0.07	0.12	0.17	0.22	0.07	0.07	0.07
SSF phytase, PU/kg	0	0	0	0	250	500	1,000
Corn	72.07	72.07	72.07	72.07	72.07	72.07	72.07
Soybean meal	25.25	25.25	25.25	25.25	25.25	25.25	25.25
Corn Starch	1.16	0.78	0.39	0.00	1.14	1.11	1.06
Monosodium phosphate	0.00	0.21	0.43	0.66	0.00	0.00	0.00
Limestone	0.82	0.99	1.16	1.32	0.82	0.82	0.82
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25
TM & Vit premix[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Antibiotic[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.84
SSF Phytase[c]	0.00	0.00	0.00	0.00	0.03	0.05	0.10
Calculated analysis							
ME, kcal/kg	3,364	3,349	3,334	3,318	3,364	3,364	3,364
Lysine, %	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Ca, %	0.41	0.47	0.53	0.59	0.41	0.41	0.41
Total P, %[d]	0.34	0.39	0.44	0.49	0.34	0.34	0.34
Available P, %	0.07	0.12	0.17	0.22	0.07	0.07	0.07
Ca:Total P	1.21	1.21	1.20	1.20	1.21	1.21	1.21
Added phytase activity, PU/kg	0	0	0	0	250	500	1,000

[a] Provided the following per kg of diet: 5,506 IU of vitamin A, 551 IU of vitamin D, 33 IU of vitamin E, 3.6 g of vitamin K (as menadione), 221 μg of biotin, 137 mg of choline, 33.04 mg of niacin, 24.78 mg of panthothenic acid (as d-pantothenate), 5.51 mg of riboflavin, 27.55 μg of vitamin B_{12}, 1.66 mg of folacin, 100 mg of Zn, 2 mg of Mn, 100 mg of Fe, 10 mg of Cu, .30 mg of I, and .30 mg of Se.

[b] Provided 55 mg of chlortetracycline per kilogram of diet.

[c] Solid-state fermented phytase (Allzyme® SSF; Alltech, Inc) contains 1,000 PU/g of product

[d] Analyzed total P were 0.37, 0.43, 0.48, 0.52, 0.37, 0.37, and 0.37 %, respectively.

Table 4.2. Effects of monosodium phosphate and solid-state fermented phytase on growth performance of pigs fed low P, corn-SBM based diets[a].

	Diet							
Total P, %	0.34	0.39	0.44	0.49	0.34	0.34	0.34	
Available P, %	0.07	0.12	0.17	0.22	0.07	0.07	0.07	
SSF phytase, PU/kg	0	0	0	0	250	500	1,000	SE
ADG, kg [bcd]	0.635	0.671	0.743	0.720	0.691	0.742	0.741	0.027
ADFI, kg	1.47	1.43	1.54	1.47	1.47	1.55	1.52	0.05
Gain:feed [bc]	0.45	0.47	0.48	0.49	0.47	0.48	0.49	0.01

[a] Least squares means for 6 pigs/trt
[b] Linear effect of added monosodium P ($P < 0.05$)
[c] Linear effect of added phytase ($P < 0.05$)
[d] None vs SSF phytase ($P < 0.01$)

Table 4.3. Effects of monosodium phosphate and solid-state fermented phytase on nutrient digestibility of pigs fed low P, corn-SBM based diets[a] (DM basis).

	Diet							
Total P, %	0.34	0.39	0.44	0.49	0.34	0.34	0.34	
Available P, %	0.07	0.12	0.17	0.22	0.07	0.07	0.07	
SSF phytase, PU/kg	0	0	0	0	250	500	1,000	SE
Phosphorus								
Intake, g/d	5.50	6.02	7.66	7.66	5.49	5.83	5.68	0.21
Feces, g/d [d]	3.06	2.98	3.42	3.00	2.48	2.36	1.68	0.15
Absorbed, g/d [cdg]	2.43	3.16	4.24	4.66	3.02	3.48	4.01	0.15
Digestibility, % [cdg]	44.07	49.67	55.75	60.72	54.75	59.45	70.54	1.61
Excretion, % [cdg]	55.9	50.3	44.2	39.2	45.2	40.5	29.5	1.6
Dry matter								
Intake, g/d [g]	1327.9	1280.6	1449.3	1351.8	1339.9	1426.9	1394.1	45.1
Feces, g/d [g]	157.3	157.7	187.3	157.4	161.7	161.8	163.9	8.9
Absorbed, g/d	1170.6	1122.9	1262.0	1194.4	1178.2	1265.0	1230.2	39.1
Digestibility, %	88.10	87.63	87.27	88.32	87.94	88.64	88.23	0.44
Energy								
Intake, kcal/d	5,906	5,631	6,335	5,911	5,939	6,307	6,069	265
Feces, kcalg/d [g]	773	772	912	756	803	807	841	55
Absorbed, kcal/d [g]	5,133	4,858	5,423	5,154	5,137	5,501	5,227	229
Digestibility, %	86.92	86.23	85.81	87.19	86.50	87.24	86.09	0.63
Nitrogen								
Intake, g/d	44.1	42.2	48.9	44.9	45.0	50.0	46.6	1.6
Feces, g/d [g]	7.2	7.2	8.7	7.2	7.5	7.4	7.5	0.4
Absorbed, g/d [f]	37.0	35.0	40.3	37.8	37.5	42.6	39.1	1.3
Digestibility, %	83.78	82.68	82.62	84.11	83.12	85.31	84.01	0.67

[a] Least squares means for 6 pigs/trt

[b] Linear effect of monosodium P ($P < 0.05$), [c] Linear effect of monosodium P ($P < 0.01$);

[d] Linear effect of SSF phytase ($P < 0.01$), [e] Quadratic effect of monosodium P ($P < 0.05$)

[f] Quadratic effect of SSF phytase ($P < 0.05$), [g] None vs SSF phytase ($P < 0.01$)

Table 4.4. Effects of monosodium P and solid-state fermented phytase on bone characteristics of pigs fed low P, corn-SBM based diets[a].

	Diet							
Total P, %	0.34	0.39	0.44	0.49	0.34	0.34	0.34	
Available P, %	0.07	0.12	0.17	0.22	0.07	0.07	0.07	
SSF phytase, PU/kg	0	0	0	0	250	500	1,000	SE
Breaking strength, kg								
Metacarpal[cehi]	38.2	46.9	54.1	69.2	48.9	59.5	63.7	2.3
Metatarsal[cegi]	33.2	45.6	56.3	70.1	48.1	59.2	67.2	3.7
Femur[cehi]	113.6	140.48	196.8	236.4	164.1	212.5	219.6	10.2
Metacarpal ash, %[cegi]	47.37	48.91	50.33	52.51	49.57	51.49	52.21	0.57
Metacarpal ash, g[cehi]	2.10	2.33	2.71	3.11	2.57	2.95	2.99	0.08
Bone weight, g								
Metacarpal [bf]	64.2	58.7	64.8	67.3	60.9	65.8	65.2	1.7
Metatarsal [cei]	73.0	75.2	78.4	83.9	77.9	81.3	82.7	1.7
Femur [cej]	159.3	159.1	167.7	181.6	161.5	177.7	180.4	3.6
Femur diameter, mm [bd]	19.2	19.5	19.4	20.8	19.4	20.6	20.5	0.4

[a] Least squares means for 6 pigs/trt
[b] Linear effect of monosodium P ($P < 0.05$)
[c] Linear effect of monosodium P ($P < 0.01$)
[d] Linear effect of SSF phytase ($P < 0.05$)
[e] Linear effect of SSF phytase ($P < 0.01$)
[f] Quadratic effect of monosodium P ($P < 0.05$)
[g] Quadratic effect of SSF phytase ($P < 0.05$)
[h] Quadratic effect of SSF phytase ($P < 0.01$)
[i] None vs SSF Phytase ($P < 0.01$)
[j] None vs SSF Phytase ($P < 0.05$)

Table 5.5. Effects of monosodium P and solid-state fermented phytase on carcass composition and tissue accretion rates for growing pigs fed low P, corn-SBM based diets[a]

	Diet							
Total P, %	0.34	0.39	0.44	0.49	0.34	0.34	0.34	
Available P, %	0.07	0.12	0.17	0.22	0.07	0.07	0.07	
SSF phytase, PU/kg	0	0	0	0	250	500	1,000	SE
Carcass composition, %								
Water	63.57	64.48	63.14	64.02	64.88	62.44	63.51	0.52
Protein (N × 6.25)	18.91	18.65	19.02	18.76	18.64	19.06	19.07	0.26
Fat	15.63	14.64	15.85	14.75	15.35	16.37	15.04	0.64
Ash [bce]	1.88	2.09	2.36	2.45	2.13	2.37	2.47	0.07
P [bce]	0.33	0.37	0.41	0.46	0.38	0.41	0.44	0.01
Accretion rate, g/d								
Water	256.0	271.0	290.8	280.3	273.6	280.4	276.0	12.5
Protein (N × 6.25)	132.2	128.5	142.5	133.6	131.5	141.3	137.0	5.2
Fat	106.5	96.5	116.4	100.9	104.6	119.0	104.8	8.5
Ash [bcde]	10.6	12.4	16.3	16.2	13.1	16.0	16.3	0.7
P [bcde]	1.46	1.77	2.33	2.63	1.88	2.32	2.44	0.13

[a] Least squares means for 6 pigs/trt
[b] Linear effect of monosodium P ($P < 0.01$)
[c] Linear effect of SSF phytase ($P < 0.01$)
[d] Quadratic effect of SSF phytase ($P < 0.05$)
[e] None vs SSF Phytase ($P < 0.01$)

Table 4.6. Improvements of P digestibility by addition of solid-state fermented phytase in corn-SBM based diets.

Item	SSF Phytase, PU/kg		
	250	500	1,000
Feed Intake, kg/d	1.47	1.55	1.52
Total P intake, g/d[a]	4.99	5.29	5.17
Assuming no phytase			
Available P intake, g/d[b]	1.03	1.09	1.06
Unavailable P intake, g/d[c]	3.96	4.20	4.11
Available of P, %	20	20	20
Resulting from phytase based on MC-MT strength			
Available P intake, g/d[d]	1.93	2.68	3.07
Unavailable P intake, g/d[e]	3.06	2.61	2.10
Available of P, %[f]	38.7	50.7	59.4
Unavailable P made available, %[g]	22.7	37.9	48.9
Available P liberated by phytase, g/d[h]	0.90	1.59	2.01
Equivalent to available P in diet, %[i]	0.06	0.10	0.13
Resulting from phytase based on femur strength			
Available P intake, g/d[d]	2.00	2.88	3.01
Unavailable P intake, g/d[e]	2.99	2.41	2.16
Available of P, %[f]	40.0	54.4	58.2
Unavailable P made available, %[g]	24.5	42.6	47.4
Available P liberated by phytase, g/d[h]	0.97	1.79	1.94
Equivalent to available P in diet, %[i]	0.07	0.12	0.13
Resulting from phytase based on average BS			
Available P intake, g/d[d]	1.98	2.81	3.03
Unavailable P intake, g/d[e]	3.01	2.48	2.14
Available of P, %[f]	39.7	53.1	58.6
Unavailable P made available, %[g]	24.0	41.0	47.9
Available P liberated by phytase, g/d[h]	0.95	1.72	1.96
Equivalent to available P in diet, %[i]	0.07	0.11	0.13

Resulting from phytase based on MC ash, g			
Available P intake, g/d[d]	2.15	3.01	3.12
Unavailable P intake, g/d[e]	2.84	2.28	2.05
Available of P, %[f]	43.1	56.9	60.3
Unavailable P made available, %[g]	28.3	45.7	47.9
Available P liberated by phytase, g/d[h]	1.12	1.93	2.06
Equivalent to available P in diet, %[i]	0.08	0.12	0.14
Resulting from phytase based on P accretion, g/d			
Available P intake, g/d[d]	1.84	2.66	2.87
Unavailable P intake, g/d[e]	3.15	2.63	2.30
Available of P, %[f]	36.8	50.3	55.5
Unavailable P made available, %[g]	20.5	37.4	44.0
Available P liberated by phytase, g/d[h]	0.81	1.57	1.81
Equivalent to available P in diet, %[i]	0.06	0.10	0.12
Average [j]			
Available P intake, %	39.9	53.4	59.1
Unavailable P made available by phytase, %	24.3	41.4	46.6
Available P liberated by phytase, g/d	0.96	1.74	1.94
Equivalent to available P in diet, %	0.07	0.11	0.13

[a] Total dietary P content in diet (%) × average daily feed intake (g/day).
[b] Total P intake × available P content (%) in corn-soybean meal (20% from NRC, 1998).
[c] Total P intake – available P intake.
[d] Based on the standard regression for MSP.
[e] Total P intake – available P intake from the standard regression.
[f] Available P intake/total P intake × 100.
[g] Additional available P intake/original unavailable P intake × 100.
[h] Value from d – value from b (i.e. 1.93- 1.03 = 0.90).
[i] Available P liberated by phytase /average daily feed intake × 100.
[j] Average of bone breaking strength and ash content in metacarpals.

Figure 4.1. Metacarpal-metatarsal breaking strength (BS) of pigs fed low P, corn-SBM based diets

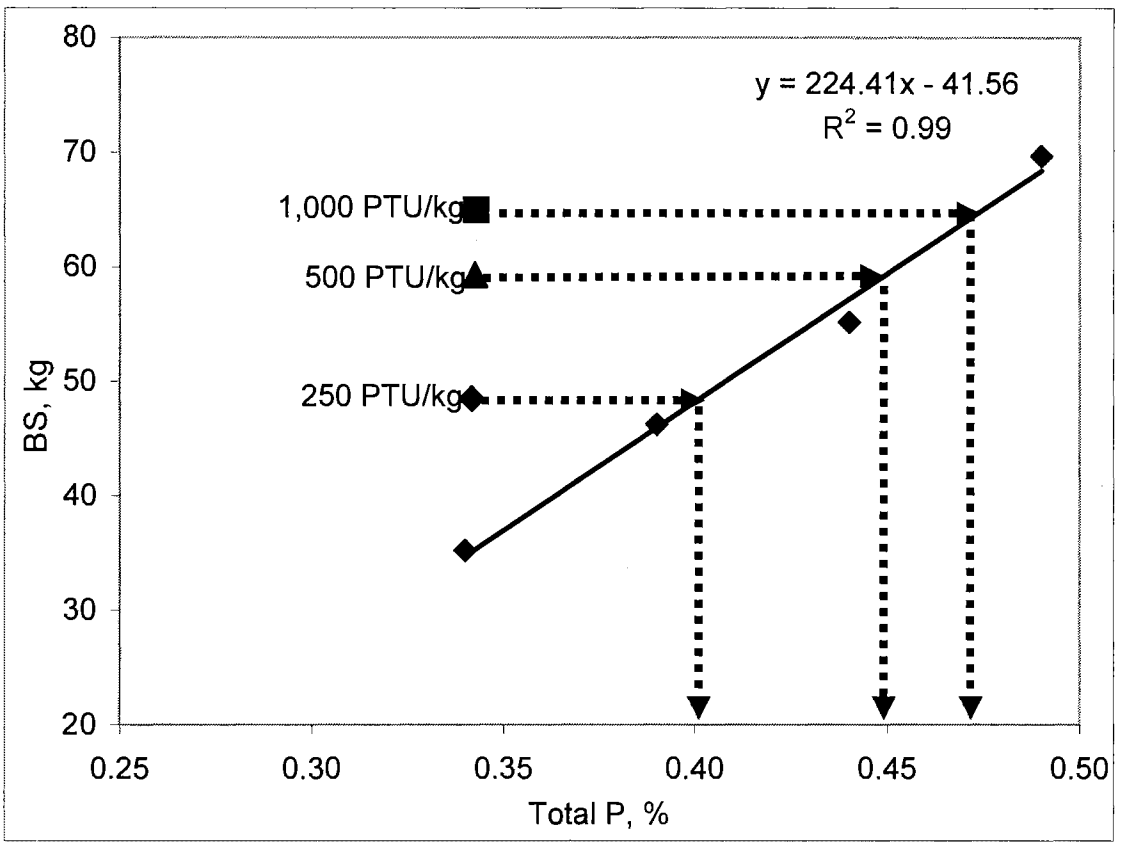
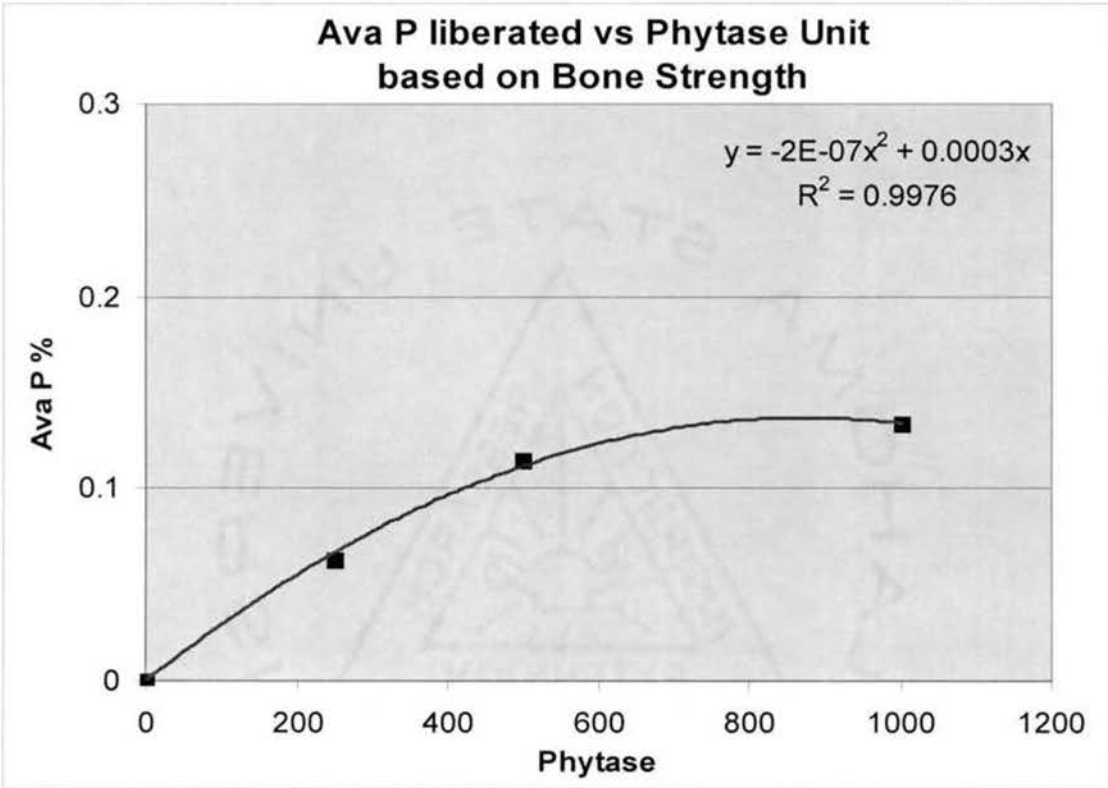


Figure 4.2. Available P liberated by solid-state fermented phytase based average bone breaking strength of pigs fed corn-SBM based diets



CHAPTER V

EXPERIMENT 2

Effects of a Solid-State Fermented Phytase on Growth Performance, Bone Traits and Phosphorus Digestibility of Growing Pigs Fed Corn-Soybean Meal Diets Containing Wheat Middlings

ABSTRACT: A total of 24 barrows was used in a 35-d study to determine the effects of the addition of a solid-state fermented phytase complex (Allzyme SSF; Alltech, Inc) to low available P, corn-soybean meal (SBM) diets containing 20% wheat middlings (WM) on growth performance, bone traits, and P utilization. Pigs were blocked by weight and ancestry, and randomly allotted to one of four dietary treatments (6 pigs/trt). A basal diet consisted of corn, SBM, and WM (20%) and was adequate in all nutrients, except available P. This diet contained 0.50% total P (0.13% available P), all of which was provided by corn, SBM, and WM. Diets 2 and 3 were the basal plus SSF to provide 250 and 500 phytase units (PU)/kg, respectively. The positive control diet (PC) was corn-SBM-based with 20% corn starch (0.50% total P, 0.24% available P). All diets were formulated to 0.77% digestible lysine and a Ca:total P of 1.2:1. Pigs were housed individually with ad libitum access to feed and water. During the 35-d study, there were two 5-d periods (d 10 to15 and d 25 to 30) for collection of feces and urine. Phytase did not affect ($P > 0.61$) ADG or ADFI, but increased ($P < 0.04$) gain:feed. Digestibility of P increased ($P < 0.03$) with SSF addition, resulting in a 10% reduction in P excretion for pigs fed 500 PU/kg. However,

digestibility of other nutrients (dry matter, protein, gross energy) were not affected (P > 0.10) by addition of SSF phytase to diets containing wheat middlings. Compared to the positive control, pigs fed 20% wheat middlings had approximately 26% greater (P < 0.01) dry matter excretion and 7.6% unit lower digestibility (P < 0.01). Bone breaking strength and ash (%) increased (P < 0.01) with SSF phytase complex. However, pigs fed PC had greater (P < 0.01) average daily gain, gain:feed, bone strength and ash compared with those fed diets containing WM. These data suggest that the addition of a solid-state fermented phytase improves P utilization of corn-soybean meal diets containing wheat middlings for growing pigs, but digestibility of other nutrients was not improved by SSF phytase complex.

Keyword: Pigs, Phytase, Bone

Introductions

Phytate (myoinositol 1,2,3,4,5,6 hexa, dihydrogen phosphate) is the major form of P in cereal grains and oilseed meals (Reddy et al., 1982). Approximately 60 to 70% of the P in corn and soybean meal is in the form of phytate (NRC 1998). Pigs cannot utilize phytate due to the lack of endogenous phytase that hydrolyzes phytic P (Peeler 1972). Supplemental microbial phytase improves the bioavailability of P in corn- (Cromwell et al., 1995), oat- (Bruce and Sundstol, 1995), wheat-, triticale (Dungelhoef et al., 1994), and barley-based (Campbell and Bedford, 1992; Valaja et al., 1998; Nasi et al., 1999) diets for swine. Many of the phytases used in previous studies were produced by submerged microbial

fermentation method. Also, most of the commercial microbial phytases on the market are produced by submerged fermentation. However, recently, the solid-state fermentation method (SSF) has been used in the production of dietary microbial phytase. A previous study in our lab (Exp. 1) reported that the addition of SSF phytase complex to low P, corn-soybean based diets improved P bioavailability, growth performance, and bone traits. Besides phytase activity, solid fermentation systems can utilize non-genetically modified microbes that often produce substantial activities of other enzymes, known as "side activities", such as α-amylase, β-glucanse, protease, xylanase, and cellulase (Filer, 2001). Therefore, the objectives of this experiment were to determine the effects of the addition of a solid-state fermented phytase complex to low P, corn-soybean meal diets containing high fiber (wheat middlings) on growth performance, nutrient digestibility, and bone traits in growing pigs.

Material and Methods

Animals, Diets, and Treatments. A total of 24 crossbred barrows with an average initial BW of 20 kg were used in a 35-d study to investigate the effects of addition of SSF phytase complex (Allzyme SSF; Alltech, Inc) on nutrient digestibility and growth performance of growing pigs fed corn-soybean meal based diets containing 20% wheat middlings. Pigs were blocked by initial body weight and randomly allotted to one of four dietary treatments in a randomized complete block design. There were 6 replications per treatment.

The basal diet (Table 5.1) was corn-soybean meal based with 20% wheat middlings (WM). This diet contained 0.77% apparent digestible lysine, 0.50% total P, 0.13% available P, and 3,262 kcal/kg ME. Diets 2 and 3 were as Diet 1 with addition of 250 or 500 phytase units/kg of diet. Diet 4 (PC) was as Diet 1 with 20% corn starch replacing the wheat middlings (0.50 % total P and 0.25 % available P). This diet was formulated to serve as a positive control. All experimental diets were formulated based on apparent ileal digestible lysine. All other nutrients met or exceeded NRC (1998) standards. The Ca: total P ratio in all diets was 1.2:1.

In this experiment, pigs were individually housed in metabolic chambers in an environmentally-controlled room. The chambers were specially designed for the total, but separate collection of feces, urine, and wasted feed. Each chamber had a galvanized steel mesh floor and one stainless steel self-feeder and one nipple waterer. Beneath the floor, a five-quart plastic container was used to collect urine. All pigs were allowed ad libitum access to feed and water. All diets were fed in meal form.

Collection and Analyses. There were two 5-d collection periods (d 10-15 and d 25-30). Feces were collected every morning from the 1-mm screen under the chamber. The collected feces were immediately weighed and placed in plastic bags and stored frozen (-20 °C) until the samples were analyzed. At the same time, refused diets were collected and weighed. For urine collection, 10 mL of 6N-HCL were added to each urine collection container to prevent N loss as

ammonia. All urine was collected daily. Urine volume was measured and a sub-sample was collected in a 100 mL container and frozen at -20 °C until lab analyses were performed.

Initially, frozen fecal samples were dried in a forced-air oven for 4 d at 55°C before grinding. Partially dried feces and diets were ground through a 1-mm screen using a Wiley Mill (Standard Model No.3; Arthur H. Thomas Co., Philadelphia, PA). Dry matter content of the diets and feces was determined by drying at 100 °C for 24 h (AOAC, 1998). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1998) using an automated analyzer (FOSS Tecator, 2020 Digestor, 2400 Kjeltec Analyzer; Hoganas, Sweden). Total phosphorus content in the diets and feces was determined by a gravimetric quinolinium molybdophosphate method (AOAC, 1998). Gross energy content in feed and fecal samples was determined by bomb calorimetry (Parr 1261 Isoperibol Calorimeter; Molin, IL). Diets and fecal samples were placed overnight at 500 °C in a muffle furnace (Sybron, Dubuque, IA) for determination of ash.

At the end of the experiment, all pigs were slaughtered at the Oklahoma State University Meat Lab. Following scalding, scraping, and evisceration, the hot carcass was weighed. The front and rear feet were removed and frozen (-20°C). Also, the femurs from each pig were excised and placed in a plastic bag and frozen (-20°C). Later, the feet were allowed to thaw and autoclaved at 120 °C and 15 psi for approximately 7 min. Following autoclaving, the MC and MT were extracted and cleaned of extraneous tissue, and frozen. Later, the MC and MT were allowed to thaw overnight before breaking strength analyses.

Breaking strength of the MC and MT were determined using an Instron Universal Testing Machine (Model 4502, Instron, Canton, MA) by procedures of Cromwell et al. (1972). Bones were placed on supports 3.8 cm apart for the MC and MT, and 8.1 cm apart for femurs. Breaking strength was defined as the amount of force (kg) required to break the bone when placed in a horizontal position. The breaking strength of MC and MT were averaged. For fat-free bone ash content, the marrow from the fourth MC of the right foot of each pig was removed and the bones were placed in a soxhelt containing petroleum ether for 48 h to allow for lipid extraction. After lipid extraction, the bones were dried overnight at 100° C, weighed, and ashed for 48 h at 600° C. Both femurs from each pig were cleaned of all extraneous tissues and weighed. The outside diameter of the femurs was measured at the midshaft by averaging two measurements taken 90° to each other, using a caliper. Breaking strength of the femurs was performed as previously described for the MC and MT.

Statistical Analyses. Data were analyzed as a randomized complete block design using procedures described by Steel et al. (1997), with initial BW as the blocking criterion. The model included the effects of block (rep), treatment, and block x treatment (error). The effects of phytase supplementation were tested for linearity and curvilinearity using orthogonal polynomial contrasts. Also, comparisons between the positive control (PC) and diets containing wheat middlings (WM) were tested. In all cases, pig served as the experimental unit.

Results

Growth Performance. Pigs fed the positive control diet had greater ($P < 0.01$) average daily feed intake, average daily gain, and feed efficiency compared to those fed diets containing 20% wheat middlings (Table 5.2). Among the pigs fed wheat middlings, there were numeric increases in average daily gain, but those differences were not significant ($P > 0.22$). Average daily feed intake was similar ($P > 0.10$) among treatments. For efficiency of gain, the addition of SSF phytase to diets containing wheat middlings improved gain:feed (linear, $P < 0.04$). When 500 PU/kg was added to diets containing wheat middlings, gain:feed was 5.1% greater than pigs fed 20% wheat middlings without SSF phytase. Even though there was improvement in growth performance for pigs by 500 PU/kg SSF phytase, those pigs had lower growth performance than those fed the positive control diet.

Nutrient Digestibility and Excretion. Phosphorus intake was similar ($P > 0.10$) among the pigs fed diets containing wheat middlings (Table 5.3). The amount of phosphorus in the feces was decreased (linear, $P < 0.05$) by addition of SSF phytase from 0 to 500 PU/kg. The addition of 500 PU/kg reduced fecal phosphorus excretion by approximately 10% compared with diets containing wheat middlings without SSF phytase. Phosphorus digestibility increased as SSF phytase level increased (linear, $P<0.01$). The pigs fed 500 PU/kg had 7.1% unit greater phosphorus digestibility compared with diets containing wheat middlings without SSF phytase.

Dry matter intake, dry matter excretion, or digestibility were not affected ($P > 0.10$) by addition of SSF phytase to diets containing wheat middlings. Compared to the positive control, pigs fed 20% wheat middlings had approximately 26% greater ($P < 0.01$) dry matter excretion and 7.6% units lower digestibility ($P < 0.01$). There was no effect ($P > 0.22$) of SSF phytase on gross energy digestibility. Pigs fed the positive control diet had greater ($P < 0.01$) energy digestibility compared to those fed diets containing 20% wheat middlings. Nitrogen excretion and digestibility were not affected ($P > 0.45$) by the addition of SSF phytase. Pigs fed the positive control diet had greater ($P < 0.01$) nitrogen digestibility than those fed diets containing wheat middlings.

Bone Characteristics. Bone breaking strength of the metacarpals and metatarsals (Table 5.4) and femurs was increased (linear, $P < 0.01$) by the addition of SSF phytase. Also, fat-free metacarpal ash content (%) increased (linear, $P < 0.01$) with SSF phytase. The addition of 500 PU/kg dramatically improved average bone breaking strength by 28%. Pigs fed the positive control diet had greater bone strength ($P < 0.01$) and fat-free MC ash content ($P < 0.01$) compared to those fed diets containing wheat middlings. Bone (femur, MT, MC) weights and femur diameter were not affected ($P > 0.58$) by the addition of SSF phytase. However, pigs fed the positive control diet had heavier bone weights and greater femur diameter ($P < 0.05$) compared with pigs fed diets containing 20% wheat middlings.

Discussion

Approximately 60 to 70% of the P in plant sources that are commonly fed to pigs is associated with phytate (NRC, 1998). Phytate-P is not available for absorption (Cromwell, 1992; Ravindran et al., 1994, 1995). An early study demonstrated phytase can release this type of phosphorus (Nelson et al., 1968). A number of studies have reported that the addition of microbial phytase catalyzed the hydrolysis of the phytate molecule, releasing P from phytate (Jongbloed et al., 1992, 1996; Cromwell et al., 1993; Kornegay 1995). Also, several phytase experiments have been conducted with different types of grain sources. Supplemental microbial phytase improves the bioavailability of P in corn- (Cromwell et al., 1995), oat- (Bruce and Sundstol, 1995), wheat-, triticale (Dungelhoef et al., 1994), and barley-based (Campbell and Bedford, 1992; Valaja et al., 1998; Nasi et al., 1999) diets for swine. Also, studies have shown that the addition of cereal phytase from wheat middlings improved phytate P utilization by growing pigs (initial average BW of 9.9 kg) (Han et al., 1998). These authors reported that pigs fed diets containing 15% wheat middlings exhibited greater growth performance, plasma inorganic P concentration, bone strength, and mobility score than pigs fed low-P, corn-soybean meal diets. Also, these authors reported that pigs fed 15% wheat middlings had similar levels of plasma inorganic P to pigs fed diets supplemented with 1,200 PU/kg. Similar results have been reported by Han et al. (1997).

In our study, the addition of microbial phytase (SSF phytase) did not improve ADG of pigs fed diets containing 20% WM. Pigs fed the positive control

had greater growth performance compared with pigs fed diets containing 20% WM. The addition of 500 PU/kg increased gain:feed by 5.1%. Even though there was improvement in growth performance of pigs by SSF phytase complex, the growth performance of pigs fed 500PU/kg did not reach the levels of growth performance observed in pigs fed the positive control diets. Previous studies with corn-soybean meal diets have shown that the addition of microbial phytase improved growth performance of pigs fed low P diets by increasing ADG with increased daily feed intake (Simons et al., 1990; Beer and Jongbloed, 1992; Kornegay and Qian, 1996; Yi et al., 1996b). The results found in our study indicate that the fiber content in the diet had a negative effect on growth performance of pigs and the addition of SSF phytase complex may not overcome the negative effects of fiber.

The amount of phosphorus in the feces was decreased when SSF phytase levels increased from 0 to 500 PU/kg. The addition of 500 PU/kg reduced fecal phosphorus excretion by approximately 10% compared with wheat middling diets without SSF phytase complex. The pigs fed 500 PU/kg had a 7.1% unit higher phosphorus digestibility compared to pigs fed control diet. A previous study in our lab (Exp. 1) suggested that the addition of SSF phytase complex at 1,000 PU/kg decreased total daily P excretion by 45%. In other studies, the addition of microbial phytase decreased P excretion by 25 to 50% by increasing P digestibility or retention and by decreasing the total level of P in the diet (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996). Also, total P digestibility is improved in pigs fed

diets containing phytase-rich cereals, such as wheat, triticale, and barley or barley by-products (Stober et al., 1980; Newton et al, 1983; Pointillart et al., 1987; Helander and Partanen, 1994). The previous study in our lab (Exp.1) reported that the addition of SSF phytase (500 PU/kg of diet) improved total P digestibility of growing pigs fed a low P, corn-soybean meal diet and the equivalency value of 500 PU/kg was 0.11% P in the diet. The present results indicate the magnitude of effect of SSF phytase complex in diets containing wheat middlings is relatively lower than that found in a low P, corn-soybean meal diet. Even though wheat middlings have intrinsic phytase activity (Pointillart, 1994), the addition of SSF phytase to diets containing 20% wheat middlings did not reach the level of P digestibility of pigs fed the positive control diet. These results suggest that the fiber content in wheat middlings may negatively affect microbial phytase activity in the gastrointestinal tract. Calvert (1991) proposed that fiber in diets may have negative effects on dietary phytase activity.

Bone breaking strength of the metacarpals and metatarsals and femurs was increased by addition of SSF phytase. Also, fat-free metacarpal ash content (%) increased with SSF phytase. There was dramatic improvement in average bone breaking strength by 28%, but pigs fed the positive control diet had greater bone strength and fat-free MC ash content compared with pigs fed diets containing 20% wheat middlings. A previous study in our lab, Park et al. (Exp. 1) reported that the addition of SSF phytase complex to low P corn-soybean meal diets improved bone traits. Also, similar results have been found in previous studies with corn-soybean meal diets supplemented with microbial phytase

(Cromwell et al., 1993; Kornegay and Qian, 1996; Yi et al., 1996b; Radcliff and Kornegay, 1998).

Digestibility of dry matter, energy, and nitrogen was not improved by SSF phytase complex, which indicates the side enzyme activity in SSF did not improve nutrient utilization by pigs fed diets containing 20% wheat middlings. However, pigs fed the positive control diet had greater nutrient digestibility and lower nutrient excretion than pigs fed diets containing 20% wheat middlings. These results may be explained by the fiber content in wheat middlings (Calvert, 1991). The phytase complex levels used in our study may not overcome the negative effect of fiber in wheat middlings.

In summary, the addition of SSF phytase complex to low P, corn-soybean meal diets containing 20% wheat middlings improved growth performance, phosphorus utilization, and bone traits. However, as mentioned above, the magnitude of effect of SSF phytase was relatively lower than that found in corn-soybean meal based diets, suggesting the fiber content in the diet may diminish the enzyme activity in the gastrointestinal tract.

Implications

The addition of a solid-state fermented phytase complex to low P, corn-soybean meal based diets containing 20% wheat middlings improved feed efficiency and bone strength of pigs. The amount of daily P excretion was reduced by 9.6% by the addition of 500 PU/kg in diets. Because wheat middlings

contain relatively high intrinsic phytase activity, the magnitude of response to dietary phytase might be lower than that previously observed for corn-soybean meal diets. Except for P, other nutrients were not affected by the addition of SSF phytase complex. These data indicate that the addition of a solid-state fermented phytase complex only improves P utilization of corn-soybean meal diets containing wheat middlings for growing pigs. The fiber content in the diet may negatively affect nutrient utilization and microbial phytase activity. Further research is needed to elucidate the effects of fiber on dietary phytase activity.

Table 5.1. Composition of Diets (as-is)

	Dietary treatments			
Total P, %	0.50	0.50	0.50	0.50
Available P, %	0.13	0.13	0.13	0.25
Phytase, PU/kg	0	250	500	0
Corn	55.01	55.01	55.01	55.01
Soybean meal	22.89	22.89	22.89	22.89
Wheat middlings	20.00	20.00	20.00	--
Corn starch	0.05	0.025	--	19.41
Dicalcium phosphate	--	--	--	1.02
Limestone	1.34	1.34	1.34	0.78
Sodium chloride	0.25	0.25	0.25	0.25
Vitamin & mineral premix [a]	0.25	0.25	0.25	0.25
Antibiotic [b]	0.20	0.20	0.20	0.20
Lysine-HCL	0	0	0	0.11
L-threonine	0	0	0	0.02
SSF Phytase [bc]	0	0.025	0.05	0
Calculated analysis				
ME, kcal/kg	3,261	3,260	3,259	3,429
Crude protein, %	18.6	18.6	18.6	15.3
App. dig. Lysine, %	0.77	0.77	0.77	0.77
Ca, %	0.60	0.60	0.60	0.60
Total P, %[d]	0.50	0.50	0.50	0.50
Available P, %	0.13	0.13	0.13	0.25
Added phytase activity, PTU/kg of diet	0	250	500	0

[a] Provided the following per kg of diet: 5,506 IU of vitamin A, 551 IU of vitamin D, 33 IU of vitamin E, 3.6 mg of vitamin K (as menadione), 221 µg of biotin, 137 mg of choline, 33.04 mg of niacin, 24.78 mg of panthothenic acid (as d-pantothenate), 5.51 mg of riboflavin, 27.55 µg of vitamin B_{12}, 1.66 mg of folacin, 100 mg of Zn, 2 mg of Mn, 100 mg of Fe, 10 mg of Cu, .30 mg of I, and .30 mg of Se.

[b] Provided 55 mg of chlortetracycline per kilogram of diet.

[c] Solid-state fermented phytase complex (Allzyme® SSF; Alltech, Inc) contains 1,000 PU/g of product.

[d] Analyzed total P were 0.49, 0.49, 0.49, and 0.51, respectively.

Table 5.2. Effects of SSF phytase complex on growth performance [a]

	Dietary treatments				
Total P, %	0.50	0.50	0.50	0.50	
Available P, %	0.13	0.13	0.13	0.25	
Phytase, PTU/kg	0	250	500	0	SE
ADG, g [c]	590	629	637	746	26
ADFI, g [c]	1,263	1,286	1,294	1,453	46
Gain/feed, g/kg [bc]	467	470	491	515	8

[a] Least squares means for 6 pigs/trt
[b] Linear effect of SSF phytase ($P < 0.05$)
[c] WM vs PC ($P < 0.01$)

Table 5.3. Effects of SSF phytase complex on nutrient digestibility of pigs fed low P, corn-SBM based diets containing wheat middlings (DM basis) [ab]

	Dietary treatments				
Total P, %	0.50	0.50	0.50	0.50	
Available P, %	0.13	0.13	0.13	0.25	
Phytase, PTU/kg	0	250	500	0	SE
Phosphorus					
Intake, g/d	6.66	6.71	6.87	8.97	0.28
Feces, g/d [c]	4.17	3.89	3.77	3.52	0.15
Absorbed, g/d	2.49	2.82	3.10	5.45	0.28
Digestibility, % [de]	37.2	41.9	44.3	60.3	1.9
Dry matter					
Intake, g/d [e]	1,196	1,253	1,231	1,521	55
Feces, g/d [e]	215	224	224	156	12
Absorbed, g/d	980	1,029	1,007	1,364	48
Digestibility, % [e]	81.9	82.5	81.7	89.6	0.5
Energy					
Intake, kcal/d [e]	5,129	5,459	5,364	6,515	243
Feces, kcal/d [e]	978	1,060	1,061	742	58
Absorbed, kcal/d [e]	4,151	4,399	4,303	5,773	214
Digestibility, % [e]	80.9	80.7	80.1	88.4	0.4
Nitrogen					
Intake, g/d [e]	41.2	42.7	42.3	47.1	1.9
Feces, g/d [e]	8.9	9.2	9.4	7.2	0.7
Absorbed, g/d [e]	32.3	33.5	32.9	39.9	1.5
Digestibility, % [e]	78.3	78.7	77.7	84.4	0.9

[a] Least squares means for 6 pigs/trt,
[b] Data were pooled (no period x treatment interaction)
[c] Linear effect of SSF phytase ($P < 0.05$)
[d] Linear effect of SSF phytase ($P < 0.01$)
[e] WM vs CS ($P < 0.01$)

Table 5.4. Effects of SSF phytase complex on bone characteristics of pigs fed low P, corn-SBM based diets containing wheat middlings [a]

	Dietary treatments				
Total P, %	0.50	0.50	0.50	0.50	
Available P, %	0.13	0.13	0.13	0.25	
Phytase, PTU/kg	0	250	500	0	SE
Breaking strength, kg					
Metacarpal [ce]	41.6	47.5	52.2	69.1	2.6
Metatarsal [ce]	33.3	43.8	45.9	67.9	3.3
Femur [bc]	140.2	171.1	177.5	256.3	12.2
Average bone strength [ce]	71.7	87.5	91.9	130.1	4.9
Metacarpal ash, % [ce]	49.1	50.9	51.9	54.9	0.5
Metacarpal ash, g [c]	2.2	2.3	2.4	3.0	0.1
Bone weight, g					
Metacarpal [d]	56.6	55.0	56.2	61.5	2.0
Metatarsal [e]	68.5	66.6	70.3	75.6	2.3
Femur [e]	161.9	158.4	168.1	180.7	3.2
Femur diameter [e], mm	19.1	19.2	18.7	20.6	0.6

[a] Least squares means for 6 pigs/trt
[b] Linear effect of SSF phytase ($P < 0.05$)
[c] Linear effect of SSF phytase ($P < 0.01$)
[d] WM vs CS ($P < 0.05$)
[e] WM vs CS ($P < 0.01$)

CHAPTER VI

EXPERIMENT 3

Effects of a Solid-State Fermented Phytase on Phosphorus and Energy Digestibility of Growing Pigs Fed Barley-Soybean Meal Based Diets

ABSTRACT: A total of 24 barrows was utilized in a 21-d digestibility study to determine the effects of the addition of a solid-state fermented phytase complex (Allzyme SSF; Alltech, Inc) to low available P, barley-soybean meal diets on growth performance, and P and energy digestibility. Pigs were blocked by weight and ancestry, and randomly allotted to one of four dietary treatments (6 pigs/trt). A basal diet consisted of barley and soybean meal and was adequate in all nutrients, except available P. This diet contained 0.42% total P (0.11% avail. P), all of which was provided by barley and SBM. Diets 2, 3, and 4 were the basal plus SSF to provide 250, 500, and 1,000 phytase units (PU)/kg, respectively. All diets were formulated to 0.77% digestible lysine and a Ca:total P of 1.2:1. Pigs were housed individually. Experimental diets were fed at 3.0 × maintenance with ad libitum access to water. There was a 7-d period (d 14 - 21) for collection of feces and urine. The addition of SSF phytase complex increased (linear, $P < 0.05$) average daily gain and gain:feed ratio. Digestibility of phosphorus, dry matter, organic matter, ash, gross energy, and nitrogen increased (linear, $P < 0.05$) with SSF phytase complex. Compared with the basal diet, digestibility of

phosphorus for pigs fed 1,000 PU/kg was 38.7% greater. These results indicate the addition of SSF phytase complex to low P, barely-soybean meal diets improved the nutrient digestibility of growing pigs with a dramatic decrease in P excretion.

Keyword: Pigs, Phytase, Digestibility

Introductions

Phytate (myoinositol 1,2,3,4,5,6 hexa, dihydrogen phosphate) is the major form of P in cereal grains and oilseed meals (Reddy et al., 1982). Approximately 70% of the P in cereal grains and oilseed protein supplements is organically bound in the form of phytate, which reduces phosphorus availability to pigs (Kornegay 1996; NRC 1998). Pigs cannot utilize phytate due to the lack of endogenous phytase that hydrolyzes phytic P (Peeler 1972). Thus, improving phytate utilization can reduce the need for inorganic P supplementation in feed, resulting in reduced P excretion in manure.

Addition of dietary microbial phytase improves P utilization and decreases P excretion (Lei et al., 1993; Cromwell et al., 1995; O'Quinn et al., 1997). Most of the experiments evaluating phytase were conducted using corn-soybean meal based diets. Also, many of the microbial phytases are produced by submerged microbial fermentation. Recently, solid-state fermentation (SSF) has been used to produce dietary microbial phytase. By using the SSF system, phytase from solid fermentation systems also contains substantial activities of other enzymes, known as "side activities", such as α-amylase, β-glucanse, protease, xylanase,

and cellulase (Filer, 2001). A previous study in our lab (Exp. 1) reported that the addition of SSF phytase to low P, corn-soybean based diets improved P utilization, growth performance, and bone traits. However, other nutrients were not affected by the addition of the SSF phytase up to 1,000 PU/kg. Because nutrient digestibility is relatively high in corn-soybean meal diets, the potential to improve digestibility of energy, protein, and dry matter is minimal. On the other hand, barley is known to have a lower feeding value for swine as compared with corn. Thus, there is greater potential to improve nutrient digestibility in barley based diets. Therefore, the objectives of this study were to determine the effects of the addition of a solid-state fermented phytase complex to low P, barley-soybean meal diets on growth performance, nutrient excretion and digestibility of growing pigs.

Material and Methods

Animals, Diets, and Treatments. Twenty four crossbred barrows with an average BW of 24.3 kg were used in a 21-d study to investigate the effects of addition of SSF phytase complex (Allzyme SSF; Alltech, Inc) on growth performance and nutrient digestibility of growing pigs fed barley-soybean meal based diets. Pigs were blocked by initial body weight and randomly allotted to one of four dietary treatments in a randomized complete block design. There were 6 replications per treatment.

The experimental diets (Table 6.1) were fed in meal form. The basal diet was barley-soybean meal containing 0.77% apparent digestible lysine, 0.50%

total P, 0.11% available P, and 2,960 kcal/kg ME. Diets 2 to 4 were as Diet 1 with addition of 250, 500, or 1,000 phytase units/kg of diet. All experimental diets were formulated based on apparent ileal digestible lysine. All other nutrients met or exceeded NRC (1998) standards. The Ca: total P ratio in all diets was 1.2:1.

In this experiment, pigs were individually housed in metabolic chambers in an environmentally-controlled room. The chambers were specially designed for the total, but separate collection of feces, urine, and wasted feed. Each chamber had a galvanized steel mesh floor and one stainless steel self-feeder and one nipple waterer. Beneath the floor, a five-quart plastic container was used to collect urine.

All pigs were fed at 3.0 × maintenance with *ad libitum* access to water. Pigs were individually fed at 0700 and 1800. The pigs were weighed weekly for calculation of the next week's feed allowance and ADG and gain:feed ratio. Feed intake was determined by the following equation (NRC, 1998):

$$106 \times BW^{0.75} \times 3.0 \text{ /ME content of the diet}$$

Collection and Analyses. During a 7-d collection period (d 14 to 21), feces were collected every morning from the 1-mm screen under the chamber. The collected feces were immediately weighed and placed in plastic bags and stored frozen (-20 °C) until the samples were analyzed. At the same time, refused feed also was collected and weighed.

Initially, all feces from 7-d collection were dried in a forced-air oven for 4 d at 50°C. Partially-dried fecal samples and diet samples were then ground in a Wiley Mill (Standard Model No.3; Arthur H. Thomas Co., Philadelphia, PA) equipped with a 1-mm screen. Dry matter content of diets and feces was determined by drying at 100°C for 24 h (AOAC, 1998). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1998) by automated analyzer (FOSS Tecator, 2020 Digestor, 2400 Kjeltec Analyzer; Hoganas, Sweden). Total phosphorus content was determined by a gravimetric quinolinium molybdophosphate method (AOAC, 1998). Gross energy content in feed and fecal samples were determined by bomb calorimetry (Parr 1261 Isoperibol Calorimeter; Molin, IL). For ash content, diets and fecal samples were ashed overnight at 500°C in a muffle furnace (Sybron, Dubuque, IA).

Statistical Analyses. Data were analyzed as a randomized complete block design using procedures described by Steel et al. (1997), with initial BW as the blocking criterion. The model included the effects of block (rep), treatment, and block x treatment (error). The effects of phytase supplementation were tested for linearity and curvilinearity using orthogonal polynomial contrasts. For the four levels of phytase, polynomial coefficients for unequally spaced treatments were generated using the ORPAL matrix function of the IML procedure of SAS. In addition, a nonorthogonal contrast was used for comparisons between diets containing SSF phytase and control diet. In all cases, pig served as the experimental unit.

Results

Growth Performance. The addition of SSF phytase complex increased (linear, $P < 0.05$) ADG and gain:feed ratio (Table 6.2). Compared with the basal diet, pigs fed 1,000 PU/kg had 19.2% and 25.5% greater ADG and gain:feed, respectively. Feed intake was not affected ($P > 0.10$) by dietary treatment.

Nutrient Excretion and Digestibility. During the 7-d collection period, feed intake increased with increased (linear, $P < 0.01$) levels of SSF phytase complex. Total phosphorus intake (Table 6.3) was linearly increased ($P < 0.01$) with SSF phytase levels due to the higher feed intake. Daily fecal phosphorus excretion was decreased (linear, $P < 0.01$) by 16.8% with SSF phytase (Figure 6.1), resulting in increased phosphorus digestibility. Pigs fed 1,000 PU/kg had approximately 63% greater phosphorus digestibility compared with that of pigs fed the diet without SSF phytase (Figure 6.2). Also, ash digestibility was increased (linear, $P < 0.01$) by the addition of SSF phytase. Pigs fed the diet supplemented with 1,000 PU/kg SSF phytase complex had much greater ash digestibility (63.76%) compared with pigs fed the diet without SSF phytase complex (51.93%).

Dry matter intake was increased (linear, $P < 0.01$) by the addition of SSF phytase. Dry matter excretion of pigs fed SSF phytase complex was higher ($P < 0.05$) than that of pigs fed control diet due to higher feed intake. However, dry matter digestibility was increased (linear, $P < 0.01$) by the addition of SSF

phytase complex. When 1,000 PU/kg was added to diet, dry matter digestibility increased by 3.2% units, compared with the diet without SSF complex.

Organic matter intake increased (quadratic, $P < 0.05$) with SSF phytase complex level. Due to the increased organic matter intake, the fecal excretion of organic matter increased (quadratic, $P < 0.01$) with SSF phytase. However, digestibility of organic matter increased (linear, $P < 0.01$) by the addition of SSF phytase complex. Pigs fed the diet containing SSF phytase complex (1,000 PU/kg) had 2.6% units greater organic matter digestibility, compared with pigs fed the control diet.

Digestibility of gross energy and nitrogen were improved ($P < 0.01$) by the addition of SSF phytase complex. Compared with pigs fed the diet without SSF phytase complex, pigs fed 1,000 PU/kg had 2.6% and 4.1% unit greater digestibility of energy and nitrogen, respectively. Digestible energy in the diet 73.5 kcal/kg greater for the diet containing 1,000 PU/kg compared with control diet (Table 6.4).

Discussion

Diets fed to pigs normally contain a high amount of phytate, which has a low digestibility (NRC 1998). The bioavailability of P from barley for pigs was reported to be about 30% (Kornegay, 1996). Phytate also combines with protein forming phytate-protein complex thus making the protein less available (Mroz and Jongbloed, 1998; Caldwell, 1992). Most of the experiments on phytase were conducted using corn-soybean meal based diets. Also, phytase used in most of

previous studies were produced by submerged fermentation process. The phytase produced from submerged fermentation usually contained only phytase activity (Filer, 2001). However, the phytase produced from the solid-state fermentation process contains significant activity of α-amylase, β-glucanase, protease, xylanase and cellulose as well as phytase (Filer, 2001); therefore, the addition of SSF phytase complex might be beneficial for other nutrients besides phosphorus.

In the present study, the addition of SSF phytase complex to barley soybean meal diets increased ADG and gain:feed ratio. Feed intake was not significantly different among the dietary treatments. Similar results were observed in previous studies from our lab (Exp.1 and Exp.2) using corn soybean meal diets with or without wheat middlings. Also, another study showed that pigs fed low P, barley-soybean meal diets supplemented with 500 PU/kg had similar growth performance compared to those fed an adequate P diet (Grandhi, 2000a). Another study by Grandhi (2000b) showed that the addition of carbohydrase to a hulless barley diet improved feed efficiency. Also, Baidoo et al., (1997) reported an improvement in feed efficiency in growing pigs fed hulless-barley diets supplemented with a mixture of different enzymes (β-glucanse, xylanase, amylase, and pectinase). In corn-soybean meal diets, Jongbloed et al (1996) reported that the addition of phytase overcame the adverse effect of feeding low P diets on pig growth performance in 11 different experiments.

The phytase used in our experiment was produced by a solid-state fermentation (SSF) process. The SSF phytase contains significant activity of α-

amylase, β-glucanase, protease, xylanase and cellulose. Phytic acid is able to inhibit α-amylase, trypsin, tyrosinase and pepsin (Nair et al., 1991; Caldwell, 1992). Therefore, the degradation of phytate by the addition of dietary phytase complex could enhance digestibility of other nutrients as well as phosphorus. In a previous study from our lab (Exp.1), the addition of SSF phytase complex to low P, corn-soybean meal diets dramatically decreased fecal excretion of phosphorus, resulting in an increase in digestibility of phosphorus, but there was no improvement in digestibility of other nutrients by SSF phytase complex. Due to the high digestibility of corn-soybean meal diets, the potential for improvement in the digestibility of other nutrients by enzymes might be minimal.

In our study, the feeding level was limited at 3.0 × maintenance. However, during the 7-d total collection period, feed intake was increased with SSF phytase complex. Pigs fed basal diets had the lowest feed intake. The reason for the lower feed intake is unclear. As a result, the amounts of daily nutrient excretion were increased with SSF phytase complex, except for P excretion. Even though P intake increased, the daily P excretion decreased due to a dramatic increase in P digestibility by SSF phytase. Nevertheless, the digestibility of each nutrient was significantly increased by the addition of SSF phytase complex. The addition of SSF phytase complex (up to 1,000 PU/kg) to low P, barley-soybean meal diets improved digestibility of P, dry matter, organic matter, nitrogen, and gross energy. Phosphorus digestibility was improved by 38.7% when 1,000 PU/kg of SSF phytase was added to a low P, barley-soybean meal diet. Also, ash digestibility was increased by the addition of SSF phytase

complex. Grandhi (2000a) reported that the addition of microbial phytase (500 PU/kg) decreased the excretion of P in pigs fed barley-soybean meal diets. In this study, the reduction in total P excretion was 26.8% compared to an adequate P diet.

Previous studies in our lab (Exp. 1 and Exp. 2) have shown no beneficial effect of SSF phytase on dry matter digestibility of pigs fed corn-soybean meal based diets or corn-soybean meal diets containing 20% wheat middlings. However, the beneficial effects of SSF phytase complex were observed in this study with barley. These results indicate that the addition of SSF phytase complex is more beneficial for energy and protein, but not for P in relatively low quality feed (low available P, low energy, high fiber content, etc.). A similar response was also observed in organic matter excretion and digestibility. However, previous studies showed that the addition of phytase did not improve digestibility of organic components in barely, corn-soybean meal or tapioca, hominy feed-soybean meal diets (Graham, 1989; Nasi and Helander, 1994; Simons et al., 1990; Jongbloed et al., 1992). The enzyme used in those studies contained only phytase. However, Nasi et al. (1995) reported that the addition of 1,000 PU/g of phytase complex to a barley-rapeseed meal diet significantly improved ash digestibility (54.9%) compared with the unsupplemented diet (52%). The phytase used in their study contained protein, starch, and pectin-degrading enzymes. These authors also found improved protein digestibility.

Digestibility of energy and nitrogen were improved by the addition of SSF phytase complex. Similar results were found by Nasi et al. (1995) who reported

that the addition of phytase complex improved crude protein digestibility of barley-rapeseed meal diets. However, a previous study from our lab (Exp.1 and Exp.2) suggested that the addition of SSF phytase to low P, corn -soybean meal diets did not improve digestibility of gross energy or nitrogen. These results indicate the response and magnitude of phytase effect varies with different types of feedstuffs.

Implications

The addition of a solid-state fermented phytase complex to low P, barley-soybean meal based diets improved feed efficiency and nutrient digestibility by growing pigs. Digestibility of total P was dramatically increased by the addition of SSF phytase complex (up to 1,000 PU/kg). Also, dry matter, energy and protein digestibility were increased by the addition of SSF phytase complex. However, such improvements were not observed in previous studies utilizing corn-soybean meal. Therefore, these data indicate that the addition of a solid-state fermented phytase complex improves nutrient digestibility as well as P utilization of pigs fed barley-soybean meal diets. Also, this study suggests the addition of SSF phytase complex to low quality feedstuffs is more beneficial for nutrient utilization of pigs.

Table 6.1. Composition of experimental diets, as fed basis (Exp.3)

	Dietary treatments			
Total P, %	0.47	0.47	0.47	0.47
Available P, %	0.11	0.11	0.11	0.11
Phytase, PTU/kg	0	250	500	1,000
Barley	76.51	76.51	76.51	76.51
Soybean meal	21.57	21.57	21.57	21.57
Corn Starch	0.10	0.08	0.05	-
Limestone	1.07	1.07	1.07	1.07
Sodium chloride	0.25	0.25	0.25	0.25
Vitamin premix [a]	0.15	0.15	0.15	0.15
Trace mineral premix[a]	0.15	0.15	0.15	0.15
Antibiotic [b]	0.20	0.20	0.20	0.20
SSF Phytase [c]	-	0.025	0.05	0.10
Calculated analysis				
ME, kcal/kg	2,960	2,959	2,958	2,956
Lysine, % (App. Dig.)	0.77	0.77	0.77	0.77
Ca, %	0.50	0.50	0.50	0.50
Total P, %	0.42	0.42	0.42	0.42
Available P, %[d]	0.11	0.11	0.11	0.11
Ca:Total P	1.2:1	1.2:1	1.2:1	1.2:1
Added phytase activity, PTU/kg of diet	0	250	500	1,000

[a] Provided the following per kg of diet: 5,506 IU of vitamin A, 551 IU of vitamin D, 33 IU of vitamin E, 3.6 mg of vitamin K (as menadione), 221 µg of biotin, 137 mg of choline, 33.04 mg of niacin, 24.78 mg of panthothenic acid (as d-pantothenate), 5.51 mg of riboflavin, 27.55 µg of vitamin B_{12}, 1.66 mg of folacin, 100 mg of Zn, 2 mg of Mn, 100 mg of Fe, 10 mg of Cu, .30 mg of I, and .30 mg of Se.

[b] Provided 55 mg of chlortetracycline per kilogram of diet.

[c] Solid-state fermented phytase complex (Allzyme® SSF; Alltech, Inc) contains 1,000 PU/g of product

[d] Analyzed total P were 0.44, 0.44, 0.44, and 0.43, respectively.

Table 6.2. Effects of SSF phytase complex on growth performance[a]

	Dietary treatments				
Total P, %	0.47	0.47	0.47	0.47	
Available P, %	0.11	0.11	0.11	0.11	
Phytase, PTU/kg	0	250	500	1,000	SE
ADG, g [b]	555	550	638	662	34
ADFI, g	1,307	1,210	1,284	1,187	40
Gain/feed, g/kg [bc]	380	432	450	477	30

[a] Least squares means for 6 pigs/trt

[b] Linear effect of SSF phytase ($P < 0.05$)

[c] None vs SSF Phytase ($P < 0.05$)

Table 6.3. Effects of SSF phytase on phosphorus, dry matter, organic matter, and ash digestibility of pigs fed low P, barley-SBM based diets (DM basis) [ab]

	Dietary treatments				
Total P, %	.47	.47	.47	.47	
Available P, %	.11	.11	.11	.11	
Phytase, PTU/kg	0	250	500	1,000	SE
Phosphorus					
Intake, g/d [bde]	4.43	5.05	5.73	5.52	.22
Feces, g/d [bd]	2.91	2.71	2.61	2.43	.09
Absorbed, g/d [bde]	1.53	2.35	3.12	3.09	.19
Digestibility, % [bde]	34.28	45.86	54.43	55.96	1.97
Dry matter					
Intake, g/d [bde]	902.9	1037.4	1150.7	1139.5	45.7
Feces, g/d [e]	216.6	240.4	243.1	237.6	9.2
Absorbed, g/d [bde]	686.3	796.6	907.6	901.9	41.5
Digestibility, % [ce]	75.97	76.54	78.84	79.16	.87
Organic Matter					
Intake, g/d [bde]	840.5	964.2	1066.9	1057.1	42.4
Feces, g/d [e]	186.6	209.4	212.1	207.8	8.3
Absorbed, g/d [bde]	653.8	754.8	854.9	849.3	38.7
Digestibility, % [c]	77.76	78.04	80.09	80.36	.83
Ash					
Intake, g/d [bde]	62.4	72.9	83.7	82.4	3.2
Feces, g/d	30.0	31.1	31.0	29.8	1.0
Absorbed, g/d [bde]	32.5	41.8	52.8	52.6	2.9
Digestibility, % [bde]	51.93	56.79	62.87	63.76	1.65

[a] Least squares means for 6 pigs/trt,
[b] Linear effect of SSF phytase ($P < 0.01$)
[c] Linear effect of SSF phytase ($P < 0.05$)
[d] Quadratic effect of SSF phytase ($P < 0.05$)
[e] None vs SSF phytase ($P < 0.05$)

Table 6.4. Effects of SSF phytase on energy and nitrogen digestibility of pigs fed low P, barley-SBM based diets (DM basis) [ab]

	Dietary treatments				
Total P, %	.47	.47	.47	.47	
Available P, %	.11	.11	.11	.11	
Phytase, PTU/kg	0	250	500	1,000	SE
Gross Energy					
Intake, kcal/d [bde]	3,968.2	4,564.6	4,982.2	4,947.7	200.1
Feces, kcal/d [e]	996.3	1128.1	1138.9	1115.5	46.1
Absorbed, kcal/d [be]	2971.9	3436.4	3843.3	3832.2	179.1
Digestibility, % [b]	74.86	75.01	77.09	77.47	.95
Digestible Energy					
kcal/kg	3290.1	3301.6	3337.9	3363.6	41.5
Nitrogen					
Intake, g/d [bde]	30.38	36.40	40.24	40.60	1.6
Feces, g/d [de]	7.86	9.50	9.40	8.88	.48
Absorbed, g/d [bde]	22.52	26.91	30.84	31.72	1.43
Digestibility, % [b]	74.07	73.69	76.51	78.18	1.23

[a] Least squares means for 6 pigs/trt,
[b] Linear effect of SSF phytase ($P < 0.01$)
[c] Linear effect of SSF phytase ($P < 0.05$)
[d] Quadratic effect of SSF phytase ($P < 0.05$)
[e] None vs SSF phytase ($P < 0.05$)

Figure 6.1. Reduction in daily fecal P excretion by the addition of SSF phytase complex.

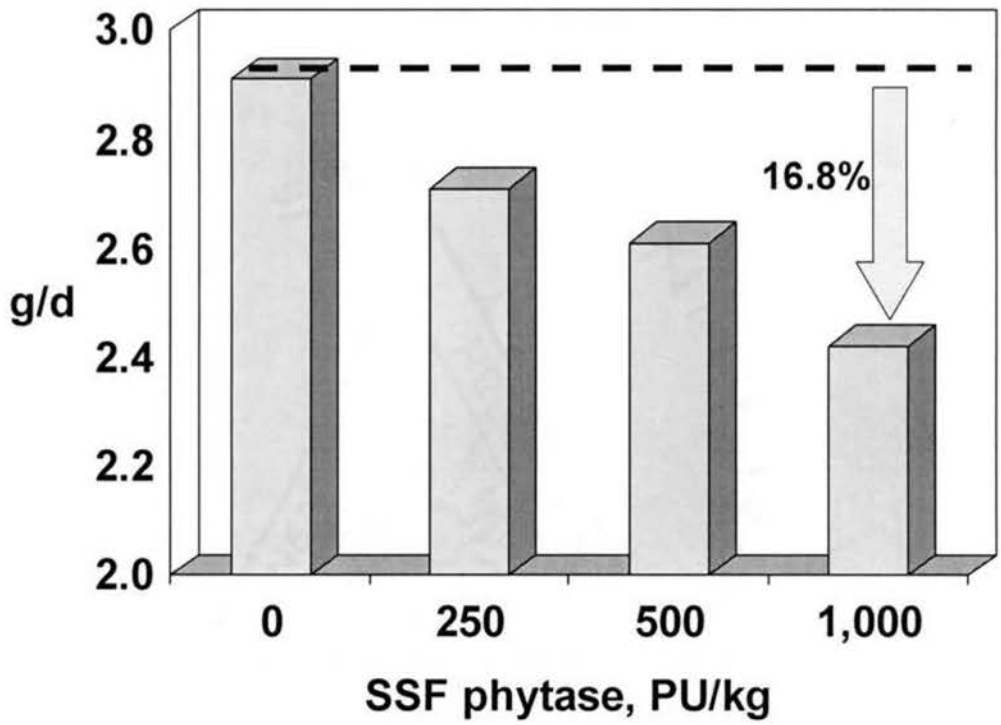
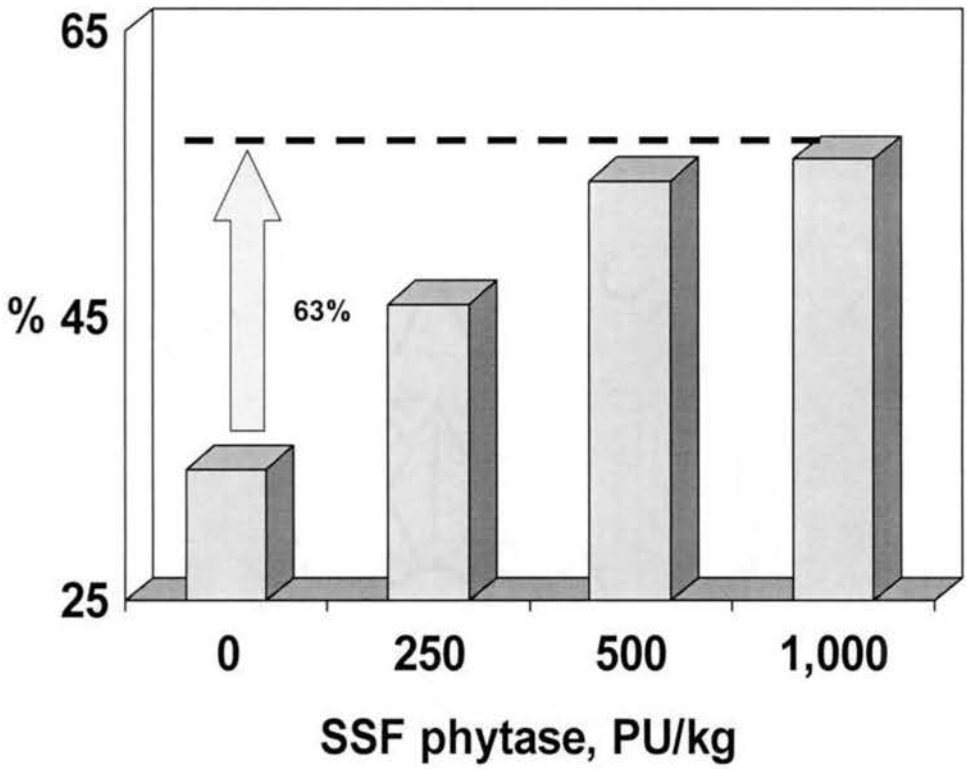


Figure 6.2. Improvement in P digestibility by the addition of SSF phytase complex.



CHAPTER VII

Summary and Conclusion

Corn-soybean meal diets are the most common diets for pigs. Phytate is the major form of phosphorus in cereal grains and oilseed meals. Approximately 60 to 70% of the phosphorus in corn and soybean meal is in the form of phytate. Pigs can not utilize the phytate-P efficiently due to the lack of endogenous phytase that hydrolyzed phytic P. Therefore, inorganic sources of phosphorus have been routinely added to diets for swine feed to supply sufficient levels of available phosphorus. This routine feed management can satisfy the requirement for animal growth, but it causes significant amounts of phosphorus excretion to the environment.

During the past decade, dietary microbial phytase has been added to swine diets to attempt to improve digestibility of phytate-P in feedstuffs for less P excretion to the environment. Because of the high nutrient content of manure and its fertilizer value, land application has been the major means of handling manure. Excess phosphorus application results in excess buildup of phosphorus in soil and in surface runoff water into streams, lake, and rivers. Phosphorus is the most limiting nutrient that regulate aquatic plant growth, so as the level of phosphorus in these bodies of water increases, so does the growth of algae and other aquatic vegetation. For these reasons, the addition of dietary phytase with

decreasing additions of inorganic phosphorus is beneficial for both animals and the environment.

There are several kinds of phytase already available in the market and new phytase sources are currently being developed. Most of phytase in the market are produced by submerged microbial fermentation (SmF). Recently, solid-state fermentation (SSF) technology has been utilized as an alternative to produce microbial phytase. This type of phytase also contains substantial activities of other enzymes, known as "side activities", such as α-amylase, β-glucanse, protease, xylanase, and cellulase. Due to its side enzyme activity, the addition of SSF phytase complex might be beneficial for other nutrients as well as P utilization by pigs. Therefore, the purpose of our studies was to determine the effects of the addition of a solid-state fermented phytase complex on growth performance, nutrient utilization, bone traits and tissue accretion rates in growing pigs fed different types of feeds.

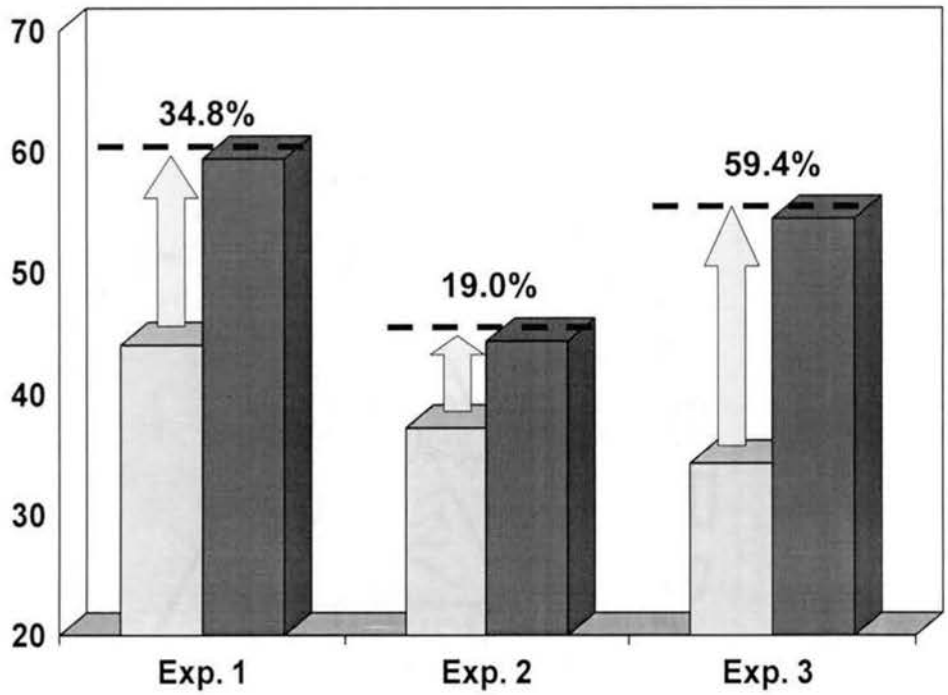
To accomplish our objectives, ninety barrows were used in 3 different experiments. In Exp. 1, forty-two barrows (avg BW = 19.9 kg) were used in a 33-d study, utilizing low P, corn-soybean meal diets. In Exp. 2, a total of 24 barrows was used in a 35-d study utilizing low P, corn-soybean meal diets containing 20% wheat middlings. For Exp. 3, twenty four barrows were utilized in a 21-d study with low available P, barley-soybean meal diets.

For growth performance, the addition of SSF phytase complex (250 to 1,000 PU/kg) did not affect ADFI in Exp.1 and 2. However, in Exp. 3, ADFI increased with SSF phytase complex. The reason for increased feed intake is

unclear. The addition of SSF phytase complex improved gain:feed ratio in all three experiments. In Exp.1, the addition of 500 or 1,000 PU/kg to the low P, corn-soybean meal diet increased gain:feed similar to that for the adequate P diet. These results indicate that the addition of SSF phytase complex is beneficial for growth performance of growing pigs fed low P diets.

Phosphorus utilization was improved by the addition of SSF phytase complex in all three experiments. However, the magnitude of improvement was somewhat different among experiments with different types of feed (Figure 7.2). In Exp 1, daily fecal P excretion was dramatically decreased by the addition of SSF phytase complex, and digestibility of P also improved by the addition of SSF phytase. Compared to the basal diet, additions of SSF decreased P excretion (3.06 vs 2.48, 2.36, 1.68 g/d) by 19.3, 23.3, and 45.4%, respectively. In Exp. 2, fecal P excretion was reduced by 10% when 500 PU/kg was added to diets containing 20% wheat middlings, which is lower than that found in Exp 1. (23.3% reduction in P excretion). In Exp. 3, even though higher feed intake during collection period was observed, the amount of daily fecal P excretion was reduced by 16.8% and digestibility of P was increased by 62%. The results from all three experiments indicate the addition of SSF phytase improves phytate-P in corn or barley-soybean diets. In the barley soybean meal diets, the improvement in P digestibility by SSF phytase complex is more prominent (Figure 7.2).

Figure 7.1. Improvements in phosphorus digestibility (%) by the addition of SSF phytase complex (500PU/kg) to different diets in Exp.1 (corn-SBM), Exp. 2 (corn-SBM-20% WM) and Exp. 3(barley-SBM).



Bone traits were measured in Exp. 1 and 2. In both experiments, bone breaking strength (BB) of metacarpal and metatarsal and femurs and ash (%) increased with increasing SSF phytase complex. In Exp.1, based on average BS and ash, addition of 250, 500, or 1,000 PU/kg was equivalent to 0.066, 0.120, and 0.140% available P, respectively. For the carcass, the contents (%) and accretion rates of water, protein, and fat were not affected by SSF phytase complex. However, the content (%) and accretion of P and ash increased with addition of SSF phytase complex. The increase in bone strength and carcass P associated with increasing SSF was similar to that for diets containing adequate P. These results from Exp 1 and 2 indicate that the addition of a solid-state fermented phytase improves P bioavailability.

As mentioned in the previous chapter, SSF phytase also contains substantial activities of other enzymes, known as "side activities", such as α-amylase, β-glucanse, protease, xylanase, and cellulase. Due to the side activity, digestibilities of other nutrients were measured in the all three experiments. Unlike P utilization, the addition of SSF phytase complex up to 1,000PU/kg did not improve digestibility of dry matter, nitrogen, or gross energy besides phosphorus (Exp. 1 and 2). In Exp.1, low P corn-soybean meal diets were fed with or without SSF phytase complex. Due to the high digestibility of dry matter and N in corn-soybean meal diet, there was no further improvement in dry matter, N, or energy digestibility. Therefore, in Exp. 2, a fiber source was added to corn-soybean meal diets to determine if the side enzymes improve digestibility of other nutrients. To accomplish these objectives, 20% wheat middlings was added to the basal diets. However, the addition of SSF phytase complex (500 PU/kg) did not improve utilization of dry matter, N or gross energy. In Exp. 3, barely was used as basal grain source to formulate a relatively poor quality diet in terms of P and energy. As we expected, the addition of SSF phytase complex improved the digestibility of dry matter, organic matter, ash, protein, especially, gross energy. The results found in three experiments indicate the effects of SSF phytase complex on nutrient utilization varies depending on type of feed. In addition, these data suggest the addition of SSF phytase is more beneficial for pigs fed barley-soybean meal diets.

In conclusion, the addition of SSF phytase improves growth performance and P utilization of pigs fed corn or barley soybean meal diets. However, the

beneficial effect of side enzyme in SSF phytase only is found with relatively low quality feed. Further study is needed to elucidate the effects of fiber on dietary phytase activity.

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APPENDIX

Pigs Means and Analysis of Variance Tables

Appendix Table 1

Pigs means for average daily gain, average daily feed intake, average daily dry matter intake, and gain:feed (Experiment 1).

PEN	TRT	REP	ADG,g	ADFI,g	G:F
2	C	1	816.33	1769.91	461.23
3	G	1	785.60	1645.70	477.37
4	E	1	611.51	1434.50	426.29
5	F	1	809.01	1731.01	467.36
6	B	1	749.03	1673.48	447.59
7	D	1	731.48	1554.43	470.57
8	F	2	735.86	1548.09	475.34
10	C	2	749.03	1495.19	500.96
11	G	2	746.10	1549.68	481.46
12	B	2	620.29	1517.25	408.83
14	D	2	713.92	1577.91	452.45
15	E	2	712.46	1598.14	445.80
16	A	2	593.96	1430.87	415.10
17	G	3	670.03	1557.48	430.20
18	B	3	617.37	1482.32	416.49
19	D	3	649.55	1532.71	423.79
20	E	3	684.66	1548.25	442.22
21	C	3	573.48	1419.06	404.12
22	F	3	816.33	1708.46	477.82
23	A	3	604.20	1457.13	414.65
23	A	3	604.20	1457.13	414.65

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Table 1. (continued)

PEN	TRT	REP	ADG,g	ADFI,g	G:F
1	B	4	812.32	1570.70	517.17
2	E	4	806.99	1629.86	495.13
3	D	4	744.30	1452.31	512.49
4	C	4	924.37	1751.21	527.85
5	F	4	781.65	1602.30	487.83
6	G	4	730.96	1486.43	491.76
7	A	4	733.63	1487.83	493.08
9	E	5	661.60	1344.99	491.90
10	B	5	678.94	1287.18	527.46
11	C	5	708.28	1521.90	465.40
12	D	5	817.66	1533.58	533.17
14	F	5	708.28	1376.46	514.57
16	G	5	689.61	1290.29	534.46
17	A	6	585.57	1219.42	480.20
18	G	6	825.66	1587.55	520.09
19	F	6	606.91	1354.87	447.95
20	D	6	670.94	1187.48	565.01
21	C	6	688.28	1272.54	540.87
22	E	6	668.27	1248.94	535.07
23	B	6	550.89	1057.77	520.80

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 2

Analysis of variance for average daily gain, average daily feed intake, average daily dry matter intake, and gain:feed (Experiment 1).

		Mean Squares		
Source	df	ADG, g	ADFI, g	G:F, g/kg
Total	39			
Error	28	4627.0285	12178.670	779.40911
Repetition	5	17927.25234	112751.2533	8617.34742
Treatment	6	8670.95296	15801.4631	841.41201
Linear MSP	1	25821.06021	22108.69521	4146.475130
Quadratic MSP	1	4417.71282	10525.95371	164.742245
Cubic MSP	1	4890.38156	18879.94340	26.418884
Linear SSF	1	28485.05649	34028.23491	3138.816136
Quadratic SSF	1	10067.65700	23817.96661	371.593744
Cubic SSF	1	474.78773	4173.34237	97.349603
None vs SSF	1	1534.41947	9541.05680	79.893803
C.V., %		9.58	7.41	5.83

Appendix Table 3

Pigs means for average dry matter intake, excretion, absorbed, and digestibility (Experiment 1).

Pen	TRT	BLK	Period	DM-intake	DM-Exc	DM-abs	DM-dig
1	A	1	1	1639.02	186.59	1452.43	88.62
2	C	1	1	1432.91	156.39	1276.52	89.09
3	G	1	1	1251.91	129.92	1121.99	89.62
4	E	1	1	1296.32	119.00	1177.32	90.82
5	F	1	1	1430.70	161.13	1269.57	88.74
6	B	1	1	1411.44	146.99	1264.45	89.59
7	D	1	1	1313.25	144.84	1168.41	88.97
9	F	2	1	1327.65	121.79	1205.86	90.83
10	C	2	1	1261.51	153.23	1108.28	87.85
11	G	2	1	1374.12	142.96	1231.16	89.60
12	B	2	1	1275.84	141.95	1133.89	88.87
14	D	2	1	1439.04	134.58	1304.46	90.65
15	E	2	1	1213.64	130.68	1082.96	89.23
16	A	2	1	1325.25	153.33	1171.92	88.43
17	G	3	1	1238.78	121.26	1117.52	90.21
18	B	3	1	1178.76	126.77	1052.00	89.25
19	D	3	1	1326.61	137.29	1189.32	89.65
20	E	3	1	1300.05	153.69	1146.36	88.18
21	C	3	1	1166.76	136.86	1029.90	88.27
22	F	3	1	1439.27	170.47	1268.80	88.16
23	A	3	1	886.34	100.18	786.16	88.70
1	A	1	2	1719.98	194.60	1525.38	88.69
2	C	1	2	1680.23	191.16	1489.07	88.62
3	G	1	2	1506.23	188.01	1318.22	87.52
4	E	1	2	1432.86	163.19	1269.68	88.61
5	F	1	2	1638.94	177.90	1461.04	89.15
6	B	1	2	1411.25	157.26	1253.99	88.86
7	D	1	2	1465.07	159.95	1305.11	89.08
9	F	2	2	1263.58	136.72	1126.86	89.18
10	C	2	2	1522.83	204.09	1318.74	86.60
11	G	2	2	1466.18	164.59	1301.59	88.77
12	B	2	2	1335.13	161.56	1173.57	87.90
14	D	2	2	1365.92	120.60	1245.32	91.17
15	E	2	2	1378.01	187.66	1190.35	86.38
16	A	2	2	1237.44	131.98	1105.46	89.33
17	G	3	2	1488.13	223.86	1264.27	84.96
18	B	3	2	1230.42	133.33	1097.10	89.16
19	D	3	2	1398.92	163.84	1235.08	88.29
20	E	3	2	1436.10	176.84	1259.26	87.69

Table 3. (continued)

Pen	TRT	BLK	Period	DM-intake	DM-Exc	DM-abs	DM-dig
21	C	3	2	1233.47	134.76	1098.71	89.07
22	F	3	2	1721.68	169.57	1552.11	90.15
23	A	3	2	1271.82	133.40	1138.42	89.51
1	B	4	1	1419.12	183.52	1235.60	87.07
2	E	4	1	1434.70	188.83	1245.87	86.84
3	D	4	1	1280.81	156.56	1124.25	87.78
4	C	4	1	1726.94	279.39	1447.55	83.82
5	F	4	1	1425.45	212.31	1213.14	85.11
6	G	4	1	1240.95	185.93	1055.02	85.02
7	A	4	1	1305.77	186.97	1118.81	85.68
9	E	5	1	1221.19	193.67	1027.52	84.14
10	B	5	1	1266.11	217.41	1048.69	82.83
11	C	5	1	1665.47	261.03	1404.44	84.33
12	D	5	1	1330.95	206.16	1124.79	84.51
14	F	5	1	1310.86	128.30	1182.57	90.21
15	A	5	1	1300.56	186.78	1113.77	85.64
16	G	5	1	1031.58	129.29	902.29	87.47
17	A	6	1	1096.42	175.00	921.41	84.04
18	G	6	1	1655.67	195.59	1460.08	88.19
19	F	6	1	1080.44	142.15	938.29	86.84
20	D	6	1	1063.45	150.45	913.00	85.85
21	C	6	1	1095.12	134.30	960.82	87.74
22	E	6	1	1043.15	130.12	913.03	87.53
23	B	6	1	866.48	115.10	751.38	86.72
1	B	4	2	1713.27	212.66	1500.60	87.59
2	E	4	2	1685.37	205.09	1480.28	87.83
3	D	4	2	1469.04	151.71	1317.34	89.67
4	C	4	2	1551.69	177.56	1374.13	88.56
5	F	4	2	1643.54	209.57	1433.98	87.25
6	G	4	2	1461.21	201.63	1259.58	86.20
7	A	4	2	1381.42	145.83	1235.59	89.44
9	E	5	2	1377.76	172.78	1204.98	87.46
10	B	5	2	1145.07	151.20	993.87	86.80
11	C	5	2	1783.06	280.48	1502.58	84.27
12	D	5	2	1523.36	227.47	1295.89	85.07
14	F	5	2	1463.93	151.25	1312.68	89.67
15	A	5	2	1498.32	187.57	1310.75	87.48
16	G	5	2	1379.70	128.18	1251.53	90.71
17	A	6	2	1273.18	105.90	1167.28	91.68
18	G	6	2	1634.78	155.56	1479.22	90.48

Table 3. (continued)

Pen	TRT	BLK	Period	DM-intake	DM-Exc	DM-abs	DM-dig
19	F	6	2	1375.60	160.49	1215.11	88.33
20	D	6	2	1245.30	135.06	1110.23	89.15
21	C	6	2	1271.84	138.37	1133.47	89.12
22	E	6	2	1259.59	118.75	1140.83	90.57
23	B	6	2	1114.79	145.08	969.71	86.99

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 4

Analysis of average dry matter intake, excretion, absorbed, and digestibility (Experiment 1).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	83				
Error	65	25062.369	1018.2214	19172.976	2.8712675
Repetition	5	140947.1643	6343.40044	103787.7979	15.77065619
Treatment	6	42069.4562	1360.88954	32437.8200	2.44594643
Period	1	438001.3086	1131.53440	394609.4544	12.43550476
Treatment x Period	6	4607.4497	518.24423	3225.7279	2.18002421
Linear MSP	1	34626.0315	527.859020	26603.41380	0.05133375
Quadratic MSP	1	7556.8574	2757.452419	1184.84813	6.83275208
Cubic MSP	1	139502.8711	4716.978000	92915.58128	0.99717042
Linear SSF	1	37148.2434	217.771262	31679.05620	0.39775129
Quadratic SSF	1	20090.6930	32.108335	18517.60510	0.68772875
Cubic SSF	1	20305.9047	23.171659	21701.61655	2.08409274
None vs SSF	1	31301.2761	235.980803	26103.24923	0.24420069
C.V., %		11.58	19.47	11.51	1.92

Appendix Table 5

Pigs means for average daily phosphorus intake, excretion, absorption, and digestibility (Experiment 1).

Pen	TRT	BLK	Period	Intake g/d	Feces g/d	Absorbed g/d	Digestibility, %
1	A	1	1	6.93	3.57	3.36	48.48
2	C	1	1	7.69	2.91	4.78	62.18
3	G	1	1	5.09	1.49	3.60	70.74
4	E	1	1	5.26	1.83	3.43	65.16
5	F	1	1	5.82	2.05	3.76	64.69
6	B	1	1	6.57	2.40	4.17	63.49
7	D	1	1	7.73	2.86	4.88	63.03
9	F	2	1	5.40	2.11	3.28	60.84
10	C	2	1	6.77	2.96	3.81	56.30
11	G	2	1	5.59	1.33	4.26	76.25
12	B	2	1	5.94	2.43	3.51	59.13
14	D	2	1	8.48	2.75	5.73	67.59
15	E	2	1	4.88	2.38	2.50	51.27
16	A	2	1	5.61	3.19	2.42	43.09
17	G	3	1	5.04	1.04	4.00	79.32
18	B	3	1	5.48	2.71	2.78	50.62
19	D	3	1	7.81	3.05	4.77	61.01
20	E	3	1	5.28	2.29	2.99	56.59
21	C	3	1	6.26	2.13	4.13	65.93
22	F	3	1	5.85	2.60	3.25	55.62
23	A	3	1	3.75	2.08	1.67	44.66
1	A	1	2	6.96	3.79	3.17	45.52
2	C	1	2	8.89	4.07	4.82	54.21
3	G	1	2	6.27	1.90	4.38	69.77
4	E	1	2	5.90	2.22	3.68	62.35
5	F	1	2	6.70	2.29	4.41	65.79
6	B	1	2	6.64	2.75	3.88	58.49
7	D	1	2	8.04	2.93	5.11	63.50
9	F	2	2	5.17	2.17	3.00	57.96
10	C	2	2	8.05	4.01	4.04	50.17
11	G	2	2	6.11	2.51	3.59	58.87
12	B	2	2	6.28	1.55	4.73	75.34
14	D	2	2	7.50	2.12	5.38	71.76
15	E	2	2	5.67	3.00	2.67	47.15
16	A	2	2	5.01	2.66	2.34	46.81
17	G	3	2	6.20	1.97	4.23	68.19

Table 5. (continued)

Pen	TRT	BLK	Period	Intake g/d	Feces g/d	Absorbed g/d	Digestibility, %
18	B	3	2	5.79	2.92	2.87	49.54
19	D	3	2	7.68	3.51	4.17	54.26
20	E	3	2	5.91	2.78	3.13	52.99
21	C	3	2	6.52	2.53	3.99	61.23
22	F	3	2	7.04	2.93	4.11	58.43
23	A	3	2	5.15	2.99	2.16	41.89
1	B	4	1	6.64	3.14	3.49	52.62
2	E	4	1	5.89	2.38	3.51	59.65
3	D	4	1	7.12	2.77	4.35	61.09
4	C	4	1	9.04	4.81	4.22	46.74
5	F	4	1	5.73	2.31	3.43	59.79
6	G	4	1	5.00	1.60	3.40	67.99
7	A	4	1	5.39	3.16	2.23	41.34
9	E	5	1	5.01	2.79	2.22	44.28
10	B	5	1	5.92	3.92	2.00	33.73
11	C	5	1	8.72	4.19	4.53	51.94
12	D	5	1	7.40	3.72	3.67	49.66
14	F	5	1	5.27	1.90	3.37	63.89
15	A	5	1	5.36	3.37	2.00	37.22
16	G	5	1	4.16	1.32	2.84	68.35
17	A	6	1	4.52	3.02	1.50	33.17
18	G	6	1	6.67	1.94	4.73	70.86
19	F	6	1	4.35	2.04	2.30	52.96
20	D	6	1	5.91	2.82	3.09	52.33
21	C	6	1	5.73	2.72	3.01	52.50
22	E	6	1	4.28	2.18	2.10	49.03
23	B	6	1	4.05	2.32	1.73	42.74
1	B	4	2	8.17	4.22	3.95	48.35
2	E	4	2	6.95	3.12	3.83	55.14
3	D	4	2	8.39	2.70	5.69	67.87
4	C	4	2	8.19	3.55	4.64	56.62
5	F	4	2	6.83	2.70	4.12	60.39
6	G	4	2	5.90	2.03	3.87	65.61
7	A	4	2	5.78	3.02	2.76	47.79
9	E	5	2	5.68	2.80	2.88	50.75
10	B	5	2	5.46	2.79	2.67	48.96
11	C	5	2	9.41	4.13	5.28	56.12
12	D	5	2	8.70	4.10	4.60	52.86
14	F	5	2	6.08	2.41	3.67	60.33
15	A	5	2	6.27	3.86	2.41	38.41
16	G	5	2	5.58	1.36	4.22	75.61
17	A	6	2	5.32	2.10	3.22	60.53

Table 5. (continued)

Pen	TRT	BLK	Period	Intake g/d	Feces g/d	Absorbed g/d	Digestibility, %
18	G	6	2	6.61	1.66	4.95	74.90
19	F	6	2	5.71	2.70	3.01	52.69
20	D	6	2	7.11	2.58	4.53	63.69
21	C	6	2	6.71	3.01	3.70	55.10
22	E	6	2	5.19	1.94	3.25	62.68
23	B	6	2	5.32	3.14	2.17	40.87

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 6

Analysis of variance for average daily phosphorus intake, excretion, absorption, and digestibility (Experiment 1)

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	83				
Error	65	0.5425315	0.28544952	0.33851084	44.069653
Repetition	5	3.18483357	0.90137619	1.86153905	137.250177
Treatment	6	11.31378016	3.95040833	7.22066230	802.877301
Period	1	8.97026786	0.95147143	4.06560000	31.721719
Treatment x Period	6	0.08633730	0.10365754	0.01034167	26.455838
Linear MSP	1	39.349880167	0.06767042	36.18490042	1730.106602
Quadratic MSP	1	0.83213333	0.13975208	0.28366875	26.048533
Cubic MSP	1	4.63148167	1.85328375	0.62730375	17.205615
Linear SSF	1	0.33943709	11.21120218	15.44642255	4205.431034
Quadratic SSF	1	0.21107291	0.10455163	0.60402160	86.810096
Cubic SSF	1	0.38113990	0.36201700	0.00011519	42.112171
None vs SSF	1	0.24337778	7.27201111	10.16015625	2757.562656
C.V., %		11.76	19.84	16.28	11.70

Appendix Table 7

Pigs means for average daily nitrogen intake, excretion, absorption, and digestibility (Experiment 1).

Pen	TRT	BLK	Period	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	A	1	1	53.72	8.09	45.63	84.93
2	C	1	1	46.39	6.47	39.92	86.06
3	G	1	1	40.94	6.28	34.66	84.66
4	E	1	1	42.54	5.25	37.29	87.66
5	F	1	1	46.83	6.88	39.95	85.32
6	B	1	1	45.84	6.53	39.31	85.76
7	D	1	1	42.56	6.15	36.42	85.56
9	F	2	1	43.46	5.44	38.01	87.47
10	C	2	1	40.84	6.96	33.88	82.96
11	G	2	1	44.93	6.33	38.60	85.91
12	B	2	1	41.43	6.82	34.62	83.55
14	D	2	1	46.64	5.61	41.03	87.98
15	E	2	1	39.82	6.05	33.77	84.80
16	A	2	1	43.44	7.64	35.80	82.42
17	G	3	1	40.51	5.63	34.88	86.11
18	B	3	1	38.28	5.61	32.67	85.35
19	D	3	1	42.99	5.85	37.15	86.40
20	E	3	1	42.66	6.98	35.68	83.64
21	C	3	1	37.77	6.51	31.26	82.77
22	F	3	1	47.11	7.21	39.90	84.69
23	A	3	1	29.05	4.11	24.94	85.84
1	A	1	2	56.22	8.15	48.07	85.51
2	C	1	2	54.15	8.28	45.87	84.71
3	G	1	2	49.70	8.65	41.05	82.60
4	E	1	2	47.52	7.14	40.38	84.97
5	F	1	2	54.15	7.81	46.33	85.57
6	B	1	2	45.76	6.81	38.95	85.11
7	D	1	2	47.80	6.86	40.94	85.64
9	F	2	2	41.74	6.04	35.71	85.54
10	C	2	2	49.08	9.21	39.87	81.23
11	G	2	2	48.38	7.35	41.03	84.81
12	B	2	2	43.30	6.85	36.45	84.18
14	D	2	2	44.57	5.64	38.93	87.35
15	E	2	2	45.70	8.34	37.36	81.75
16	A	2	2	40.44	5.92	34.52	85.36
17	G	3	2	49.10	10.13	38.97	79.36

Table 7 (continued).

Pen	TRT	BLK	Period	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
18	B	3	2	39.90	5.70	34.20	85.72
19	D	3	2	45.65	7.42	38.23	83.75
20	E	3	2	47.63	8.34	39.28	82.48
21	C	3	2	39.76	6.16	33.60	84.52
22	F	3	2	56.88	7.00	49.88	87.69
23	A	3	2	41.57	5.54	36.03	86.68
1	B	4	1	47.84	8.54	39.30	82.15
2	E	4	1	49.28	8.43	40.86	82.90
3	D	4	1	43.88	6.93	36.95	84.22
4	C	4	1	61.22	12.34	48.88	79.84
5	F	4	1	53.45	9.25	44.20	82.69
6	G	4	1	42.24	8.67	33.57	79.48
7	A	4	1	44.50	9.08	35.41	79.59
9	E	5	1	41.95	9.07	32.88	78.38
10	B	5	1	42.68	10.21	32.47	76.07
11	C	5	1	59.04	12.41	46.64	78.99
12	D	5	1	45.60	9.78	35.82	78.55
14	F	5	1	49.16	6.34	42.82	87.11
15	A	5	1	44.32	8.47	35.85	80.90
16	G	5	1	35.11	4.51	30.60	87.14
17	A	6	1	37.36	8.01	29.35	78.55
18	G	6	1	56.35	8.81	47.55	84.37
19	F	6	1	40.52	6.30	34.22	84.46
20	D	6	1	36.43	6.30	30.14	82.72
21	C	6	1	38.82	5.64	33.18	85.46
22	E	6	1	35.83	6.08	29.75	83.04
23	B	6	1	29.21	5.61	23.60	80.79
1	B	4	2	57.21	9.70	47.51	83.04
2	E	4	2	57.28	9.64	47.64	83.17
3	D	4	2	49.58	7.63	41.95	84.62
4	C	4	2	53.89	9.20	44.70	82.94
5	F	4	2	61.12	10.46	50.66	82.89
6	G	4	2	49.42	9.18	40.24	81.42
7	A	4	2	46.46	6.78	39.68	85.40
9	E	5	2	46.82	8.01	38.81	82.88
10	B	5	2	38.24	7.12	31.12	81.37
11	C	5	2	61.93	13.83	48.10	77.67
12	D	5	2	51.41	11.35	40.06	77.93
14	F	5	2	54.44	7.83	46.61	85.62
15	A	5	2	50.40	8.67	41.72	82.79
16	G	5	2	46.67	6.13	40.54	86.87
17	A	6	2	42.82	5.39	37.43	87.41

Table 7 (continued).

Pen	TRT	BLK	Period	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
18	G	6	2	55.29	8.10	47.19	85.35
19	F	6	2	51.16	7.86	43.29	84.63
20	D	6	2	42.03	6.44	35.59	84.69
21	C	6	2	44.17	6.96	37.21	84.24
22	E	6	2	42.81	6.23	36.58	85.45
23	B	6	2	37.23	7.41	29.82	80.10

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 8

Analysis of variance for average daily nitrogen intake, excretion, absorption, and digestibility (Experiment 1).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	39				
Error	28	29.379604	2.2032804	75.098734	5.2674575
Repetition	5	181.6985219	17.06496762	106.1791279	28.9818898
Treatment	6	88.8185714	3.34678254	72.7209024	9.9542159
Period	1	461.2617333	6.90263333	355.0218583	3.7592012
Treatment x Period	6	7.4845444	1.76240000	4.9223833	6.6983317
Linear MSP	1	47.4014817	1.26005042	33.3238538	0.44118375
Quadratic MSP	1	12.5460750	7.57635208	0.6279187	19.01341875
Cubic MSP	1	223.4554017	10.86727042	135.7360004	0.37052042
Linear SSF	1	52.2087780	0.42497546	43.2804287	1.82556123
Quadratic SSF	1	111.6427877	0.13508678	103.9776913	7.26382059
Cubic SSF	1	74.5322727	0.25038841	83.3270700	15.09984530
None vs SSF	1	80.4011111	0.72250000	65.9479340	1.94602500
C.V., %		11.79	19.77	11.67	2.74

Appendix Table 9

Pigs means for average daily gross energy intake, excretion, absorption, and digestibility (Experiment 1).

Pen	TRT	Rep	Intake kcal/d	Feces kcal/d	Absorbed, kcal/d	Digestibility %
1	A	1	7441.6	893.4	6548.2	87.99
2	C	1	6836.8	826.5	6010.4	87.91
3	G	1	5992.7	821.3	5171.4	86.29
4	E	1	6080.9	675.6	5405.3	88.89
5	F	1	6796.2	835.6	5960.5	87.70
6	B	1	6199.9	728.0	5471.9	88.26
7	D	1	6094.5	702.3	5392.1	88.48
9	F	2	5737.0	611.4	5125.6	89.34
10	C	2	6114.8	851.2	5263.6	86.08
11	G	2	6171.2	768.7	5402.5	87.54
12	B	2	5734.9	743.2	4991.7	87.04
14	D	2	6152.9	620.6	5532.3	89.91
15	E	2	5774.5	822.4	4952.1	85.76
16	A	2	5677.4	719.5	4957.9	87.33
17	G	3	5924.8	913.8	5011.1	84.58
18	B	3	5291.7	648.4	4643.3	87.75
19	D	3	5978.7	719.7	5259.0	87.96
20	E	3	6096.5	799.4	5297.0	86.89
21	C	3	5271.2	657.2	4614.0	87.53
22	F	3	6998.3	824.0	6174.3	88.23
23	A	3	4781.2	573.0	4208.3	88.02
1	B	4	6892.9	987.6	5905.3	85.67
2	E	4	6878.9	977.8	5901.1	85.79
3	D	4	5990.4	734.5	5255.9	87.74
4	C	4	7135.0	1123.4	6011.5	84.25
5	F	4	6771.0	1095.3	5675.7	83.82
6	G	4	5892.7	1009.8	4882.9	82.86
7	A	4	5999.0	852.0	5147.0	85.80
9	E	5	5730.0	929.1	4800.9	83.79
10	B	5	5305.8	906.7	4399.2	82.91
11	C	5	7504.7	1358.6	6146.1	81.90
12	D	5	6218.0	1078.4	5139.6	82.66
14	F	5	6121.9	727.1	5394.8	88.12
15	A	5	6248.3	920.6	5327.8	85.27
16	G	5	5258.4	656.9	4601.5	87.51
17	A	6	5290.0	681.2	4608.8	87.12
18	G	6	7175.6	880.1	6295.6	87.74

Table 9. (continued)

Pen	TRT	Rep	Intake kcal/d	Feces kcal/d	Absorbed, kcal/d	Digestibility %
19	F	6	5418.7	745.6	4673.1	86.24
20	D	6	5029.5	685.1	4344.4	86.38
21	C	6	5151.0	659.2	4491.8	87.20
22	E	6	5076.9	614.1	4462.7	87.90
23	B	6	4359.8	622.4	3737.4	85.72

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 10

Analysis of variance for average daily gross energy intake, excretion, absorption, and digestibility (Experiment 1).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	41				
Error	30	422525.13	18447.520	315598.47	2.4090951
Repetition	5	1371579.374	93518.7048	984700.051	13.21341952
Treatment	6	367060.177	17183.7794	268207.980	1.88927222
Linear MSP	1	154657.200	2452.55208	118095.5021	0.04485333
Quadratic MSP	1	33525.375	36200.43375	51.3338	6.44806667
Cubic MSP	1	1335419.008	57137.85208	840097.0021	0.68101333
Linear SSF	1	133915.895	13450.40358	62500.1930	1.51215459
Quadratic SSF	1	248601.126	70.20859	240186.7546	0.99819901
Cubic SSF	1	212908.611	629.75785	236750.0562	2.03074879
None vs SSF	1	178373.690	8643.93347	108430.4835	0.43555556
C.V., %		10.80	16.77	10.79	1.79

Appendix Table 11

Pigs means for metatarsal (MT), metacarpal (MC), and femur strength (Experiment 1).

Pen	TRT	Rep	MT,kg	MC, kg	MT-MC,kg	Femur, kg
1	A	1	29.0	43.2	36.1	115.2
2	C	1	65.9	55.7	60.8	225.6
3	G	1	68.4	69.2	68.8	232.9
4	E	1	55.7	52.0	53.8	164.9
5	F	1	58.6	62.2	60.4	200.5
6	B	1	59.2	59.0	59.1	146.4
7	D	1	82.4	69.1	75.7	233.7
9	F	2	61.8	69.9	65.9	226.1
10	C	2	55.5	57.1	56.3	194.3
11	G	2	74.3	71.4	72.8	225.0
12	B	2	45.4	51.7	48.6	155.1
14	D	2	78.6	79.9	79.3	285.0
15	E	2	49.5	51.4	50.4	194.0
16	A	2	37.0	41.1	39.1	116.2
17	G	3	57.6	53.3	55.4	229.3
18	B	3	42.0	41.7	41.9	150.1
19	D	3	65.6	68.7	67.1	233.0
20	E	3	52.3	48.7	50.5	193.1
21	C	3	57.4	49.1	53.2	181.2
22	F	3	50.3	46.2	48.2	202.6
23	A	3	40.6	39.7	40.2	154.2
1	B	4	49.9	50.8	50.4	162.6
2	E	4	56.8	60.2	58.5	161.2
3	D	4	77.0	72.8	74.9	270.0
4	C	4	51.7	47.1	49.4	185.8
5	F	4	52.4	63.5	58.0	199.9
6	G	4	55.6	62.8	59.2	201.3
7	A	4	39.1	43.7	41.4	118.5
9	E	5	36.7	41.8	39.3	124.9
10	B	5	39.9	39.9	39.9	108.7
11	C	5	61.6	66.2	63.9	218.4
12	D	5	59.2	63.7	61.5	193.3
14	F	5	69.1	54.9	62.0	222.4
15	A	5	23.3	26.0	24.7	69.2
16	G	6	54.6	55.5	55.0	164.7
17	A	6	28.0	31.9	29.9	107.8
18	G	6	90.8	66.5	78.7	265.3
19	F	6	62.8	60.2	61.5	223.7

Table 11. (continued)

Pen	TRT	Rep	MT,kg	MC, kg	MT-MC,kg	Femur, kg
20	D	6	57.9	60.9	59.4	203.2
21	C	6	45.5	49.1	47.3	175.5
22	E	6	37.4	39.3	38.4	146.6
23	B	6	37.3	38.0	37.6	120.0

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 12

Analysis of variance for metatarsal (MT), metacarpal (MC), and femur strength (Experiment 1).

		Mean Squares			
Source	df	MT,kg	MC, kg	MT-MC,kg	Femur, kg
Total	41				
Error	30	81.980349	34.918746	46.274429	572.26778
Repetition	5	111.593571	183.222524	137.595095	1500.41467
Treatment	6	1010.943016	689.609127	837.839286	12252.46111
Linear MSP	1	4501.8750000	3118.140750	3773.286750	54153.50533
Quadratic MSP	1	1.706667	51.920417	18.200417	238.14000
Cubic MSP	1	8.533333	29.900083	17.864083	637.56300
Linear SSF	1	3532.150707	2029.897415	2723.760738	34970.61884
Quadratic SSF	1	400.201679	334.683672	366.963103	8365.28400
Cubic SSF	1	0.236717	12.907883	4.272045	376.36480
None vs SSF	1	2858.940139	1722.845000	2251.205000	32729.61125
C.V., %		16.73	10.90	12.56	13.04

Appendix Table 13

Pigs means for weights of metatarsal (MT), metacarpal (MC), and femur strength and femur diameter (Experiment 1).

Pen	TRT	Rep	MT Wt,g	MC Wt,g	Femur Wt,g	Fem Dia, mm
1	A	1	76.8	64	180.0	21.3
2	C	1	84.2	66.3	171.4	19.7
3	G	1	83.3	66.9	181.6	19.8
4	E	1	86.5	67.1	177.1	19.2
5	F	1	90	75.4	200.9	21.7
6	B	1	78.7	66.5	165.9	20.6
7	D	1	86.8	67.7	199.2	22.5
9	F	2	91.7	71.4	198.4	21.7
10	C	2	79.4	62.1	167.4	18.8
11	G	2	82	63.9	175.8	19.1
12	B	2	80	63.4	165.3	21.2
14	D	2	85	68.1	191.6	23.0
15	E	2	81.8	57.3	164.8	20.2
16	A	2	69.1	65.5	165.8	18.8
17	G	3	78.7	61.8	171.7	21.9
18	B	3	77.5	56.8	156.1	18.6
19	D	3	83.6	64.5	175.2	20.1
20	E	3	71.1	57.9	154.3	19.4
21	C	3	78.7	60.5	164.3	19.0
22	F	3	70.3	56.6	158.7	19.9
23	A	3	69.7	56	148.9	17.0
1	B	4	74.9	60.5	184.0	20.5
2	E	4	79.4	62.5	171.3	19.8
3	D	4	84	72.8	188.1	21.0
4	C	4	78.3	74.5	184.3	21.1
5	F	4	78.9	72	178.5	20.8
6	G	4	88.3	76.1	197.0	20.9
7	A	4	83.9	68	157.7	20.9
9	E	5	73.1	63.7	160.1	19.8
10	B	5	74.8	57.7	158.6	19.2
11	C	5	73.1	66.3	167.0	19.7
12	D	5	79.5	66.7	170.4	19.9
14	F	5	79.3	62.2	167.2	20.1
15	A	5	65.3	69.2	139.4	17.7
16	G	5	85.5	55.6	179.3	21.1
17	A	6	73.9	57.1	152.9	18.7

Table. 13 (continued)

Pen	TRT	Rep	MT Wt,g	MC Wt,g	Femur Wt,g	Fem Dia, mm
18	G	6	78.8	61.6	165.9	19.4
19	F	6	77.6	57.4	162.5	19.6
20	D	6	84.4	64.2	165.3	18.6
21	C	6	76.7	58.9	151.7	18.6
22	E	6	75.7	56.8	141.3	18.1
23	B	6	65.3	47.3	124.5	17.2

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 14

Analysis of variance for weights of metatarsal (MT), metacarpal (MC), and femur (FM) strength and femur diameter (Experiment 1).

		Mean Squares			
Source	df	MT Wt,g	MC Wt,g	FM Wt,g	FMDia, mm
Total	39				
Error	28	19.402683	15.953540	77.14086	1.07155841
Repetition	5	87.2485714	151.4460952	1017.049667	4.87753952
Treatment	6	93.8532540	52.2799206	616.924147	2.80783175
Linear MSP	1	378.0750000	99.00833333	1976.814187	8.17452000
Quadratic MSP	1	17.3400000	77.04166667	227.858438	1.08375000
Cubic MSP	1	0.4083333	60.20833333	0.858521	1.07163000
Linear SSF	1	278.3003387	15.36303626	1681.727438	6.469880760
Quadratic SSF	1	51.8585333	1.18572637	206.440358	2.02626313
Cubic SSF	1	0.0458305	61.27453235	252.898078	1.48153256
None vs SSF	1	256.5112500	0.64222222	1031.715312	5.12000000
C.V., %		5.58	6.28	5.19	5.20

Appendix Table 15

Pigs means for fat-free metacarpal ash weight and percentage ash (Experiment 1).

Pen	TRT	Rep	Ash Wt.	%ash
1	A	1	2.39	49.30
2	C	1	3.22	53.51
3	G	1	3.01	52.39
4	E	1	2.93	52.07
5	F	1	3.35	52.12
6	B	1	2.95	51.65
7	D	1	3.18	53.01
9	F	2	3.28	51.89
10	C	2	2.71	52.62
11	G	2	3.15	53.87
12	B	2	2.51	50.49
14	D	2	3.52	55.11
15	E	2	2.88	51.93
16	A	2	2.02	48.50
17	G	3	2.94	53.91
18	B	3	2.22	49.55
19	D	3	3.03	53.30
20	E	3	2.23	50.66
21	C	3	2.43	51.35
22	F	3	2.64	52.54
23	A	3	2.07	46.85
1	B	4	2.47	48.83
2	E	4	2.55	49.25
3	D	4	3.29	52.38
4	C	4	2.92	44.27
5	F	4	2.94	50.01
6	G	4	3.18	50.34
7	A	4	2.36	45.56
9	E	5	2.65	46.78
10	B	5	2.13	46.35
11	C	5	2.52	49.53
12	D	5	2.70	49.46
14	F	5	2.83	51.17
15	A	5	1.82	47.21

Table 15. (continued)

Pen	TRT	Rep	Ash Wt.	%ash
16	G	6	2.68	50.03
17	A	6	1.96	46.82
18	G	6	3.01	52.70
19	F	6	2.66	51.20
20	D	6	2.91	51.81
21	C	6	2.46	50.68
22	E	6	2.16	46.75
23	B	6	1.72	46.59

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 16

Analysis of variance fat-free metacarpal ash weight and percent ash(Experiment 1).

		Mean Squares	
Source	df	Ash Wt.	%ash
Total	41		
Error	30	0.03487127	2.0033605
Repetition	5	0.42259238	17.9016171
Treatment	6	0.81921508	20.9532024
Linear MSP	1	3.43070083	84.99150083
Quadratic MSP	1	0.04083750	0.63050417
Cubic MSP	1	0.00494083	0.23674083
Linear SSF	1	2.39894335	72.09558962
Quadratic SSF	1	0.67694900	12.00467582
Cubic SSF	1	0.01265457	0.27777151
None vs SSF	1	2.42366806	62.14266806
C.V., %		6.96	2.81

Appendix Table 17

Pigs means for carcass composition, % (Experiment 1).

Pen	TRT	Rep	% water	% protein	% Fat	%Ash	%P
1	A	1	62.79	19.03	16.77	1.94	0.32
6	B	1	65.34	17.47	14.33	2.14	0.37
2	C	1	59.76	19.96	19.47	2.82	0.46
7	D	1	62.84	17.19	15.93	2.58	0.49
4	E	1	65.07	17.25	14.15	2.31	0.44
5	F	1	63.12	19.41	16.21	2.38	0.42
3	G	1	63.04	19.74	16.20	2.17	0.38
16	A	2	63.84	18.91	15.07	2.15	0.37
12	B	2	64.46	18.82	14.37	2.43	0.41
10	C	2	63.16	18.34	16.57	2.13	0.37
14	D	2	63.02	18.90	15.69	2.73	0.51
15	E	2	62.71	18.89	17.08	2.25	0.37
9	F	2	63.87	18.39	14.49	2.66	0.42
11	G	2	61.58	18.35	17.52	2.42	0.44
23	A	3	63.61	18.98	15.76	1.97	0.38
18	B	3	63.18	18.41	16.44	1.87	0.41
21	C	3	64.53	19.03	14.15	2.46	0.42
19	D	3	64.20	19.23	14.54	2.49	0.48
20	E	3	63.59	18.57	16.18	2.32	0.37
22	F	3	60.20	18.21	19.79	2.23	0.38
17	G	3	63.60	19.24	14.37	2.77	0.48
1	B	4	63.74	19.20	15.21	2.03	0.33
2	E	4	62.45	19.90	15.29	2.05	0.37
3	D	4	64.84	18.95	13.85	2.24	0.42
4	C	4	62.93	19.61	15.52	2.28	0.40
5	F	4	61.77	19.73	16.08	2.47	0.46
6	G	4	65.02	19.37	12.72	2.54	0.45
7	A	4	64.24	19.30	14.40	1.79	0.31
9	E	5	65.48	18.92	13.75	1.91	0.35
10	B	5	65.41	19.51	13.07	2.06	0.36
11	C	5	64.54	18.78	14.14	2.33	0.43
12	D	5	63.72	19.16	15.08	2.39	0.42
14	F	5	64.04	19.38	14.66	2.33	0.40
15	A	5	62.71	18.05	17.78	1.77	0.30
16	G	5	65.46	19.56	12.39	2.63	0.44

Table 17. (continued)

Pen	TRT	Rep	% water	% protein	% Fat	%Ash	%P
17	A	6	64.28	19.23	14.38	1.69	0.30
18	G	6	62.36	18.16	17.01	2.30	0.42
19	F	6	61.67	19.26	17.01	2.14	0.40
20	D	6	65.55	19.14	13.46	2.28	0.41
21	C	6	63.94	18.42	15.28	2.18	0.35
22	E	6	63.99	18.35	15.68	1.96	0.35
23	B	6	64.75	18.54	14.46	2.03	0.33

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 18

Analysis of variance for for carcass composition, % (Experiment 1).

		Mean Squares		
Source	df	water	protein	Fat
Total	41			
Error	30	1.63124381	0.43118619	2.4669019
Repetition	5	1.77068381	0.71134286	3.39997524
Treatment	6	2.58580476	0.20912619	2.35395238
Linear MSP	1	0.00005333	0.00300000	0.76480333
Quadratic MSP	1	0.00041667	0.00001667	0.00375000
Cubic MSP	1	5.96748000	0.46875000	6.23808000
Linear SSF	1	0.30285991	0.23400032	0.76683876
Quadratic SSF	1	2.19838187	0.01766404	2.12102993
Cubic SSF	1	4.57500504	0.45451834	3.02335065
None vs SSF	1	0.40350139	0.00045000	0.05013889
C.V., %		2.00	3.48	10.20

Table 18 (Continued)

		Mean Squares	
Source	df	Ash	P
Total	41		
Error	30	0.03114190	0.00104016
Repetition	5	0.08902857	0.00280238
Treatment	6	0.28874286	0.01093254
Linear MSP	1	1.16821333	0.05084083
Quadratic MSP	1	0.02281667	0.00020417
Cubic MSP	1	0.01925333	0.00006750
Linear SSF	1	1.07930023	0.03420191
Quadratic SSF	1	0.14462537	0.00403128
Cubic SSF	1	0.00582054	0.00006682
None vs SSF	1	0.86900139	0.02722222
C.V., %		7.83	8.12

Appendix Table 19

Pigs means for carcass accretion rate(g/d) (Experiment 1).

Pen	TRT	Rep	Water	Protein	Fat	Ash	P
1	A	1	296.02	153.46	134.98	12.04	1.61
2	C	1	286.03	168.17	167.09	21.81	3.17
3	G	1	295.68	156.20	124.91	14.13	2.19
4	E	1	234.96	111.19	88.10	12.68	2.19
5	F	1	306.84	157.68	129.17	16.64	2.63
6	B	1	311.94	134.30	107.29	13.77	2.06
7	D	1	292.97	131.65	122.82	18.22	3.22
9	F	2	228.28	119.87	89.70	15.65	2.01
10	C	2	290.20	138.24	123.99	13.40	1.96
11	G	2	269.88	136.92	131.64	15.95	2.60
12	B	2	238.31	122.92	88.05	13.72	1.93
14	D	2	261.56	135.75	109.32	17.89	3.10
15	E	2	271.13	140.54	126.51	14.10	1.93
16	A	2	234.13	123.79	93.87	11.48	1.58
17	G	3	232.60	126.39	88.09	16.56	2.51
18	B	3	244.97	123.85	108.80	9.59	2.04
19	D	3	252.18	130.10	92.26	14.80	2.62
20	E	3	280.73	133.05	113.09	14.45	1.89
21	C	3	229.91	120.27	82.83	13.53	1.95
22	F	3	278.73	138.31	153.91	14.77	2.13
23	A	3	280.11	136.91	109.71	11.38	1.99
1	B	4	352.95	163.79	126.18	15.52	1.93
2	E	4	307.32	160.20	118.56	14.61	2.17
3	D	4	314.44	145.26	100.26	15.96	2.55
4	C	4	358.19	170.77	131.35	18.86	2.77
5	F	4	267.94	146.28	116.12	17.58	2.72
6	G	4	285.97	139.31	82.99	17.76	2.57
7	A	4	272.29	134.14	94.36	10.19	1.27
9	E	5	277.74	128.09	86.73	11.11	1.58
10	B	5	246.20	123.26	74.52	11.42	1.54
11	C	5	304.84	136.20	96.91	16.34	2.47
12	D	5	274.19	130.76	97.99	15.82	2.23
14	F	5	346.29	152.95	109.93	17.99	2.43
15	A	5	273.15	121.44	119.02	10.41	1.22
16	G	5	254.91	121.05	67.73	16.19	2.10

Table 19. (continued)

Pen	TRT	Rep	Water	Protein	Fat	Ash	P
17	A	6	234.40	123.67	87.00	8.13	1.07
18	G	6	316.81	142.33	133.66	17.46	2.66
19	F	6	252.75	132.60	115.34	13.46	2.02
20	D	6	286.28	128.32	83.01	14.60	2.06
21	C	6	275.43	121.51	96.39	13.70	1.63
22	E	6	269.93	116.22	94.79	11.53	1.54
23	B	6	231.89	102.97	74.08	10.52	1.14

Trt A: Corn-soybean meal diets + 0% monosodium phosphate
Trt B: Corn-soybean meal diets + 0.21% monosodium phosphate
Trt C: Corn-soybean meal diets + 0.43% monosodium phosphate
Trt D: Corn-soybean meal diets + 0.66% monosodium phosphate
Trt E: Corn-soybean meal diets + 250 SSF phytase
Trt F: Corn-soybean meal diets + 500 SSF phytase
Trt G: Corn-soybean meal diets + 1,000 SSF phytase

Appendix Table 20

Analysis of variance for carcass accretion rate (g/d) (Experiment 1).

		Mean Squares		
Source	df	water	protein	Fat
Total	41			
Error	30	930.93962	162.721210	437.83202
Repetition	5	2943.05871	763.466766	851.012454
Treatment	6	400.85572	163.255916	391.309666
Linear MSP	1	1286.420083	99.6634133	3.267000
Quadratic MSP	1	409.530817	40.0416667	45.045600
Cubic MSP	1	578.602083	495.23907000	1281.840333
Linear SSF	1	331.069183	126.3250498	0.449433
Quadratic SSF	1	394.731236	81.1384837	398.491529
Cubic SSF	1	6.391659	164.8499633	459.300845
None vs SSF	1	601.987168	86.5708681	40.725312
C.V., %		11.02	9.43	19.56

Table 20. (Continued)

		Mean Squares	
Source	df	Ash	P
Total	41		
Error	30	3.1478698	0.09728746
Repetition	5	9.5017810	0.44114857
Treatment	6	33.2301746	1.06099127
Linear MSP	1	128.2987200	4.97354083
Quadratic MSP	1	5.2828167	0.00020417
Cubic MSP	1	10.5850800	0.06960083
Linear SSF	1	105.4992203	3.03962044
Quadratic SSF	1	23.3800325	0.54254640
Cubic SSF	1	2.6144898	0.03524656
None vs SSF	1	92.7749014	2.58781250
C.V., %		12.30	14.72

Appendix Table 21

Pigs means for average daily gain, average daily feed intake, average daily dry matter intake, and gain:feed (Experiment 2).

PEN	TRT	REP	ADG,g	ADFI,g	G:F, g/kg
1	D	1	817.6	1592.0	513.6
2	B	1	500.2	1114.5	448.8
3	C	1	597.3	1191.7	501.2
4	A	1	645.3	1342.8	480.6
5	C	2	690.6	1406.3	491.1
6	A	2	646.6	1299.0	497.7
7	B	2	743.8	1636.5	454.5
8	D	2	716.6	1348.2	531.5
9	B	3	659.5	1313.3	502.2
10	C	3	566.2	1182.4	478.9
11	D	3	736.0	1403.7	524.3
12	A	3	561.1	1231.4	455.6
13	B	4	453.5	1134.0	399.9
14	A	4	541.6	1205.8	449.2
15	D	4	815.0	1606.0	507.5
16	C	4	716.6	1473.8	486.2
17	B	5	606.4	1296.2	467.8
18	D	5	699.7	1335.2	524.0
19	C	5	641.4	1259.4	509.3
20	A	5	618.1	1234.2	500.8
21	C	6	609.0	1249.3	487.5
22	D	6	697.1	1433.6	486.3
23	A	6	530.0	1264.5	419.1
24	B	6	602.5	1309.2	460.2

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch)

Appendix Table 22

Analysis of variance for average daily gain, average daily feed intake, average daily dry matter intake, and gain:feed (Experiment 2).

		Mean Squares		
Source	df	ADG	ADFI	G:F
Total	39			
Error	28	3977.0904	11065.7723	347.07203
Repetition	5	4354.46420	7552.2767	803.134722
Treatment	6	26919.65042	44014.1779	2660.790648
Linear SSF	1	6380.02637	2882.2560	1905.120000
Quadratic SSF	1	810.43589	153.0398	212.558034
WM vs CS	1	72446.53167	125798.8337	6055.017558
C.V., %		9.698813	7.951996	3.82

Appendix Table 23

Pigs means for average dry matter intake, excretion, absorbed, and digestibility (Experiment 2).

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	D	1	1	1357.32	149.85	1207.46	88.96
2	B	1	1	1070.87	186.16	884.71	82.62
3	C	1	1	1310.48	247.99	1062.49	81.08
4	A	1	1	1302.90	227.23	1075.67	82.56
5	C	1	2	1231.90	225.98	1005.92	81.66
6	A	1	2	996.10	172.23	823.87	82.71
7	B	1	2	1362.00	234.71	1127.29	82.77
8	D	1	2	1136.87	139.86	997.01	87.70
9	B	1	3	1159.73	198.47	961.25	82.89
10	C	1	3	930.94	177.26	753.68	80.96
11	D	1	3	1302.11	118.46	1183.65	90.90
12	A	1	3	1135.65	220.23	915.42	80.61
13	B	1	4	953.05	165.77	787.27	82.61
14	A	1	4	819.49	143.74	675.76	82.46
15	D	1	4	1622.60	173.06	1449.54	89.33
16	C	1	4	1132.42	211.41	921.01	81.33
17	B	1	5	1109.06	214.34	894.72	80.67
18	D	1	5	1262.78	149.27	1113.51	88.18
19	C	1	5	1135.35	227.81	907.54	79.93
20	A	1	5	1225.16	246.54	978.63	79.88
21	C	1	6	931.97	172.73	759.24	81.47
22	D	1	6	1371.29	134.69	1236.60	90.18
24	B	1	6	1187.39	213.01	974.39	82.06
1	D	2	1	1772.56	196.52	1576.04	88.91
2	B	2	1	1246.70	219.95	1026.74	82.36
3	C	2	1	1080.11	218.95	861.16	79.73
4	A	2	1	1305.64	248.77	1056.87	80.95
5	C	2	2	1694.51	286.60	1407.92	83.09
6	A	2	2	1514.22	275.87	1238.35	81.78
7	B	2	2	1880.67	407.32	1473.35	78.34
8	D	2	2	1537.97	150.55	1387.42	90.21
9	B	2	3	1343.57	213.39	1130.18	84.12
10	C	2	3	1384.51	224.13	1160.38	83.81
11	D	2	3	2033.31	149.69	1883.63	92.64
12	A	2	3	1238.57	218.87	1019.70	82.33

Table 23. (continued)

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
13	B	2	4	1150.65	223.10	927.55	80.61
14	A	2	4	1357.53	214.18	1143.35	84.22
15	D	2	4	1738.35	203.99	1534.36	88.27
16	C	2	4	1605.93	282.72	1323.21	82.40
17	B	2	5	1372.32	237.13	1135.19	82.72
18	D	2	5	1535.22	158.49	1376.73	89.68
19	C	2	5	1095.96	215.68	880.29	80.32
20	A	2	5	1106.14	219.97	886.17	80.11
21	C	2	6	1242.93	197.61	1045.32	84.10
22	D	2	6	1584.93	156.29	1428.64	90.14
23	A	2	6	1266.18	207.62	1058.56	83.60
24	B	2	6	1202.33	175.90	1026.43	85.37

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch

Appendix Table 24

Analysis of average dry matter intake, excretion, absorbed, and digestibility (Experiment 2).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	44				
Error	32	31575.850	764.36358	24526.426	1.3840497
Repetition	5	13167.2896	1219.83907	11884.671	5.6010799
Period	1	663910.3978	7463.50458	530591.859	6.7833639
Treatment	3	296508.9504	10691.49301	413162.012	167.2779658
Trt X Period	3	18772.1719	96.37027	18508.679	0.3173457
Linear SSF	1	9099.1869	539.66850	5206.739	0.6871480
Quadratic SSF	1	3955.5990	1530.77788	948.498	4.1929823
WM vs CS	1	883186.0408	29705.266.9	1236833.299	490.7798348
C.V., %		13.76	13.81	14.36	1.39

Appendix Table 25

Pigs means for average daily phosphorus intake, excretion, absorption, and digestibility (Experiment 2).

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	D	1	1	8.01	3.22	4.78	59.73
2	B	1	1	6.10	3.45	2.64	43.35
3	C	1	1	7.31	3.32	3.98	54.51
4	A	1	1	7.25	4.00	3.25	44.78
5	C	1	2	6.87	3.98	2.89	42.06
6	A	1	2	5.54	3.40	2.14	38.63
7	B	1	2	7.75	4.17	3.59	46.27
8	D	1	2	6.71	3.20	3.51	52.35
9	B	1	3	6.60	3.48	3.12	47.21
10	C	1	3	5.19	2.99	2.20	42.44
11	D	1	3	7.68	2.79	4.89	63.72
12	A	1	3	6.32	4.23	2.09	33.06
13	B	1	4	5.42	3.41	2.01	37.08
14	A	1	4	4.56	2.86	1.70	37.23
15	D	1	4	9.57	3.60	5.97	62.39
16	C	1	4	6.32	3.45	2.86	45.35
17	B	1	5	6.31	4.16	2.16	34.17
18	D	1	5	7.45	3.23	4.22	56.67
19	C	1	5	6.33	4.00	2.33	36.76
20	A	1	5	6.82	4.82	2.00	29.29
21	C	1	6	5.20	3.42	1.77	34.10
22	D	1	6	8.09	3.27	4.82	59.54
24	B	1	6	6.76	4.39	2.37	35.08
1	D	2	1	10.46	4.15	6.31	60.34
2	B	2	1	7.10	3.43	3.66	51.63
3	C	2	1	6.02	3.56	2.47	40.93
4	A	2	1	7.27	4.84	2.43	33.45
5	C	2	2	9.45	5.27	4.18	44.28
6	A	2	2	8.43	5.31	3.12	36.99
7	B	2	2	10.70	7.23	3.47	32.42
8	D	2	2	9.07	3.85	5.22	57.55
9	B	2	3	7.65	3.91	3.74	48.88
10	C	2	3	7.72	3.59	4.13	53.44
11	D	2	3	11.99	3.51	8.49	70.75
12	A	2	3	6.89	3.93	2.97	43.03

Table 25. (continued)

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
13	B	2	4	6.55	4.01	2.54	38.75
14	A	2	4	7.55	4.30	3.26	43.09
15	D	2	4	10.25	4.08	6.18	60.23
16	C	2	4	8.96	4.14	4.81	53.73
17	B	2	5	7.81	4.44	3.37	43.12
18	D	2	5	9.06	3.67	5.39	59.52
19	C	2	5	6.11	3.80	2.32	37.89
20	A	2	5	6.16	4.12	2.03	33.02
21	C	2	6	6.93	3.75	3.18	45.94
22	D	2	6	9.35	3.63	5.72	61.20
23	A	2	6	7.05	4.33	2.71	38.52
24	B	2	6	6.84	3.94	2.90	42.44

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch

Appendix Table 26

Analysis of variance for average daily phosphorus intake, excretion, absorption, and digestibility (Experiment 2)

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	44				
Error	32	1.0185035	0.20826342	0.54630581	21.341576
Repetition	5	0.42205990	0.36676480	1.05994946	89.302097
Period	1	21.66255499	2.43478280	9.62475643	125.501512
Treatment	3	14.4914621	0.84104855	21.33124634	1173.316955
Trt X Period	3	0.71694803	0.11117273	0.48582697	6.953198
Linear SSF	1	0.31958776	0.87073015	2.23514597	292.445300
Quadratic SSF	1	0.00004835	0.00873989	0.01009990	7.877461
WM vs CS	1	43.21296404	1.68772730	62.0641394	3244.06748
C.V., %		13.71	11.92	20.94	9.56

Appendix Table 27

Pigs means for average daily nitrogen intake, excretion, absorption, and digestibility (Experiment 2).

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	D	1	1	41.99	7.74	34.25	81.57
2	B	1	1	36.47	7.88	28.59	78.40
3	C	1	1	44.97	10.74	34.23	76.12
4	A	1	1	44.91	9.49	35.42	78.88
5	C	1	2	42.27	10.53	31.74	75.08
6	A	1	2	34.34	7.35	26.98	78.59
7	B	1	2	46.38	9.91	36.47	78.63
8	D	1	2	35.17	6.93	28.24	80.28
9	B	1	3	39.49	7.92	31.57	79.94
10	C	1	3	31.95	7.76	24.19	75.71
11	D	1	3	40.28	4.70	35.58	88.33
12	A	1	3	39.15	8.90	30.24	77.26
13	B	1	4	32.46	6.29	26.17	80.62
14	A	1	4	28.25	6.73	21.52	76.18
15	D	1	4	50.20	7.62	42.57	84.81
16	C	1	4	38.86	9.64	29.22	75.19
17	B	1	5	37.77	9.81	27.96	74.04
18	D	1	5	39.07	7.83	31.24	79.96
19	C	1	5	38.96	9.20	29.77	76.40
20	A	1	5	42.23	10.26	31.97	75.70
21	C	1	6	31.98	6.90	25.08	78.43
22	D	1	6	42.42	6.19	36.23	85.41
24	B	1	6	40.44	8.69	31.74	78.50
1	D	2	1	54.84	8.84	45.99	83.87
2	B	2	1	42.46	8.31	34.14	80.42
3	C	2	1	37.07	8.28	28.78	77.65
4	A	2	1	45.01	10.57	34.44	76.52
5	C	2	2	58.15	12.19	45.96	79.03
6	A	2	2	52.19	11.62	40.58	77.75
7	B	2	2	64.05	17.44	46.60	72.76
8	D	2	2	47.58	6.67	40.91	85.97
9	B	2	3	45.76	8.30	37.45	81.85
10	C	2	3	47.51	8.99	38.52	81.08
11	D	2	3	62.90	6.22	56.69	90.11
12	A	2	3	42.69	8.47	34.22	80.16

Table 27. (continued)

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
13	B	2	4	39.19	9.32	29.87	76.22
14	A	2	4	46.79	8.37	38.42	82.11
15	D	2	4	53.78	8.95	44.83	83.35
16	C	2	4	55.11	12.64	42.47	77.06
17	B	2	5	46.73	10.31	36.43	77.95
18	D	2	5	47.49	7.57	39.93	84.07
19	C	2	5	37.61	8.43	29.18	77.60
20	A	2	5	38.13	9.85	28.27	74.16
21	C	2	6	42.65	7.18	35.47	83.16
22	D	2	6	49.03	7.12	41.91	85.47
23	A	2	6	43.64	7.57	36.07	82.65
24	B	2	6	40.95	6.24	34.70	84.76

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch

Appendix Table 28

Analysis of variance for average daily nitrogen intake, excretion, absorption, and digestibility (Experiment 2).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	44				
Error	32	35.165679	1.8600488	25.182466	4.1271265
Repetition	5	14.7490486	5.48188820	15.5905398	24.874333
Period	1	725.506453	5.18421320	608.1355201	46.9108197
Treatment	3	101.8138735	10.22321631	163.7979437	111.8583515
Trt X Period	3	14.2047629	0.30068556	13.6842241	1.961275
Linear SSF	1	8.0012207	1.61180357	2.4471146	2.3230154
Quadratic SSF	1	9.4937899	2.82001627	1.9780331	8.0245746
WM vs CS	1	293.4706346	24.98854830	489.9503052	318.4174190
C.V., %		13.82	16.10	14.57	2.54

Appendix Table 29

Pigs means for average daily gross energy intake, excretion, absorption, and digestibility (Experiment 2).

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	D	1	1	5813.12	739.83	5073.29	87.27
2	B	1	1	4664.39	912.84	3751.55	80.43
3	C	1	1	5708.24	1214.63	4493.60	78.72
4	A	1	1	5764.41	1072.78	4691.62	81.39
5	C	1	2	5365.94	1120.51	4245.43	79.12
6	A	1	2	4407.06	863.37	3543.69	80.41
7	B	1	2	5932.47	1104.82	4827.65	81.38
8	D	1	2	4868.99	686.16	4182.83	85.91
9	B	1	3	5051.44	929.12	4122.31	81.61
10	C	1	3	4055.01	837.95	3217.05	79.34
11	D	1	3	5576.67	560.62	5016.05	89.95
12	A	1	3	5024.44	1039.25	3985.19	79.32
13	B	1	4	4151.21	782.78	3368.43	81.14
14	A	1	4	3625.69	698.41	2927.27	80.74
15	D	1	4	6949.26	813.92	6135.34	88.29
16	C	1	4	4932.61	991.99	3940.62	79.89
17	B	1	5	4830.76	1069.78	3760.98	77.85
18	D	1	5	5408.25	736.93	4671.33	86.37
19	C	1	5	4945.39	1084.25	3861.14	78.08
20	A	1	5	5420.49	888.82	4531.67	83.60
21	C	1	6	4059.50	844.37	3215.13	79.20
22	D	1	6	5872.98	660.07	5212.90	88.76
24	B	1	6	5171.94	1016.92	4155.02	80.34
1	D	2	1	7591.54	895.67	6695.88	88.20
2	B	2	1	5430.25	1013.68	4416.57	81.33
3	C	2	1	4704.77	1013.05	3691.72	78.47
4	A	2	1	5776.53	1143.22	4633.31	80.21
5	C	2	2	7381.01	1346.88	6034.13	81.75
6	A	2	2	6699.35	1239.29	5460.06	81.50
7	B	2	2	8191.65	1899.63	6292.02	76.81
8	D	2	2	6586.80	713.80	5873.00	89.16
9	B	2	3	5852.18	993.15	4859.03	83.03
10	C	2	3	6030.69	1053.11	4977.58	82.54
11	D	2	3	8708.28	700.95	8007.34	91.95
12	A	2	3	5479.82	1021.43	4458.39	81.36

Table 29. (continued)

Pen	TRT	Period	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
13	B	2	4	5011.90	1060.63	3951.27	78.84
14	A	2	4	6006.12	1013.51	4992.61	83.13
15	D	2	4	7445.00	940.63	6504.37	87.37
16	C	2	4	6995.14	1340.27	5654.87	80.84
17	B	2	5	5977.41	1113.54	4863.88	81.37
18	D	2	5	6575.04	742.68	5832.36	88.70
19	C	2	5	4773.82	966.23	3807.59	79.76
20	A	2	5	4893.88	1067.80	3826.08	78.18
21	C	2	6	5414.00	917.00	4497.00	83.06
22	D	2	6	6787.96	718.17	6069.79	89.42
23	A	2	6	5601.95	939.19	4662.76	83.23
24	B	2	6	5237.00	821.01	4415.98	84.32

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch

Appendix Table 30

Analysis of variance for average daily gross energy intake, excretion, absorption, and digestibility (Experiment 2).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	44				
Error	32	5.7587.66	15084.305	468455.71	2.0746267
Repetition	5	248570.01	30012.3724	198048.02	6.2714195
Period	1	12523219.05	118824.5810	10202387.47	15.0038944
Treatment	3	4545820.69	226989.0150	6666921.65	177.0271184
Trt X Period	3	328211.73	7326.3469	342540.21	2.1768584
Linear SSF	1	48486.23	36003.0508	927.44	8.6112176
Quadratic SSF	1	142184.39	8776.1811	80309.50	0.5488184
WM vs CS	1	13567859.72	620122.8749	19989358.29	513.7760863
C.V., %		13.77	13.05	14.64	1.74

Appendix Table 31

Pigs means for metatarsal (MT), metacarpal (MC), and femur strength (Experiment 2).

Pen	TRT	Rep	MC,kg	MT, kg	Femur, kg	Average, kg
1	D	1	80.64	63.78	258.85	134.42
2	B	1	47.28	47.10	137.92	77.43
3	C	1	50.12	42.00	219.44	103.86
4	A	1	33.19	17.94	109.57	53.57
5	C	2	61.40	45.86	191.81	99.69
6	A	2	50.04	51.90	164.17	88.71
7	B	2	58.38	49.82	225.82	111.34
8	D	2	78.51	77.14	241.83	132.49
9	B	3	44.06	42.47	194.05	93.53
10	C	3	39.41	40.30	158.62	79.44
11	D	3	50.19	48.17	232.60	110.32
12	A	3	42.75	37.58	122.32	67.55
13	B	4	40.97	42.96	155.10	79.68
14	A	4	43.54	39.25	159.08	80.62
15	D	4	73.58	83.67	309.94	155.73
16	C	4	53.49	55.11	186.97	98.52
17	B	5	43.71	34.56	119.87	66.05
18	D	5	68.96	67.87	217.71	118.18
19	C	5	51.49	48.05	149.39	82.98
20	A	5	38.33	30.40	153.72	74.15
21	C	6	57.35	44.05	158.87	86.76
22	D	6	63.01	66.57	259.16	129.58
23	A	6	41.81	22.98	132.05	65.61
24	B	6	50.41	45.85	193.95	96.73

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch

Appendix Table 32

Analysis of variance for metatarsal (MT), metacarpal (MC), and femur strength (Experiment 2).

		Mean Squares			
Source	df	MC,kg	MT, kg	Femur, kg	Average, kg
Total	23				
Error	15	40.358032	65.001678	888.11559	146.71388
Repetition	5	133.169937	159.238707	1157.58746	318.68321
Treatment	3	842.226406	1262.747828	13860.85813	3685.01898
Linear SSF	1	337.080000	472.758533	4188.42967	1220.890133
Quadratic SSF	1	1.246944	69.722500	603.60300	128.671211
WM vs CS	1	2188.352272	3245.762450	36790.54170	9705.495606
C.V., %		12.07	16.89	16.06	12.71

Appendix Table 33

Pigs means for weights of metatarsal (MT), metacarpal (MC), and femur (FM) and FM diameter (Experiment 2).

Pen	TRT	Rep	MT, g	MC, g	FM, g	FM, mm
1	D	1	64.6	54.8	161.8	18.2
2	B	1	67.6	56.3	151.0	17.9
3	C	1	78.9	61.7	157.5	19.0
4	A	1	60.9	52.9	143.9	17.9
5	C	2	72.6	62.5	179.5	18.9
6	A	2	78.0	61.8	185.2	21.1
7	B	2	77.5	65.5	183.4	22.0
8	D	2	84.8	69.4	203.6	22.0
9	B	3	56.9	44.0	147.5	18.0
10	C	3	66.7	48.5	173.3	18.8
11	D	3	71.8	59.2	181.1	20.8
12	A	3	69.9	59.0	164.9	19.5
13	B	4	72.9	58.8	163.9	19.5
14	A	4	73.4	57.6	164.9	17.9
15	D	4	83.5	63.1	181.0	20.9
16	C	4	73.4	57.0	177.9	19.0
17	B	5	67.7	57.6	157.5	19.3
18	D	5	68.7	55.2	164.5	18.5
19	C	5	66.4	54.4	164.4	18.0
20	A	5	66.0	56.1	158.7	20.5
21	C	6	64.0	53.2	156.2	18.4
22	D	6	80.0	67.0	192.4	23.4
23	A	6	63.0	52.0	153.8	17.8
24	B	6	57.1	48.0	147.1	18.2

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch)

Appendix Table 34

Analysis of variance for weights of metatarsal (MT), metacarpal (MC), and femur strength and femur diameter (Experiment 2).

		Mean Squares			
Source	df	MT, g	MC, g	FM, g	FM, mm
Total	23				
Error	15	32.906306	22.8877778	62.193889	1.83986111
Repetition	5	113.6187500	71.178667	557.407667	3.20941667
Treatment	3	88.8426389	48.1411111	579.037222	4.35486111
Linear SSF	1	9.7200000	0.3675000	116.563333	0.56333333
Quadratic SSF	1	31.7344444	7.3802778	175.121111	0.25000000
WM vs CS	1	225.0734722	136.6755556	1445.427222	12.25125000
C.V., %		8.16	8.35	4.71	6.99

Appendix Table 35

Pigs means for fat-free metacarpal ash weight and percent ash (Experiment 2).

Pen	TRT	BLK	Ash Wt, g	%ash
1	D	1	2.69	54.62
2	B	1	2.36	49.71
3	C	1	2.53	52.37
4	A	1	1.75	48.56
5	C	2	2.75	53.16
6	A	2	2.68	52.80
7	B	2	2.76	52.76
8	D	2	3.26	54.17
9	B	3	2.05	50.47
10	C	3	1.99	50.49
11	D	3	2.96	53.88
12	A	3	2.40	47.22
13	B	4	2.34	49.21
14	A	4	2.26	48.88
15	D	4	3.59	56.74
16	C	4	2.62	51.57
17	B	5	2.07	50.55
18	D	5	2.68	54.85
19	C	5	2.37	51.68
20	A	5	1.97	47.50
21	C	6	2.26	52.29
22	D	6	3.10	55.06
23	A	6	1.91	49.77
24	B	6	2.15	52.84

Trt A: Control diet (Corn-soybean meal + 20% wheat middlings)
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Positive control (Corn-soybean meal + 20% corn starch

Appendix Table 36

Analysis of variance fat-free metacarpal ash weight and percent ash (Experiment 2).

		Mean Squares	
Source	df	Ash Wt, g	%ash
Total	23		
Error	15	0.05151444	1.4860575
Repetition	5	0.23451667	3.8490442
Treatment	3	0.92556111	34.9127708
Linear SSF	1	0.20020833	23.60407500
Quadratic SSF	1	0.00002500	0.63733611
WM vs CS	1	2.57645000	80.49690139
C.V., %		9.15	2.35

Appendix Table 37

Pigs means for average daily gain, average daily feed intake, average daily dry matter intake, and gain:feed (Experiment 3).

PEN	TRT	REP	ADG,g	ADFI,g	G:F, g/kg
1	A	1	628	1231	458
2	C	1	622	1332	503
3	B	1	466	1261	335
4	D	1	706	1185	491
5	C	2	758	1247	490
6	D	2	622	1336	452
7	B	2	564	1220	446
8	A	2	441	1493	267
9	D	3	797	1246	459
10	A	3	693	1290	373
11	C	3	590	1430	397
12	B	3	492	1264	325
13	C	4	661	1204	501
14	D	4	622	1234	441
15	B	4	674	1080	580
16	A	4	505	1198	454
17	D	5	667	1018	589
18	B	5	570	1315	441
19	A	5	603	1294	380
20	C	5	615	1376	373
21	B	6	544	1125	473
22	C	6	577	1119	437
23	A	6	460	1340	353
24	D	6	551	1104	429

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 38

Analysis of variance for average daily gain, average daily feed intake, average daily dry matter intake, and gain:feed (Experiment 3).

		Mean Squares		
Source	df	ADG	ADFI	G:F
Total	23			
Error	15	7.118.6111	9682.5314	5263.0556
Repetition	5	5507.50000	15948.69422	5054.16667
Treatment	3	19581.94444	19981.64218	9993.05556
Linear SSF	1	48002.96863	28541.59410	26409.64751
Quadratic SSF	1	500.43694	39.64170	3127.72454
Cubic SSF	1	10242.40062	31363.73693	441.82114
None vs SSF	1	17112.50000	28832.80934	23834.72222
C.V., %		14.03	7.89	16.69

Appendix Table 39

Pigs means for average dry matter intake, excretion, absorbed, and digestibility (Experiment 3).

Pen	TRT	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	A	1	846.28	211.57	634.71	75.00
2	C	1	1297.95	277.98	1019.97	78.58
3	B	1	1070.27	275.65	794.62	74.25
4	D	1	1129.30	282.60	846.70	74.98
5	C	2	1112.39	262.10	850.29	76.44
6	D	2	1165.88	247.86	918.01	78.74
7	B	2	978.63	244.18	734.45	75.05
8	A	2	845.06	217.50	627.56	74.26
9	D	3	1056.17	206.10	850.07	80.49
10	A	3	956.98	226.34	730.65	76.35
11	C	3	1097.92	225.07	872.85	79.50
12	B	3	921.70	204.53	717.17	77.81
13	C	4	1161.20	252.61	908.59	78.25
14	D	4	1127.15	205.26	921.89	81.79
15	B	4	1348.10	262.30	1085.80	80.54
16	A	4	919.93	230.68	689.25	74.92
17	D	5	1242.15	252.90	989.26	79.64
18	B	5	827.31	215.28	612.03	73.98
19	A	5	926.53	237.27	689.26	74.39
20	C	5	1204.59	214.64	989.95	82.18
21	B	6	1076.16	240.69	835.48	77.63
22	C	6	1030.16	225.93	804.23	78.07
23	A	6	922.88	176.38	746.51	80.89

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 40

Analysis of average dry matter intake, excretion, absorbed, and digestibility (Experiment 3).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	23				
Error	15	12505.0477	511.85816	10337.6797	4.5914667
Repetition	5	9136.9180	1176.826830	6985.3671	8.14172667
Treatment	3	79443.5341	875.530700	65537.3028	15.46756667
Linear SSF	1	167127.9194	888.521726	143640.1435	38.19520594
Quadratic SSF	1	69488.4188	1586.25483	50081.0539	3.36733477
Cubic SSF	1	1714.3838	152.813741	2890.7962	4.84015496
None vs SSF	1	191225.7710	2538.043756	14698.4164	22.00055556
C.V., %		10.57	9.65	12.35	2.76

Appendix Table 41

Pigs means for average daily organic matter intake, excretion, absorption, and digestibility (Experiment 3).

Pen	TRT	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	A	1	787.72	184.55	603.18	76.57
2	C	1	786.59	186.48	600.11	76.29
3	B	1	890.77	193.67	697.10	78.26
4	D	1	856.27	199.22	657.05	76.73
5	C	2	862.42	206.30	656.12	76.08
6	D	2	859.03	149.49	709.53	82.60
7	B	2	995.07	243.37	751.70	75.54
8	A	2	909.87	212.07	697.80	76.69
9	D	3	856.94	177.75	679.19	79.26
10	A	3	1253.38	226.54	1026.84	81.93
11	C	3	769.18	185.46	583.72	75.89
12	B	3	1000.55	211.02	789.53	78.91
13	C	4	1203.50	245.22	958.28	79.62
14	D	4	1031.44	229.31	802.13	77.77
15	B	4	1018.02	196.34	821.68	80.71
16	A	4	1076.70	218.53	858.17	79.70
17	D	5	1116.93	186.45	930.48	83.31
18	B	5	955.19	196.60	758.59	79.42
19	A	5	1047.60	248.64	798.96	76.27
20	C	5	1081.53	216.71	864.82	79.96
21	B	6	979.76	178.66	801.10	81.76
22	C	6	1045.61	178.13	867.48	82.96
23	A	6	1152.29	223.49	928.79	80.60

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 42

Analysis of variance for average daily organic matter intake, excretion, absorption, and digestibility (Experiment 3)

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	23				
Error	15	10796.9427	416.77545	8970.8595	4.1529911
Repetition	5	7885.7667	1021.671904	6174.1753	8.31776667
Treatment	3	66412.8620	820.960115	53894.7264	10.97809444
Linear SSF	1	139626.8329	935.215875	117707.6198	26.57896973
Quadratic SSF	1	58338.7888	1388.956214	41724.4094	1.79362493
Cubic SSF	1	1273.0658	138.710061	2252.2211	4.56168197
None vs SSF	1	160639.6221	2406.445312	123723.2768	13.60680556
C.V., %		10.58	10.00	12.17	2.58

Appendix Table 43

Pigs means for average daily ash intake, excretion, absorption, and digestibility (Experiment 3).

Pen	TRT	BLK	Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
1	A	1	58.56	27.02	31.53	53.85
2	C	1	94.45	32.76	61.69	65.31
3	B	1	75.20	32.28	42.92	57.08
4	D	1	81.70	33.96	47.74	58.43
5	C	2	80.95	32.79	48.16	59.49
6	D	2	84.35	31.15	53.20	63.07
7	B	2	68.76	32.11	36.65	53.30
8	A	2	58.47	31.02	27.45	46.94
9	D	3	76.41	27.44	48.97	64.09
10	A	3	66.22	32.67	33.55	50.66
11	C	3	79.90	28.73	51.17	64.04
12	B	3	64.76	26.78	37.98	58.65
13	C	4	84.50	34.08	50.42	59.67
14	D	4	81.55	27.13	54.42	66.73
15	B	4	94.72	35.76	58.96	62.25
16	A	4	63.65	31.46	32.19	50.58
17	D	5	89.87	29.40	60.46	67.28
18	B	5	58.13	29.82	28.30	48.69
19	A	5	64.11	30.97	33.14	51.69
20	C	5	87.66	28.19	59.47	67.84
21	B	6	75.61	29.67	45.95	60.77
22	C	6	74.96	29.33	45.64	60.88
23	A	6	63.86	26.88	36.97	57.90

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 44

Analysis of variance for average daily ash intake, excretion, absorption, and digestibility (Experiment 3).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	23				
Error	15	62.732111	6.4086408	50.404805	16.4089342
Repetition	5	46.049684	8.76301417	27.647154	11.4194675
Treatment	3	585.022337	2.48217083	570.470149	184.7529042
Linear SSF	1	1235.489254	0.59893351	1290.418817	452.5110410
Quadratic SSF	1	486.824376	6.50530253	381.189495	88.9950920
Cubic SSF	1	32.754147	0.34227234	39.802760	12.2127423
None vs SSF	1	1331.710035	1.75781250	1237.116701	381.1100347
C.V., %		10.51	8.31	15.81	6.88

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 45

Pigs means for average daily phosphorus energy intake, excretion, absorption, and digestibility (Experiment 3).

Pen	TRT	BLK	Intake kcal/d	Feces kcal/d	Absorbed kcal/d	Digestibility %
1	A	1	4.15	2.71	1.45	34.81
2	C	1	6.46	3.03	3.44	53.17
3	B	1	5.22	2.90	2.32	44.40
4	D	1	5.47	2.60	2.86	52.38
5	C	2	5.54	2.71	2.83	51.04
6	D	2	5.65	2.56	3.09	54.71
7	B	2	4.77	2.82	1.95	40.96
8	A	2	4.15	3.01	1.14	27.39
9	D	3	5.11	2.29	2.83	55.32
10	A	3	4.70	3.04	1.66	35.27
11	C	3	5.47	2.52	2.95	53.90
12	B	3	4.49	2.23	2.26	50.26
13	C	4	5.78	2.88	2.90	50.22
14	D	4	5.46	2.35	3.11	57.03
15	B	4	6.57	3.21	3.36	51.11
16	A	4	4.52	3.01	1.50	33.31
17	D	5	6.02	2.28	3.74	62.11
18	B	5	4.03	2.56	1.47	36.44
19	A	5	4.55	3.06	1.49	32.75
20	C	5	6.00	2.22	3.78	62.98
21	B	6	5.25	2.52	2.73	52.00
22	C	6	5.13	2.29	2.83	55.27
23	A	6	4.53	2.62	1.91	42.13

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 46

Analysis of variance for average daily phosphorus intake, excretion, absorption, and digestibility (Experiment 3).

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	23				
Error	15	0.30033417	0.05354889	0.21590528	23.205028
Repetition	5	0.21877417	0.11720000	0.10877417	25.361657
Treatment	3	1.98230417	0.24187222	3.24038194	594.079287
Linear SSF	1	3.63663507	0.69603864	7.51464703	1396.812426
Quadratic SSF	1	2.14891331	0.02483140	2.63574270	383.080233
Cubic SSF	1	0.16136688	0.00474688	0.11076076	2.346054
None vs SSF	1	4.51501250	0.48347222	7.95340139	1426.848200
C.V., %		10.57	8.69	18.42	10.11

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 47

Pigs means for average daily nitrogen intake, excretion, absorption, and digestibility (Experiment 3).

Pen	TRT	BLK	Intake g/d	Feces gl/d	Absorbed gl/d	Digestibility %
1	A	1	28.48	7.13	21.35	74.98
2	C	1	28.44	8.41	20.03	70.41
3	B	1	32.21	8.11	24.10	74.82
4	D	1	30.96	8.90	22.06	71.26
5	C	2	31.18	8.54	22.64	72.61
6	D	2	31.06	6.10	24.95	80.35
7	B	2	37.57	11.21	26.36	70.17
8	A	2	34.35	8.92	25.43	74.03
9	D	3	32.35	8.21	24.14	74.63
10	A	3	47.32	10.36	36.96	78.10
11	C	3	29.04	8.07	20.96	72.19
12	B	3	37.77	10.20	27.58	73.01
13	C	4	45.39	10.34	35.05	77.22
14	D	4	38.90	9.99	28.92	74.33
15	B	4	38.40	8.69	29.71	77.37
16	A	4	40.61	9.26	31.35	77.19
17	D	5	42.13	8.97	33.16	78.72
18	B	5	36.03	9.15	26.87	74.60
19	A	5	40.24	11.26	28.97	72.01
20	C	5	41.54	8.61	32.93	79.28
21	B	6	37.63	6.99	30.64	81.43
22	C	6	40.16	7.42	32.74	81.51
23	A	6	44.26	10.20	34.06	76.96
24	D	6	39.78	8.81	30.97	77.86
1	A	1	28.48	7.13	21.35	74.98
2	C	1	28.44	8.41	20.03	70.41
3	B	1	32.21	8.11	24.10	74.82

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 48

Analysis of variance for average daily nitrogen intake, excretion, absorption, and digestibility (Experiment 3)

Source	df	Mean Squares			
		Intake g/d	Feces g/d	Absorbed g/d	Digestibility %
Total	23				
Error	15	15.3486578	1.36422417	12.2855753	9.0724878
Repetition	5	11.2860467	1.69188417	8.0588842	8.24077667
Treatment	3	135.0634778	3.35030417	106.2433486	27.12437778
Linear SSF	1	300.5309586	1.43923136	260.3530371	70.57440444
Quadratic SSF	1	104.6227284	7.33040433	56.6256400	0.16373090
Cubic SSF	1	0.0369729	1.28128147	1.7514988	10.63494985
None vs SSF	1	340.0832000	8.74316806	239.8415014	19.34420000
C.V., %		10.61	13.11	12.52	3.98

Appendix Table 49

Pigs means for average daily gross energy intake, excretion, absorption, and digestibility (Experiment 3).

Pen	TRT	BLK	Intake kcal/d	Feces kcal/d	Absorbed kcal/d	Digestibility %
1	A	1	3719.21	953.41	2765.80	74.37
2	C	1	3713.86	980.51	2733.35	73.60
3	B	1	4205.72	1031.09	3174.64	75.48
4	D	1	4042.86	1065.12	2977.73	73.65
5	C	2	4071.90	1129.83	2942.07	72.25
6	D	2	4055.86	817.91	3237.95	79.83
7	B	2	4710.87	1287.17	3423.69	72.68
8	A	2	4307.53	1127.94	3179.59	73.81
9	D	3	4056.92	951.72	3105.20	76.54
10	A	3	5933.74	1228.90	4704.84	79.29
11	C	3	3641.45	1006.76	2634.69	72.35
12	B	3	4736.81	1166.24	3570.57	75.38
13	C	4	5619.73	1286.25	4333.47	77.11
14	D	4	4816.30	1235.66	3580.64	74.34
15	B	4	4753.65	1042.65	3711.00	78.07
16	A	4	5027.66	1162.95	3864.71	76.87
17	D	5	5215.49	1040.29	4175.20	80.05
18	B	5	4460.27	1065.63	3394.65	76.11
19	A	5	4903.28	1320.35	3582.92	73.07
20	C	5	5062.09	1135.49	3926.60	77.57
21	B	6	4585.75	958.56	3627.19	79.10
22	C	6	4893.95	952.70	3941.25	80.53
23	A	6	5393.27	1210.09	4183.18	77.56

Trt A: Control diet
Trt B: Control diet + SSF 250 PU/kg
Trt C: Control diet + SSF 500 PU/kg
Trt D: Control diet + SSF 1,000 PU/kg

Appendix Table 50

Analysis of variance for average daily gross energy intake, excretion, absorption, and digestibility (Experiment 3)

Source	df	Mean Squares			
		Intake kcal/d	Feces kcal/d	Absorbed kcal/d	Digestibility %
Total	23				
Error	15	240261.299	12727.4648	192380.985	5.3981578
Repetition	5	175480.494	21596.4820	133762.848	7.77602667
Treatment	3	1332695.312	26366.0476	1018694.131	11.16221111
Linear SSF	1	2770353.735	28792.44904	2234285.080	27.33131895
Quadratic SSF	1	1221660.633	44786.94622	798625.769	1.12491544
Cubic SSF	1	6073.808	5518.80523	23172.999	5.03038908
None vs SSF	1	3353303.258	77448.01650	2411515.450	12.40020000
C.V., %		10.62	10.30	12.46	3.05

VITA (2)

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