NUTRIENT DIGESTIBILITY AND PERFORMANCE OF WEANLING PIGS FED DIETS CONTAINING FERMENTED SOYBEAN MEAL AND PHYTASE

Laura Merriman

50 Pages May 2011

This thesis investigated the growth performance and nutrient digestibility of weanling piglets fed diets containing fermented soybean meal and supplemental phytase.

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This thesis was an investigation of the use of fermented soybean meal and the application of supplemental phytase to diets fed to weaned piglets. Element emissions from animals fed plant-based diets have contributed to nutrient overload in surface waters, resulting in algal bloom. Detrimental consequences to the environment such as oxygen depletion, compromised natural habitat, and decreased water quality may result from excess phosphorus supplementation in swine diets. Consequently, research was conducted through the use of weaned pig feeding trials to assess nutrient excretions of nitrogen and phosphorus that can accumulate in local water sources.

First, a metabolism trial was conducted to determine if any differences exist in the dry matter, nitrogen, and energy digestibility, as well as phosphorus excretion of weaned piglets fed either a conventional diet consisting of corn and solvent extracted soybean meal or a modified conventional diet replacing seven percent of the soybean constituent with fermented soybean meal. Fermented soybean meal is known for its ability to make phytate-bound phosphorus more readily available to weanling pigs. Results of this study

suggested that the utilization of fermented soybean meal at seven percent of the total diet had no effect on digestibility values or phosphorus excretion in weanling pigs.

A second trial was conducted to evaluate the growth performance of weanling pigs fed the same control diet in experiment 1 compared to three varied diets containing fermented soybean meal and phytase. Results from this second study suggest that early application of phytase with or without fermented soybean meal improves growth performance and encourages early feed consumption in weaned pigs.

These trials indicated that increasing the availability of phosphorus from the phytate found naturally in plant seeds is beneficial for pig growth. The first trial revealed that fermented soybean meal has no detrimental effects on digestibility of dry matter, nitrogen, or phosphorus, and no effect on energy metabolism. The second trial was effective in demonstrating that fermented soybean meal and phytase, either alone or in combination, improves feed efficiency during the first week following weaning compared to a conventional corn-soybean diet. The results suggest that further research on diet formulation utilizing alternative ingredients and feed additives to reduce phosphorus excretion from weaned pigs is warranted.

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LAURA MERRIMAN

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LAURA MERRIMAN

THESIS APPROVED:

Date	Kerry Tudor, Chair
Date	Jonathan P. Holt
Date	Paul Walker

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L.A.M

CONTENTS

TABLES

CHAPTER I

LITERATURE REVIEW

Feral piglets gradually wean from mother's milk to solid feeds between eight and twenty weeks of age (Jensen and Recen, 1989). The piglets are exposed to solid feeds early, but the gradual weaning allows time for the gut to mature. Commercially raised piglets are not provided this same lengthy transition period. While some facilities provide creep feeding, which offers exposure to solid feeds, weaning is often an abrupt change from the farrowing room to the nursery room (Pajor et al., 1991; Puppe and Tuchscherer, 2000). Transportation to a new environment where feed in the form of a solid is separate from water means the feed supply has changed from mothers' readily available source to foreign material unknown to the piglet. In addition, the piglet must adapt to change in ingesta digestibility. To compound the problem, transformation immunity to diseases naturally provided by mothers' milk is no longer available, making the weanling vulnerable to illnesses while it matures.

Newly weaned piglets must adapt immediately to diet change and consume adequate feed and nutrients to not only maintain their health status, but also encourage substantial early growth. It is commonly accepted that a growth depression in feed consumption occurs shortly after weaning because adaptability to the non-maternal feed source requires time (Bark et al., 1986). Pluske et al. (1996) found that a decline in feed consumption is connected with gastrointestinal tract atrophy and an elevated sensitivity to inflammation in the immature pig. Often pigs that demonstrate a decline in feed intake following weaning are prone to inabilities to tolerate proteins found in legumes, resulting in diarrhea (McCracken et al, 1999).

Animal proteins have proven to be the best protein source for diets fed to young pigs. Animal proteins are highly digestible (Kim and Easter, 2001; Gottlob et al., 2006), palatable (Hansen et al., 1993; Ermer et al., 1994), and contain macronutrients and minerals in a form that are biologically usable for pigs. In addition, animal proteins contain very low or no anti-nutritional factors (Liener, 1981; Li et al., 1991; Anderson and Wolf, 1995), which makes animal proteins superior to plant-based proteins. Animal proteins are also advantageous for swine diets because they may contain immune stimulating components. Recently, the prices of animal proteins have risen dramatically, leading to a search for alternative feed ingredients dictated by least cost diet formulation. Due to the discovery of Vitamin B and the common practice of supplementing vitamins, high priced animal proteins are no longer required in swine diets. Plant-based diets have become the preferred alternative because of the widespread availability to United States swine producers.

Due to increased costs of animal-based feeds and concern for potential zoonotic diseases that can occur with animal protein based feeds, plant-based diets are the leading protein sources in swine rations (Baker, 2000). Soybeans are the ideal plant source in these feeds for many reasons. First, they are consistent in quality and composition, and an excellent source of lysine, the first limiting amino acid in swine (NRC, 1998). In addition, soybeans, in combination with cereal grains, provide a diet that offers a desirable amino acid profile and requires very little supplementation. However, young piglets express a transient hypersensitivity to soybean (Hong et al., 2004) due to an immature gut that results in poor absorption of nutrients, deterioration of the intestinal villi, and crypt hyperplasia (Li et al., 1990). The result is reduced nutrient absorption. The primary storage proteins found in soybeans, glycinin and β-conglycinin, are antigens responsible for the transient hypersensitivity in the young piglet (Holzhauser et al., 2009; Li et al., 1990, Li et al., 1991). The initial response occurs between the third and fourth day of ingestion of the proteins, with symptoms abating between seven to ten days after initiation of the feed (Stokes et al., 1986). Introducing soybean proteins through introductory creep feeding reduces transient hypersensitivity (Bruininx et al., 2002a; English, 1981). However, some studies suggest that piglets weaned prior to twenty-five days of age do not achieve an oral tolerance to the proteins found in soybeans prior to weaning (Tokach et al., 2003).

Consumption of plant-based feed by swine is limited due to plant structures called anti-nutritional factors. Anti-nutritional factors are compounds that interfere with the absorption of nutrients and include saponins, trypsin inhibitors, phytic acid, lectins, alkaloids, glucosinolates, polyphenolic compounds, and isoflavones (Anderson, 1995; de Lange et al., 2000). Phytate, or phytic acid, is the primary storage form of phosphorus and is used by the seed during germination. Phytate is a negatively charged molecule that contains six highly reactive phosphate groups that interact over a broad pH range. Because of this negative charge, phytate has a high affinity for positively charged particles such as proteins (Cheryan, 1980). Furthermore, phytate has been shown to reduce the solubility of calcium, magnesium, iron, zinc, copper, manganese, molybdenum, and cobalt by forming complexes, which results in reduced availability to the animal (Erdman, 1979; Torre et al., 1991; Anderson, 1995). The bioavailability of elements is dependent upon many factors including pH, mineral concentration, phytate concentration, and the interactions of phytate with the macronutrients (Cheryan, 1980; Torre et al., 1991).

Phosphorus & the Young Pig

Phosphorus is the second most common element required by pigs and the third most expensive (Silva et al., 2008). Phosphorus is an essential nutrient that can be found in protein. This element serves many critical functions throughout the metabolic processes of all organisms, including the pig. Some roles include energy metabolism, nucleic acid synthesis, membrane function, acid-base homeostasis, and bone mineralization. Phosphorus has a very important and close relationship with calcium. Swine diets are optimal with approximately a 1.2:1 ratio of calcium to phosphorus (Jurgens, 1974; Cromwell et al., 1979). This element plays a critical role in maintaining proper bone density and formation while also being closely related to calcium metabolism and vitamin D regulation.

In order to meet daily requirements, phosphorus is generally supplemented into swine diets with inorganic sources such as monocalcium phosphate and dicalcium phosphate. For nursery pigs (5-10kg), the requirement for phosphorus is 3.25 g/d for total phosphorus or 2.00 g/d for available phosphorus (NRC, 1998). Modern swine diets are commonly formulated for total phosphorus concentration in the feed ingredients rather than available phosphorus, resulting in formulations above the pigs' requirement. However, increased demand for inorganic phosphorus for other purposes has resulted in increased production costs. Consequently, swine producers must consider more cost effective means of supplying phosphorus in pig diets.

As a rule, only fifty-five percent of the phosphorus found in grains in their natural, unprocessed state is available for use by the animal, while the remainder is biologically unavailable as phytate (Jergens, 1974). According to the National Research Council (1998), the biological availability of phosphorus to swine is 14 % for corn (*Zea mays*) and 31 % for solvent-extracted soybean (*Glycine max*). Phosphorus is bound in the form of phytate or phytic acid in complexes that require the enzyme phytase to hydrolyze the phosphorus (Nelson et al., 1968; Lolas et al., 1976). Non-ruminants, animals with a simple stomach such as swine and chickens, do not produce significant amounts of the phytase enzyme (Taylor, 1965; Peeler, 1972; Cromwell, 1979). This suggests that non-ruminants are unable to efficiently utilize the phosphorus provided in the standard corn and soybean meal based diet. Ruminants, on the other hand, naturally produce ample amounts of phytase to utilize the phytate phosphorus because of phytase production by the microorganisms found in the rumen (Reid et al., 1947). Removing or reducing these poorly digestible components found in plant ingredients through additive ingredients or feed processing makes plant-based diets a suitable alternative to swine diets based on costly animal products.

Phosphorus & the Environment

To ensure that dietary requirements for phosphorus have been met with plant-based diets, swine producers have commonly supplemented phosphorus in excess of the NRC requirements (Wilt and Carlson, 2005). The practice of excess supplementation is not only costly, but also has negative consequences for the environment due to excess nutrients in the excreta. Eutrophication is a term used to describe the natural process where excess nutrients, specifically nitrogen and phosphorus, accumulate in surface waters. The limiting nutrient for algae growth is phosphorus. Considerable amounts of phosphorus can accumulate in water causing “eutrification,” a condition where algae grows rampant limiting the amount of oxygen available to other aquatic life forms. The blue green algae are problematic to both humans and animals because they produce toxins. Aquatic ecosystems with excess nitrogen and phosphorus lose many organisms to oxygen deprivation. While eutrophication is a natural process, the accelerated rate and degree of eutrophication that is harmful to the natural ecosystem can be blamed, in part, on excess nutrients from swine excreta (Kotak et al., 1993).

According to the United States Department of Agriculture (USDA; 2009), one hog produces approximately 10 pounds of nitrogen, 1.7 pounds of phosphorus, and 4.4 pounds of potassium in 1,200 pounds of manure before it is marketed. In the United States, swine annually excrete 460,000 tons of phosphorus (Sweeten, 1992). While waste can be used as plant fertilizer and for composting, excess nutrient application to soils is potentially harmful to the environment. Furthermore, excess phosphorus and nitrogen, as well as other nutrients, can affect the productivity of the land and contribute pollution to water sources. When minerals are supplemented at very high levels in the diet, and animal production is concentrated, the effects can be extremely detrimental to the environment. In Illinois, more than fifty percent of soil samples tested for phosphorus exceeded 50ppm, which is a dangerously high level (Sharpley et al, 2003). Many of these sites were located near water sources. Crenshaw and Johansen (1995) reported that twenty-three percent of phosphorus from animal production came directly from pigs. The average phosphorus in swine manure collected from Arkansas producers was 5.68 lb/ 1000 gallons (Daniel et al., 1998). Soils can only maintain a specific level of minerals and any amounts beyond this level migrate to the soil surface where they are easily transferred during soil and water erosion. Swine fecal waste is used primarily for corn crops at an application rate of 125 pounds/acre (USDA 2009). This would require 280 acres for only 3000 finishing pigs, significantly increasing the expenses associated with removing this waste. The industry has asked for solutions to alleviate this problem. To answer this call, researchers are investigating methods of increasing the availability of phosphorus to the pig, which will lead to lower mineral excretions.

Phytase

A proposed solution to excessive phosphorus excretion is to manipulate the ability of the pig to absorb bound phosphorus from phytate and decrease the need for phosphorus supplementation. Efforts to reduce the amount of nutrients excreted by animals have included: diet formulation, phase feeding, split sex feeding, dietary enzyme addition, reduction in feed supplements, use of growth promotants, reduced feed wastage, and feeding more highly digestible feeds (USDA, 2003). The focus of this discussion is enzyme supplementation and feed processing. Researchers are interested in improving phosphorus availability and alleviating the impacts of the anti-nutritional factors present in plants. To achieve this, diets have been supplemented with phytase. Phytase is the common name for the molecule myo-inositol hexakisphosphate phosphohydrolase, an enzyme that hydrolyzes the phytate molecule, freeing phosphorus for absorption and yielding inositol and o-phosphates (Reddy et al., 1982). Phytase has been used in both poultry and swine diets to improve digestion of phosphorus from phytate rich grains. Nelson et al. first suggested the idea of phytase supplementation in 1968. In their study, chicks were fed low available phosphorus diets that contained soybeans treated with

Aspergillus ficcum. The authors observed significant improvements in growth. Then in 1971, Nelson et al. added phytase directly to low phosphorus diets fed to chicks and found results similar to the 1968 findings. Despite phytase's success, the methods for producing the enzyme were originally inefficient and not cost effective for feeding animals. Advances in recombinant DNA technology have made phytase use more cost effective.

An enzyme is a protein that catalyzes or speeds up a chemical reaction by lowering the energy required to carry out the reaction. In the case of phytase, it is the reaction required to make phosphorus available from phytate. Various amounts of phytase are found in plants, and yeasts, fungi, and bacteria produced phytase (Patwardham, 1937). Some plants such as wheat, rye, and barley have relatively large amounts of phytase while oats, corn, and soybeans contain very small amounts of phytase (McCance and Widdowson, 1944; Mollgaard, 1946). Adding exogenous phytase during feed mixing is a convenient approach to the problem of phytates in plant-based diets from a producer's standpoint because costs and labor required are relatively low. Phytase is often reported as phytase units per kg of feed (FTU/kg), which describes the amount of phosphorus released from the feed. Phytase has been shown to reduce the amount of fecal phosphorus from finisher pigs that consume diets with low phosphorus levels (Pierce et al., 1997). Furthermore, using microbial phytase has the potential to eliminate the use of supplemental phosphorus for finisher pigs (McNaughton and Barnes, 2004; Bryant et al., 2004).

Two commercially available phytase products are Natuphos and Ronozyme, which target the third and sixth phosphate groups, respectively, and work at pH levels of 4.0 to

4.5 (Augspurger et al., 2003). OptiPhos (Enzyvia LLC, Sheridan, IN) is a commercially available brand of phytase that provides phytase at 1,000 FTU/kg. It is a recombinant enzyme produced by *Pichia pastoris* expressing the AppA2 gene from *Escherichia coli.* Optiphos is classified as a 6-phytase, which is indicative of the location where the enzyme attaches to the phytate molecule. *E. coli* phytases have proven more effective at releasing phosphorus from soybean meal than Natuphos (Rodriguez et al., 1999). *Aspergillus* phytases are commonly used for improving phosphorus availability and have greatest activity at pH levels between 5.0 and 5.5 (Simons et al., 1990). The argument has been made that yeast phytases, which work best in the pH range of 4.0 to 4.5 (Nakamura et al., 2000) are better than *Aspergillus* phytases in swine diets because the yeast phytase range falls within normal swine stomach pH levels of 3.8 (Yi and Kornegray, 1996) and 4.5 (Dintzis et al, 1995). However, Matsui et al. (2000) refuted this statement, suggesting that *Aspergillus* phytases have greater stability compared to yeast types.

Exogenous phytase supplementation has been studied extensively for use in both poultry and swine diets. Recall that the negatively charged phytate is very reactive with many minerals. With that in mind, it is not surprising that phyase supplementation can result in improved availability and retention of those minerals. Namely, phytase has been shown to enhance the availability of manganese (Schoner et al., 1991; Sobastian et al., 1996), zinc (Yi et al., 1996), and copper (Sobastian et al., 1996) to nonruminant species because of its ability to ionically chelate (Erdman, 1979). Furthermore, retention of phosphorus, calcium, copper, zinc, and manganese (Lan et al., 2002) are greater in animals supplemented with phytase. Inconsistencies in the bioavailability of some minerals have been observed across studies of phytase supplementation. However, studies have consistently indicated that phosphorus availability increases with phytase supplementation (Simons et al., 1990; Jongbloed et al., 1992; Cromwell et al., 1993; Lie et al., 1993; Kornegray and Quian, 1996). Phosphorus retention was shown to increase from 52 to 64% for pigs fed corn and soybean meal diets with phytase inclusion levels at 1,000FTU/kg (Kornegay, 1999). The addition of phytase enzymes to poultry and swine diets deficient in phosphorus has been shown to improve carcass characteristics, growth performance, and bone strength (Adeola et al., 2004; Cowieson et al., 2004; Rillai et al., 2006). With regard to growth performance traits, studies have suggested an increase average daily feed intake and an improvement in gain: feed (Jalal and Scheideler, 2001) for chickens fed corn and soybean meal diets with low levels of available phosphorus. Studies have shown that poultry consuming a corn and soybean meal diet deficient in total phosphorus exhibit the best performance with phytase supplementation levels of 12,000FTU/kg (Dilger et al., 2004; Shirley and Edwards, 2003). This level of supplementation is much higher than levels currently recommended by enzyme manufacturers. Watson et al (2006) observed greater average daily feed intake and average daily gain for chicks fed diets both adequate and deficient in available phosphorus and calcium, which the authors attributed to increased transit time through the digestive tract. Similar results have arisen from swine studies. Phytase supplementation leads to declines in required levels of energy, protein, and minerals in the diet (Zanella et al., 1999).

Feed Processing

Feed processing alters the natural composition of feed ingredients to make them more digestible in animals. Examples of feed processing include heating, extrusion, solvent extraction, and fermentation. Heat treatment is required to inactivate the anti-nutritional factors and denature the trypsin inhibitor, which is a protein that does not allow the uptake of trypsin (Anderson, 1995). Solvent extraction is used to extract or remove the oils found in soybeans. The oils are then further processed for sale to humans as vegetable oil. The byproduct of this oil production is solvent extracted soybean meal. During the extraction process, the soybeans are subjected to heat. This destroys some of the anti-nutritional factors present in raw soybeans, thus providing an excellent protein source for pigs.

Fermentation is another means of improving nutrient availability. Fermentation is a natural process that takes place in the gastrointestinal tract of both non-ruminant and ruminant animals. Fermentation in the ruminant occurs primarily in the rumen and is of benefit to the animal. Fermentation in the non-ruminant takes place primarily in the large intestine and provides few beneficial outcomes for the pig or chick. The benefits of fermentation include improved micro flora health, utilization of potentially harmful ammonia, and production of volatile fatty acids. Fermented feed ingredients have the potential to replace antibiotics (Williams, 2001). Humans have utilized fermentation to prepare food for many years. Fermentation causes bread to rise and it is required for brewing beer. Fermented soy foods consumed by humans include fermented tofu, tempeh (fermented with *Rhizopus oligosporus*), miso (fermented with *Bacillus subtilis*), soy sauces, natto (fermented with *Aspergillus oryzae*), and soy milk products (Goblitz, 1995). Miso is a soybean paste that is often combined with wheat, barley, or rice in soups in Asian countries. This product has recently been introduced into United States specialty food markets and has become a popular product for health conscious cooks (Golbitz, 1995). Tempeh (Noute and Kiers, 2005) is a fermented food that comes mostly from soybeans. This Asian soy product is popular for its flavor, texture, and digestibility.

Two types of fermentation occur, solid state and submerged. Solid state fermentation is defined as fermentation in the near absence of water compared to submerged fermentation, where an aqueous solution is used. The general process of fermenting soybeans begins with taking soybeans, often with the hulls already removed, and soaking with water. After the soybeans have been soaked, yeast, bacteria, or fungus is introduced onto the moistened soybeans. Following the introduction of the inoculant, an incubation period occurs. Depending on the final product desired, the soybeans are then dried and packaged for sale (Goblitz, 1995; Hong et al., 2004). Hong et al. (2004) fermented soybeans using *Aspergillus oryzae* GB-107 and found an increase in protein content, a reduction in trypsin inhibitors, and a reduction in the peptide size in soybeans and soybean meal. As a result, fermented foods are recommended not only for human diets, but also for use in livestock rations.

Similar to preparing human foods, fermentation has been utilized as a means to process animal feeds. By introducing phytase-producing strains of bacteria to feed ingredients through the fermentation process, the digestibility of phosphorus can be improved. The fungus *Aspergillus oryzae* is often used for the fermentation of soybean meal. *Aspergillus oryzae* has proven to be a very safe and effective means of fermenting feeds while yielding no aflotoxins. In fact, *Aspergillus oryzae* has been used extensively in processing products for human consumption. Furthermore, *Aspergillus oryzae* has been studied thoroughly, uncovering the entire genetic sequence and specific biochemical mechanisms. Jongbloed et al. (1992) used *Aspergillus niger* phytase to determine the digestibility of phosphorus. This study made use of cannulated growing pigs to determine not only if phosphorus became more available, but also where the unbound phosphorus was utilized. They found that phosphorus digestibility improved by nearly thirty percent with a reduction of phytate in both the ileum and duodenum. Cromwell et al. (1993) observed similar results with the *Aspergillus niger* phytase, finding an improvement in availability of phosphorus by a factor of three. Ilas et al. (1995) used *Aspergillus usamii* to ferment soybean meal. The authors observed that most of the phosphorus was freed in the fermented product. Further studies with poultry have suggested that *Aspergillus usamii* increased the availability of phosphorus and eliminated the need for phosphorus supplementation (Matsui et al. 1996). Evidence clearly suggests that fermented soybean products make phosphorus more available due to altered phytate levels in raw soybeans and soybean products.

Fermenting soybeans not only frees up the phosphorus for the pig, but also appears to have additional benefits for young pigs. Wang et al. (2007) and Kiers et al. (2003) concluded that fermented soybean products help the early weanling pig adjust from mother's milk to conventional weanling diets by reducing the incidence of diarrhea. Additionally, folate was found to be significantly higher with fermented soybean meal when compared to boiled soybeans (Ginting & Arcot, 2004). An increase in folate is beneficial for early development because folate has roles in cellular development and is critical for the fetus *in-utero*. Studies suggest significant improvements in overall productivity and health of swine, especially for weanling piglets that consume fermented soybean meal products. Pigs fed soybeans fermented with *Bacillus subtilis* had greater feed intake, weight gain, and feed efficiency relative to de-hulled full fat toasted soybeans. Zemora and Veum (1979) analyzed the effects of heating and fermenting soybean meal with *Aspergillus oryzae.* Their findings suggested that the addition of fermentation to the heating process resulted in an increase in feed efficiency, better utilization of energy, and greater nitrogen metabolism when fed to growing piglets. The availability of threonine, methionine, leucine and lysine was slightly greater in soybeans subjected to fermentation. Hong et al. (2004) found that the reduction in peptide size in soybean meal from fermentation can benefit newly weaned pigs that possess small amounts of gastric hydrochloric acid needed for protein digestion (Cranwell, 1985).

PepSoyGen (NutraFerma, North Sioux City, SD) is a fermented soybean meal product commercially available to swine producers. This product is being used as a protein source for weanling pigs. PepSoyGen is the product of solid state fermentation of soybean meal in the presence of *Apergillus oryzae* and *Bacillus subtilis*. PepSoyGen has been the subject of many research projects. According to company product information, PepSoyGen has at least 52.0% crude protein with a maximum of 7.5% crude ash, 6.0% crude fiber, and 12.0% moisture. Furthermore, product benefits include an increase in feed intake, reduced peptide size, low anti-nutritional factors, reduced diarrhea, longer shelf life, undetectable levels of trypsin inhibitor, stachyose, raffinose, and beneficial microbial enzymes. Nutraferma© claims that weaned piglets fed PepSoyGen perform similar to pigs fed diets with fishmeal or dried skim milk. The recommended inclusion rate for PepSoyGen is 3% to 8% of the total diet. The manufacturing of this product takes place by soaking de-hulled soybean meal and then subjecting it to low temperature sterilization. At that point, the soybeans are inoculated by micro-organisms. Fermentation occurs and microbial enzymatic hydrolysis occurs. This product is then dried at low temperatures and packaged for sale.

Research has suggested that PepSoyGen reduces antigens and anti-nutritional factors. In addition, oligosaccharides and sugars are removed through the fermentation process (Hong et al., 2004; Yang et al., 2007; Pahm, 2008). A reduction in peptide size is observed in PepSoyGen proteins when compared to conventional soybeans due to the hydrolyzation that occurs during fermentation (Hong et al., 2004). Reducing the peptide size increases the surface area of the feed, thus increasing absorption capacity in the gastrointestinal tract. Kim et al. (2010) observed 40% destruction of the large polypeptides, conglycinin and β-conglycinin, present within the soybeans after fermentation with *Aspergillus oryzae*. They also observed a reduction in phytic acid by 35% in the fermented soybean meal when compared to the unfermented soybean meal.

Producers are looking for feed that satisfies nutrient requirements at a relatively low cost while providing consistent weight gain in a healthy pig. The swine industry is also seeking feeds that result in decreased excretion nitrogen and phosphorus. Research is attempting to show that utilization of phytase and/or fermented soybean meal increases the absorption of plant phosphorus by the developing pig. This would decrease the need for excess phosphorus supplementation; thereby decreasing excreted nutrients.

CHAPTER II

NUTRIENT DIGESTIBILITY AND PERFORMANCE OF WEANLING PIGS FED DIETS CONTAINING FERMENTED SOYBEAN MEAL AND PHYTASE*

Abstract

The inclusion of fermented soybean meal and the addition of enzymes during mixing may reduce nutrient excretion while maintaining weaned pig performance. Two experiments were conducted to determine the effects of replacing conventional soybean meal with fermented soybean meal on growth performance and digestibility in weanling pigs. In the metabolism experiment, barrows (n=14, BW 9.35 ± 0.16 kg) were placed into metabolism crates and randomly assigned to one of two experimental diets containing either conventional soybean meal (CON) or fermented soybean meal (FSBM) at 7 percent inclusion of the diet on an as fed basis. Experimental diets were fed in 2 phases. Total urine and feces were collected during each phase for 3 days following a 7-day adjustment period. Gross Energy of feed, feces, and urine was determined by bomb calorimetry, and N was determined using the combustion method. No differences ($P >$ 0.05) were observed in DM digestibility, N digestibility, or DE of pigs fed either phase of the experimental diets. Experiment 2 was a growth assay with 328 pigs (BW 6.28 ± 0.06 kg) blocked by weight and sex. Pigs were randomly assigned within block to 44 nursery pens and fed one of four experimental diets for 28 days. Diets consisted of a standard corn/soy diet (CON), a diet containing 6% inclusion of fermented soybean meal (PSG), a CON + phytase diet (PHY), and a PSG + phytase diet (PP). Diets were fed in 3 phases. Pig weights and feed disappearance (kg) were measured weekly to determine ADG, ADFI, and G:F. Average daily gain (kg) of pigs consuming PHY (0.16kg) and PP (0.15) was greater ($P < 0.05$) compared to PSG (0.11) or CON (0.09) during phase 1. Average daily feed intake (kg) of pigs fed PHY (.44) was greater ($P < 0.05$) than CON (0.39), PP (0.42), or PSG (0.37) during phase 2. No differences in ADG, ADFI, or G:F were observed during phase 3. Diets containing phytase promoted increased ADG and ADFI during the early nursery stage. Fermented soybean meal is a suitable protein source for weaned pigs when used as a partial replacement for conventional soybean meal.

Keywords: Phytase, swine, fermented soybean meal

Introduction

The biological availability of phosphorus (P) to swine is 14 % for corn (*Zea mays*) and 31 % for solvent extracted soybean meal (*Glycine max*) (NRC, 1998). P in plants is bound in phytate complexes that require the enzyme phytase to hydrolyze the phytate molecule and release the P (Nelson et al., 1968; Lolas et al., 1976). Non-ruminants do not produce sufficient amounts of phytase to break down phytate bound P in a plant diet (Cromwell, 1979). To ensure that dietary requirements for P have been met, swine producers typically supplement P in excess of the NRC requirements (Wilt and Carlson, 2005). This practice has negative consequences for the environment because excess nutrients are not absorbed by the animals and are excreted in the feces.

Including exogenous phytase during feed mixing aids swine in the digestion of phytate rich grains. The enzyme, naturally found in yeasts, fungi, and bacteria, hydrolyzes the phytate molecule into a more available form of P. Phytase has been shown to reduce the amount of fecal P in finisher pigs consuming diets with low levels of P (Pierce et al, 1997). Using microbial phytase has the potential to eliminate the use of supplemental P for finisher pigs.

Processing changes the chemical structure of feed ingredients to eliminate poorly digested and harmful compounds while improving the availability of beneficial and necessary metabolic components. Hong et al. (2004) found an improvement in the protein content of soybeans as a result of the fermentation process, suggesting a nutritional benefit for humans and animals. Zemora and Veum (1979) analyzed the effects of heating and fermenting soybean meal with *Aspergillus oryzae*. Their findings suggested that fermentation with heating resulted in an improvement in metabolizable nitrogen and utilization of energy from soybean meal when fed to growing pigs compared to unfermented whole soybeans.

The objectives of this study were to 1) determine the nutrient digestibility of diets with or without fermented soybean meal fed to weaned pigs; 2) quantify P excretion of weaned pigs fed diets containing fermented soybean meal; and 3) assess growth performance of weaned pigs fed diets containing fermented soybean meal and phytase.

Materials & Methods

General. All experiments were performed with approval of and conducted according to the standards of the Illinois State University Institutional Animal Care and Use Committee (IACUC). Both experiments were conducted at the Illinois State University Farm at Lexington swine research facility.

Experiment 1

Animal Management. Fourteen crossbred barrows (initial BW 9.35kg ± 0.16kg) of Chester White, Yorkshire, and Duroc cross from the Illinois State University Farm were utilized in experiment 1. Upon weaning, piglets were moved from the farrowing room to the nursery where they were provided a common pre-starter pellet for four days. On the fourth day, fourteen barrows with similar weights were selected and randomly assigned to 14 metabolism cages. Pigs were individually housed in raised metabolism cages (0.8m x 0.71m) that allowed for total urine and fecal collection. Two rooms at the opposite ends of one facility were used to house the pigs. Both rooms were environmentally controlled with 24-hour light period. Treatment allocation was random and littermates received different experimental treatments.

Table 1. Composition of Diets Fed to Pigs in Experiment 1 Table should follow reference

	Treatment			
	Phase 1		Phase 2	
Ingredient, %	CON	FSBM	CON	FSBM
Corn	64.1	64.1	66.7	66.7
Soybean Meal	28.1	21.1	29.3	22.3
PepSoyGen	0.0	7.0	0.0	7.0
Vit/Min Premix[1]	7.5	7.5	3.8	3.8
Chromic Oxide	0.3	0.3	0.3	0.3
Nutrients Analyzed				
ME kcal/kg	3476.5	3337.6	3487.5	3370.7
CP, %	25.4	22.7	22.9	23.6
Calcium (%)	0.7	0.8	0.7	0.9
Phosphorus (%)	0.7	0.7	0.7	0.7

[1] Vitamin Mineral Premix offered proprietary amounts of Calcium, Phosphorus, Magnesium, Potassium, sodium, zinc, copper, Manganese, Iodine, Selenium, Iron, Molybdenum, Chlorine, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin B12, Biotin, Choline, Folate, Niacin, Pantothenic Acid, Vitamin B6, Vitamin B2, and Vitamin B1.

Experimental Treatments. Two experimental diets were formulated to contain either a conventional soybean meal (CON) diet or a fermented soybean meal (FSBM) diet (Table 1). Seven pigs were fed the CON diet, which consisted of a typical Midwest corn and soybean meal diet with a mineral premix included. The remaining seven pigs were provided the experimental diet, which included fermented soybean meal at seven percent of the diet at the expense of the conventional soybean meal. The fermented soybean meal was PepSoyGen, a commercially available product produced by Nutraferma (Sioux City, IA). Vitamins and minerals were added to the diets to meet or exceed current NRC (1998) recommendations for weanling pigs. Diets were fed in two phases, each being 14 d in length. Both the CON and the FSBM diet contained an indigestible marker, chromic oxide (Cr_2O_3), to determine the passage of the diets. Pigs were allowed *ad libitum* access to feed and water through a Rotecna® Grow Feeder Mini (Lleida, Spain).

Sample Collection. Pigs were allowed a seven d adjustment period to the experimental diets. Following this adjustment period, total urine and fecal samples were collected twice daily at twelve-hour intervals (600h and 1800h) for a period of three d (d 8, 9, and 10). In order to eliminate urinary nitrogen loss through evaporation, 50 ml of 10% hydrochloric acid was added to the urine collection pail prior to each twelve-hour collection period. Total urine was measured and a 20% subsample was stored at -25°C for further analysis subsequent to filtering through cheese cloth to eliminate foreign particles. Each subsample was stored in 1000 ml, NALGENE bottle (Thermo Fisher Scientific International, Rochester, NY). Samples were thawed, pooled, and homogenized prior to analysis. Total fecal matter was collected from each pen twice daily and placed into Ziploc bags using a gloved hand. Crates were equipped with screens to prevent feces from escaping the collection area or falling into the urine collection pale. Waste feed was collected daily and was allowed to dry through ambient air current. The waste feed was then weighed and used to determine actual feed consumption. At the initiation and conclusion of each phase, feeders were emptied and weights of both feed and feeders were recorded.

Laboratory Analysis. Immediately following collection, samples were stored in holding freezers at -25°C. Fecal samples were thawed and homogenized. Immediately after thawing and mixing samples, dry matter was determined by placing aliquots into containers and drying in forced air drying ovens (Iso Temp®, Fisher Scientific

International, Rochester, NY) at 50ºC until sample mass was relatively constant with less than 10% difference in moisture within a twenty-four hour period. Upon completion of the drying process, samples were ground through a 1.0 mm screen using a laboratory mill (Thomas Scientific, Model 4, USA). Samples were then analyzed for phosphorus, nitrogen, and energy. Urine samples were retrieved from the freezers and allowed to thaw to room temperature. Thawed urine samples were then further filtered using filter paper to eliminate possible contaminants. P was analyzed for each diet and feces and urine samples by first digesting and then running through the Inductively Coupled Plasma (ICP; Thermo Jarrell Ash, Waltham, Massachusettes). Nitrogen composition was established by the combustion method using a Leco FP-528 (Leco Corporation, St. Joseph, Michigan) and AOAC official method 990.03, 2006 with EDTA as the standard for feed and feces and glycine as the standard for the filtered liquid urine. The energy in the feed, feces, and urine samples was then analyzed using bomb calorimentry. First, a pellet was formed by a Pellet Press (Parr Instrument Company, Moline IL, USA) and then the pelleted sample was analyzed using bomb calorimetry (C2000 version, IKA WERKE, Staufen, Germany) to establish GE. To use bomb calorimetry for determining energy composition of the urine, the urine was first processed to allow for pellet formation. Solkafloc®, a cellulose product, was weighed and then dried in a forced draft oven at 100 °C for 24 hours. After 24 hours, it was then weighed again to determine dry matter. A 2.0 ml aliquot of urine and 0.5g of dry Sulkafloc® were placed into a 100 ml graduated cylinder and dried in a drying oven at 50 °C for 24 h. Once dried, the mixture of urine and Sulkafloc® was formed into a pellet with a pellet press (Parr Instrument Company, Moline IL, USA). A similar procedure was used for feed and feces.

All chemical analyses of urine and feces were performed in the Illinois State University Department of Agriculture nutrition laboratory. Dairyland Laboratories, Inc. (Arcadia, WI) conducted proximate analysis of experimental diets.

Experiment 2

Animal Management. Upon weaning, 322 crossbred weaned pigs (initial BW: 6.28 ± 0.06kg) were transitioned from the farrowing room to the nursery where they were blocked by sex and weight and assigned to one of four experimental diets. Pigs were housed in one of two nursery rooms, accounting for two different weaning groups, each approximately one week apart in age.

Housing. Pigs were housed in pens with an area of 2.2 square meters and plastic interlocking slatted floors. A 2-station stainless steel feeder provided feed, and a single nipple water cup provided water. Rooms were environmentally controlled with twenty-four hours of artificial lighting period per day. Room temperature was initially set at 27.2°C and then reduced by approximately .6°C per week for the four wks until a temperature of 24.2°C was reached.

Experimental Treatments. All pigs were provided a common commercial pre-starter for four days. Experimental diets consisted of a standard corn/soy diet (CON), a diet containing 6% inclusion of a commercially available fermented soybean meal (PSG), CON + phytase (PHY), or PSG + phytase (PP). A vitamin/mineral premix provided the phytase at 1,000FTU/kg. Each of the 4 experimental treatments had eleven replicates. Each diet was fed in three phases with phases lasting 7 d, 7 d, and 14 d, respectively, for a total of 28 d.

Table 2. Composition (%) and Nutrient Analysis of diets fed to pigs in Experiment 2.

	Treatment[1]											
	Phase 1				Phase 2				Phase 3			
	CON[2]	PSG[3]	PHY[4]	PP[5]	CON[2]	PSG[3]	PHY[4]	PP[5]	CON[2]	PSG[3]	PHY[4]	PP[5]
Ingredient, %												
Corn	49.59	49.59	49.37	49.37	62.32	62.32	63.32	63.32	63.17	63.17	63.38	63.38
Soybean Meal	28.46	22.46	30.63	24.63	27.67	21.67	29.18	23.18	32.94	26.94	33.62	27.62
Limestone	1.02	1.02			1.21	1.21			0.14	0.14		
Mono-cal	0.93	0.93			1.30	1.30						
Vit/Min Premix[6]	20.00	20.00	20.00	20.00	7.50	7.50	7.50	7.50	3.75	3.75	3.00	3.00
PepSoyGen		6.00		6.00		6.00		6.00		6.00		6.00
Analyzed Values												
ME kcal/kg	3542.63	3498.54	3447.84	3463.27	3372.89	3516.18	3520.59	3505.16	3522.79	3443.43	3516.18	3529.40
CP, %	26.90	27.21	25.94	26.38	23.60	24.88	23.79	23.45	22.99	23.52	23.67	22.99
Calcium (%)	0.83	0.92	0.91	0.95	0.89	0.87	0.82	0.82	0.67	0.91	0.64	0.78
Phosphorus (%)	0.73	0.72	0.74	0.79	0.77	0.79	0.64	0.61	0.68	.072	0.60	0.63

[1]Phase 1 was fed during wk 1, phase 2 was fed during wk 2, and phase 3 was fed during wk 3 & 4.
[2]CON = control group fed a diet containing no fermented SBM or phytase.
[3]PSG = test group fed diet containining fermented SBM at 7% of the diet.
[4]PHY = test group fed diet containing phytase at 1000FTU/kg of feed
[5]PP = test group fed diet containing fermented SBM at 7% of the diet and phytase at 1000 FTU/kg of feed
[6]Vitamin Premix offered proprietary amounts of: Calcium, Phosphorus, Magnesium, Potassium, sodium, zinc, copper, Manganese, Iodine, Selenium, Iron, Molybdenum, Chlorine, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin B12, Biotin, Choline, Folate, Niacin, Pantothenic Acid, Vitamin B6, Vitamin B2, and Vitamin B1.

All experimental diets were formulated to meet or exceed NRC requirements for energy and lysine. All pigs were allowed *ad libitum* access to water and feed. Pig BW and feed disappearance were recorded weekly (d 0, d 7, d 14, d 21, and d 28). Growth performance was determined by calculating ADFI, ADG, and G:F.

Statistical Analysis

Data from experiment 1 were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data were analyzed as a completely randomized design two-way factorial analysis of variance. Dry matter, nitrogen, and energy digestibility, plus phosphorus excretion were analyzed by least squares ANOVA. The statistical model included effects of diet, room, and the interaction between room and diet. Tukey's test was used to correct the alpha level of multiple statistical tests. The pig was the experimental unit for all analyses, and the alpha level was set at 0.05 to assess significance among means. All means are reported as least squares means.

The data in Experiment 2 were analyzed as a completely randomized block design using PROC GLM (SAS Institute Inc., Cary, NC) as an ANOVA. Treatment means for ADFI, ADG, and G: F were compared using LS MEANS statement with differences assessed using the PDIFF option. Repeated measures (MANOVA) were performed on all three growth performance measurements over the four weeks. The repeated measures assignment created a split plot design with sex, trial, diet treatment, and all significant interactions as the whole plot design. The subplot was then created with the main effects and significant interactions against the designated time. Insignificant interactions were eliminated from the model. Contrasts were then reported to determine differences over time. A factorial ANOVA was performed using the GLM procedure to assess differences in dietary treatment for the third phase and for the overall trial. The initial statistical models for analyses included the main effects of trial, sex, dietary treatment, and all interactions. Insignificant interactions were removed from the model and those factors were pooled into the residual error term. The pen, presented as the average of the pigs within the pen, was the experimental unit for all analyses. The alpha level was set at 0.05 to assess significance among least squares means.

Results & Discussion

Experiment 1

The level of feed wastage in this study appeared to be very high. Water was provided to the pig by a nipple that was placed inside the feeder, and it was noted that some pigs used water excessively. This left wet feed in the bottom of the feeders. After this unconsumed, wet feed was collected, it was allowed to dry at ambient temperature, and weights were recorded when feed appeared to be dry. It is possible that some of the unconsumed feed never fully dried. This could have caused variation in the feed intake values, which would have affected differences between CON and FSBM.

The current study revealed no difference ($P > 0.05$) in P excretion between CON and FSBM. P excretion in phase one was 807 ± 305 ppb for CON and 923 ± 349 ppb for FSBM. In the second phase, no difference ($P > 0.05$) in P excretion was observed between CON (21,566 ± 8,151 ppb) and FSBM (22,666 ± 8,567 ppb). However, P excretion was higher ($P < 0.05$) in the second phase (22116.4 ± 764.80 ppb) when compared to the first phase (864.8 ± 86.05 ppb) regardless of treatment. The higher levels of excretion in the second phase were likely due to over supplementation of P in the phase 2 diets.

All diets in this study were formulated using total P because an available P value was not available for the commercial fermented soybean product at the time the diets were formulated. The results of this study suggest that an available P value must be determined to more accurately formulate diets with this ingredient. Diets formulated on available phosphorus would more precisely meet the needs of the pig, thus reducing excess phosphorus in the diet and theoretically reducing phosphorus excretion.

Digestibilities of DM, N, and energy were not different ($P > 0.05$) between CON and FSBM diets during phase 1 of the experimental period, but there was a trend ($P < 0.10$) toward higher digestibility values for the CON diet for DM, N, and energy during phase 2. This trend toward greater digestibilities for pigs fed the CON diet in the second phase is likely due to greater ME being supplied by the CON diets, which could have led to a decline in ADFI. Generally, as ADFI decreases, the digestibility of nutrients increases. The CON pigs consumed smaller quantities of feed, which could explain the trend toward higher digestibilities. Digestibilty results are summarized in Table 3.

Differences may not have been observed in the digestibility of N during the first phase because the CON diet was higher in crude protein (25.4%) compared to the FSBM diet (22.7%) (Table1). The results of this study are consistent with findings by Zamora and Veum (1988). In that study, neonate piglets fed soybeans fermented with *Aspergillus oryzae* or *Rhizopus oligosporus* had protein and energy digestibilities that were similar to those of pigs fed soybeans, heated soybeans, or dried skim milk. Other researchers, however, have observed improvements in energy and nitrogen digestibility after feeding fermented soybeans to growing pigs (Zamora and Veum, 1979).

Table 3. LSMeans (±SE) of Dry Matter, Energy, and Nitrogen Digestibility of Experimental Diets Fed to Weaned Pigs.

	Treatment[1]		
Digestibility	CON[2]	FSBM[3]	P Value
Dry Matter (%)			
Phase 1	94.7 ± 0.4	94.8 ± 0.9	0.87
Phase 2	95.2 ± 1.6	91.3 ± 1.6	0.06
Energy (%)			
Phase 1	94.4 ± 1.1	94.7 ± 2.6	0.81
Phase 2	94.9 ± 1.5	90.7 ± 7.6	0.06
Nitrogen (%)			
Phase 1	93.2 ± 1.4	93.0 ± 2.7	0.89
Phase 2	93.4 ± 1.2	88.6 ± 9.8	0.1

[1]Phase 1 was feed during wk 1 and wk 2. Phase 2 was fed in wk 3 and wk 4.
[2]CON= diet without the fermented soybean meal
[3]FSBM= diet containing fermented soybean meal at 7% of the diet

Diets were formulated to contain a Ca:P ratio of approximately 1.01:1.00. However, after diets were analyzed, the Ca: P ratio was 1.03 for the phase 1 CON diet, 1.01 for the phase 1 FSBM Diet, 1.04 for the phase 2 CON diet, and 1.32 for the phase 2 FSBM diet. High Ca:P ratios have been shown to cause a reduction in growth. This difference in diet composition may be due to variation within the commercial fermented soybean product. Nitrogen retention has been shown to be affected by dietary levels of calcium and phosphorus. High calcium to phosphorus ratios may lead to a reduction in the retention of nitrogen (Vipperman et al., 1974). In this study, digestibility of nitrogen may have been affected by inconsistent ratios of calcium and phosphorus levels.

Experiment 2

Growth performance data (ADFI, ADG, G/F) for pigs fed in experiment 2 are reported in Table 4. Initial BW of pigs fed the PHY diet was greater compared to pigs fed the CON and PSG diets, with PP being intermediate. An interaction between sex, trial, and time was significant ($P < 0.05$) during all 4 wks of experiment 2 (Table 5). Initial BW of gilts from trial 2 were greater ($P < 0.05$) than BW of gilts from trial 1, which were greater than barrows from either trial 1 or trial 2.

Studies have suggested that exposure to soybeans prior to weaning may alleviate the sensitivity expressed by the young piglet to the soybean proteins (Bruininx et al., 2002a; English, 1981). All pigs in this study were offered a common pre-starter feed for 4 d following weaning. It is possible that this early exposure to soybean proteins contributed to the lack of differences in growth between the control and fermented soybean meal diet.

Results from the present growth trial showed that fermented soybean meal significantly improved the feed efficiency of piglets when compared to the control diet. Similar growth promoting results were reported by Liu et al. (2007), who observed an 8.33% increase in ADG and a 5.56% increase in feed conversion for pigs fed soybeans fermented with *Aspergillus oryzae* in place of conventional soybean meal. Several other studies have suggested an improvement in growth performance in pigs fed supplemental phosphorus (Kies et al., 2006; Veum et al., 2006; Lei et al., 1993). Liu et al. (2007) observed a decrease in Immunoglobulin G, which they suggest resulted from the denaturing of soybean antigens and a reduction in the intensity of the immune response. In the current trial, however, FSBM was included at only 6%.

Feng et al. (2007) conducted a study to determine the growth performance and digestibility of weaned pigs fed *Aspergillus oryzae* fermented soybean meal at 24.5% of the diet. In that study, sixty crossbred piglets were fed fermented soybean meal after weaning, and the results showed an improvement in ADG and G: F by pigs fed the fermented soy diet over the control diet. In addition, the pigs fed the fermented diet had greater dry matter, protein and energy digestibility values. Unlike the present study, these piglets were placed on trial after a thirty-five day weaning age, rather than the twenty day weaning age used in this study. Therefore, the piglets in the present study may not have developed an oral tolerance to the proteins present within the soybean by weaning time. In this study, pigs consuming the PSG diet might have had improved feed efficiency in the first week compared to pigs fed the CON diet because fermentation has been shown to reduce the levels of soy proteins that elicit the transient hypersensitivity response.

In a similar study, Kim et al. (2010) assessed the growth performance of weaned pigs fed experimental diets that contained various levels of soybeans fermented with *Aspergillus oryzae*. In one experiment, ADG from the diet with 3% FSBM was superior ($P < 0.05$) to the control (standard corn and soybean meal mixture), whereas ADG from the diet with 6% FSBM was intermediate during the first week. There were no differences during the other weeks. Pigs fed the 6% FSBM diet had greater ($P < 0.05$) ADFI when compared to the control during wk 2 and wk 3. As a result, feed efficiency was superior ($P < 0.05$) in the 6% FSBM diet over the control during wk 1, wk 2, wk 3, and the duration of the trial. In the same study, a second experiment assessed the growth performance of pigs fed FSBM at 3%, 6%, and 9% of the diet. Both the ADFI and ADG of pigs consuming the 9% FSBM diet were greater than the control during wk 1 and wk 2 of the trial.

Jongbloed (1987) determined that none of the phosphorus bound as phytic acid in corn and soybean meal diets was absorbed by the growing pig. Studies have shown that the addition of phytase to swine and poultry diets significantly affects growth performance and digestibility of diets containing low levels of phosphorus. Phytase added to phosphorus deficient diets has been shown to match or even outperform diets without phytase (Brana et al., 2006). It is well accepted that phytase improves phosphorus availability for both swine and poultry. In general, the improvements in growth observed with phytase supplemented diets suggest that the phytic acid is hydrolyzed and more phosphorus is available. The improvements in ADG and G: F associated with phytase supplementation can be partially attributed to the release of minerals from phytate complexes, utilization of the released phytate components, and increased starch digestibility (Crawley and Mitchell, 1968; Knuckles and Betschart, 1987).

It is interesting that no additive effects were observed for phytase and fermented soybean meal in the current study. In general, the inclusion of phytase was the primary factor that enhanced growth in this study given that pigs fed the PHY only diet performed numerically, though not statistically better, than pigs fed the combination diet. Because diets that contained phytase showed significant improvements over the control and fermented only diets with regard to growth, phytase is considered the primary contributor to growth improvements.

With regard to growth, it is not known why weight gain was reduced during the third week of the experiment, but then recovered during the final week. The lag in growth might have been related to the hypersensitivity of the young piglet, though this would have been more likely to have occurred during the first week with recovery during the second week of trial. The ADFI was consistently rising throughout the four weeks, eliminating the possibility that the pigs were challenged by disease and went off feed. A trial by sex by time interaction was significant for growth performance during the second experiment. The initial and final weights of the gilts were greater than the weights of the barrows in both trials. The significant interaction is likely due to the unexplained growth reduction that occurred during the third week of the trial for all individuals.

In experiment 2, all diets were formulated to contain a Ca: P ratio of approximately 1.1:1. Studies have consistently shown that growth performance may be reduced by a wide Ca: P ratio (Lei et al., 1994; Quian et al., 1996; Liu et al., 1996) . Quian et al. (1996) observed a reduction in growth performance of weanling piglets when Ca: P ratios were increased from 1.1 to 1.6:1, and then to 2:1. The ratios of Ca: P in the present study were in the range of 1.3:1 to 1.0:1. These varying values of Ca: P may offer a partial explanation for the minimal growth response observed by pigs fed the PSG diet during phase 1 and phase 3.

Table 4. Growth performance of nursery pigs fed diets containing fermented soybean meal and phytase in Experiment 2.

	Dietary Treatment					
	CON[1]	PSG[2]	PHY[3]	PP[4]	*P* value	SEM[5]
n	81	80	81	80		
Initial BW	6.90 ± 0.14	7.05 ± 0.15	7.38 ± 0.15	7.30 ± 0.15		
Final BW	14.86 ± 0.26	14.50 ± 0.29	15.26 ± 0.27	15.33 ± 0.32		
ADFI						
wk 1	0.22	0.21	0.24	0.24	0.12	0.01
wk 2	0.39^{AB}	0.37^{B}	0.44^{A}	0.42^{AB}	0.02	0.02
wk 3	0.55	0.53	0.55	0.56	0.83	0.02
wk 4	0.79	0.73	0.75	0.76	0.63	0.03
wk 3 & 4	0.67	0.63	0.65	0.65	0.71	0.02
Overall	0.63	0.45	0.45	0.49	0.37	0.02
ADG						
wk 1	0.09^{B}	0.11^{B}	0.16^{A}	0.15^{A}	<0.01	0.02
wk 2	0.27	0.26	0.30	0.29	0.13	0.02
wk 3	0.34	0.35	0.31	0.33	0.21	0.01
wk 4	0.53	0.47	0.52	0.53	0.06	0.02
wk 3 & 4	0.44	0.41	0.42	0.43	0.58	0.01
Overall	0.30	0.29	0.31	0.31	0.22	0.01
G:F						
wk 1	0.39^{C}	0.52^{B}	0.64^{A}	0.62^{A}	<0.01	0.03
wk 2	0.68	0.70	0.69	0.69	0.97	0.02
wk 3	0.61^{AB}	0.67^{A}	0.56^{B}	0.59^{AB}	0.01	0.02
wk 4	0.68	0.64	0.70	0.70	0.09	0.02
wk 3 & 4	0.65	0.66	0.64	0.66	0.79	0.02
Overall	0.59^{B}	0.63^{AB}	0.64^{AB}	$.65^{A}$	0.03	0.02

[1]CON = control group fed a diet containing no fermented SBM or phytase.
[2]PSG = test group fed diet containining fermented SBM at 7% of the diet.
[3]PHY = test group fed diet containing phytase at 1000FTU/kg of feed
[4]PP = test group fed diet containing fermented SBM at 7% of the diet and phytase at 1000 FTU/kg of feed
[5]Pooled SEM
[ABC] Least squares means within the same row lacking a common superscript differ ($P < 0.05$)

Table 5. Growth performance of barrows and gilts in Experiment 2.

	Trial 1		Trial 2		
	Barrows	Gilts	Barrows	Gilts	SEM[1]
n	83	92	91	56	
Initial BW	6.91 ± 0.14	7.08 ± 0.13	6.80 ± 0.14	8.23 ± 0.14	
Final BW	14.84 ± 0.26	15.10 ± 0.24	13.96 ± 0.28	16.67 ± 0.30	
ADFI					
wk 1	0.20^{B}	0.22^{B}	0.23^{B}	0.26^{A}	0.01
wk 2	0.37^{C}	0.41^{B}	0.39^{BC}	0.45^{A}	0.02
wk 3	0.56^{B}	0.61^{A}	0.48^{C}	0.56^{B}	0.02
wk 4	0.73^{B}	0.82^{A}	0.70^{B}	0.79^{A}	0.03
ADG					
wk 1	0.11^{C}	0.11^{C}	0.13^{B}	0.15^{A}	0.01
wk 2	0.25^{C}	0.26^{C}	0.28^{B}	0.32^{A}	0.01
wk 3	0.36^{A}	0.36^{A}	0.24^{B}	0.37^{A}	0.01
wk 4	0.51^{AB}	0.52^{A}	0.49^{B}	0.53^{A}	0.02
G:F					
wk 1	0.53^{B}	0.48^{C}	0.57^{A}	0.60^{A}	0.03
wk 2	0.66^{B}	0.65^{B}	0.73^{A}	0.71^{A}	0.02
wk 3	0.66^{A}	0.59^{B}	0.51^{C}	0.66^{A}	0.02
wk 4	0.70^{A}	0.65^{B}	0.70^{A}	0.67^{B}	0.02

[1]Pooled SEM

[ABC] Least squares means within the same row lacking a common superscript differ ($P < 0.05$)

Conclusions & Implications

Neither growth of pigs nor digestibility of experimental diets was hindered by the inclusion of fermented soybean meal. In fact, feed efficiency was improved by the addition of either fermented soybean meal, or phytase, or a combination of the two compared to the control diet. Therefore, fermented soybean meal is a suitable protein source for weaned pigs and can be included in the diet at up to 7% without any detrimental effects on growth performance or nutrient digestibility.

In the present study, supplemental phytase combined with fermented soybean meal at six percent of the diet significantly improved overall average daily gain during the first week post weaning and the gain to feed ratio for the entire 28 days on feed. This strategy may allow swine producers to reduce P supplementation, which may lower feed expenses and reduce the risk of environmental pollution from P. More research regarding diet formulation with alternative ingredients and feed additives to reduce phosphorus excretion of weaned pigs is warranted.

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